

17 May, 2013

Validation report for the international validation study on ROS
(Reactive Oxygen Species) assay as a test evaluating phototoxic
potential of chemicals
(Atlas Suntest version)

Conducted by:
ROS assay Validation Management Team

This version of validation report was prepared for peer review. Revisions in the text parts from the previous January 15th, 2013 version are shown in blue.

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INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING PHOTOTOXIC POTENTIAL OF CHEMICALS (VERSION 1.0)

- Attachment 2 Protocol for phase 2 study
INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING PHOTOTOXIC POTENTIAL OF CHEMICALS (ATLAS VERSION 3.02)

1. Background

The use of a reactive oxygen species (ROS) assay to detect chemicals with phototoxicity potential is expected to conform to the ICH framework.

The aim of this study was to validate the ROS assay method for between-laboratory variability and transferability in order to incorporate this assay for photoreactivity testing of drug candidates into an ICH framework. The ROS assay multi-study validation trials were undertaken in accordance with:

- i. the principles and criteria documented in the OECD No. 34 guidance document on the validation and international acceptance of new or updated test methods for hazard assessment [OECD, 2005],
- ii. the modular approach to validation [Hartung et al., 2004], and
- iii. discussions on multi-study validation trials with participation of good laboratory practice (GLP) test facilities [Cooper-Hannan et al., 1999] where the whole concept of multi-study validation trials was described in the context of GLP.

The studies part of multi-study trials should ideally be performed in accordance with GLP and should include but not necessarily be limited to the use of standard operating procedures (SOP) as well as adequate data recording, reporting, and record keeping.

A general conceptional framework [Hartung et al., 2004; OECD, 2005] was used for documenting the entire validation of a test method, which is called a “modular approach” to validation. In this approach, the information needed to support the validity of the method is organized into modules that provide the following information:

Module 1: Test definition

Module 2: Within-laboratory repeatability and reproducibility

Module 3: Between-laboratory transferability

Module 4: Between-laboratory reproducibility

Module 5: Predictive capacity

Module 6: Applicability domain

Module 7: Performance standards

The modular approach as introduced by Hartung et al., allows the use of datasets from various sources and studies, and we took advantage of this approach in assessing the scientific validity of the ROS assay.

2. Objective of the study

The multi-study validation trial assessed the reliability (reproducibility within and between laboratories) and relevance (predictive capacity) of the ROS assay with a challenging set of test chemicals for which high quality in vivo data are available.

3. Test Method

3-1. ROS assay

The ROS assay was developed by Onoue et al. [2008a] and is a high-throughput and high-performance system for predicting the phototoxic potential of pharmaceutical substances. This assay is a multiwell plate-based study using a quartz reaction container, the advantages of which include reduced sample volumes, improved assay productivity, and highly-uniform irradiation.

In this study, the generation of ROS, including superoxide and singlet oxygen was detected by spectrophotometric measurement. Singlet oxygen was measured by monitoring the bleaching of p-nitrosodimethyl aniline (RNO) at 440 nm using imidazole as a selective acceptor of singlet oxygen. Two hundred microliters of samples containing the test chemical, RNO, and imidazole were transferred to the wells of a plastic 96-well plate before light exposure. The plate was subjected to measurement of absorbance at 440 nm using a

microplate spectrophotometer. The plate was then fixed in the quartz reaction container with a quartz cover and irradiated with simulated sunlight for 1 hour. After agitation on a plate shaker, UV absorbance at 440 nm was measured. Superoxide was measured by irradiating samples containing the test chemical and nitroblue tetrazolium (NBT) with simulated sunlight for 1 hour, and then measuring the reduction in NBT by observing the increase in absorbance at 560 nm in the same manner as the singlet oxygen determination.

3-2. Sunlight simulator

An Atlas Suntest CPS series (CPS plus or CPS; Atlas Material Technology LLC, Chicago, IL, USA) equipped with a 1500-W xenon arc lamp was used for solar simulator. The irradiation test was carried out at 25°C with an irradiance of ca. 2.0 mW/cm² as determined using a calibrated UVA detector (Dr Hönle 0037, München, Germany).

4. Validation Management Structure

This validation study was performed by the Japanese Center for the Validation of Alternative Methods (JaCVAM). The management structure is shown in Figure 1.

4-1. Validation Management Team

The validation management team (VMT) comprised individuals with the collective expertise in the underlying science to perform the scientific design, management, and evaluation of this study. The VMT played a central role in overseeing the validation study, including:

- 1) Goal statement
- 2) Project plan including objective
- 3) Study protocol/amendments
- 4) Outcome of QC audits
- 5) Test chemicals
- 6) Data management procedures
- 7) Timeline/study progression
- 8) Study interpretation and conclusions
- 9) Reports and publication

Final determination of which laboratories would participate in the validation study was the responsibility of the VMT.

Members:

Hajime Kojima; JaCVAM, VMT chairperson
Kazuhiro Hosoi; Santen Pharmaceutical Co., Ltd., VMT co-chair
Satomi Onoue; University of Shizuoka, Lead laboratory
Kazuichi Nakamura; Shionogi & Co., Ltd.
Tsuguto Toda; Shionogi & Co., Ltd.
Yasuhiro Matsumoto; ASKA Pharmaceutical Co., Ltd.
Manfred Liebsch; German Centre for the Documentation and Validation of
Alternative Methods (ZEBET)
Hironori Takagi; Taisho Pharmaceutical Co., Ltd.
Naoto Osaki; Taisho Pharmaceutical Co., Ltd.
Satoru Kawakami; Asahi Kasei Pharma Co.
Valerie Zang; ECVAM
Warren Casey; ICCVAM

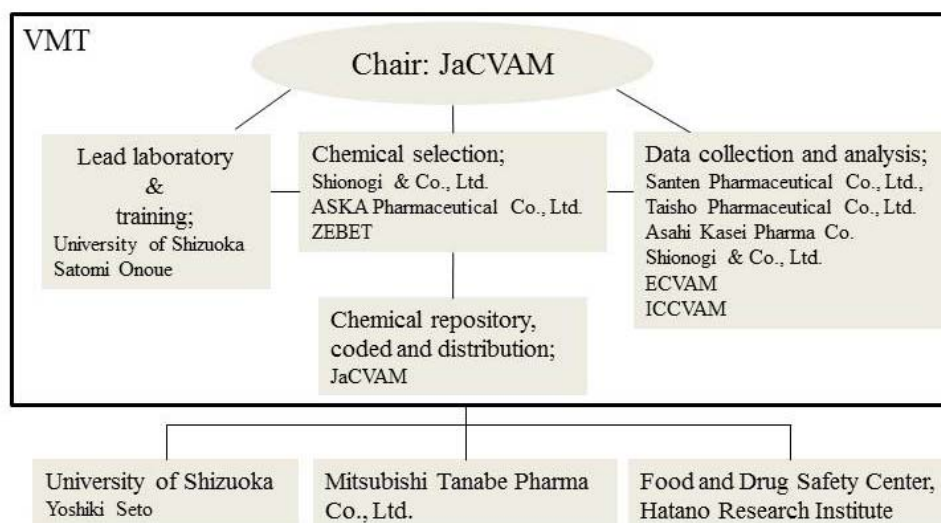


Figure 1 Management structure of the ROS assay validation study

4-2. Chemical selection, acquisition, coding and distribution

1) Definition of selection criteria

2) Chemical selection

Members:

Tsuguto Toda; Shionogi & Co., Ltd.

Yasuhiro Matsumoto; ASKA Pharmaceutical Co., Ltd.

Manfred Liebsch; ZEBET

(1) Liaise with suppliers

(2) Final check of chemicals provided

(3) Acquisition

(4) Coding

(5) Distribution

Member:

Hajime Kojima; JaCVAM

4-3. Independent biostatistician

1) Approve spreadsheets

2) Data collection

3) Data analysis

Members:

Kazuhiro Hosoi; Santen Pharmaceutical Co., Ltd.

Naoto Osaki; Taisho Pharmaceutical Co., Ltd.

Satoru Kawakami; Asahi Kasei Pharma Co.

Kazuichi Nakamura; Shionogi & Co., Ltd.

Hironori Takagi; Taisho Pharmaceutical Co., Ltd.

Valerie Zang; ECVAM

Warren Casey; ICCVAM

4-4. Participating laboratory

The laboratories participating in the study were defined as shown in Figure 1.

The following three laboratories participated in the validation study for the evaluation of the ROS assays:

- Laboratory 1: University of Shizuoka (Yoshiki Seto)
- Laboratory 2: Food and Drug Safety Center, Hatano Research Institute (Shinobu Wakuri)
- Laboratory 3: Mitsubishi Tanabe Pharma Co. (Toshinobu Yamamoto)

Although both the lead laboratory (Satomi Onoue) and Laboratory 1 (Yoshiki Seto) are located at the University of Shizuoka, Laboratory 1 participated in this validation study independently of the lead laboratory.

Each laboratory also was responsible for complying with GLP and specifying QA aspects.

5. Study Design

Before validation studies, a training course using Atlas Suntest CPS series was performed by the lead laboratory in March 2011. All technicians at each laboratory participated in this training course, which used quinine as a positive control and sulisobenzone as a negative control. After the training course, the two phases of the validation study were performed.

In the phase 1 study, within-laboratory repeatability and reproducibility were assessed using 11 new chemicals (5-fluorouracil, 8-methoxy psoralen, amiodarone, chlorpromazine, diclofenac, doxycycline, furosemide, ketoprofen, levofloxacin, norfloxacin, and omeprazole), the positive control, and the negative control (Table 1). These tests were conducted between April and May 2011 at three laboratories.

In the phase 2 study, between-laboratory reproducibility and predictivity were assessed using 42 coded chemicals, the positive control, and the negative control (Table 2). This study was conducted between August and October 2011 at the three laboratories which had participated in both the training course and the phase 1 study.

6. Test Chemicals

6-1. Chemical selection

6-1-1. Chemicals for the phase 1 study

Chemicals selected for the phase 1 study are listed in Table 1-1 and 1-2. Twelve phototoxic chemicals and one non-phototoxic chemical were used. Chemicals for the phase 1 study were not coded.

Quinine (No. I-12) was selected as positive control and sulisobenzone (No. I-13) as negative control in the ROS assay according to the method established by Onoue et al. [2008a]. Quinine HCl was classified as phototoxic positive in humans, according to the publication by Ljunggren et al [1986]. Sulisobenzone was classified as phototoxic negative, since human data for this chemical was reported as negative in the publication for the in vitro 3T3 neutral red uptake phototoxicity test (3T3NRU-PT) validation study [Spielmann et al, 1998b].

5-fluorouracil (5-FU, No. I-1) was reported as being phototoxic in humans [Dillaha et al., 1983], but was negative in the 3T3NRU-PT [Kleinman et al., 2010 and Onoue et al., 2010]. Reported causes of 5-FU phototoxicity in humans include photocytotoxicity induced by UV-B alone [Kirkup M.E. et al., 2003 and Andersen K.E. et al., 1984] and/or ROS generation triggered by UV-B induced photodegradation [Miolo G. et al., 2011]. 5-FU absorbs mainly UV-B (290–320 nm) within the range of natural sunlight (Appendix 8), so UV-B irradiation might be essential for photochemical activation of 5-FU. Therefore it was unknown whether 5-FU was phototoxic or not, and high quality human data was not available. 5-FU was selected in order to provide information on the limits of ROS assay.

The remaining 10 chemicals (Nos. I-2, I-3, I-4, I-5, I-6, I-7, I-8, I-9, I-10 and I-11) were selected from the typical phototoxic chemicals.

8-MOP (No. I-2), amiodarone HCl (No. I-3), chlorpromazine (No. I-4), doxycycline HCl (No. I-6), furosemide (No. I-7), ketoprofen (No. I-8) and norfloxacin (No. I-10) were selected from the list of phototoxic positive chemicals used in the 3T3NRU-PT validation study. Human data for these chemicals were described as phototoxic positive in the publication for 3T3NRU-PT validation study [Spielmann et al, 1994a and 1998a].

Diclofenac (No. I-5), levofloxacin (No. I-9) and omeprazole (No. I-11) were selected as phototoxic positive in humans, according to the publication by Przybilla et al [1987], Boccumini et al [2000] and Dam et al [2008], respectively.

6-1-2. Chemicals for the phase 2 study

Chemicals selected for the phase 2 study are listed in Table 2-1 and 2-2. Phototoxic positive and negative chemicals were evenly selected (approximately 1:1). Chemicals for the phase 2 study were coded.

1) Phototoxic positive chemicals

Twenty-three (23) positive chemicals (18 drugs and 5 non-drug chemicals) were selected based on the results in humans and 3T3 NRU-PT.

Twenty-one (21) chemicals (Nos. II-1, II-2, II-3, II-4, II-5, II-6, II-7, II-8, II-9, II-10, II-11, II-12, II-13, II-14, II-15, II-16, II-18, II-19, II-21, II-22 and II-23) out of the 23 positive chemicals were selected from the list of positive chemicals used in the 3T3NRU-PT validation study. Human data for these chemicals were described as being positive in the publication for 3T3 NRU-PT validation study [Spielmann et al, 1994a and 1998a].

Acridine (No. II-1) and acridine HCl (No. II-2) or nalidixic acid (II-11) and nalidixic acid (Na salt) (II-12) were assessed both as a free form and as a salt, respectively, in order to test whether the aqueous solubility of chemicals would limit the predictive power of ROS assay.

Rosiglitazone (No. II-17) was selected because 3T3 NRU-PT result was positive, but high quality human data regarding its phototoxicity was not available.

Avobenzone was reported to be photoallergy negative by a photopatch test according to the publication by Szczurko C et al. [1994] and Trevisi P et al. [1994], but was reported to have induced photoallergic reactions according to the publication by Schauder, S. et al. [1997]. Therefore, avobenzone was classified as phototoxic positive because we were not able to completely determine whether avobenzone was phototoxic negative or not.

2) Phototoxic negative chemicals

Nineteen (19) phototoxic negative chemicals (5 drugs and 14 non-drug chemicals) were selected, mainly based on the negative results of 3T3 NRU-PT, because clinical information was not available for many of the chemicals.

We searched for human data for these chemicals but were unable to find reliable phototoxicity data.

Five (5) chemicals (Nos. II-27, II-31, II-34, II-40 and II-41) were selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a and 1998a]. Negative results in humans and animals were reported for chlorhexidine (No. II-31) and PABA (II-40), respectively, in the publication for 3T3 NRU-PT validation study [Spielmann et al, 1998a].

Seven (7) chemicals (Nos. II-29, II-33, II-35, II-36, II-38, II-39 and II-42) were selected from among UV absorbers. Three (Nos. II-35, II-38 and II-39) (3) were included in the list of negative chemicals in the 3T3 NRU-PT validation study, and human data of these chemicals were described as negative in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998b]. We believed that human data for four UV absorbers (Nos. II-29, II-33, II-36 and II-42) would be provided. However, human data was not available for these

chemicals. Therefore, after confirming negative results in 3T3 NRU-PT, these chemicals were classified as phototoxic negative.

Four (4) chemicals (Nos. II-24, II-25, II-26 and II-28) were selected based on negative results in 3T3 NRU-PT, in reference to the publication by Onoue et al. [2010].

As chemical No. II-32, we were planning to register cinnamic aldehyde, the main ingredient of cinnamon oil and the ingredient of cinnamon fragrance. This chemical was used in the 3T3 NRU-PT validation study. However, when we registered the chemical name on the list, we registered cinnamic acid instead of cinnamic aldehyde. Cinnamic acid is a substance known to form a dimer by light irradiation in solid state. The difference between cinnamic aldehyde and cinnamic acid is the side chain being aldehyde or carboxyl. In addition, we conducted 3T3NRU-PT for cinnamic acid, and the result was negative, the same as cinnamic aldehyde. Therefore, we believe that the mix-up would not affect the results for the ROS assay validation study, because the structure of cinnamic acid resembles that of cinnamic aldehyde, and the 3T3 NRU-PT results are the same.

Chemical No. II-30 and II-37 were registered at first as benzylindene camphor sulphonic acid and octyl methoxycinnamate, respectively. These were UV absorbers used in the 3T3 NRU-PT validation study. However, we conducted the phase 2 study without noticing the transcription error in CAS number when we made the final chemical list. Because we noticed the error after the phase 2 study, these chemicals were classified as phototoxic negative again after confirming the negative results in 3T3 NRU-PT and low molar extinction coefficient (MEC).

6-2. Chemical coding, distribution and disclosure of code

Coding and distribution of chemicals were performed by JaCVAM. The coded chemicals were sent to the safety officer, who is not involved in the experiments, together with a sealed envelope containing the material safety data sheets (MSDS). Since the chemicals were coded, the laboratories did not know their identity and therefore all chemicals were treated as hazardous chemicals. The disclosure of codes was performed at a VMT meeting on 11 October, 2011, after the data had been finalized per QC confirmation.

Table 1-1 List of rational for chemical selection in phase 1 study

NO.	Compound	Rational for chemical selection
I-1	5-Fluorouracil (5-FU)	5-FU was reported as being phototoxic in humans [Dillaha et al., 1983], but it was negative in the 3T3NRU-PT [Kleinman et al., 2010 and Onoue et al., 2010]. Reported causes of 5-FU phototoxicity in humans include photocytotoxicity induced by UV-B alone [Kirkup M.E. et al., 2003 and Andersen K.E. et al., 1984] and/or ROS generation triggered by UV-B induced photodegradation [Miolo G. et al., 2011]. 5-FU absorbs mainly UV-B (290–320 nm) within the range of natural sunlight (Appendix 8), so UV-B irradiation might be essential for photochemical activation of 5-FU. Therefore it was unknown whether 5-FU was phototoxic or not, and high quality human data was not available. 5-FU was selected in order to provide information on the limits of the ROS assay.
I-2	8-Methoxy psoralen (8-MOP)	8-MOP was selected from the list of phototoxic positive chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as phototoxic positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
I-3	Amiodarone HCl	Amiodarone HCl was selected from the list of phototoxic positive chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as phototoxic positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
I-4	Chlorpromazine HCl	Chlorpromazine HCl was selected from the list of phototoxic positive chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as phototoxic positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
I-5	Diclofenac	Diclofenac was selected as a phototoxic positive in humans, according to the publication by Przybilla et al [1987].
I-6	Doxycycline HCl	Doxycycline HCl was selected from the list of phototoxic positive chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as phototoxic positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
I-7	Furosemide	Furosemide was selected from the list of phototoxic positive chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as phototoxic positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
I-8	Ketoprofen	Ketoprofen was selected from the list of phototoxic positive chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as phototoxic positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
I-9	Levofloxacin	Levofloxacin was selected as a phototoxicity positive in humans, according to the publication by Boccumini et al [2000].
I-10	Norfloxacin	Norfloxacin was selected from the list of phototoxic positive chemicals used in the 3T3NRU-PT validation study. Human data for this chemical was described as phototoxic positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
I-11	Omeprazole	Omeprazole was selected as a phototoxic positive in humans, according to the publication by Dam et al [2008].
I-12	Quinine HCl	Quinine HCl was selected as positive control according to the method established by Onoue et al. [2008a]. Quinine HCl was classified as a phototoxic positive in humans, according to the publication by Ljunggren et al [1986].
I-13	Sulisobenzone	Sulisobenzone was selected as negative control according to the method established by Onoue et al. [2008a]. Sulisobenzone was classed as a phototoxic negative, since human data for this chemical was reported as negative in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998b].

Table 1-2 Test chemicals for the phase 1 study

No.	Chemical name	CAS No. ^{a)}	UV/vis absorption ^{b)}		ROS assay	3T3 NRU	<i>in vivo</i>	
			MEC (L/mol/cm)	λ_{max} (nm)			Animal	Human
I-1	5-FU	51-21-8	1800 ^{c)}	290 ^{d)}	- ¹⁾	- ^{2,3)}	NA	+? ⁴⁾
I-2	8-MOP	298-81-7	3631	300	+ ¹⁾	+ ⁵⁾	+ ⁵⁾	+ ⁵⁾
I-3	Amiodarone HCl	19774-82-4	5400	290 ^{d)}	+ ³⁾	+ ⁵⁾	+ ⁵⁾	+ ⁵⁾
I-4	Chlorpromazine HCl	69-09-0	1746	304	+ ¹⁾	+ ⁵⁾	+ ⁵⁾	+ ⁵⁾
I-5	Diclofenac	15307-79-6	7800 ^{c)}	290 ^{d)}	+ ¹⁾	+ ³⁾	+ ⁶⁾	+ ⁷⁾
I-6	Doxycycline HCl	10592-13-9	3715	290 ^{d)}	+ ¹⁾	+ ⁵⁾	+ ⁵⁾	+ ⁵⁾
I-7	Furosemide	54-31-9	2650	290 ^{d)}	+ ¹⁾	+/- ^{3,8,9)}	NA	+ ⁸⁾
I-8	Ketoprofen	22071-15-4	2092	290 ^{d)}	+ ¹⁾	+ ⁸⁾	- ⁸⁾	+ ⁸⁾
I-9	Levofloxacin	100986-85-4	13000 ^{c)}	333	+ ¹⁰⁾	+ ¹⁰⁾	+ ¹¹⁾	+ ¹²⁾
I-10	Norfloxacin	70458-96-7	3562	323	+ ¹⁾	+ ³⁾	+ ⁸⁾	+ ⁸⁾
I-11	Omeprazole	73590-58-6	15000 ^{c)}	301	+ ¹⁾	+/- ³⁾	NA	+ ¹³⁾
I-12	Quinine HCl	6119-47-7	1938	330	+ ¹⁾	+ ³⁾	+ ⁶⁾	+ ¹⁴⁾
I-13	Sulisobenzone	4065-45-6	3519	290 ^{d)}	- ¹⁾	- ³⁾	NA	- ¹⁵⁾

5-FU: 5-fluorouracil, 8-MOP: 8-methoxy psoralen

+ : Positive, - : Negative, +/- : Equivocal, NA : Not available, ? : unclear

a) CAS No.: Chemical abstracts service registry number, b) The UV/vis absorbance (290-700 nm) of chemicals was measured in 20 mM phosphate buffer (pH 7.4). Test chemicals were dissolved in dimethylsulfoxide (DMSO) at 10 mM and diluted with 20 mM phosphate buffer (pH 7.4). Final concentration of DMSO was unified to 0.5%. c) Molar extinction coefficient (MEC) of 5-FU, diclofenac, levofloxacin, and omeprazole were extracted from the articles of Onoue et al. (2008a) and Seto et al. (2011).

d) λ_{max} (nm) was a wavelength at which the UV/vis absorbance shows a peak between 290 and 700 nm. λ_{max} (nm) was indicated as 290 nm in the case where the peak absorption is located below 290 nm and the maximum absorption is at 290 nm.

1) Onoue et al., 2008a, 2) Kleinman et al., 2010, 3) Onoue et al., 2010, 4) Dillaha et al., 1983, 5) Spielmann et al., 1994a, 6) Spielmann et al., 1994b, 7) Przybilla et al., 1987, 8) Spielmann et al., 1998a, 9) Peters et al., 2002, 10) Seto et al., 2011, 11) Wagai et al., 1992, 12) Boccumini et al., 2000, 13) Dam et al., 2008, 14) Ljunggren et al., 1986, 15) Spielmann et al., 1998b

Table 2-1 List of rational for chemical selection in phase 2 study

NO.	Chemical name	Rational for chemical selection
Phototoxic drugs		
II-1	Acridine	Acridine was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Acridine (No. II-1) and acridine HCl (No. II-2) were assessed both as a free form and as a salt, respectively, in order to test whether the aqueous solubility of chemicals would limit the predictive power of the ROS assay.
II-2	Acridine HCl	Acridine HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Acridine (No. II-1) and acridine HCl (No. II-2) were assessed both as a free form and as a salt, respectively, in order to test whether the aqueous solubility of chemicals would limit the predictive power of the ROS assay.
II-3	Amiodarone HCl	Amiodarone HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-4	Chlorpromazine HCl	Chlorpromazine HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-5	Doxycycline HCl	Doxycycline HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-6	Fenofibrate	Fenofibrate was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-7	Furosemide	Furosemide was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-8	Ketoprofen	Ketoprofen was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-9	6-Methylcoumarine	6-Methylcoumarine was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-10	8-MOP	8-MOP was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-11	Nalidixic acid	Nalidixic acid was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Nalidixic acid (II-11) and nalidixic acid (Na salt) (II-12) were assessed both as a free form and as a salt, respectively, in order to test whether the aqueous solubility of chemicals would limit the predictive power of the ROS assay.
II-12	Nalidixic acid (Na salt)	Nalidixic acid (Na salt) was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a]. Nalidixic acid (II-11) and nalidixic acid (Na salt) (II-12) were assessed both as a free form and as a salt, respectively, in order to test whether the aqueous solubility of chemicals would limit the predictive power of the ROS assay.
II-13	Norfloxacin	Norfloxacin was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-14	Ofloxacin	Ofloxacin was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-15	Piroxicam	Piroxicam was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].

II-16	Promethazine HCl	Promethazine HCl was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-17	Rosiglitazone	Rosiglitazone was selected because 3T3 NRU-PT result was positive, but high quality human data regarding its phototoxicity was not available.
II-18	Tetracycline	Tetracycline was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
Phototoxic non-drug chemicals		
II-19	Anthracene	Anthracene was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-20	Avobenzone	Avobenzone was reported to be photoallergy negative by a photopatch test according to the publication of Szczurko C et al. [1994] and Trevisi P et al. [1994], but was reported to have induced photoallergic reactions according to the publication by Schauder, S. et al. [1997]. Therefore, avobenzone was classified as a phototoxic positive chemical because we were not able to completely determine whether avobenzone was a phototoxic negative or not.
II-21	Bithionol	Bithionol was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-22	Hexachlorophene	Hexachlorophene was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Masuda et al., 1971 and Spielmann et al, 1998a].
II-23	Rose bengal	Rose bengal was selected from the list of positive chemicals used in the 3T3 NRU-PT validation study. Human data for this chemical was described as being positive in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
Non-phototoxic drugs		
II-24	Aspirin	Aspirin was selected based on negative results in 3T3 NRU-PT, in reference to the publication by Onoue et al. [2010].
II-25	Benzocaine	Benzocaine was selected based on negative results in 3T3 NRU-PT in reference to the publication by Onoue et al. [2010].
II-26	Erythromycin	Erythromycin was selected based on negative results in 3T3 NRU-PT in reference to the publication by Onoue et al. [2010].
II-27	Penicillin G	Penicillin G was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-28	Phenytoin	Phenytoin was selected based on negative results in 3T3 NRU-PT in reference to the publication by Onoue et al. [2010].
Non-phototoxic non-drug chemicals		
II-29	Bumetrizole	Bumetrizole was selected from among UV absorbers. We believed that human data for this UV absorber would be provided. However, human data was not available for this chemical. Therefore, after confirming negative result in 3T3 NRU-PT, this chemical was classified as a phototoxic negative chemical.
II-30	Camphor sulfonic acid	Chemical No. II-30 was registered at first as benzylindene camphor sulphonic acid. This was UV absorber used in the 3T3 NRU-PT validation study. However, we conducted the phase 2 study without noticing the transcription error in CAS number when we made the final chemical list. Because we noticed the error after the phase 2 study, these chemicals were classified as phototoxic negative again after confirming the negative result in 3T3 NRU-PT and low molar extinction coefficient (MEC).
II-31	Chlorhexidine	Chlorhexidine was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a and 1998a]. Negative result in humans was reported for this chemical in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-32	Cinnamic acid	As chemical No. II-32, we were planning to register cinnamic aldehyde, the main ingredient of cinnamon oil and the ingredient of cinnamon fragrance. This chemical was used in the 3T3 NRU-PT validation study. However, when we registered the chemical name on the list, we registered cinnamic acid instead of cinnamic aldehyde. Cinnamic acid is a substance known to form a dimer by light irradiation in solid state. The difference between cinnamic aldehyde and cinnamic acid is the side chain being aldehyde or carboxyl. In addition, we conducted 3T3NRU-PT for cinnamic acid, and the result was negative, the same as cinnamic aldehyde. Therefore, we believe that the mix-up would not affect the results for the ROS assay validation study, because the structure of cinnamic acid resembles that of cinnamic aldehyde, and the 3T3 NRU-PT

		results are the same.
II-33	Drometrizole	Drometrizole was selected from among UV absorbers. We believed that human data for this UV absorber would be provided. However, human data was not available for this chemical. Therefore, after confirming negative result in 3T3 NRU-PT, this chemical was classified as a phototoxic negative chemical.
II-34	L-Histidine	L-Histidine was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-35	Methylbenzylidene camphor	Methylbenzylidene camphor was selected from among UV absorbers. This was included in the list of negative chemicals in the 3T3 NRU-PT validation study, and human data of this chemical was described as negative in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998b].
II-36	Octrizole	Octrizole was selected from among UV absorbers. We believed that human data for this UV absorber would be provided. However, human data was not available for this chemical. Therefore, after confirming negative result in 3T3 NRU-PT, this chemical was classified as a phototoxic negative chemical.
II-37	Octyl methacrylate	Chemical No. II-37 was registered at first as octyl methoxycinnamate. This was UV absorber used in the 3T3 NRU-PT validation study. However, we conducted the phase 2 study without noticing the transcription error in CAS number when we made the final chemical list. Because we noticed the error after the phase 2 study, these chemicals were classified as phototoxic negative again after confirming the negative result in 3T3 NRU-PT and low molar extinction coefficient (MEC).
II-38	Octyl methoxycinnamate	Octyl methoxycinnamate was selected from among UV absorbers. This was included in the list of negative chemicals in the 3T3 NRU-PT validation study, and human data of this chemical was described as negative in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998b].
II-39	Octyl salicylate	Octyl salicylate was selected from among UV absorbers. This was included in the list of negative chemicals in the 3T3 NRU-PT validation study, and human data of this chemical was described as negative in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998b].
II-40	PABA	PABA was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a and 1998a]. Negative result in animals was reported for this chemical in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998a].
II-41	SDS	SDS was selected from the list of negative chemicals used in the 3T3 NRU-PT validation study [Spielmann et al, 1994a].
II-42	UV-571	UV-571 was selected from among UV absorbers. We believed that human data for this UV absorber would be provided. However, human data was not available for this chemical. Therefore, after confirming negative result in 3T3 NRU-PT, this chemical was classified as a phototoxic negative chemical.
Positive/Negative controls		
PC	Quinine HCl	Quinine HCl was selected as positive control according to the method established by Onoue et al. [2008a]. Quinine HCl was classified as a phototoxic positive in humans, according to the publication by Ljunggren et al [1986].
NC	Sulisobenzone	Sulisobenzone was selected as negative control according to the method established by Onoue et al. [2008a]. Sulisobenzone was classed as a phototoxic negative, since human data for this chemical was reported as negative in the publication for the 3T3 NRU-PT validation study [Spielmann et al, 1998b].

Table 2-2 Test chemicals for phase 2 study and code list

No.	Chemical name	CAS No. ^{a)}	UV/vis absorption ^{b)}		3T3 NRU	in vivo		Laboratory		
			MEC (L/mol/cm)	λ_{max} (nm)		Animal	Human	1	2	3
Phototoxic drugs										
II-1	Acridine	260-94-6	2773	354	+ ¹⁾	+ ¹⁾	+ ¹⁾	C-130	B-090	A-005
II-2	Acridine HCl	17784-47-3	2635	354	+ ¹⁾	+ ¹⁾	+ ¹⁾	C-126	B-086	A-001
II-3	Amiodarone HCl	19774-82-4	5400	290 ^{c)}	+ ²⁾	+ ²⁾	+ ²⁾	C-127	B-087	A-002
II-4	Chlorpromazine HCl	69-09-0	1746	304	+ ²⁾	+ ²⁾	+ ²⁾	C-106	B-066	A-026
II-5	Doxycycline HCl	10592-13-9	3715	290 ^{c)}	+ ²⁾	+ ²⁾	+ ²⁾	C-116	B-076	A-036
II-6	Fenofibrate	49562-28-9	3514	290 ^{c)}	+ ¹⁾	NA	+ ¹⁾	C-139	B-054	A-014
II-7	Furosemide	54-31-9	2650	290 ^{c)}	+/- ^{1,3,4)}	NA	+ ¹⁾	C-141	B-056	A-016
II-8	Ketoprofen	22071-15-4	2092	290 ^{c)}	+ ¹⁾	- ¹⁾	+ ¹⁾	C-128	B-088	A-003
II-9	6-Methylcoumarine	92-48-8	3219	290 ^{c)}	+ ¹⁾	+ ¹⁾	+ ¹⁾	C-113	B-073	A-033
II-10	8-MOP	298-81-7	3631	300	+ ²⁾	+ ²⁾	+ ²⁾	C-131	B-091	A-006
II-11	Nalidixic acid	389-08-2	3192	331	+ ¹⁾	+ ¹⁾	+ ¹⁾	C-137	B-052	A-012
II-12	Nalidixic acid (Na salt)	3374-05-8	3019	333	+ ¹⁾	+ ¹⁾	+ ¹⁾	C-134	B-094	A-009
II-13	Norfloxacin	70458-96-7	3562	323	+ ³⁾	+ ¹⁾	+ ¹⁾	C-110	B-070	A-030
II-14	Ofloxacin	82419-36-1	8443	290 ^{c)}	+ ¹⁾	+ ¹⁾	+ ¹⁾	C-112	B-072	A-032
II-15	Piroxicam	36322-90-4	3304	352	- ²⁾	- ²⁾	+ ²⁾	C-135	B-095	A-010
II-16	Promethazine HCl	58-33-3	1558	297	+ ²⁾	NA	+ ²⁾	C-101	B-061	A-021
II-17	Rosiglitazone	122320-73-4	1765	311	+ ^{d)}	NA	NA	C-117	B-077	A-037
II-18	Tetracycline	60-54-8	3842	290 ^{c)}	+ ²⁾	+ ²⁾	+ ²⁾	C-102	B-062	A-022
Phototoxic non-drug chemicals										
II-19	Anthracene	120-12-7	2315	355	+ ¹⁾	+ ¹⁾	+ ¹⁾	C-121	B-081	A-041
II-20	Avobenzone	70356-09-1	7686	354	+ ⁵⁾	- ⁶⁾	+ ⁷⁾	C-109	B-069	A-029
II-21	Bithionol	97-18-7	2462	321	+ ²⁾	+ ²⁾	+ ²⁾	C-115	B-075	A-035
II-22	Hexachlorophene	70-30-4	2431	300	- ¹⁾	- ¹⁾	+ ^{1,8)}	C-107	B-067	A-027
II-23	Rose bengal	632-69-9	19269	549	+ ¹⁾	- ¹⁾	+ ¹⁾	C-104	B-064	A-024
Non-phototoxic drugs										
II-24	Aspirin	50-78-2	80	290 ^{c)}	- ³⁾	NA	NA	C-140	B-055	A-015
II-25	Benzocaine	94-09-7	4273	290 ^{c)}	- ³⁾	NA	NA	C-114	B-074	A-034
II-26	Erythromycin	114-07-8	0	290 ^{c)}	- ³⁾	NA	NA	C-119	B-079	A-039
II-27	Penicillin G	113-98-4	0	290 ^{c)}	- ²⁾	NA	NA	C-118	B-078	A-038
II-28	Phenytoin	57-41-0	0	290 ^{c)}	- ³⁾	NA	NA	C-145	B-060	A-020

Table 2-2 Test chemicals for phase 2 study and code list (continued)

No.	Chemical name	CAS No. ^{a)}	UV/vis absorption ^{b)}		3T3 NRU	in vivo		Laboratory		
			MEC (L/mol/cm)	λ_{max} (nm)		Animal	Human	1	2	3
Non-phototoxic non-drug chemicals										
II-29	Bumetizole	3896-11-5	3873	306	- ^{d)}	NA	NA	C-138	B-053	A-013
II-30	Camphor sulfonic acid	3144-16-9	0	290 ^{c)}	- ^{d)}	NA	NA	C-132	B-092	A-007
II-31	Chlorhexidine	55-56-1	1338	290 ^{c)}	- ¹⁾	NA	- ¹⁾	C-144	B-059	A-019
II-32	Cinnamic acid	140-10-3	3373	290 ^{c)}	- ^{d)}	NA	NA	C-123	B-083	A-043
II-33	Drometizole	2440-22-4	3946	295	- ^{d)}	NA	NA	C-129	B-089	A-004
II-34	L-Histidine	71-00-1	0	290 ^{c)}	- ²⁾	NA	NA	C-111	B-071	A-031
II-35	Methylbenzylidene camphor	36861-47-9	9200	304	- ⁹⁾	- ⁹⁾	- ⁹⁾	C-136	B-051	A-011
II-36	Octrizole	3147-75-9	3958	296	- ^{d)}	NA	NA	C-133	B-093	A-008
II-37	Octyl methacrylate	688-84-6	0	290 ^{c)}	- ^{d)}	NA	NA	C-105	B-065	A-025
II-38	Octyl methoxycinnamate	5466-77-3	3000	290 ^{c)}	- ⁹⁾	- ⁹⁾	- ⁹⁾	C-142	B-057	A-017
II-39	Octyl salicylate	118-60-5	1500	290 ^{c)}	- ⁹⁾	- ⁹⁾	- ⁹⁾	C-120	B-080	A-040
II-40	PABA	150-13-0	2404	290 ^{c)}	- ²⁾	- ¹⁾	NA	C-124	B-084	A-044
II-41	SDS	151-21-3	0	290 ^{c)}	- ²⁾	NA	NA	C-125	B-085	A-045
II-42	UV-571	125304-04-3	1900	290 ^{c)}	- ^{d)}	NA	NA	C-122	B-082	A-042
Positive/Negative controls										
PC	Quinine HCl	6119-47-7	1938	330	+ ³⁾	+ ¹⁰⁾	+ ¹¹⁾	PC	PC	PC
NC	Sulisobenzone	4065-45-6	3519	290 ^{c)}	- ³⁾	NA	- ⁹⁾	NC	NC	NC

8-MOP: 8-methoxy psoralen, PABA: *p*-aminobenzoic acid, SDS: sodium dodecyl sulfate

+ : Positive, - : Negative, +/- : Equivocal, NA : Not available, PC : Positive control, NC : Negative control

a) CAS No.: Chemical abstracts service registry number, b) The UV/vis absorbance (290-700 nm) of most chemicals was measured in 20 mM phosphate buffer (pH 7.4). However, the UV/vis absorbance of chemical Nos. II-19, II-20, II-29, II-33 and II-36 were measured in methanol, because these chemicals were not solved in 20 mM phosphate buffer (pH 7.4). In the each case, test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM phosphate buffer (pH 7.4) or methanol. Final concentration of DMSO was unified to 0.5%. c) λ_{max} (nm) was a wavelength at which the UV/vis absorbance shows a peak between 290 and 700 nm. λ_{max} (nm) was indicated as 290 nm in the case where the peak absorption is located below 290 nm and the maximum absorption is at 290 nm. d) In vitro phototoxicity was assessed by the 3T3 NRU PT in the participating laboratories, according to the OECD 432 guideline.

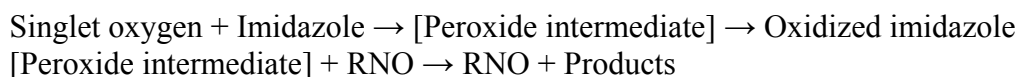
1) Spielmann et al., 1998a, 2) Spielmann et al., 1994a, 3) Onoue et al., 2010, 4) Peters et al., 2002, 5) Gaspar et al., 2012, 6) ZEBET in house data, 7) Schauder et al., 1997, 8) Masuda et al., 1971, 9) Spielmann et al., 1998b, 10) Spielmann et al., 1994b, 11) Ljunggren et al., 1986

7. Protocols

The detailed test protocols used in this study is described in Attachment 1 and 2.

7-1. Prediction model of photoreactivity

In the ROS assay, generation of singlet oxygen is detected by spectrophotometric measurement of *p*-nitrosodimethyl aniline (RNO) bleaching, followed by decreased absorbance of RNO at 440 nm. Although singlet oxygen does not react chemically with RNO, the RNO bleaching is a consequence of singlet oxygen capture by the imidazole ring, resulting in the formation of a trans-annular peroxide intermediate capable of inducing the bleaching of RNO as follows:



The generation of superoxide is detected by the reduction of nitroblue tetrazolium (NBT) by superoxide anion via a one-electron transfer reaction, yielding partially reduced (2 e⁻) monoformazan (NBT^{•-}) as a stable intermediate. Thus, superoxide reduces NBT to NBT^{•-}, whose formation can be monitored spectrophotometrically at 560 nm.



7-2. Protocol of ROS assay

7-2-1. Apparatus

In the present validation study, an Atlas Suntest CPS series (CPS plus or CPS; Atlas Material Technology LLC, Chicago, IL, USA) equipped with a 1500-W xenon arc lamp was used for solar simulator. The irradiation tests were carried out at 25°C with an irradiance of ca. 2.0 mW/cm² as determined using the calibrated UVA detector (Dr Hönle 0037, München, Germany) provided by the VMT. Quartz reaction containers for the ROS assay were manufactured by Ozawa Science (Aichi, Japan) and provided by the VMT.

7-2-2. Preparation of test chemicals and controls

The stock solutions were thawed just before use and used within the day. The coded test chemicals were dissolved in dimethylsulfoxide (DMSO) or 20 mM sodium phosphate buffer (NaPB, pH7.4) at concentrations of 0.1, 1, or 10 mM just before use under UV-cut illumination or shade. All preparations were protected from light. [Detailed information on preparation of test chemicals are shown in Appendix 9.](#) The stock solutions of quinine for positive control and sulisobenzone for negative control were prepared at 10 mM in DMSO and kept frozen in tubes for up to 1 month. According to a chromatographic analysis, these stock solutions were stable for at least 1 month under the storage condition.

7-2-3. ROS assay procedure

Singlet oxygen was measured in an aqueous solution by spectrophotometrically monitoring the bleaching of RNO at 440 nm using imidazole as a selective acceptor of singlet oxygen. Samples containing the tested chemical (2–200 μM), RNO (50 μM) and imidazole (50 μM) in 20 mM NaPB were mixed in a tube. Two hundred microliters of the sample were transferred to a well in a plastic 96-well plate (clear, non-treated, flat-bottom). The plate was subjected to measurement of absorbance at 440 nm using a microplate spectrophotometer. The plate was fixed in the quartz reaction container with a quartz cover, and then irradiated with simulated sunlight for 1 hour. After agitation on plate shaker, UV absorbance at 440 nm was measured. For the determination of superoxide, samples containing the test chemical (2–200 μM) and NBT (50 μM) in 20 mM NaPB were irradiated with the simulated sunlight for 1 hour, and the reduction in NBT was measured by the increase in absorbance at 560 nm in the same manner as the singlet oxygen determination.

7-3. Data collection, handling, and criteria

7-3-1. Data collection

In the phase 1 study, experiments were performed in triplicate wells in three independent runs. As the final concentrations, 20 μM and 200 μM of test chemical solutions were subjected to the ROS assay. When questionable data (*e.g.* technical error) were obtained, each testing facility could perform an additional experiment using the questionable chemical(s) and the positive/negative control chemicals.

In the phase 2 study, experiments were performed in triplicate wells in three independent runs. As the final concentrations, 200 μM of test chemical solutions were subjected to the ROS assay. However, when precipitation could be observed at 200 μM under the optical microscope ($\times 100$) before light exposure, additional experiments were performed at 20 μM . Further experiments should be performed at 2 μM when precipitation was still observed at 20 μM . When precipitation was observed at 2 μM in the reaction mixture, further experimentation was not performed. When questionable data (*e.g.* technical error) were obtained, each testing facility could perform an additional experiment using the questionable chemical(s) and the positive/negative chemicals.

7-3-2. Data handling

The study report and all raw data from this study were retained according to the protocol in each testing facility. All raw data and the results were submitted to the VMT for review.

7-3-3. Criteria for data acceptance and judgment

The acceptance criteria for a valid assay were:

- i. No precipitation of the test chemical in the reaction mixture before light exposure,
- ii. No missing data for the positive control, negative control, blanks, or chemicals; and
- iii. Net absorbance of 0.02–1.5 in the controls and the chemicals.
- iv. Positive control value at 200 μM (mean of 3 wells)
Singlet oxygen: 150 or more
Superoxide anion: 200 or more
- v. Negative control value at 200 μM (mean of 3 wells)
Singlet oxygen: less than 25
Superoxide anion: less than 20

According to the results (mean of triplicate determinations) of the ROS assay, the photoreactivity on each test chemical was judged to be:

- i. Positive with singlet oxygen value ($\Delta A_{440\text{ nm}} \times 10^3$) of 25 or more and/or superoxide value ($\Delta A_{560\text{ nm}} \times 10^3$) of 20 or more; or
- ii. Negative with singlet oxygen value of less than 25 and superoxide value of less than 20.

Every assay result was classified based on these classification criteria. Final judgments about chemicals were performed on the following four draft criteria at each laboratory:

- A. Final judgment of positive when positive results were obtained in at least one of three assays.
- B. Final judgment based on the mean value of three assays.
- C. Final judgment based on the majority of three assay results.
- D. Final judgment based on the first assay results.

7-4. Quality assurance

Assays and quality assurance were carried out in the spirit of GLP. Two of three test facilities were GLP certified even though tests were not performed under GLP. The participating laboratories conducted the experiments in accordance with the protocol provided by the VMT. All raw data and data analysis sheets were pre-checked for quality in each laboratory and then reviewed by the VMT quality assurance group. The results accurately reflect the raw data.

8. Results

8-1. Phase 1 study

8-1-1. Within- and between laboratory variation assessments in the phase 1 study

Results of within-laboratory variation, which comprise intra-day variation and inter-day variation of the positive and negative controls at Laboratories 1, 2, and 3 are shown in Table 3. Individual positive and negative control values are shown in Appendix 1. Parameters for intra-day variations were calculated based on results from the date on which the most assays were performed at each laboratory. Parameters for inter-day variations were calculated based on the results of an assay of the day. Intra-day variation and inter-day variation were evaluated using the coefficient of variation (CV) of the positive control as well as values for mean and standard deviation of the positive and negative controls at each laboratory. The CVs of the positive control at each laboratory were below 10%, and variations of each control value were sufficiently small to suggest good within-laboratory reproducibility.

Parameters for between-laboratory variations were calculated from the average values from all of the assay results for the positive and negative controls of each laboratory (Table 4). The CVs of positive control in the three laboratories were 12.2% (singlet oxygen) and 26.6% (superoxide anion). Negative control was shown to be inactive in all assays.

8-1-2. Results and judgments in the phase 1 study

Results of the phase 1 study were shown (Table 5 and Appendix 2). According to the results (mean values of triplicate determinations) of the ROS assay, the photoreactivity on each test chemical was judged to be

- i. Positive with singlet oxygen value ($\Delta A_{440 \text{ nm}} \times 10^3$) of 25 or more and/or superoxide value ($\Delta A_{560 \text{ nm}} \times 10^3$) of 20 or more or
- ii. Negative with singlet oxygen value of less than 25 and superoxide value of less than 20.

Although data for precipitation were not recorded, precipitations were observed for amiodarone.

In order to select criteria for final judgment in a recommendation protocol for the ROS assay, four different draft criteria for final judgments were used in this validation study.

Draft criteria A: Final judgment of positive when positive results were obtained in at least one of three assays (Table 6A).

Draft criteria B: Final judgment based on the mean value of three assays (Table 6B).

Draft criteria C: Final judgment based on the majority of three assay results (Table 6C).

Draft criteria D: Final judgment on the first assay results (Table 6D).

As for the final judgments at 20 μM , there was no inconsistency between the three laboratories under draft criteria A or B, but there was one inconsistency (furosemide) under draft criteria C or D. In addition, 8-MOP showed negative results for all laboratories at 20 μM . However, there was no inconsistency in the final judgment between the three laboratories at 200 μM .

8-1-3. Contingency tables in the phase 1 study

Inconsistencies of final judgments were assessed using contingency tables at 20 μM (Tables 7A to 7D) and at 200 μM (Table 7E).

At a concentration of 20 μM , sensitivities were 83.3% under draft criteria A or B and 75.0% or 83.3% under the draft criteria C and D at each laboratory. Although there was only one non-phototoxic chemical, specificities were 100% under all criteria at all laboratories.

Positive predictivities were also 100% under all criteria at all laboratories. Negative predictivities were 33.3% under criteria A or B and 25.0 or 33.3% under criteria C or D. Accuracies were 84.6% under criteria A or B and 76.9% or 84.6% under criteria C or D. At a concentration of 200 μM , sensitivities were 91.7%, specificities and positive predictivities were 100%, negative predictivities were 50.0%, and accuracies were 92.3% at all laboratories regardless of criteria. A false negative result was obtained for one of 12 phototoxic chemicals (5-FU).

8-2. Phase 2 study

8-2-1. Irradiance and temperature during the irradiation

The irradiances and temperatures at the beginning and the end of each irradiation are shown in Table 8 and Appendix 3. Irradiance A was measured by each laboratory with its own UVA detector. Irradiance B was a standardized irradiance calculated using values obtained using the calibrated UVA detector (Dr. Hönle), which was transported to each laboratory and conversion factors for standardized irradiance were prepared. Irradiance in each laboratory (1.93 to 2.07 mW/cm^2) were within the specified range of values, and there was no significant inconsistency between facilities. There was no apparent variation in either irradiance or temperature between the facilities.

8-2-2. Within- and between laboratory variation for phase 2 study

Results of within-laboratory variation, which comprise intra-day variation and inter-day variation of the positive and negative controls at Laboratories 1, 2 and, 3, are shown in Table 9 and Appendix 4. Parameters for intra-day variations were calculated based on results from the date on which the most assays were performed at each laboratory. Parameters for inter-day variations were calculated based on the results of the first assay of the day. Intra-day variation and inter-day variation were evaluated using the CV of the positive control as well as values for mean and standard deviation of the positive and negative controls of each laboratory. The CVs of the positive control at each laboratory were below 10%, and variations of each control value were sufficiently small to suggest good within-laboratory reproducibility.

Parameters for between-laboratory variations were calculated from the average value from all of the assay results for the positive and negative controls of each facility (Table 10). The CVs of positive control in the three laboratories were 20.6% (singlet oxygen) and 20.1% (superoxide anion). Negative control was shown to be inactive in all assays.

8-2-3. Results and judgments in the phase 2 study

The photochemical reactivities of 42 coded chemicals, comprising 23 known phototoxins and 19 non-phototoxic drugs/compounds, were assessed using the ROS assay at Laboratories 1, 2, and 3 (Table 11 and Appendix 5). Assessment of between 25 and 28 chemicals (60–67% of total) were made at a concentration of 200 μM , and that of between 14 and 17 chemicals had to be diluted to a final concentration of 2 or 20 μM due to limited solubility in aqueous media. In particular, assays of amiodarone HCl (No. II-3), anthracene (No. II-19), avobenzone (II-20), Ocotrizole (No. II-36) and UV-571 (No. II-42) were available only at 2 μM in most laboratories. For rose bengal (No. II-23), only singlet oxygen could be measured, since the intense UV absorption at 560 nm interfered directly with determination of superoxide anion. All three participating laboratories found that all phototoxins demonstrated potent ability to generate singlet oxygen, superoxide, or both under UV-vis exposure at concentrations of 20 and 200 μM . Nalidixic acid (No. II-11) and its sodium salt (No. II-12) were selected to evaluate the influence of the free form and Na salt form which may affect the solubility profile. The results of nalidixic acid and its Na salt were positive at 200 μM in all assays,

and the values for singlet oxygen and superoxide anion were similar in each laboratory. For some phototoxins, however, generation of ROS was negligible at 2 μM . Similar photochemical reactivity was also seen for test chemicals that were non-phototoxic drugs/compounds, although some exhibited potent photoreactivity in a few laboratories.

Classification criteria for positive, negative and inconclusive ROS assays are:

Positive: Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 20 or 2 μM

Negative: Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM

Inconclusive: The results do not meet the positive or negative criteria

Every ROS assay was classified based on these criteria, and final judgments were based on four draft criteria shown in Tables 12A to 12D. The draft criteria for the final judgment are followings:

- A. Final judgment of positive when positive results were obtained in at least one of three assays.
- B. Final judgment based on the mean value of three assays.
- C. Final judgment based on the majority of three assay results.
- D. Final judgment based on the first assay results.

Three chemicals at Laboratories 1 and 2 gave inconsistent results in the three independent repeat assays, as did one chemical at Laboratory 3.

Ten chemicals gave inconsistent final judgments between the laboratories for draft criteria A (chemical Nos. II-6, 19, 25, 26, 27, 31, 32, 36, 37, and 41), as did nine chemicals for draft criteria B and C (chemical Nos. 3, 6, 19, 27, 31, 32, 36, 37, and 41), and eight chemicals for draft criteria D (chemical Nos. 6, 19, 27, 31, 32, 36, 37, and 41).

8-2-4. Contingency tables in the phase 2 study

Inconsistent final judgments were assessed using contingency tables in the phase 2 study (Tables 13A to 13D).

Sensitivities and negative predictivities were 100% in each laboratory regardless of the four different criteria. Specificities were 33.3% to 81.8% under draft criteria A, 41.7% to 81.8% under draft criteria B and C, and 58.3% to 81.8% under draft criteria D. Positive predictivities were 74.2% to 91.7% under draft criteria A, 75.9% to 91.7% under draft criteria B and C, and 82.1% to 91.7% under draft criteria D. Accuracies were 77.1% to 93.9% under draft criteria A, 79.4% to 93.9% under draft criteria B and C, and 85.7% to 93.9% under draft criteria D. There were no false negatives. There were between two and seven false positives in 11 or 12 negative chemicals under draft criteria A, between two and eight false positives in 10 to 12 negative chemicals under draft criteria B or C, and between two and five false positives in 10 to 12 negative chemicals under draft criteria D.

8-3. Combined results of phase 1 and phase 2

8-3-1. Results and judgments for phase 1 and 2 combined results

The results of four phototoxic chemicals (200 μM) evaluated only in the phase 1 study were combined with the phase 2 results (diclofenac, 5-FU, levofloxacin, omeprazole; Tables 14A to 14 D). The results and the final judgments of these four chemicals were consistent between the three laboratories.

8-3-2. Contingency tables for phase 1 and 2 combined results

Inconsistencies of final judgments were assessed using contingency tables for the combined results (Tables 15A to 15D).

Sensitivities were 96.0% to 96.3% in each laboratory regardless of the four different criteria. Specificities were 33.3% to 81.8% under draft criteria A, 41.7% to 81.8% under draft criteria B and C, and 58.3% to 81.8% under draft criteria D. Variation in specificities was basically dependent on facility. Specificities were 33.3% to 58.3%, 36.4% to 60.0%, and 81.8% in Laboratories 1, 2 and 3, respectively. Positive predictivities were 76.5% to 92.6% under draft criteria A, 78.1% to 92.6% under draft criteria B and C, and 83.9% to 92.6% under the draft criteria D. Negative predictivities were 80.0% to 90.0% under draft criteria A, 83.3% to 90.0% under draft criteria B and C, and 85.7% to 90.0% under draft criteria D. Accuracies were 76.9% to 91.1% under draft criteria A, 79.0% to 91.9% under draft criteria B and C, and 84.6% to 91.9% under draft criteria D.

5-FU showed a false negative under all criteria, but no other chemicals showed false negatives under any criteria. There were between two and eight false positives in 11 or 12 negative chemicals under draft criteria A, between two and seven false positives in 10 to 12 negative chemicals under draft criteria B or C, and between two and five false positives in 10 to 12 negative chemicals under draft criteria D.

8-4. Contingency tables for integrated judgment results

Parameters in the contingency tables for the phase 1 study (20 and 200 μ M), the phase 2 study, and the combined results are shown in Tables 16-1 to 16-4. Integrated final judgments were based on a majority of laboratory judgments. Parameters in the contingency tables of integrated judgment results for the phase 1 study (200 μ M), the phase 2 study, and the combined results are shown in Table 17.

The parameters in phase 1 at 200 μ M were the same regardless of the draft criteria for judgment. For the phase 2 and the combined results, whoever, specificities were lower than the other parameters, especially under draft criteria A. The other parameters showed no apparent inconsistency regardless of draft criteria, although some were slightly lower under draft criteria A and slightly higher under draft criteria D.

8-5. Data re-analysis after receiving the comments from the peer review committee

After issuing the validation report, the peer reviewers reviewed the report in a meeting held from 27th February to 2nd March, 2013. Major comments from the peer reviewers are as follows:

- 1) It might have been better to limit data to just blind phase 2 study. The basis for overall evaluation of sensitivity, specificity, positive and negative predictivity, and overall accuracy (performance criteria) should focus on this data. In this data set, 100% negative predictivity is highly encouraging, even though refers to chemicals that provided conclusive data. Re-examine whether the human data for 5-FU phototoxicity could be regarded as “high quality human data” or not.
- 2) The database could be enlarged by taking solubility into account and accepting negative results at 20 μ M.
- 3) Low specificity of data is problematic. If possible, widen the borderline for the acceptance criteria based on validation study data. (from 20 to x for superoxide)

According to comment No. 1, the data re-analysis conducted below is focused on the phase 2 study, which was conducted under the masked condition. The VMT members re-examined the information on 5-FU phototoxicity in human. The conclusion after the re-examination was as follows; it is unknown whether 5-FU was phototoxic or not, and high quality human data was not available (See, Table 1-1).

Regarding comment No. 2, accepting negative results at 20 μ M did not cause any false negatives in validation studies with Atlas Suntest CPS/CPS+ or Seric SXL2500-V2. Therefore the negative results at 20 μ M were included in this data re-analysis.

Regarding comment No. 3, when the borderline for superoxide anion changed from 20 to 70, no false negatives were found in the validation study results using Atlas Suntest CPS/CPS+ or Seric SXL2500-V2. If we use the borderline 25 for singlet oxygen and 70 for superoxide anion, amlodipine, amoxapine, bufexamac and haloperidol would be below the borderline [Onoue et al., 2008a]. However, the phototoxicity of these drugs is not very noticeable, and it seems that the adverse events which are possibly related to the phototoxic potential of these drugs are not common events. It is considered that the chemicals, whose singlet oxygen values are less than 25 and superoxide anion values are between 20 to 70, are “Weakly photoreactive”. So, the new judgment criteria were established for the proposed protocol as follows:

Judgment criteria

Each test chemical will be judged as follows;

Judgment ¹⁾	Concentration judged	SO (mean of 3 wells)	SA (mean of 3 wells)
Photoreactive	20 and/or 200 μ M ²⁾	≥ 25	and ≥ 70
		< 25 and/or P ³⁾	and ≥ 70
		≥ 25	and < 70 and/or P
Weakly photoreactive	20 and 200 μ M ²⁾	< 25	and $\geq 20, < 70$
Non-photoreactive	20 and 200 μ M ²⁾	< 25	and < 70
Inconclusive	The results do not meet the above-mentioned criterion. ⁴⁾		

Notes

- 1) It can be judged based on results of one experiment because the ROS assay shows good reproducibility in the validation studies.
- 2) It would be judged at 20 μ M only when precipitation is observed at 200 μ M.
- 3) Precipitation before irradiation.
- 4) When precipitation is observed at 20 and 200 μ M before irradiation, the compound is regarded incompatible with the ROS assay.

The phase 2 study results were re-analyzed based the above criteria as described in the following sections. Lower incidence of “false negatives” is more important than higher incidence of “false positives”, since the ROS assay is intended for screening photoreactivity during initial photosafety evaluation. Although all chemicals which were classified as “weakly photoreactive” in the validation studies were non-phototoxic drugs or non-phototoxic non-drug chemicals, some drugs which could be related to clinical photosensitivity, such as amlodipine, amoxapine, bufexamac and haloperidol would be classified as “weakly photoreactive” [Onoue et al., 2008a]. Follow-up tests for non-clinical and/or clinical photosafety should be considered if a drug candidate is classified as “weakly photoreactive”. Therefore, in this data re-analysis, both cases are evaluated: when a weakly photoreactive chemical is defined as non-phototoxic, and when it is defined as phototoxic.

8-5-1. Data re-analysis based on the criteria for the proposed protocol: Results and judgments of phase 2 study

The results of the phase 2 study were classified based on the criteria in the proposed protocol, and final judgments were based on four draft criteria as shown in Tables 18A to 18D. The draft criteria for the final judgment are as follows:

- A. Final judgment of positive when positive results were obtained in at least one of three assays.
- B. Final judgment based on the mean value of three assays.
- C. Final judgment based on the majority of three assay results.
- D. Final judgment based on the first assay result.

For the phototoxic chemicals which were evaluated at 20 μ M and 200 μ M, all of the judgments, the final judgments and the integrated judgments were classified as “photoreactive”. For the non-phototoxic chemicals, 2 of 3 assay results for one non-drug chemical (No. II-37, Octyl methacrylate) were classified as “photoreactive” at Laboratory 1. With the exception of this case, all of the non-phototoxic chemicals were classified as “non-photoreactive” or “weakly photoreactive”.

8-5-2. Data re-analysis based on the criteria for the proposed protocol: Contingency tables of phase 2 study results

Contingency tables are shown in Tables 19A to 19D. One to four phototoxic chemicals and one to five non-phototoxic chemicals were not evaluated due to precipitation at 20 μ M and 200 μ M. Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of each laboratory based on the original criteria and the criteria for the proposed protocol are summarized in Table 20. Sensitivity, specificity, positive predictivity, negative predictivity and accuracy of the integrated judgments based on the original criteria and the criteria for the proposed protocol are summarized in Table 21.

9. Discussion

9-1. Reliability

Variability was assessed using quinine for a positive control and sulisobenzone for a negative control in the phase 1 study. Within-laboratory variations were sufficiently small to suggest high repeatability and reproducibility. In addition, preliminary findings show good between-laboratory transferability.

Between-laboratory transferability was assessed using 12 positive and 1 negative chemical at 20 and 200 μ M. All chemicals showed the same final judgments in all criteria and facilities at 200 μ M. One chemical, however, showed different results at Laboratory 3 depending on the draft criteria for final judgment at 20 μ M, which suggests that weak ROS generative chemicals could show obscure result at 20 μ M. In addition, sensitivities were lower at 20 μ M than 200 μ M for all criteria at all laboratories. Thus, we decided to accept negative results only at 200 μ M in the phase 2 study, although we did accept positive results at any concentration. However, the peer review committee recommended the inclusion of negative results at 20 μ M and widening of the borderline, in order to reduce inconclusive results and false positives from the validation study results. Additional data analysis was conducted for the results of phase 2 study, which was conducted under masked condition. The data re-analysis did not affect the reliability of the ROS assay.

9-2. Between-laboratory reproducibility

In the phase 2 study conducted with 42 coded test chemicals and 2 control chemicals, as shown in Table 20, the sensitivities, specificities, positive predictivities, negative predictivities and accuracies from each laboratory, based on the criteria for the proposed protocol, were 100%, 81.3% to 100%, 88.8% to 100%, 100%, and 92.1% to 100% when the weakly positive chemicals were defined as “non-phototoxic” chemicals; 100%, 56.3% to 88.2%, 75.9% to 91.3%, 100%, and 81.6% to 94.7% when the weakly positive chemicals were defined as “phototoxic” chemicals. Specificities exhibited some fluctuation per laboratory. Four non-phototoxic drugs/compounds — penicillin G (No. II-27), chlorhexidine (No. II-31), cinnamic acid (No. II-32), and SDS (No. II-41) — were judged to be negative in all criteria at Laboratory 3 but were each judged differently depending on criteria at Laboratories 1 and 2, resulting in high specificity at Laboratory 3 and low specificity at laboratories 1 and 2 in all decision criteria. Values for the positive control were somewhat higher at Laboratories 1 and 2 than at Laboratory 3, suggesting that conditions were more conducive to ROS generation at Laboratories 1 and 2. This ROS assay protocol is intended for use in screening phototoxicity potential and therefore requires high sensitivity. High sensitivity is more important than specificity in acquiring reliable photoreactivity assessments without false negatives, so these variations in specificity are acceptable.

9-3. Predictivity

In phase 2 results, as shown in Table 20, the sensitivity of each laboratory was 100%. In theory, the ROS assay is designed to capture all photochemically active substances that can be detected as type I and/or II photochemical reactions induced by irradiated chemicals. These photochemical reactions were observed at a very early stage of chemical-induced phototoxic cascades, so that the ROS assay had been thought effective for photosafety evaluation of pharmaceuticals. There is, however, a good probability that some photolabile substances would also be recognized as phototoxic by the ROS assay because of significant ROS generation during the photodegradation processes. [Based on the validation study results, the criteria for the ROS assay results was revised in the proposed protocol as stated in Section 8-5. According to the original criteria,](#) some of the false positives observed in the phase 2 study, which included phenytoin [Chen Y. et al., 2009] (No. II-28), penicillin G [Ray R. S. et al., 1996] (No. II-27), chlorhexidine [Information from manufacture] (No. II-31), cinnamic acid [Marin M. L. et al., 2007] (No. II-32), L-histidine [Huvaere K. et al., 2009] (No. II-34), and octyl methacrylate [Information from manufacture] (No. II-37), had previously been reported to be photodegradable and photoreactive, the mechanisms of which included radical reactions and/or electron transfer. This could explain in part the data discrepancy observed between the ROS assay and in vitro/in vivo phototoxicity, and a better understanding of this limitation would be of great help in avoiding overestimation or misleading conclusions. [However, after adopting the criteria for the proposed protocol, phenytoin, penicillin G, chlorhexidine, cinnamic acid and L-histidine were classified as “weakly photoreactive”.](#) Lower incidence of false negatives is more important than higher incidence of false positives, since ROS assay is intended to evaluate photoreactivity during initial photosafety evaluation. Although all chemicals, which were classified as “weakly photoreactive” in the validation studies, were non-phototoxic drugs or non-phototoxic non-drug chemicals, some drugs which could be related to clinical photosensitivity, such as amlodipine, amoxapine, bufexamac and haloperidol would be classified as “weakly photoreactive” [Onoue et al., 2008a]. Follow-up non-clinical and/or clinical photosafety studies should be considered, when a drug candidate is classified as “weakly photoreactive”.

As observed in the present validation study, poorly water-soluble chemicals did satisfy neither positive nor negative criteria, leading to inconclusive results, and it appears that photosafety

assessment of poorly water-soluble chemicals will require some modification of the protocols. Although analysis throughput would be decreased, the use of micellar solution systems could be effective for poorly soluble chemicals [Onoue S. et al., 2008b]. In order to overcome limitations of poorly water-soluble chemicals, a modified ROS assay system has been developed using bovine serum albumin in the lead laboratory. Careful elucidation of predictivity will of course be made for modified protocols, if such protocols are intended to use in regulatory decisions. [In this validation study, volatile substances were not included as test chemicals. It was found that water droplets attached to the inside of the quartz plate and sealed by the quartz plate, but were not dispersed from the wells during the ROS assay; chemicals with volatility to some extent can be applied to the ROS assay and may not interfere other assay results in the same reaction container.](#)

As shown in Table 21, sensitivities were the same under all criteria but specificities, positive predictivities, negative predictivities, and accuracies were lowest under draft criteria A. There was no apparent inconsistency of parameters among the other criteria, although a small advantage was found under draft criteria D. Draft criteria D also has an advantage in terms of throughput. Optimal criteria for final judgment will be determined in a comprehensive manner using results of another validation running parallel with this one but with a different solar simulator.

Performance standards were not set for the ROS assays based on the present validation study.

10. Conclusion

On the basis of the standardized protocol with Atlas solar simulator, robustness of the ROS assay was assessed using 27 phototoxic and 19 non-phototoxic chemicals at three facilities. Before assessment of predictivity, high within- and between-laboratory reproducibility and transferability of methods were confirmed. Experimental problems arising from limited solubility had a major impact on assay performance and applicability. Data analytical methods for final judgments compared four criteria, of which criteria D, in which the final judgment was made based on the first assay result from three independent assays, showed the best results. [We also propose having a standard protocol with defined data analytical methods, taking into consideration the results from other validation studies using a different solar simulator.](#)

11. References

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Table 3 Within-laboratory variation of phase 1 study

ROS assay Validation data (atlas)			Lab 1				Lab 2				Lab 3			
Chemicals			N	Mean	SD	CV (%)	N	Mean	SD	CV (%)	N	Mean	SD	CV (%)
Intra-day														
Positive Control	Quinine	SO	6	462	14.4	3.1	4	402	14.8	3.7	4	359	6.9	1.9
		SA	6	372	11.1	3.0	4	343	28.7	8.4	4	217	14.2	6.5
Negative Control	Sulisobenzone	SO	6	2	12.2	-	4	1	2.4	-	4	-7	1.0	-
		SA	6	-12	2.3	-	4	-7	2.4	-	4	-2	0.5	-
Inter-day														
Positive Control	Quinine	SO	3	455	4.5	1.0	3	401	18.6	4.6	3	359	7.6	2.1
		SA	3	349	16.2	4.6	3	335	17.8	5.3	3	223	10.0	4.5
Negative Control	Sulisobenzone	SO	3	3	8.7	-	3	-1	1.0	-	3	-5	3.0	-
		SA	3	-14	6.4	-	3	-7	4.6	-	3	-2	1.0	-

SO : Singlet oxygen

SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Intra-day variations were calculated by selecting the date on which a number of assay results were obtained most.

Inter-day variations were calculated based on the results of an assay of the day.

Table 4 Between-laboratory variation of phase 1 study

ROS assay Validation data (atlas)		N	Mean	SD	CV (%)	
Positive Control	Quinine	SO	3	410	50.0	12.2
		SA	3	312	82.9	26.6
Negative Control	Sulisobenzone	SO	3	-2	2.0	-
		SA	3	-7	5.0	-

SO : Singlet oxygen

SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Between-laboratory variations were calculated from the average value of the results of each facility.

Table 5 Results of the ROS assay multi-center variation phase 1 study

ROS assay Validation data (atlas)		Lab 1								Lab 2						Lab 3							
No.	Chemicals Name	Conc (µM)	Assay			Mean	SD	CV (%)	Conc (µM)	Assay			Mean	SD	CV (%)	Conc (µM)	Assay			Mean	SD	CV (%)	
			1st	2nd	3rd					1st	2nd	3rd					1st	2nd	3rd				
I - 1	5-FU	SO	20	2	-1	-1	0	1.7	-	20	4	-1	-9	-2	6.6	-	20	-4	-4	-1	-3	1.7	-
		SA	20	-18	-13	-9	-13	4.5	-	20	-1	-2	-3	-2	1	-	20	0	1	8	3	4.4	-
I - 2	8-MOP	SO	20	9	7	9	8	1.2	-	20	9	6	13	9	3.5	-	20	7	6	9	7	1.5	-
		SA	20	-34	-32	-37	-34	2.5	-	20	7	6	1	5	3.2	-	20	3	4	2	3	1	-
I - 3	Amiodarone ^a	SO	20	135	129	126	130	4.6	3.53	20	93	65	86	81	14.6	17.92	20	82	82	85	83	1.7	2.09
		SA	20	-27	-18	-16	-20	5.9	-	20	-4	3	-5	-2	4.4	-	20	5	8	7	7	1.5	-
I - 4	Chlorpromazine	SO	20	94	89	89	91	2.9	3.18	20	75	75	60	70	8.7	12.37	20	92	100	101	98	4.9	5.05
		SA	20	-21	-14	-14	-16	4	-	20	21	21	19	20	1.2	5.68	20	12	13	12	12	0.6	-
I - 5	Diclofenac	SO	20	163	160	161	161	1.5	0.95	20	101	113	90	101	11.5	11.35	20	155	147	145	149	5.3	3.55
		SA	20	8	14	14	12	3.5	-	20	9	8	5	7	2.1	-	20	8	10	9	9	1	-
I - 6	Doxycycline	SO	20	85	67	94	82	13.7	16.77	20	79	57	66	67	11.1	16.43	20	50	51	56	52	3.2	6.14
		SA	20	20	29	31	27	5.9	21.97	20	67	35	56	53	16.3	30.87	20	45	45	44	45	0.6	1.29
I - 7	Furosemide	SO	20	35	35	42	37	4	10.83	20	30	31	19	27	6.7	24.97	20	24	24	31	26	4	15.35
		SA	20	-15	-14	-15	-15	0.6	-	20	6	8	-2	4	5.3	-	20	5	6	5	5	0.6	-
I - 8	Ketoprofen	SO	20	41	30	48	40	9.1	22.88	20	30	31	33	31	1.5	4.88	20	41	40	44	42	2.1	5
		SA	20	-29	-31	-7	-22	13.3	-	20	4	3	6	4	1.5	-	20	1	2	2	2	0.6	-
I - 9	Levofloxacin	SO	20	33	46	36	38	6.8	17.76	20	28	30	20	26	5.3	20.35	20	35	34	39	36	2.6	7.35
		SA	20	172	161	150	161	11	6.83	20	191	209	181	194	14.2	7.33	20	162	134	141	146	14.6	10
I - 10	Norfloxacin	SO	20	82	77	122	94	24.7	26.33	20	60	59	55	58	2.6	4.56	20	58	53	56	56	2.5	4.52
		SA	20	4	3	7	5	2.1	-	20	21	28	23	24	3.6	15.02	20	18	15	16	16	1.5	-
I - 11	Omeprazole	SO	20	66	71	68	68	2.5	3.68	20	57	65	59	60	4.2	6.9	20	49	47	55	50	4.2	8.27
		SA	20	12	8	8	9	2.3	-	20	36	35	36	36	0.6	1.62	20	22	14	19	18	4	-
I - 12	Quinine	SO	20	124	121	113	119	5.7	4.77	20	117	115	109	114	4.2	3.66	20	74	81	84	80	5.1	6.44
		SA	20	10	8	21	13	7	-	20	65	65	59	63	3.5	5.5	20	34	26	31	30	4	13.32
I - 13	Sulisobenzone	SO	20	-11	-2	-10	-8	4.9	-	20	-1	3	-9	-2	6.1	-	20	-3	-2	2	-1	2.6	-
		SA	20	-6	-7	-22	-12	9	-	20	-3	-2	-4	-3	1	-	20	-2	-1	-1	-1	0.6	-

SO : Singlet oxygen SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the phase 2 study, it is considered that the precipitation have been formed at 20µM and 200µM in this study.

Table 5 Results of the ROS assay multi-center variation phase 1 study (continued)

ROS assay Validation data (atlas)			Lab 1							Lab 2							Lab 3						
No.	Chemicals Name	Conc (µM)	Assay				Mean	SD	CV (%)	Conc (µM)	Assay			Mean	SD	CV (%)	Conc (µM)	Assay			Mean	SD	CV (%)
			1st	2nd	3rd	1st					2nd	3rd	1st					2nd	3rd				
I - 1	5-FU	SO	200	0	-2	1	0	1.5	-	200	1	-2	0	0	1.5	-	200	-6	-2	-1	-3	2.6	-
		SA	200	-8	-9	-7	-8	1	-	200	-3	0	-5	-3	2.5	-	200	0	0	0	0	0	-
I - 2	8-MOP	SO	200	121	123	122	122	1	0.82	200	60	65	58	61	3.6	5.91	200	42	55	51	49	6.7	13.5
		SA	200	-2	5	4	2	3.8	-	200	64	76	50	63	13	20.55	200	15	18	15	16	1.7	-
I - 3	Amiodarone ^a	SO	200	259	192	247	233	35.7	15.35	200	379	311	443	378	66	17.48	200	319	393	362	358	37.2	10.38
		SA	200	-230	-196	-232	-219	20.2	-	200	-59	-194	-159	-137	70.1	-	200	-114	20	-82	-59	70	-
I - 4	Chlorpromazine	SO	200	-3	-12	-7	-7	4.5	-	200	-36	-35	-37	-36	1	-	200	16	19	9	15	5.1	-
		SA	200	80	78	78	79	1.2	1.47	200	90	113	98	100	11.7	11.64	200	78	74	76	76	2	2.63
I - 5	Diclofenac	SO	200	312	318	321	317	4.6	1.45	200	241	263	234	246	15.1	6.15	200	330	330	328	329	1.2	0.35
		SA	200	364	362	365	364	1.5	0.42	200	263	305	302	290	23.4	8.08	200	303	286	300	296	9.1	3.06
I - 6	Doxycycline	SO	200	113	111	117	114	3.1	2.69	200	198	227	186	204	21.1	10.35	200	175	161	170	169	7.1	4.21
		SA	200	321	321	323	322	1.2	0.36	200	394	411	368	391	21.7	5.54	200	308	315	314	312	3.8	1.21
I - 7	Furosemide	SO	200	190	196	198	195	4.2	2.14	200	129	138	131	133	4.7	3.56	200	110	117	117	115	4	3.52
		SA	200	102	99	94	98	4	4.11	200	60	76	65	67	8.2	12.22	200	42	44	43	43	1	2.33
I - 8	Ketoprofen	SO	200	252	248	255	252	3.5	1.4	200	210	202	242	218	21.2	9.71	200	204	197	209	203	6	2.96
		SA	200	90	94	94	93	2.3	2.49	200	107	95	109	104	7.6	7.3	200	58	52	51	54	3.8	7.05
I - 9	Levofloxacin	SO	200	204	188	191	194	8.5	4.38	200	125	127	134	129	4.7	3.67	200	116	118	125	120	4.7	3.95
		SA	200	371	390	379	380	9.5	2.51	200	455	472	441	456	15.5	3.4	200	440	451	443	445	5.7	1.28
I - 10	Norfloxacin	SO	200	211	208	213	211	2.5	1.19	200	154	148	159	154	5.5	3.58	200	155	143	155	151	6.9	4.59
		SA	200	116	119	115	117	2.1	1.78	200	148	164	151	154	8.5	5.51	200	121	117	102	113	10	8.84
I - 11	Omeprazole	SO	200	-29	-31	-24	-28	3.6	-	200	-46	-56	-49	-50	5.1	-	200	-36	-30	-59	-42	15.3	-
		SA	200	118	109	116	114	4.7	4.13	200	141	147	149	146	4.2	2.86	200	141	131	144	139	6.8	4.91
I - 12	Quinine	SO	200	433	432	456	440	13.6	3.08	200	406	405	415	409	5.5	1.35	200	346	337	345	343	4.9	1.44
		SA	200	387	394	379	387	7.5	1.94	200	395	396	372	388	13.6	3.5	200	215	209	195	206	10.3	4.97
I - 13	Sulisobenzone	SO	200	4	7	2	4	2.5	-	200	-1	-1	4	1	2.9	-	200	-9	-4	-4	-6	2.9	-
		SA	200	-13	-9	-16	-13	3.5	-	200	-6	-6	-6	-6	0	-	200	-1	-3	-3	-2	1.2	-

SO : Singlet oxygen SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the phase 2 study, it is considered that the precipitation have been formed at 20µM and 200µM in this study.

Table 6A Judgment from the phase 1 results: Final judgment of positive when positive results were obtained in at least one of three assays
20 µM

ROS assay Validation data (atlas) pattern A		Lab 1				Lab 2				Lab 3				Integrated Judgment ^b
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	-	-	-	-	-	-	-	-	-	-	-	-	-
I-3	Amiodarone ^a	+	+	+	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	-	+	-	-	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
I-12	Quinine	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-

200 µM

ROS assay Validation data (atlas) pattern A		Lab 1				Lab 2				Lab 3				Integrated Judgment ^b
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone ^a	+	+	+	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
I-12	Quinine	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20)

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the phase 2 study, it is considered that the precipitation have been formed at 20µM and 200µM in this study.

b : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 6B Judgment from the phase 1 results: Final judgment based on the mean value of three assays

20 μ M

ROS assay Validation data (atlas) pattern B		Lab 1				Lab 2				Lab 3				Integrated Judgment ^b
Chemicals		Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
No.	Name	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	-	-	-	-	-	-	-	-	-	-	-	-	-
I-3	Amiodarone ^a	+	+	+	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	-	+	-	-	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
I-12	Quinine	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-

200 μ M

ROS assay Validation data (atlas) pattern B		Lab 1				Lab 2				Lab 3				Integrated Judgment ^b
Chemicals		Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
No.	Name	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I-1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
I-2	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
I-3	Amiodarone ^a	+	+	+	+	+	+	+	+	+	+	+	+	+
I-4	Chlorpromazine	+	+	+	+	+	+	+	+	+	+	+	+	+
I-5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
I-6	Doxycycline	+	+	+	+	+	+	+	+	+	+	+	+	+
I-7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
I-8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I-9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I-10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I-11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
I-12	Quinine	+	+	+	+	+	+	+	+	+	+	+	+	+
I-13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20)

a : Since the precipitation was observed at a concentration of 20 μ M of Amiodarone in the phase 2 study, it is considered that the precipitation have been formed at 20 μ M and 200 μ M in this study.

b : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 6C Judgment from the phase 1 results: Final judgment based on the majority of three assay results

20 µM

ROS assay Validation data (atlas) pattern C		Lab 1				Lab 2				Lab 3				Integrated Judgment ^b
Chemicals		Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
No.	Name	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I - 1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
I - 2	8-MOP	-	-	-	-	-	-	-	-	-	-	-	-	-
I - 3	Amiodarone ^a	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 4	Chlorpromazine	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 6	Doxycycline	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 7	Furosemide	+	+	+	+	+	+	-	+	-	-	+	-	+
I - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 12	Quinine	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-

200 µM

ROS assay Validation data (atlas) pattern C		Lab 1				Lab 2				Lab 3				Integrated Judgment ^b
Chemicals		Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
No.	Name	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I - 1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
I - 2	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 3	Amiodarone ^a	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 4	Chlorpromazine	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 6	Doxycycline	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 12	Quinine	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20)

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the phase 2 study, it is considered that the precipitation have been formed at 20µM and 200µM in this study.

b : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 6D Judgment from the phase 1 results: Final judgment on the first assay results
20 µM

ROS assay Validation data (atlas) pattern D		Lab 1				Lab 2				Lab 3				Integrated Judgment ^b
Chemicals		Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
No.	Name	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I - 1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
I - 2	8-MOP	-	-	-	-	-	-	-	-	-	-	-	-	-
I - 3	Amiodarone ^a	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 4	Chlorpromazine	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 6	Doxycycline	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 7	Furosemide	+	+	+	+	+	+	-	+	-	-	+	-	+
I - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 12	Quinine	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-

200 µM

ROS assay Validation data (atlas) pattern D		Lab 1				Lab 2				Lab 3				Integrated Judgment ^b
Chemicals		Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
No.	Name	1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
I - 1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
I - 2	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 3	Amiodarone ^a	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 4	Chlorpromazine	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 6	Doxycycline	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 10	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 12	Quinine	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 13	Sulisobenzone	-	-	-	-	-	-	-	-	-	-	-	-	-

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20)

a : Since the precipitation was observed at a concentration of 20 µM of Amiodarone in the phase 2 study, it is considered that the precipitation have been formed at 20µM and 200µM in this study.

b : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 7A Contingency table for the phase 1 results at 20 μ M: Final judgment of positive when positive results were obtained in at least one of three assays

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 1		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 2		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 3		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Table 7B Contingency table for the phase 1 results at 20 μ M: Final judgment based on the mean value of three assays

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 1		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 2		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100%(10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 3		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Table 7C Contingency table for the phase 1 results at 20 μ M: Final judgment based on the majority of three assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 1		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 2		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 3		ROS		Total
		+	-	
Phototoxic	+	9	3	12
	-	0	1	1
Total		9	4	13

Sensitivity : 75.0% (9/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (9/9)
 Negative predictivity : 25.0% (1/4)
 Accuracy : 76.9% (10/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Table 7D Contingency table for the phase 1 results at 20 μ M: Final judgment on the first assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 20 μ M

Lab 1		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 2		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Lab 3		ROS		Total
		+	-	
Phototoxic	+	9	3	12
	-	0	1	1
Total		9	4	13

Sensitivity : 75.0% (9/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (9/9)
 Negative predictivity : 25.0% (1/4)
 Accuracy : 76.9% (10/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	10	2	12
	-	0	1	1
Total		10	3	13

Sensitivity : 83.3% (10/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (10/10)
 Negative predictivity : 33.3% (1/3)
 Accuracy : 84.6% (11/13)

Table 7E Contingency table for the phase 1 results at 200 μ M: The final judgments were the same in all of the analysis methods

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Concentration: 200 μ M

Lab 1		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Lab 2		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Lab 3		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Integrated Judgment		ROS		Total
		+	-	
Phototoxic	+	11	1	12
	-	0	1	1
Total		11	2	13

Sensitivity : 91.7% (11/12)
 Specificity : 100% (1/1)
 Positive predictivity : 100% (11/11)
 Negative predictivity : 50.0% (1/2)
 Accuracy : 92.3% (12/13)

Table 8 Irradiance and temperature during the irradiation in the phase 2 study

			Lab 1					Lab 2					Lab 3				
			Mean	SD	CV (%)	Max	Min	Mean	SD	CV (%)	Max	Min	Mean	SD	CV (%)	Max	Min
Irradiance (mW/cm ²)	Beginning of Irradiation	A	1.8	0	-	1.8	1.8	1.4	0.01	0.7	1.4	1.4	1.2	0.06	5.0	1.2	1.0
		B	2.1	0	-	2.1	2.1	1.9	0.05	2.6	2.0	1.8	2.1	0.12	5.7	2.2	1.8
	End of Irradiation	A	1.8	0	-	1.8	1.8	1.4	0.01	0.7	1.5	1.4	1.2	0.05	4.2	1.2	1.1
		B	2.1	0	-	2.1	2.1	1.9	0.06	3.2	2.0	1.8	2.1	0.10	4.8	2.2	2.0
Temperature (°C)	Beginning of Irradiation		26.3	1.46	5.6	29	24	24.5	0.55	2.2	25.4	22.8	24.6	1.21	4.9	27.6	21.9
	End of Irradiation		26.1	1.58	6.1	29	24	25.0	0.12	0.5	25.2	24.8	25.1	1.43	5.7	28.3	23.0

A : Irradiances which were measured with each test facility's UVA detector.

B : Standardized irradiances which were calculated as the irradiances by the calibrated UVA detector Dr. Hönle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the values from the UVA detectors.

CV : Coefficient of variation

Table 9 Within-laboratory variation of phase 2 study

ROS assay Validation data (atlas)			Lab 1				Lab 2				Lab 3			
Chemicals			N	Mean	SD	CV (%)	N	Mean	SD	CV (%)	N	Mean	SD	CV (%)
Intra-day														
Positive Control	Quinine	SO	7	553	14.4	2.6	6	438	10.7	2.4	3	366	5.0	1.4
		SA	7	424	30.8	7.3	6	305	11.7	3.8	3	306	26.7	8.7
Negative Control	Sulisobenzone	SO	7	5	9.6	-	6	0	3.2	-	3	-2	1.2	-
		SA	7	-13	6.6	-	6	-12	3.0	-	3	-5	0	-
Inter-day														
Positive Control	Quinine	SO	5	532	11.9	2.2	8	430	6.1	1.4	11	359	9.8	2.7
		SA	5	408	8.4	2.1	8	276	24.2	8.8	11	295	15.9	5.4
Negative Control	Sulisobenzone	SO	5	2	4.8	-	8	1	3.3	-	11	0	1.7	-
		SA	5	-14	7.8	-	8	-11	3.3	-	11	-6	1.2	-

SO : Singlet oxygen

SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Intra-day variations were calculated by selecting the date on which a number of assay results were obtained most.

Inter-day variations were calculated based on using the assay results of the first assay of the each assay day.

Table 10 Between-laboratory variation of phase 2 study

ROS assay Validation data (atlas)		N	Mean	SD	CV (%)	
Positive Control	Quinine	SO	3	445	91.8	20.6
		SA	3	323	64.9	20.1
Negative Control	Sulisobenzone	SO	3	2	2.6	-
		SA	3	-11	5.3	-

SO : Singlet oxygen

SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Between-laboratory variations were calculated from the average value of the results of each facility.

Table 11 Results of the ROS assay multi-center validation phase 2 study

ROS assay Validation data (atlas)			Lab 1							Lab 2							Lab 3						
N0.	Chemicals		Conc (µM)	Assay			Mean	SD	CV (%)	Conc (µM)	Assay			Mean	SD	CV (%)	Conc (µM)	Assay			Mean	SD	CV (%)
	Name			1st	2nd	3rd					1st	2nd	3rd					1st	2nd	3rd			
II - 1	Acridine	SO	200	225	233	222	227	5.7	2.51	200	229	218	214	220	7.8	3.53	200	223	221	223	222	1.2	0.52
		SA	200	228	231	215	225	8.5	3.79	200	191	209	190	197	10.7	5.44	200	192	172	168	177	12.9	7.25
II - 2	Acridine HCl	SO	200	224	214	223	220	5.5	2.5	200	226	216	224	222	5.3	2.38	200	215	215	218	216	1.7	0.8
		SA	200	211	215	215	214	2.3	1.08	200	198	212	181	197	15.5	7.88	200	176	181	164	174	8.7	5.03
II - 3	Amiodarone HCl	SO	2	33	9	22	21	12	-	2	25	38	28	30	6.8	22.44	2	36	51	48	45	7.9	17.64
		SA	2	-16	-13	-5	-11	5.7	-	2	3	5	-5	1	5.3	-	2	0	2	0	1	1.2	-
II - 4	Chlorpromazine HCl	SO	200	-18	-41	-15	-25	14.2	-	200	-2	-13	-6	-7	5.6	-	200	-22	-32	-23	-26	5.5	-
		SA	200	84	106	97	96	11.1	11.56	200	97	99	109	102	6.4	6.32	200	87	84	78	83	4.6	5.52
II - 5	Doxycycline HCl	SO	200	271	269	262	267	4.7	1.77	200	222	234	222	226	6.9	3.07	200	166	160	160	162	3.5	2.14
		SA	200	353	486	342	394	80.2	20.36	200	413	437	411	420	14.5	3.44	200	247	249	261	252	7.6	3
II - 6	Fenofibrate	SO	20	124	202	173	166	39.4	23.7	2	5	18	11	11	6.5	-	20	161	161	161	161	0	0
		SA	20	0	-39	-31	-23	20.6	-	2	-6	17	-9	1	14.2	-	20	-9	-12	-12	-11	1.7	-
II - 7	Furosemide	SO	200	227	238	224	230	7.4	3.21	200	138	131	133	134	3.6	2.69	200	145	146	144	145	1	0.69
		SA	200	115	121	102	113	9.7	8.62	200	62	67	67	65	2.9	4.42	200	52	50	54	52	2	3.85
II - 8	Ketoprofen	SO	200	358	362	368	363	5	1.39	200	245	259	240	248	9.8	3.97	200	224	220	206	217	9.5	4.36
		SA	200	130	122	137	130	7.5	5.79	200	107	117	109	111	5.3	4.77	200	80	88	87	85	4.4	5.13
II - 9	6-methylcoumarine	SO	200	114	111	120	115	4.6	3.98	200	103	122	142	122	19.5	15.94	200	106	96	99	100	5.1	5.11
		SA	200	109	128	130	122	11.6	9.47	200	87	110	100	99	11.5	11.65	200	62	70	67	66	4	6.09
II - 10	8-MOP	SO	200	83	101	78	87	12.1	13.85	200	81	79	60	73	11.6	15.8	200	65	77	70	71	6	8.53
		SA	200	76	138	113	109	31.2	28.62	200	87	103	92	94	8.2	8.71	200	23	30	31	28	4.4	15.57
II - 11	Nalidixic acid	SO	200	348	185	182	238	95	39.85	200	147	144	145	145	1.5	1.05	200	134	130	119	128	7.8	6.08
		SA	200	355	271	264	297	50.6	17.07	200	254	206	252	237	27.2	11.44	200	294	356	314	321	31.6	9.85
II - 12	Nalidixic acid (Na salt)	SO	200	183	165	183	177	10.4	5.87	200	144	140	149	144	4.5	3.12	200	125	124	119	123	3.2	2.62
		SA	200	287	233	203	241	42.6	17.66	200	218	192	211	207	13.5	6.5	200	299	341	320	320	21	6.56
II - 13	Norfloxacin	SO	200	215	219	214	216	2.6	1.22	200	188	222	200	203	17.2	8.48	200	164	171	169	168	3.6	2.15
		SA	200	145	139	113	132	17	12.85	200	132	120	119	124	7.2	5.85	200	149	149	139	146	5.8	3.96
II - 14	Ofloxacin	SO	200	193	203	192	196	6.1	3.1	200	132	137	149	139	8.7	6.27	200	126	125	117	123	4.9	4.02
		SA	200	351	228	274	284	62.1	21.86	200	288	292	292	291	2.3	0.79	200	420	439	446	435	13.5	3.09
II - 15	Piroxicam	SO	200	191	210	232	211	20.5	9.72	200	207	230	221	219	11.6	5.28	200	168	160	153	160	7.5	4.68
		SA	20	28	38	26	31	6.4	20.96	20	56	47	43	49	6.7	13.68	20	4	3	7	5	2.1	-
II - 16	Promethazine HCl	SO	200	70	62	84	72	11.1	15.47	200	91	89	103	94	7.6	8.03	200	43	46	39	43	3.5	8.23
		SA	200	67	86	86	80	11	13.77	200	43	51	48	47	4	8.54	200	35	33	36	35	1.5	4.41
II - 17	Rosiglitazone	SO	200	118	104	131	118	13.5	11.48	200	90	87	93	90	3	3.33	200	57	54	54	55	1.7	3.15
		SA	20	41	36	29	35	6	17.06	20	27	29	35	30	4.2	13.73	20	15	17	15	16	1.2	-
II - 18	Tetracycline	SO	200	200	194	200	198	3.5	1.75	200	167	160	166	164	3.8	2.3	200	134	129	129	131	2.9	2.21
		SA	200	197	216	218	210	11.6	5.51	200	240	255	239	245	9	3.66	200	146	101	123	123	22.5	18.24

SO : Singlet oxygen SA : Superoxide anion

CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Table 11 Results of the ROS assay multi-center validation phase 2 study (continued)

ROS assay Validation data (atlas)			Lab 1							Lab 2							Lab 3								
No.	Chemicals Name	Conc (µM)	Assay					Mean	SD	CV (%)	Conc (µM)	Assay					Mean	SD	CV (%)	Conc (µM)	Assay				
			1st	2nd	3rd	1st	2nd					3rd	1st	2nd	3rd	1st					2nd	3rd			
II - 19	Anthracene	SO	20	261	340	272	291	42.8	14.7	2	-2	7	6	4	4.9	-	2	5	3	4	4	1	-		
		SA	2	39	48	1	29	24.9	85.05	2	3	8	-3	3	5.5	-	2	4	4	4	4	0	-		
II - 20	Avobenzene	SO	20	142	121	117	127	13.4	10.6	2	0	12	10	7	6.4	-	20	58	60	65	61	3.6	5.91		
		SA	2	52	19	32	34	16.6	48.42	2	29	36	25	30	5.6	18.56	20	13	9	18	13	4.5	-		
II - 21	Bithionol	SO	200	81	113	114	103	18.8	18.28	200	137	143	140	140	3	2.14	200	81	68	72	74	6.7	9.04		
		SA	20	21	28	24	24	3.5	14.43	2	13	15	15	14	1.2	-	200	34	30	33	32	2.1	6.44		
II - 22	Hexachlorophene	SO	200	318	333	355	335	18.6	5.55	200	246	255	258	253	6.2	2.47	200	227	226	217	223	5.5	2.47		
		SA	200	6	22	7	12	9	-	2	-6	15	-3	2	11.4	-	200	10	8	6	8	2	-		
II - 23	Rose bengal	SO	200	682	685	679	682	3	0.44	200	631	634	667	644	20	3.1	200	608	589	607	601	10.7	1.78		
		SA	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
II - 24	Aspirin	SO	200	1	2	4	2	1.5	-	200	1	-1	1	0	1.2	-	200	-2	3	1	1	2.5	-		
		SA	200	-12	-15	10	-6	13.7	-	200	0	-6	-4	-3	3.1	-	200	-1	-1	0	-1	0.6	-		
II - 25	Benzocaine	SO	200	6	3	-2	2	4	-	200	9	1	-12	-1	10.6	-	200	0	4	3	2	2.1	-		
		SA	200	9	20	0	10	10	-	200	-4	12	6	5	8.1	-	200	0	1	0	0	0.6	-		
II - 26	Erythromycin	SO	200	-16	5	8	-1	13.1	-	200	6	-1	13	6	7	-	200	0	-4	-3	-2	2.1	-		
		SA	200	4	8	6	6	2	-	200	14	4	35	18	15.8	-	200	1	4	2	2	1.5	-		
II - 27	Penicillin G	SO	200	17	6	9	11	5.7	-	200	0	-1	4	1	2.6	-	200	0	5	4	3	2.6	-		
		SA	200	11	26	40	26	14.5	56.5	200	37	36	34	36	1.5	4.28	200	4	11	16	10	6	-		
II - 28	Phenytoin	SO	200	1	19	3	8	9.9	-	200	0	-4	2	-1	3.1	-	200	2	6	4	4	2	-		
		SA	200	63	55	32	50	16.1	32.19	200	52	53	48	51	2.6	5.19	200	35	17	26	26	9	34.62		
II - 29	Bumetizole	SO	20	-7	-11	-11	-10	2.3	-	2	-5	10	0	2	7.6	-	20	-6	-16	-19	-14	6.8	-		
		SA	2	-8	9	-6	-2	9.3	-	2	-7	0	-7	-5	4	-	20	-2	2	9	3	5.6	-		
II - 30	Camphor sulfonic acid	SO	200	7	-2	8	4	5.5	-	200	-1	3	2	1	2.1	-	200	3	1	4	3	1.5	-		
		SA	200	-4	-12	-22	-13	9	-	200	-4	5	-2	0	4.7	-	200	-4	-1	0	-2	2.1	-		
II - 31	Chlorhexidine	SO	200	-5	8	-12	-3	10.1	-	200	11	-9	8	3	10.8	-	200	23	22	22	22	0.6	-		
		SA	200	28	35	6	23	15.1	65.79	200	13	21	16	17	4	-	200	13	7	10	10	3	-		
II - 32	Cinnamic acid	SO	200	6	0	0	2	3.5	-	200	5	0	-8	-1	6.6	-	200	0	-1	2	0	1.5	-		
		SA	200	61	70	36	56	17.6	31.65	200	52	36	37	42	9	21.51	200	9	10	7	9	1.5	-		
II - 33	Drometizole	SO	20	7	-4	-7	-1	7.4	-	20	2	6	8	5	3.1	-	20	8	2	4	5	3.1	-		
		SA	20	-11	13	-8	-2	13.1	-	20	8	3	2	4	3.2	-	20	6	7	7	7	0.6	-		
II - 34	L-Histidine	SO	200	12	15	7	11	4	-	200	4	9	-3	3	6	-	200	4	3	4	4	0.6	-		
		SA	200	61	73	51	62	11	17.86	200	55	-4	68	40	38.4	96.73	200	51	48	48	49	1.7	3.53		
II - 35	Methylbenzylidene camphor	SO	20	-7	-3	-4	-5	2.1	-	20	-3	-1	-1	-2	1.2	-	20	-4	-3	-8	-5	2.6	-		
		SA	20	-6	5	-4	-2	5.9	-	20	4	7	-5	2	6.2	-	20	-2	-3	-1	-2	1	-		
II - 36	Octrizole	SO	2	4	-6	1	0	5.1	-	2	-3	3	-3	-1	3.5	-	20	-13	-11	-17	-14	3.1	-		
		SA	2	56	47	22	42	17.6	42.28	2	2	20	6	9	9.5	-	20	4	6	11	7	3.6	-		
II - 37	Octyl methacrylate	SO	200	17	26	52	32	18.2	57.39	20	1	-1	4	1	2.5	-	20	10	4	6	7	3.1	-		
		SA	200	-3	-25	-38	-22	17.7	-	20	-6	-1	-7	-5	3.2	-	20	0	0	-1	0	0.6	-		
II - 38	Octyl methoxycinnamate	SO	20	6	2	5	4	2.1	-	2	-4	6	2	1	5	-	20	3	2	4	3	1	-		
		SA	20	-17	-15	-11	-14	3.1	-	2	-5	2	1	-1	3.8	-	20	-5	-5	-4	-5	0.6	-		
II - 39	Octyl salicylate	SO	20	0	14	2	5	7.6	-	20	1	7	7	5	3.5	-	20	-3	-3	-3	-3	0	-		
		SA	20	12	2	1	5	6.1	-	20	7	2	-4	2	5.5	-	20	0	0	0	0	0	-		
II - 40	PABA	SO	200	18	-8	5	5	13	-	200	2	2	7	4	2.9	-	200	-5	-3	3	-2	4.2	-		
		SA	200	10	-5	8	4	8.1	-	200	-9	-2	-1	-4	4.4	-	200	-1	0	-1	-1	0.6	-		
II - 41	SDS	SO	200	18	17	15	17	1.5	-	200	5	8	12	8	3.5	-	200	5	5	8	6	1.7	-		
		SA	20	16	5	3	8	7	-	2	1	-4	1	-1	2.9	-	200	5	6	14	8	4.9	-		
II - 42	UV-571	SO	20	-16	-19	-15	-17	2.1	-	2	-10	7	-4	-2	8.6	-	2	-5	-4	-4	-4	0.6	-		
		SA	2	10	4	15	10	5.5	-	2	2	8	0	3	4.2	-	2	1	2	1	1	0.6	-		

SO : Singlet oxygen SA : Superoxide anion ND : no data
 CV : CV (coefficient of variation) values are presented when the assay results of SA or SO were judged positive based on the mean values.

Table 12A Judgment from the phase 2 results: Final judgment of positive when positive results were obtained in at least one of three assays

ROS assay Validation data (atlas) pattern A		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	+	I	I	+	+	+	+	+	+	+	+	+	+
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	+
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloracin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	I	I	I	I	I	I	I	I	I
II - 20	Avobenzene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 12A Judgment from the phase 2 results: Final judgment of positive when positive results were obtained in at least one of three assays (continued)

ROS assay Validation data (atlas) pattern A		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	+	-	+	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	+	+	-	-	-	-	-
II - 27	Penicillin G	-	+	+	+	+	+	+	+	-	-	-	-	+
II - 28	Phenytoin	+	+	+	+	+	+	+	+	+	-	+	+	+
II - 29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	+	+	-	+	-	+	-	+	-	-	-	-	+
II - 32	Cinnamic acid	+	+	+	+	+	+	+	+	-	-	-	-	+
II - 33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 34	L-Histidine	+	+	+	+	+	-	+	+	+	+	+	+	+
II - 35	Methylbenzylidene camphor	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 36	Octrizole	+	+	+	+	I	+	I	+	I	I	I	I	+
II - 37	Octyl methacrylate	-	+	+	+	I	I	I	I	I	I	I	I	I
II - 38	Octyl methoxycinnamate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 39	Octyl salicylate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	I	I	I	I	I	I	I	I	-	-	-	-	I
II - 42	UV-571	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 12B Judgment from the phase 2 results: Final judgment based on the mean value of three assays

ROS assay Validation data (atlas) pattern B		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	+	I	I	I	+	+	+	+	+	+	+	+	+
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	+
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	I	I	I	I	I	I	I	I	I
II - 20	Avobenzone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 12B Judgment from the phase 2 results: Final judgment based on the mean value of three assays (continued)

ROS assay Validation data (atlas) pattern B		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	+	-	-	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	+	-	-	-	-	-	-
II - 27	Penicillin G	-	+	+	+	+	+	+	+	-	-	-	-	+
II - 28	Phenytoin	+	+	+	+	+	+	+	+	+	-	+	+	+
II - 29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	+	+	-	+	-	+	-	-	-	-	-	-	-
II - 32	Cinnamic acid	+	+	+	+	+	+	+	+	-	-	-	-	+
II - 33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 34	L-Histidine	+	+	+	+	+	-	+	+	+	+	+	+	+
II - 35	Methylbenzylidene camphor	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 36	Octrizole	+	+	+	+	I	+	I	I	I	I	I	I	I
II - 37	Octyl methacrylate	-	+	+	+	I	I	I	I	I	I	I	I	I
II - 38	Octyl methoxycinnamate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 39	Octyl salicylate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	I	I	I	I	I	I	I	I	-	-	-	-	I
II - 42	UV-571	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 12C Judgment from the phase 2 results: Final judgment based on the majority of three assay results

ROS assay Validation data (atlas) pattern C		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	+	I	I	I	+	+	+	+	+	+	+	+	+
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	+
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloracin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	I	I	I	I	I	I	I	I	I
II - 20	Avobenzone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 12C Judgment from the phase 2 results: Final judgment based on the majority of three assay results (continued)

ROS assay Validation data (atlas) pattern C		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	+	-	-	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	+	-	-	-	-	-	-
II - 27	Penicillin G	-	+	+	+	+	+	+	+	-	-	-	-	+
II - 28	Phenytoin	+	+	+	+	+	+	+	+	+	-	+	+	+
II - 29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	+	+	-	+	-	+	-	-	-	-	-	-	-
II - 32	Cinnamic acid	+	+	+	+	+	+	+	+	-	-	-	-	+
II - 33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 34	L-Histidine	+	+	+	+	+	-	+	+	+	+	+	+	+
II - 35	Methylbenzylidene camphor	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 36	Octrizole	+	+	+	+	I	+	I	I	I	I	I	I	I
II - 37	Octyl methacrylate	-	+	+	+	I	I	I	I	I	I	I	I	I
II - 38	Octyl methoxycinnamate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 39	Octyl salicylate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	I	I	I	I	I	I	I	I	-	-	-	-	I
II - 42	UV-571	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 12D Judgment from the phase 2 results: Final judgment on the first assay results

ROS assay Validation data (atlas) pattern D		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	+	I	I	+	+	+	+	+	+	+	+	+	+
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	+
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	I	I	I	I	I	I	I	I	I
II - 20	Avobenzone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 12D Judgment from the phase 2 results: Final judgment on the first assay results (continued)

ROS assay Validation data (atlas) pattern D		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	+	-	-	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	+	-	-	-	-	-	-
II - 27	Penicillin G	-	+	+	-	+	+	+	+	-	-	-	-	-
II - 28	Phenytoin	+	+	+	+	+	+	+	+	+	-	+	+	+
II - 29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	+	+	-	+	-	+	-	-	-	-	-	-	-
II - 32	Cinnamic acid	+	+	+	+	+	+	+	+	-	-	-	-	+
II - 33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 34	L-Histidine	+	+	+	+	+	-	+	+	+	+	+	+	+
II - 35	Methylbenzylidene camphor	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 36	Octrizole	+	+	+	+	I	+	I	I	I	I	I	I	I
II - 37	Octyl methacrylate	-	+	+	-	I	I	I	I	I	I	I	I	I
II - 38	Octyl methoxycinnamate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 39	Octyl salicylate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	I	I	I	I	I	I	I	I	-	-	-	-	I
II - 42	UV-571	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)
 - : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)
 I : inconclusive(The results does not meet the positive or negative criterion)
 a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 13A Contingency table for phase 2 results: Final judgment of positive when positive results were obtained in at least one of three assays

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	-	I	
Phototoxic	+	23	0	(0)	23(23)
	-	8	4	(7)	12(19)
Total		31	4	(7)	35(42)

Sensitivity : 100% (23/23)
 Specificity : 33.3% (4/12)
 Positive predictivity : 74.2% (23/31)
 Negative predictivity : 100% (4/4)
 Accuracy : 77.1% (27/35)

Lab 2		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	7	4	(8)	11(19)
Total		28	4	(10)	32(42)

Sensitivity : 100% (21/21)
 Specificity : 36.4% (4/11)
 Positive predictivity : 75.0% (21/28)
 Negative predictivity : 100% (4/4)
 Accuracy : 78.1% (25/32)

Lab 3		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	2	9	(8)	11(19)
Total		24	9	(9)	33(42)

Sensitivity : 100% (22/22)
 Specificity : 81.8% (9/11)
 Positive predictivity : 91.7% (22/24)
 Negative predictivity : 100% (9/9)
 Accuracy : 93.9% (31/33)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	6	5	(8)	11(19)
Total		28	5	(9)	33(42)

Sensitivity : 100% (22/22)
 Specificity : 45.5% (5/11)
 Positive predictivity : 78.6% (22/28)
 Negative predictivity : 100% (5/5)
 Accuracy : 81.8% (27/33)

Table 13B Contingency table for phase 2 results: Final judgment based on the mean value of three assays

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	7	5	(7)	12(19)
Total		29	5	(8)	34(42)

Sensitivity : 100% (22/22)
 Specificity : 41.7% (5/12)
 Positive predictivity : 75.9% (22/29)
 Negative predictivity : 100% (5/5)
 Accuracy : 79.4% (27/34)

Lab 2		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	4	6	(9)	10(19)
Total		25	6	(11)	31(42)

Sensitivity : 100% (21/21)
 Specificity : 60.0% (6/10)
 Positive predictivity : 84.0% (21/25)
 Negative predictivity : 100% (6/6)
 Accuracy : 87.1% (27/31)

Lab 3		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	2	9	(8)	11(19)
Total		24	9	(9)	33(42)

Sensitivity : 100% (22/22)
 Specificity : 81.8% (9/11)
 Positive predictivity : 91.7% (22/24)
 Negative predictivity : 100% (9/9)
 Accuracy : 93.9% (31/33)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	4	6	(9)	10(19)
Total		26	6	(10)	32(42)

Sensitivity : 100% (22/22)
 Specificity : 60.0% (6/10)
 Positive predictivity : 84.6% (22/26)
 Negative predictivity : 100% (6/6)
 Accuracy : 87.5% (28/32)

Table 13C Contingency table for phase 2 results: Final judgment based on the majority of three assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	7	5	(7)	12(19)
Total		29	5	(8)	34(42)

Sensitivity : 100% (22/22)
 Specificity : 41.7% (5/12)
 Positive predictivity : 75.9% (22/29)
 Negative predictivity : 100% (5/5)
 Accuracy : 79.4% (27/34)

Lab 2		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	4	6	(9)	10(19)
Total		25	6	(11)	31(42)

Sensitivity : 100% (21/21)
 Specificity : 60.0% (6/10)
 Positive predictivity : 84.0% (21/25)
 Negative predictivity : 100% (6/6)
 Accuracy : 87.1% (27/31)

Lab 3		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	2	9	(8)	11(19)
Total		24	9	(9)	33(42)

Sensitivity : 100% (22/22)
 Specificity : 81.8% (9/11)
 Positive predictivity : 91.7% (22/24)
 Negative predictivity : 100% (9/9)
 Accuracy : 93.9% (31/33)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	4	6	(9)	10(19)
Total		26	6	(10)	32(42)

Sensitivity : 100% (22/22)
 Specificity : 60.0% (6/10)
 Positive predictivity : 84.6% (22/26)
 Negative predictivity : 100% (6/6)
 Accuracy : 87.5% (28/32)

Table 13D Contingency table for phase 2 results: Final judgment on the first assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	-	I	
Phototoxic	+	23	0	(0)	23(23)
	-	5	7	(7)	12(19)
Total		28	7	(7)	35(42)

Sensitivity : 100% (23/23)
 Specificity : 58.3% (7/12)
 Positive predictivity : 82.1% (23/28)
 Negative predictivity : 100% (7/7)
 Accuracy : 85.7% (30/35)

Lab 2		ROS			Total
		+	-	I	
Phototoxic	+	21	0	(2)	21(23)
	-	4	6	(9)	10(19)
Total		25	6	(11)	31(42)

Sensitivity : 100% (21/21)
 Specificity : 60.0% (6/10)
 Positive predictivity : 84.0% (21/25)
 Negative predictivity : 100% (6/6)
 Accuracy : 87.1% (27/31)

Lab 3		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	2	9	(8)	11(19)
Total		24	9	(9)	33(42)

Sensitivity : 100% (22/22)
 Specificity : 81.8% (9/11)
 Positive predictivity : 91.7% (22/24)
 Negative predictivity : 100% (9/9)
 Accuracy : 93.9% (31/33)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	22	0	(1)	22(23)
	-	3	7	(9)	10(19)
Total		25	7	(10)	32(42)

Sensitivity : 100% (22/22)
 Specificity : 70.0% (7/10)
 Positive predictivity : 88.0% (22/25)
 Negative predictivity : 100% (7/7)
 Accuracy : 90.6% (29/32)

Table 14A Judgment from the phase 1 and 2 results: Final judgment of positive when positive results were obtained in at least one of three assays

ROS assay Validation data (atlas) pattern A		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	+	I	I	+	+	+	+	+	+	+	+	+	+
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	+
I - 1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketopofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	I	I	I	I	I	I	I	I	I
II - 20	Avobenzene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 14A Judgment from the phase 1 and 2 results: Final judgment of positive when positive results were obtained in at least one of three assays (continued)

ROS assay Validation data (atlas) pattern A		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	+	-	+	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	+	+	-	-	-	-	-
II - 27	Penicillin G	-	+	+	+	+	+	+	+	-	-	-	-	+
II - 28	Phenytoin	+	+	+	+	+	+	+	+	+	-	+	+	+
II - 29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	+	+	-	+	-	+	-	+	-	-	-	-	+
II - 32	Cinnamic acid	+	+	+	+	+	+	+	+	-	-	-	-	+
II - 33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 34	L-Histidine	+	+	+	+	+	-	+	+	+	+	+	+	+
II - 35	Methylbenzylidene camphor	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 36	Octrizole	+	+	+	+	I	+	I	+	I	I	I	I	+
II - 37	Octyl methacrylate	-	+	+	+	I	I	I	I	I	I	I	I	I
II - 38	Octyl methoxycinnamate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 39	Octyl salicylate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	I	I	I	I	I	I	I	I	-	-	-	-	I
II - 42	UV-571	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 14B Judgment from the phase 1 and 2 results: Final judgment based on the mean value of three assays

ROS assay Validation data (atlas) pattern B		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	+	I	I	I	+	+	+	+	+	+	+	+	+
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	+
I - 1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketopofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	I	I	I	I	I	I	I	I	I
II - 20	Avobenzone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 14B Judgment from the phase 1 and 2 results: Final judgment based on the mean value of three assays (continued)

ROS assay Validation data (atlas) pattern B		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	+	-	-	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	+	-	-	-	-	-	-
II - 27	Penicillin G	-	+	+	+	+	+	+	+	-	-	-	-	+
II - 28	Phenytoin	+	+	+	+	+	+	+	+	+	-	+	+	+
II - 29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	+	+	-	+	-	+	-	-	-	-	-	-	-
II - 32	Cinnamic acid	+	+	+	+	+	+	+	+	-	-	-	-	+
II - 33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 34	L-Histidine	+	+	+	+	+	-	+	+	+	+	+	+	+
II - 35	Methylbenzylidene camphor	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 36	Octrizole	+	+	+	+	I	+	I	I	I	I	I	I	I
II - 37	Octyl methacrylate	-	+	+	+	I	I	I	I	I	I	I	I	I
II - 38	Octyl methoxycinnamate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 39	Octyl salicylate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	I	I	I	I	I	I	I	I	-	-	-	-	I
II - 42	UV-571	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 14C Judgment from the phase 1 and 2 results: Final judgment based on the majority of three assay results

ROS assay Validation data (atlas) pattern C		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	+	I	I	I	+	+	+	+	+	+	+	+	+
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	+
I - 1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketopofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	I	I	I	I	I	I	I	I	I
II - 20	Avobenzone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+: positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

-: negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I: inconclusive(The results does not meet the positive or negative criterion)

a: Integrated judgments were made by the majority of each laboratory's final judgment.

Table 14C Judgment from the phase 1 and 2 results: Final judgment based on the majority of three assay results (continued)

ROS assay Validation data (atlas) pattern C		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	+	-	-	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	+	-	-	-	-	-	-
II - 27	Penicillin G	-	+	+	+	+	+	+	+	-	-	-	-	+
II - 28	Phenytoin	+	+	+	+	+	+	+	+	+	-	+	+	+
II - 29	Bumetrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	+	+	-	+	-	+	-	-	-	-	-	-	-
II - 32	Cinnamic acid	+	+	+	+	+	+	+	+	-	-	-	-	+
II - 33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 34	L-Histidine	+	+	+	+	+	-	+	+	+	+	+	+	+
II - 35	Methylbenzylidene camphor	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 36	Octrizole	+	+	+	+	I	+	I	I	I	I	I	I	I
II - 37	Octyl methacrylate	-	+	+	+	I	I	I	I	I	I	I	I	I
II - 38	Octyl methoxycinnamate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 39	Octyl salicylate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	I	I	I	I	I	I	I	I	-	-	-	-	I
II - 42	UV-571	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Compound Numbers from the phase1 study were shown in the brackets.

Table 14D Judgment from the phase 1 and 2 results: Final judgment on the first assay results

ROS assay Validation data (atlas) pattern D		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	+	I	I	+	+	+	+	+	+	+	+	+	+
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 5	Diclofenac	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	I	I	I	I	+	+	+	+	+
I - 1	5-FU	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketopofen	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 9	Levofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
I - 11	Omeprazole	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	I	I	I	I	I	I	I	I	I
II - 20	Avobenzone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)
 - : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)
 I : inconclusive(The results does not meet the positive or negative criterion)
 a : Integrated judgments were made by the majority of each laboratory's final judgment.
 Compound Numbers from the phase1 study were shown in the brackets.

Table 14D Judgment from the phase 1 and 2 results: Final judgment on the first assay results (continued)

ROS assay Validation data (atlas) pattern D		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	+	-	-	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	+	-	-	-	-	-	-
II - 27	Penicillin G	-	+	+	-	+	+	+	+	-	-	-	-	-
II - 28	Phenytoin	+	+	+	+	+	+	+	+	+	-	+	+	+
II - 29	Bumetizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	+	+	-	+	-	+	-	-	-	-	-	-	-
II - 32	Cinnamic acid	+	+	+	+	+	+	+	+	-	-	-	-	+
II - 33	Drometrizole	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 34	L-Histidine	+	+	+	+	+	-	+	+	+	+	+	+	+
II - 35	Methylbenzylidene camphor	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 36	Octrizole	+	+	+	+	I	+	I	I	I	I	I	I	I
II - 37	Octyl methacrylate	-	+	+	-	I	I	I	I	I	I	I	I	I
II - 38	Octyl methoxycinnamate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 39	Octyl salicylate	I	I	I	I	I	I	I	I	I	I	I	I	I
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	I	I	I	I	I	I	I	I	-	-	-	-	I
II - 42	UV-571	I	I	I	I	I	I	I	I	I	I	I	I	I

+ : positive(Singlet oxygen results ≥ 25 or Superoxide results ≥ 20 at 200, 20 or 2 μM)

- : negative(Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 μM)

I : inconclusive(The results does not meet the positive or negative criterion)

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Compound Numbers from the phase1 study were shown in the brackets.

Table 15A Contingency table for phase 1 and 2 results: Final judgment of positive when positive results were obtained in at least one of three assays

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	-	I	
Phototoxic	+	26	1	(0)	27(27)
	-	8	4	(7)	12(19)
Total		34	5	(7)	39(46)

Sensitivity : 96.3% (26/27)
 Specificity : 33.3% (4/12)
 Positive predictivity : 76.5% (26/34)
 Negative predictivity : 80.0% (4/5)
 Accuracy : 76.9% (30/39)

Lab 2		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	7	4	(8)	11(19)
Total		31	5	(10)	36(46)

Sensitivity : 96.0% (24/25)
 Specificity : 36.4% (4/11)
 Positive predictivity : 77.4% (24/31)
 Negative predictivity : 80.0% (4/5)
 Accuracy : 77.8% (28/36)

Lab 3		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	2	9	(8)	11(19)
Total		27	10	(9)	37(46)

Sensitivity : 96.2% (25/26)
 Specificity : 81.8% (9/11)
 Positive predictivity : 92.6% (25/27)
 Negative predictivity : 90.0% (9/10)
 Accuracy : 91.9% (34/37)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	6	5	(8)	11(19)
Total		31	6	(9)	37(46)

Sensitivity : 96.2% (25/26)
 Specificity : 45.5% (5/11)
 Positive predictivity : 80.7% (25/31)
 Negative predictivity : 83.3% (5/6)
 Accuracy : 81.1% (30/37)

Table 15B Contingency table for phase 1 and 2 results: Final judgment based on the mean value of three assays

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	7	5	(7)	12(19)
Total		32	6	(8)	38(46)

Sensitivity : 96.2% (25/26)
 Specificity : 41.7% (5/12)
 Positive predictivity : 78.1% (25/32)
 Negative predictivity : 83.3% (5/6)
 Accuracy : 79.0% (30/38)

Lab 2		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	4	6	(9)	10(19)
Total		28	7	(11)	35(46)

Sensitivity : 96.0% (24/25)
 Specificity : 60.0% (6/10)
 Positive predictivity : 85.7% (24/28)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 85.7% (30/35)

Lab 3		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	2	9	(8)	11(19)
Total		27	10	(9)	37(46)

Sensitivity : 96.2% (25/26)
 Specificity : 81.8% (9/11)
 Positive predictivity : 92.6% (25/27)
 Negative predictivity : 90.0% (9/10)
 Accuracy : 91.9% (34/37)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	4	6	(9)	10(19)
Total		29	7	(10)	36(46)

Sensitivity : 96.2% (25/26)
 Specificity : 60.0% (6/10)
 Positive predictivity : 86.2% (25/29)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 86.1% (31/36)

Table 15C Contingency table for phase 1 and 2 results: Final judgment based on the majority of three assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	7	5	(7)	12(19)
Total		32	6	(8)	38(46)

Sensitivity : 96.2% (25/26)
 Specificity : 41.7% (5/12)
 Positive predictivity : 78.1% (25/32)
 Negative predictivity : 83.3% (5/6)
 Accuracy : 79.0% (30/38)

Lab 2		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	4	6	(9)	10(19)
Total		28	7	(11)	35(46)

Sensitivity : 96.0% (24/25)
 Specificity : 60.0% (6/10)
 Positive predictivity : 85.7% (24/28)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 85.7% (30/35)

Lab 3		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	2	9	(8)	11(19)
Total		27	10	(9)	37(46)

Sensitivity : 96.2% (25/26)
 Specificity : 81.8% (9/11)
 Positive predictivity : 92.6% (25/27)
 Negative predictivity : 90.0% (9/10)
 Accuracy : 91.9% (34/37)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	4	6	(9)	10(19)
Total		29	7	(10)	36(46)

Sensitivity : 96.2% (25/26)
 Specificity : 60.0% (6/10)
 Positive predictivity : 86.2% (25/29)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 86.1% (31/36)

Table 15D Contingency table for phase 1 and 2 results: Final judgment on the first assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	-	I	
Phototoxic	+	26	1	(0)	27(27)
	-	5	7	(7)	12(19)
Total		31	8	(7)	39(46)

Sensitivity : 96.3% (26/27)
 Specificity : 58.3% (7/12)
 Positive predictivity : 83.9% (26/31)
 Negative predictivity : 87.5% (7/8)
 Accuracy : 84.6% (33/39)

Lab 2		ROS			Total
		+	-	I	
Phototoxic	+	24	1	(2)	25(27)
	-	4	6	(9)	10(19)
Total		28	7	(11)	35(46)

Sensitivity : 96.0% (24/25)
 Specificity : 60.0% (6/10)
 Positive predictivity : 85.7% (24/28)
 Negative predictivity : 85.7% (6/7)
 Accuracy : 85.7% (30/35)

Lab 3		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	2	9	(8)	11(19)
Total		27	10	(9)	37(46)

Sensitivity : 96.2% (25/26)
 Specificity : 81.8% (9/11)
 Positive predictivity : 92.6% (25/27)
 Negative predictivity : 90.0% (9/10)
 Accuracy : 91.9% (34/37)

Integrated Judgment		ROS			Total
		+	-	I	
Phototoxic	+	25	1	(1)	26(27)
	-	3	7	(9)	10(19)
Total		28	8	(10)	36(46)

Sensitivity : 96.2% (25/26)
 Specificity : 70.0% (7/10)
 Positive predictivity : 89.3% (25/28)
 Negative predictivity : 87.5% (7/8)
 Accuracy : 88.9% (32/36)

Table 16-1 Contingency table for phase 1 results at 20 μM

Concentration: 20 μM

Lab 1	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	83.3% (10/12)	83.3% (10/12)	83.3% (10/12)	83.3% (10/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)
Negative predictivity	33.3% (1/3)	33.3% (1/3)	33.3% (1/3)	33.3% (1/3)
Accuracy	84.6% (11/13)	84.6% (11/13)	84.6% (11/13)	84.6% (11/13)

Lab 2	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	83.3% (10/12)	83.3% (10/12)	83.3% (10/12)	83.3% (10/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (10/10)	100% (10/10)	100% (10/10)	100% (10/10)
Negative predictivity	33.3% (1/3)	33.3% (1/3)	33.3% (1/3)	33.3% (1/3)
Accuracy	84.6% (11/13)	84.6% (11/13)	84.6% (11/13)	84.6% (11/13)

Lab 3	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	83.3% (10/12)	83.3% (10/12)	75.0% (9/12)	75.0% (9/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (10/10)	100% (10/10)	100% (9/9)	100% (9/9)
Negative predictivity	33.3% (1/3)	33.3% (1/3)	25.0% (1/4)	25.0% (1/4)
Accuracy	84.6% (11/13)	84.6% (11/13)	76.9% (10/13)	76.9% (10/13)

a : A : Final judgment of positive when positive results were obtained in at least one of three assays.

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results

D : Final judgment on the first assay results

Table 16-2 Contingency table for phase 1 results at 200 μM

Concentration: 200 μM

Lab 1	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Lab 2	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Lab 3	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

a : A : Final judgment of positive when positive results were obtained in at least one of three assays.

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results

D : Final judgment on the first assay results

Table 16-3 Contingency table for phase 2 results

Lab 1	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	100% (23/23)	100% (22/22)	100% (22/22)	100% (23/23)
Specificity	33.3% (4/12)	41.7% (5/12)	41.7% (5/12)	58.3% (7/12)
Positive predictivity	74.2% (23/31)	75.9% (22/29)	75.9% (22/29)	82.1% (23/28)
Negative predictivity	100% (4/4)	100% (5/5)	100% (5/5)	100% (7/7)
Accuracy	77.1% (27/35)	79.4% (27/34)	79.4% (27/34)	85.7% (30/35)

Lab 2	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	36.4% (4/11)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)
Positive predictivity	75.0% (21/28)	84.0% (21/25)	84.0% (21/25)	84.0% (21/25)
Negative predictivity	100% (4/4)	100% (6/6)	100% (6/6)	100% (6/6)
Accuracy	78.1% (25/32)	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)

Lab 3	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)
Specificity	81.8% (9/11)	81.8% (9/11)	81.8% (9/11)	81.8% (9/11)
Positive predictivity	91.7% (22/24)	91.7% (22/24)	91.7% (22/24)	91.7% (22/24)
Negative predictivity	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)
Accuracy	93.9% (31/33)	93.9% (31/33)	93.9% (31/33)	93.9% (31/33)

a : A : Final judgment of positive when positive results were obtained in at least one of three assays.

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results

D : Final judgment on the first assay results

Table 16-4 Contingency table for phase 1 and 2 results

Lab 1	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	96.3% (26/27)	96.2% (25/26)	96.2% (25/26)	96.3% (26/27)
Specificity	33.3% (4/12)	41.7% (5/12)	41.7% (5/12)	58.3% (7/12)
Positive predictivity	76.5% (26/34)	78.1% (25/32)	78.1% (25/32)	83.9% (26/31)
Negative predictivity	80.0% (4/5)	83.3% (5/6)	83.3% (5/6)	87.5% (7/8)
Accuracy	76.9% (30/39)	79.0% (30/38)	79.0% (30/38)	84.6% (33/39)

Lab 2	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	96.0% (24/25)	96.0% (24/25)	96.0% (24/25)	96.0% (24/25)
Specificity	36.4% (4/11)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)
Positive predictivity	77.4% (24/31)	85.7% (24/28)	85.7% (24/28)	85.7% (24/28)
Negative predictivity	80.0% (4/5)	85.7% (6/7)	85.7% (6/7)	85.7% (6/7)
Accuracy	77.8% (28/36)	85.7% (30/35)	85.7% (30/35)	85.7% (30/35)

Lab 3	Draft criteria for the final judgment ^a			
	A	B	C	D
Sensitivity	96.2% (25/26)	96.2% (25/26)	96.2% (25/26)	96.2% (25/26)
Specificity	81.8% (9/11)	81.8% (9/11)	81.8% (9/11)	81.8% (9/11)
Positive predictivity	92.6% (25/27)	92.6% (25/27)	92.6% (25/27)	92.6% (25/27)
Negative predictivity	90.0% (9/10)	90.0% (9/10)	90.0% (9/10)	90.0% (9/10)
Accuracy	91.9% (34/37)	91.9% (34/37)	91.9% (34/37)	91.9% (34/37)

a : A : Final judgment of positive when positive results were obtained in at least one of three assays.

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results

D : Final judgment on the first assay results

Table 17 Contingency table for integrated judgment results

Phase 1 (200 µM)	Draft criteria for the final judgment ^a			
	A ^b	B	C	D
Sensitivity	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)	91.7% (11/12)
Specificity	100% (1/1)	100% (1/1)	100% (1/1)	100% (1/1)
Positive predictivity	100% (11/11)	100% (11/11)	100% (11/11)	100% (11/11)
Negative predictivity	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)	50.0% (1/2)
Accuracy	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)

Phase 2	Draft criteria for the final judgment ^a			
	A ^c	B	C	D
Sensitivity	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)
Specificity	45.5% (5/11)	60.0% (6/10)	60.0% (6/10)	70.0% (7/10)
Positive predictivity	78.6% (22/28)	84.6% (22/26)	84.6% (22/26)	88.0% (22/25)
Negative predictivity	100% (5/5)	100% (6/6)	100% (6/6)	100% (7/7)
Accuracy	81.8% (27/33)	87.5% (28/32)	87.5% (28/32)	90.6% (29/32)

Phase 1 and 2	Draft criteria for the final judgment ^a			
	A ^d	B	C	D
Sensitivity	96.2% (25/26)	96.2% (25/26)	96.2% (25/26)	96.2% (25/26)
Specificity	45.5% (5/11)	60.0% (6/10)	60.0% (6/10)	70.0% (7/10)
Positive predictivity	80.7% (25/31)	86.2% (25/29)	86.2% (25/29)	89.3% (25/28)
Negative predictivity	83.3% (5/6)	85.7% (6/7)	85.7% (6/7)	87.5% (7/8)
Accuracy	81.1% (30/37)	86.1% (31/36)	86.1% (31/36)	88.9% (32/36)

Integrated judgments were made by the majority of each laboratory's final judgment.

a : A : Final judgment of positive when positive results were obtained in at least one of three assays.

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results

D : Final judgment on the first assay results

b : See, tables 6A to 6D

c : See, tables 12A to 12D

d : See, tables 14A to 14D

Table 18A Re-analysis based on the criteria for the proposed protocol: Judgment from the phase 2 results: Final judgment of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

ROS assay Validation data (atlas) pattern A		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	(-)	(-)	(-)	(-)	+	+	+	+	+
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	+
II - 20	Avobenzene	+	+	+	+	(±)	(±)	(±)	(±)	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : Photoreactive (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70 at 200 or 20 μM)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70 at 200 or 20 μM)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 or 20 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the photoreactive criteria (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the weakly photoreactive criteria (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the Non-photoreactive criteria (Singlet oxygen results < 25 and Superoxide results < 20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

I : inconclusive (The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)

ND: not determined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 18A Re-analysis based on the criteria for the proposed protocol: Judgment from the phase 2 results: Final judgment of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays (continued)

ROS assay Validation data (atlas) pattern A		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judg- ment	Assay			Final Judg- ment	Assay			Final Judg- ment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	±	-	±	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	±	±	-	-	-	-	-
II - 27	Penicillin G	-	±	±	±	±	±	±	±	-	-	-	-	±
II - 28	Phenytoin	±	±	±	±	±	±	±	±	±	-	±	±	±
II - 29	Bumetrizole	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	±	±	-	±	-	±	-	±	-	-	-	-	±
II - 32	Cinnamic acid	±	+	±	+	±	±	±	±	-	-	-	-	I
II - 33	Drometrizole	-	-	-	-	-	-	-	-	(-)	(-)	(-)	(-)	-
II - 34	L-Histidine	±	+	±	+	±	-	±	±	±	±	±	±	±
II - 35	Methylbenzylidene camphor	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 36	Octrizole	(±)	(±)	(±)	(±)	(-)	(±)	(-)	(±)	-	-	-	-	-
II - 37	Octyl methacrylate	-	+	+	+	-	-	-	-	-	-	-	-	-
II - 38	Octyl methoxycinnamate	-	-	-	-	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 39	Octyl salicylate	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	-	-	-	-	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 42	UV-571	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	ND

+ : Photoreactive (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70 at 200 or 20 μM)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70 at 200 or 20 μM)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 or 20 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the photoreactive criteria (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the weakly photoreactive criteria (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the Non-photoreactive criteria (Singlet oxygen results < 25 and Superoxide results < 20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

I : inconclusive (The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)

ND: not determined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 18B Re-analysis based on the criteria for the proposed protocol: Judgment from the phase 2 results: Final judgment based on the mean value of three assays

ROS assay Validation data (atlas) pattern B		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	(-)	(-)	(-)	(-)	+	+	+	+	+
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	+
II - 20	Avobenzene	+	+	+	+	(±)	(±)	(±)	(±)	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : Photoreactive (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70 at 200 or 20 μM)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70 at 200 or 20 μM)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 or 20 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the photoreactive criteria (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the weakly photoreactive criteria (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the Non-photoreactive criteria (Singlet oxygen results < 25 and Superoxide results < 20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

I : inconclusive (The results does not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)

ND: not determined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 18B Re-analysis based on the criteria for the proposed protocol: Judgment from the phase 2 results: Final judgment based on the mean value of three assays (continued)

ROS assay Validation data (atlas) pattern B		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	±	-	-	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	±	-	-	-	-	-	-
II - 27	Penicillin G	-	±	±	±	±	±	±	±	-	-	-	-	±
II - 28	Phenytoin	±	±	±	±	±	±	±	±	±	-	±	±	±
II - 29	Bumetrizole	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	±	±	-	±	-	±	-	-	-	-	-	-	-
II - 32	Cinnamic acid	±	+	±	±	±	±	±	±	-	-	-	-	±
II - 33	Drometrizole	-	-	-	-	-	-	-	-	(-)	(-)	(-)	(-)	-
II - 34	L-Histidine	±	+	±	±	±	-	±	±	±	±	±	±	±
II - 35	Methylbenzylidene camphor	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 36	Octrizole	(±)	(±)	(±)	(±)	(-)	(±)	(-)	(-)	-	-	-	-	-
II - 37	Octyl methacrylate	-	+	+	+	-	-	-	-	-	-	-	-	-
II - 38	Octyl methoxycinnamate	-	-	-	-	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 39	Octyl salicylate	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	-	-	-	-	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 42	UV-571	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	ND

+ : Photoreactive (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70 at 200 or 20 μM)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70 at 200 or 20 μM)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 or 20 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the photoreactive criteria (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the weakly photoreactive criteria (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the Non-photoreactive criteria (Singlet oxygen results < 25 and Superoxide results < 20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

I : inconclusive (The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)

ND: not determined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 18C Re-analysis based on the criteria for the proposed protocol: Judgment from the phase 2 results: Final judgment based on the majority of three assay results

ROS assay Validation data (atlas) pattern C		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	(-)	(-)	(-)	(-)	+	+	+	+	+
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	+
II - 20	Avobenzene	+	+	+	+	(±)	(±)	(±)	(±)	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : Photoreactive (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70 at 200 or 20 μM)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70 at 200 or 20 μM)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 or 20 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the photoreactive criteria (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the weakly photoreactive criteria (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the Non-photoreactive criteria (Singlet oxygen results < 25 and Superoxide results < 20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

I : inconclusive (The results does not meet the photoreactive, weakly photoreactive or Non-photoreactive criterion)

ND: not determined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 18C Re-analysis based on the criteria for the proposed protocol: Judgment from the phase 2 results: Final judgment based on the majority of three assay results (continued)

ROS assay Validation data (atlas) pattern C		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	±	-	-	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	±	-	-	-	-	-	-
II - 27	Penicillin G	-	±	±	±	±	±	±	±	-	-	-	-	±
II - 28	Phenytoin	±	±	±	±	±	±	±	±	±	-	±	±	±
II - 29	Bumetrizole	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	±	±	-	±	-	±	-	-	-	-	-	-	-
II - 32	Cinnamic acid	±	+	±	±	±	±	±	±	-	-	-	-	±
II - 33	Drometrizole	-	-	-	-	-	-	-	-	(-)	(-)	(-)	(-)	-
II - 34	L-Histidine	±	+	±	±	±	-	±	±	±	±	±	±	±
II - 35	Methylbenzylidene camphor	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 36	Octrizole	(±)	(±)	(±)	(±)	(-)	(±)	(-)	(-)	-	-	-	-	-
II - 37	Octyl methacrylate	-	+	+	+	-	-	-	-	-	-	-	-	-
II - 38	Octyl methoxycinnamate	-	-	-	-	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 39	Octyl salicylate	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	-	-	-	-	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 42	UV-571	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	ND

+ : Photoreactive (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70 at 200 or 20 μM)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70 at 200 or 20 μM)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 or 20 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the photoreactive criteria (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the weakly photoreactive criteria (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the Non-photoreactive criteria (Singlet oxygen results < 25 and Superoxide results < 20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

I : inconclusive (The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)

ND: not determined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 18D Re-analysis based on the criteria for the proposed protocol: Judgment from the phase 2 results: Final judgment on the first assay results

ROS assay Validation data (atlas) pattern D		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 1	Acridine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 2	Acridine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 3	Amiodarone HCl	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	ND
II - 4	Chlorpromazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 5	Doxycycline HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 6	Fenofibrate	+	+	+	+	(-)	(-)	(-)	(-)	+	+	+	+	+
II - 7	Furosemide	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 8	Ketoprofen	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 9	6-methylcoumarine	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 10	8-MOP	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 11	Nalidixic acid	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 12	Nalidixic acid (Na salt)	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 13	Norfloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 14	Ofloxacin	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 15	Piroxicam	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 16	Promethazine HCl	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 17	Rosiglitazone	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 18	Tetracycline	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 19	Anthracene	+	+	+	+	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	+
II - 20	Avobenzene	+	+	+	+	(±)	(±)	(±)	(±)	+	+	+	+	+
II - 21	Bithionol	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 22	Hexachlorophene	+	+	+	+	+	+	+	+	+	+	+	+	+
II - 23	Rose bengal	+	+	+	+	+	+	+	+	+	+	+	+	+

+ : Photoreactive (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70 at 200 or 20 μM)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70 at 200 or 20 μM)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 or 20 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the photoreactive criteria (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the weakly photoreactive criteria (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the Non-photoreactive criteria (Singlet oxygen results < 25 and Superoxide results < 20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

I : inconclusive (The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)

ND: not determined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 18D Re-analysis based on the criteria for the proposed protocol: Judgment from the phase 2 results: Final judgment on the first assay results (continued)

ROS assay Validation data (atlas) pattern D		Lab 1				Lab 2				Lab 3				Integrated Judgment ^a
No.	Chemicals Name	Assay			Final Judgment	Assay			Final Judgment	Assay			Final Judgment	
		1st	2nd	3rd		1st	2nd	3rd		1st	2nd	3rd		
II - 24	Aspirin	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 25	Benzocaine	-	±	-	-	-	-	-	-	-	-	-	-	-
II - 26	Erythromycin	-	-	-	-	-	-	±	-	-	-	-	-	-
II - 27	Penicillin G	-	±	±	-	±	±	±	±	-	-	-	-	-
II - 28	Phenytoin	±	±	±	±	±	±	±	±	±	-	±	±	±
II - 29	Bumetrizole	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 30	Camphor sulfonic acid	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 31	Chlorhexidine	±	±	-	±	-	±	-	-	-	-	-	-	-
II - 32	Cinnamic acid	±	+	±	±	±	±	±	±	-	-	-	-	±
II - 33	Drometrizole	-	-	-	-	-	-	-	-	(-)	(-)	(-)	(-)	-
II - 34	L-Histidine	±	+	±	±	±	-	±	±	±	±	±	±	±
II - 35	Methylbenzylidene camphor	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 36	Octrizole	(±)	(±)	(±)	(±)	(-)	(±)	(-)	(-)	-	-	-	-	-
II - 37	Octyl methacrylate	-	+	+	-	-	-	-	-	-	-	-	-	-
II - 38	Octyl methoxycinnamate	-	-	-	-	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 39	Octyl salicylate	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 40	PABA	-	-	-	-	-	-	-	-	-	-	-	-	-
II - 41	SDS	-	-	-	-	(-)	(-)	(-)	(-)	-	-	-	-	-
II - 42	UV-571	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	ND

+ : Photoreactive (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70 at 200 or 20 μM)

±: Weakly photoreactive (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70 at 200 or 20 μM)

- : Non-photoreactive (Singlet oxygen results < 25 and Superoxide results < 20 at 200 or 20 μM)

(+): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the photoreactive criteria (Singlet oxygen results ≥ 25 or Superoxide results ≥ 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(±): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the weakly photoreactive criteria (Singlet oxygen results < 25 and Superoxide results ≥ 20 and less than 70), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

(-): Ros assay was conducted at 2 μM due to precipitation at 20 μM and 200 μM . Although, the results met the Non-photoreactive criteria (Singlet oxygen results < 25 and Superoxide results < 20), the results shown in the parenthesis since the results at 2 μM were not used for the judgment of integrated results or data analyses.

I : inconclusive (The results does not meet the photoreactive , weakly photoreactive or Non-photoreactive criterion)

ND: not determined due to precipitation

a : Integrated judgments were made by the majority of each laboratory's final judgment.

Table 19A Re-analysis based on the criteria for the proposed protocol: Contingency table for phase 2 results: Final judgment of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	±	-	
Phototoxic	+	22	0	0	22 ^a
	-	3	4	9	16 ^b
Total		25	4	9	38

a: One of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl).
 b: 3 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Bumetrizole, Octizole, UV-571).
 When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
 Sensitivity : 100% (22/22) Specificity : 81.3% (13/16)
 Positive predictivity : 88.0% (22/25) Negative predictivity : 100% (13/13)
 Accuracy : 92.1% (35/38)
 When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
 Sensitivity : 100% (22/22) Specificity : 56.3% (9/16)
 Positive predictivity : 75.9%(22/29) Negative predictivity : 100% (9/9)
 Accuracy : 81.6%(31/38)

Lab 2		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	0	6	8	14 ^d
Total		19	6	8	33

c: 4 of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
 d: 5 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).
 When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
 Sensitivity : 100% (19/19) Specificity : 100% (14/14)
 Positive predictivity : 100% (19/19) Negative predictivity : 100% (14/14)
 Accuracy : 100% (33/33)
 When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
 Sensitivity : 100% (19/19) Specificity : 57.1%(8/14)
 Positive predictivity : 76.0%(19/25) Negative predictivity : 100% (8/8)
 Accuracy : 81.8%(27/33)

Lab 3		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^e
	-	0	2	15	17 ^f
Total		21	2	15	38

e: 2 of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl, Anthracene)
 f: 2 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Drometrizole, UV-571).
 When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
 Sensitivity : 100% (21/21) Specificity : 100% (17/17)
 Positive predictivity : 100% (21/21) Negative predictivity : 100% (17/17)
 Accuracy : 100% (38/38)
 When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
 Sensitivity : 100% (21/21) Specificity : 88.2%(15/17)
 Positive predictivity : 91.3%(21/23) Negative predictivity : 100% (15/15)
 Accuracy : 94.7%(36/38)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	22	0	0	22 ^g
	-	0	4	13	17 ^h
Total		22	4	13	39

g: One of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl) .
 h: 2 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Cinnamic acid, UV-571).
 When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
 Sensitivity : 100% (22/22) Specificity : 100% (17/17)
 Positive predictivity : 100% (22/22) Negative predictivity : 100% (17/17)
 Accuracy : 100% (39/39)
 When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
 Sensitivity : 100% (22/22) Specificity : 76.5%(13/17)
 Positive predictivity : 84.6%(22/26) Negative predictivity : 100% (13/13)
 Accuracy : 89.7%(35/39)

Table 19C Re-analysis based on the criteria for the proposed protocol: Contingency table for phase 2 results: Final judgment based on the majority of three assay results Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	±	-	
Phototoxic	+	22	0	0	22 ^a
	-	1	5	10	16 ^b
Total		23	5	10	38

a: One of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl).
b: 3 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Bumetizole, Octizole, UV-571).
When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (22/22) Specificity : 93.8% (15/16)
Positive predictivity : 95.7% (22/23) Negative predictivity : 100% (15/15)
Accuracy : 97.4% (37/38)
When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (22/22) Specificity : 62.5% (10/16)
Positive predictivity : 78.6%(22/28) Negative predictivity : 100% (10/10)
Accuracy : 84.2%(32/38)

Lab 2		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	0	4	10	14 ^d
Total		19	4	10	33

c: 4 of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).
d: 5 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Bumetizole, Octizole, Octyl methoxycinnamate, SDS, UV-571).
When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 100% (14/14)
Positive predictivity : 100% (19/19) Negative predictivity : 100% (14/14)
Accuracy : 100% (33/33)
When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 71.4%(10/14)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (10/10)
Accuracy : 87.9%(29/33)

Lab 3		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^e
	-	0	2	15	17 ^f
Total		21	2	15	38

e: 2 of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl, Anthracene)
f: 2 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Drometizole, UV-571).
When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (17/17)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (17/17)
Accuracy : 100% (38/38)
When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 88.2%(15/17)
Positive predictivity : 91.3%(21/23) Negative predictivity : 100% (15/15)
Accuracy : 94.7%(36/38)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	22	0	0	22 ^g
	-	0	4	14	18 ^h
Total		22	4	14	40

g: One of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl) .
h: One of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (UV-571).
When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (22/22) Specificity : 100% (18/18)
Positive predictivity : 100% (22/22) Negative predictivity : 100% (18/18)
Accuracy : 100% (40/40)
When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (22/22) Specificity : 77.8%(14/18)
Positive predictivity : 84.6%(22/26) Negative predictivity : 100% (14/14)
Accuracy : 90.0%(36/40)

Table 19D Re-analysis based on the criteria for the proposed protocol: Contingency table for phase 2 results: Final judgment on the first assay results

Sensitivity, Specificity, Positive predictivity, Negative predictivity, Accuracy (Phototoxic vs. ROS assay)

Lab 1		ROS			Total
		+	±	-	
Phototoxic	+	22	0	0	22 ^a
	-	0	4	12	16 ^b
Total		22	4	12	38

a: One of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl).

b: 3 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Bumetrizole, Octizole, UV-571).

When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (22/22) Specificity : 100% (16/16)
Positive predictivity : 100% (22/22) Negative predictivity : 100% (16/16)
Accuracy : 100% (38/38)

When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (22/22) Specificity : 75.0% (12/16)
Positive predictivity : 84.6%(22/26) Negative predictivity : 100% (12/12)
Accuracy : 89.5%(34/38)

Lab 2		ROS			Total
		+	±	-	
Phototoxic	+	19	0	0	19 ^c
	-	0	4	10	14 ^d
Total		19	4	10	33

c: 4 of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl, Fenofibrate, Anthracene, Avobenzone).

d: 5 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Bumetrizole, Octrizole, Octyl methoxycinnamate, SDS, UV-571).

When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 100% (14/14)
Positive predictivity : 100% (19/19) Negative predictivity : 100% (14/14)
Accuracy : 100% (33/33)

When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (19/19) Specificity : 71.4%(10/14)
Positive predictivity : 82.6%(19/23) Negative predictivity : 100% (10/10)
Accuracy : 87.9%(29/33)

Lab 3		ROS			Total
		+	±	-	
Phototoxic	+	21	0	0	21 ^e
	-	0	2	15	17 ^f
Total		21	2	15	38

e: 2 of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl, Anthracene)

f: 2 of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Drometrizole, UV-571).

When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 100% (17/17)
Positive predictivity : 100% (21/21) Negative predictivity : 100% (17/17)
Accuracy : 100% (38/38)

When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (21/21) Specificity : 88.2%(15/17)
Positive predictivity : 91.3%(21/23) Negative predictivity : 100% (15/15)
Accuracy : 94.7%(36/38)

Integrated Judgment		ROS			Total
		+	±	-	
Phototoxic	+	22	0	0	22 ^g
	-	0	3	15	18 ^h
Total		22	3	15	40

g: One of 23 phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (Amiodarone HCl) .

h: One of 19 non-phototoxic chemicals were not evaluated at 20 or 200µM due to precipitation (UV-571).

When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals;
Sensitivity : 100% (22/22) Specificity : 100% (18/18)
Positive predictivity : 100% (22/22) Negative predictivity : 100% (18/18)
Accuracy : 100% (40/40)

When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals;
Sensitivity : 100% (22/22) Specificity : 83.3%(15/18)
Positive predictivity : 88.0%(22/25) Negative predictivity : 100% (15/15)
Accuracy : 92.5%(37/40)

Table 20 Re-analysis based on the criteria for the proposed protocol: Contingency table for phase 2 results

Lab 1	Draft criteria for the final judgment ^a											
	Original				Re-analysis based on the criteria for the proposed protocol							
					When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals				When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (23/23)	100% (22/22)	100% (22/22)	100% (23/23)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)
Specificity	33.3% (4/12)	41.7% (5/12)	41.7% (5/12)	58.3% (7/12)	81.3% (13/16)	93.8% (15/16)	93.8% (15/16)	100% (16/16)	56.3% (9/16)	62.5% (10/16)	62.5% (10/16)	75.0% (12/16)
Positive predictivity	74.2% (23/31)	75.9% (22/29)	75.9% (22/29)	82.1% (23/28)	88.0% (22/25)	95.7% (22/23)	95.7% (22/23)	100% (22/22)	75.9% (22/29)	78.6% (22/28)	78.6% (22/28)	84.6% (22/26)
Negative predictivity	100% (4/4)	100% (5/5)	100% (5/5)	100% (7/7)	100% (13/13)	100% (15/15)	100% (15/15)	100% (16/16)	100% (9/9)	100% (10/10)	100% (10/10)	100% (12/12)
Accuracy	77.1% (27/35)	79.4% (27/34)	79.4% (27/34)	85.7% (30/35)	92.1% (35/38)	97.4% (37/38)	97.4% (37/38)	100% (38/38)	81.6% (31/38)	84.2% (32/38)	84.2% (32/38)	89.5% (34/38)

Lab 2	Draft criteria for the final judgment ^a											
	Original				Re-analysis based on the criteria for the proposed protocol							
					When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals				When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)
Specificity	36.4% (4/11)	60.0% (6/10)	60.0% (6/10)	60.0% (6/10)	100% (14/14)	100% (14/14)	100% (14/14)	100% (14/14)	57.1% (8/14)	71.4% (10/14)	71.4% (10/14)	71.4% (10/14)
Positive predictivity	75.0% (21/28)	84.0% (21/25)	84.0% (21/25)	84.0% (21/25)	100% (19/19)	100% (19/19)	100% (19/19)	100% (19/19)	76.0% (19/25)	82.6% (19/23)	82.6% (19/23)	82.6% (19/23)
Negative predictivity	100% (4/4)	100% (6/6)	100% (6/6)	100% (6/6)	100% (14/14)	100% (14/14)	100% (14/14)	100% (14/14)	100% (8/8)	100% (10/10)	100% (10/10)	100% (10/10)
Accuracy	78.1% (25/32)	87.1% (27/31)	87.1% (27/31)	87.1% (27/31)	100% (33/33)	100% (33/33)	100% (33/33)	100% (33/33)	81.8% (27/33)	87.9% (29/33)	87.9% (29/33)	87.9% (29/33)

a : A : Original : Final judgment of positive when positive results were obtained in at least one of three assays

Re-analysis based on the criteria for the proposed protocol : Final judgment of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results

D : Final judgment on the first assay results

Table 20 Re-analysis based on the criteria for the proposed protocol: Contingency table for phase 2 results (continued).

Lab 3	Draft criteria for the final judgment ^a											
	Original				Re-analysis based on the criteria for the proposed protocol							
	A	B	C	D	When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals				When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals			
	A	B	C	D	A	B	C	D	A	B	C	D
Sensitivity	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)
Specificity	81.8% (9/11)	81.8% (9/11)	81.8% (9/11)	81.8% (9/11)	100% (17/17)	100% (17/17)	100% (17/17)	100% (17/17)	88.2% (15/17)	88.2% (15/17)	88.2% (15/17)	88.2% (15/17)
Positive predictivity	91.7% (22/24)	91.7% (22/24)	91.7% (22/24)	91.7% (22/24)	100% (21/21)	100% (21/21)	100% (21/21)	100% (21/21)	91.3% (21/23)	91.3% (21/23)	91.3% (21/23)	91.3% (21/23)
Negative predictivity	100% (9/9)	100% (9/9)	100% (9/9)	100% (9/9)	100% (17/17)	100% (17/17)	100% (17/17)	100% (17/17)	100% (15/15)	100% (15/15)	100% (15/15)	100% (15/15)
Accuracy	93.9% (31/33)	93.9% (31/33)	93.9% (31/33)	93.9% (31/33)	100% (38/38)	100% (38/38)	100% (38/38)	100% (38/38)	94.7% (36/38)	94.7% (36/38)	94.7% (36/38)	94.7% (36/38)

a : A : Original : Final judgment of positive when positive results were obtained in at least one of three assays

Re-analysis based on the criteria for the proposed protocol : Final judgment of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

B : Final judgment based on the mean value of three assays.

C : Final judgment based on the majority of three assay results

D : Final judgment on the first assay results

Table 21 Re-analysis based on the criteria for the proposed protocol: Contingency table for integrated judgment results.

Phase 2	Draft criteria for the final judgment ^a												
	Original				Re-analysis based on the criteria for the proposed protocol								
					When the “±: Weakly photoreactive” chemicals were defined as “non-phototoxic” chemicals				When the “±: Weakly photoreactive” chemicals were defined as “phototoxic” chemicals				
	A ^c	B	C	D	A ^b	B	C	D	A ^b	B	C	D	
Sensitivity	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)
Specificity	45.5% (5/11)	60.0% (6/10)	60.0% (6/10)	70.0% (7/10)	100% (17/17)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	76.5% (13/17)	77.8% (14/18)	77.8% (14/18)	83.3% (15/18)
Positive predictivity	78.6% (22/28)	84.6% (22/26)	84.6% (22/26)	88.0% (22/25)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	100% (22/22)	84.6% (22/26)	84.6% (22/26)	84.6% (22/26)	88.0% (22/25)
Negative predictivity	100% (5/5)	100% (6/6)	100% (6/6)	100% (7/7)	100% (17/17)	100% (18/18)	100% (18/18)	100% (18/18)	100% (18/18)	100% (13/13)	100% (14/14)	100% (14/14)	100% (15/15)
Accuracy	81.8% (27/33)	87.5% (28/32)	87.5% (28/32)	90.6% (29/32)	100% (39/39)	100% (40/40)	100% (40/40)	100% (40/40)	100% (40/40)	89.7% (35/39)	90.0% (36/40)	90.0% (36/40)	92.5% (37/40)

Integrated judgments were made by the majority of each laboratory’s final judgment.

a : A : Original : Final judgment of positive when positive results were obtained in at least one of three assays

Re-analysis based on the criteria for the proposed protocol : Final judgment of photoreactive or weakly photoreactive when photoreactive or weakly photoreactive results were obtained in at least one of three assays

B : Final judgment based on the mean value of three assays.

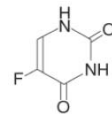
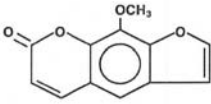
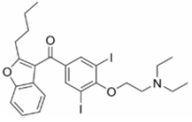
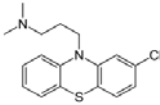
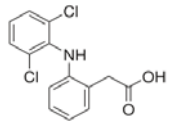
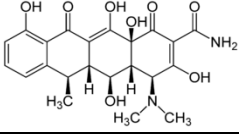
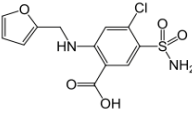
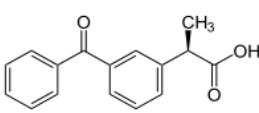
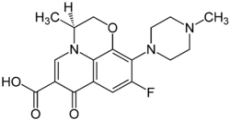
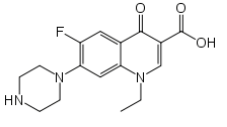
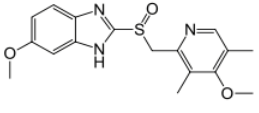
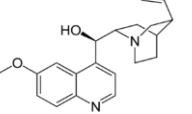
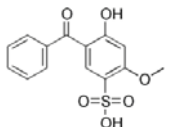
C : Final judgment based on the majority of three assay results

D : Final judgment on the first assay results

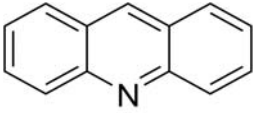
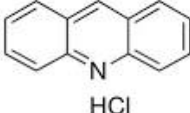
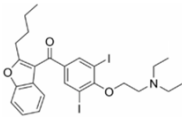
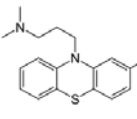
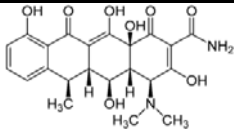
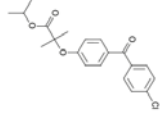
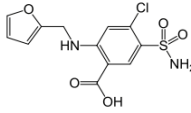
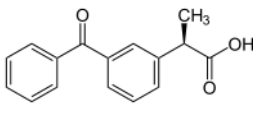
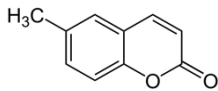
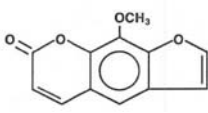
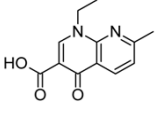
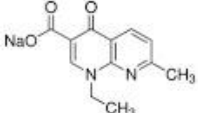
b : See, tables 18A to 18D

c : See, tables 12A to 12D

Appendix 1 Chemical structures of the test chemicals for the phase 1 study

No.	Chemical name	CAS No.	Molecular weight	Chemical structure
I-1	5-FU	51-21-8	130.1	
I-2	8-MOP	298-81-7	216.2	
I-3	Amiodarone HCl	19774-82-4	681.8	
I-4	Chlorpromazine HCl	69-09-0	355.3	
I-5	Diclofenac	15307-79-6	318.1	
I-6	Doxycycline HCl	10592-13-9	512.9	
I-7	Furosemide	54-31-9	330.7	
I-8	Ketoprofen	22071-15-4	254.3	
I-9	Levofloxacin	100986-85-4	361.4	
I-10	Norfloxacin	70458-96-7	319.3	
I-11	Omeprazole	73590-58-6	345.4	
I-12	Quinine HCl	6119-47-7	396.9	
I-13	Sulisobenzone	4065-45-6	308.3	

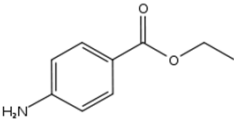
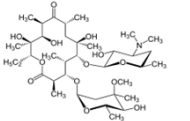
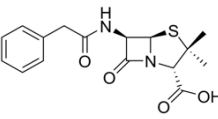
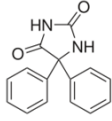
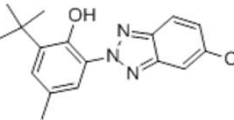
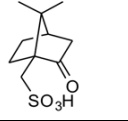
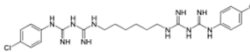
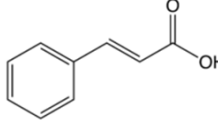
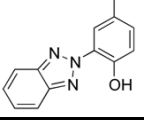
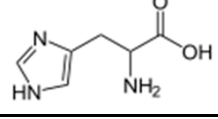
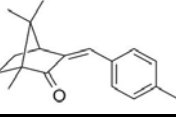
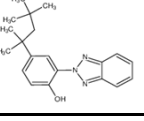
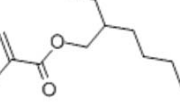
Appendix 2 Chemical structures of the test chemicals for the phase 2 study

NO.	Chemical name	CAS No. ^{a)}	Molecular weight	Chemical structure
Phototoxic drugs				
II-1	Acridine	260-94-6	179.2	
II-2	Acridine HCl	17784-47-3	215.7	
II-3	Amiodarone HCl	19774-82-4	681.8	
II-4	Chlorpromazine HCl	69-09-0	355.3	
II-5	Doxycycline HCl	10592-13-9	480.9	
II-6	Fenofibrate	49562-28-9	360.8	
II-7	Furosemide	54-31-9	330.7	
II-8	Ketoprofen	22071-15-4	254.3	
II-9	6-Methylcoumarine	92-48-8	160.2	
II-10	8-MOP	298-81-7	216.2	
II-11	Nalidixic acid	389-08-2	232.2	
II-12	Nalidixic acid (Na salt)	3374-05-8	254.2	

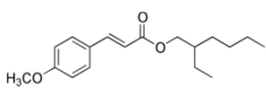
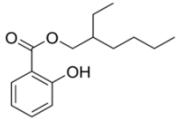
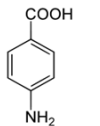
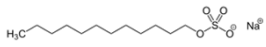
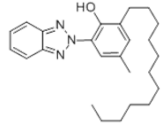
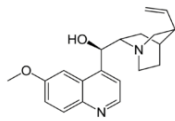
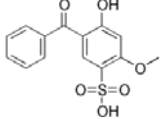
Appendix 2 Chemical structures of the test chemicals for the phase 2 study

II-13	Norfloxacin	70458-96-7	319.3	
II-14	Ofloxacin	82419-36-1	361.4	
II-15	Piroxicam	36322-90-4	331.4	
II-16	Promethazine HCl	58-33-3	320.9	
II-17	Rosiglitazone	122320-73-4	357.4	
II-18	Tetracycline	60-54-8	444.4	
Phototoxic non-drug chemicals				
II-19	Anthracene	120-12-7	178.2	
II-20	Avobenzene	70356-09-1	310.39	
II-21	Bithionol	97-18-7	356.1	
II-22	Hexachlorophene	70-30-4	406.9	
II-23	Rose bengal	632-69-9	1017.6	
Non-phototoxic drugs				
II-24	Aspirin	50-78-2	180.2	

Appendix 2 Chemical structures of the test chemicals for the phase 2 study

II-25	Benzocaine	94-09-7	165.2	
II-26	Erythromycin	114-07-8	733.9	
II-27	Penicillin G	113-98-4	372.5	
II-28	Phenytoin	57-41-0	252.3	
Non-phototoxic non-drug chemicals				
II-29	Bumetizole	3896-11-5	315.8	
II-30	Camphor sulfonic acid	3144-16-9	232.3	
II-31	Chlorhexidine	55-56-1	505.5	
II-32	Cinnamic acid	140-10-3	148.2	
II-33	Drometrizole	2440-22-4	225.25	
II-34	L-Histidine	71-00-1	155.2	
II-35	Methylbenzylidene camphor	36861-47-9	254.4	
II-36	Octrizole	3147-75-9	323.43	
II-37	Octyl methacrylate	688-84-6	198.3	

Appendix 2 Chemical structures of the test chemicals for the phase 2 study

II-38	Octyl methoxycinnamate	5466-77-3	290.4	
II-39	Octyl salicylate	118-60-5	250.3	
II-40	PABA	150-13-0	137.1	
II-41	SDS	151-21-3	288.4	
II-42	UV-571	125304-04-3	393.56	
Positive/Negative control				
PC	Quinine HCl	6119-47-7	396.9	
NC	Sulizobenzone	4065-45-6	308.3	

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 1
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	0.869	0.873	0.394	0.867	469	1	1	0.036	0.032	0.394	0.036	350
	2	0.875	0.880	0.411	0.873	458		2	0.034	0.047	0.406	0.040	363
	3	0.871	0.882	0.416	0.875	449		3	0.045	0.030	0.411	0.061	357
	Mean	0.872	0.878	0.407	0.872	459		Mean	0.038	0.036	0.404	0.045	357
2	1	0.867	0.880	0.407	0.869	451	2	1	0.037	0.033	0.396	0.036	348
	2	0.878	0.889	0.418	0.882	451		2	0.035	0.052	0.399	0.050	353
	3	0.877	0.879	0.420	0.871	447		3	0.041	0.033	0.415	0.066	362
	Mean	0.874	0.883	0.415	0.874	450		Mean	0.038	0.039	0.403	0.051	354
3	1	0.890	0.886	0.395	0.877	486	3	1	0.035	0.033	0.415	0.039	371
	2	0.884	0.891	0.408	0.885	469		2	0.035	0.048	0.412	0.044	367
	3	0.879	0.892	0.412	0.884	459		3	0.042	0.032	0.425	0.057	373
	Mean	0.884	0.890	0.405	0.882	471		Mean	0.037	0.037	0.417	0.047	370
4	1	0.860	0.871	0.390	0.864	463	4	1	0.036	0.033	0.387	0.058	332
	2	0.860	0.870	0.405	0.864	449		2	0.034	0.035	0.383	0.041	329
	3	0.873	0.875	0.413	0.867	453		3	0.041	0.030	0.389	0.058	328
	Mean	0.864	0.872	0.403	0.865	455		Mean	0.037	0.033	0.386	0.053	330
5	1	0.859	0.859	0.399	0.850	452	5	1	0.041	0.030	0.394	0.038	342
	2	0.852	0.876	0.406	0.868	438		2	0.035	0.058	0.386	0.056	340
	3	0.866	0.871	0.415	0.863	441		3	0.041	0.031	0.406	0.057	354
	Mean	0.859	0.869	0.407	0.860	444		Mean	0.039	0.039	0.395	0.050	345
6	1	0.853	0.868	0.382	0.861	464	6	1	0.040	0.029	0.393	0.036	344
	2	0.850	0.865	0.398	0.858	445		2	0.035	0.045	0.403	0.042	359
	3	0.864	0.865	0.401	0.857	455		3	0.043	0.031	0.414	0.055	362
	Mean	0.856	0.866	0.394	0.858	455		Mean	0.039	0.035	0.403	0.044	355

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

The optical absorbance values were rounded to the third decimal place. If the calculated "results" are different from the numerical results in the raw data sheets by the number of significant digits in the data sheet and the appendices are different, the data in the raw data sheet is used in the appendices.

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 1
 Chemical Name : Qunine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.846	0.871	0.380	0.862	457	7	1	0.037	0.028	0.402	0.037	353
	2	0.851	0.877	0.395	0.868	447		2	0.035	0.046	0.404	0.042	359
	3	0.857	0.880	0.401	0.871	447		3	0.040	0.031	0.415	0.061	364
	Mean	0.851	0.876	0.392	0.867	450		Mean	0.037	0.035	0.407	0.046	359
8	1	0.824	0.853	0.372	0.849	444	8	1	0.038	0.033	0.425	0.037	375
	2	0.840	0.859	0.391	0.852	442		2	0.036	0.048	0.423	0.039	375
	3	0.845	0.861	0.398	0.853	440		3	0.040	0.032	0.427	0.074	375
	Mean	0.836	0.858	0.387	0.851	442		Mean	0.038	0.038	0.425	0.050	375
9	1	0.865	0.884	0.386	0.878	473	9	1	0.043	0.032	0.396	0.037	343
	2	0.848	0.886	0.398	0.879	444		2	0.034	0.043	0.408	0.037	364
	3	0.867	0.880	0.407	0.873	455		3	0.040	0.030	0.424	0.061	374
	Mean	0.860	0.883	0.397	0.877	457		Mean	0.039	0.035	0.409	0.045	360
10	1	0.870	0.865	0.379	0.856	483	10	1	0.054	0.033	0.434	0.036	370
	2	0.855	0.880	0.389	0.874	458		2	0.045	0.033	0.438	0.039	384
	3	0.880	0.873	0.400	0.865	472		3	0.061	0.029	0.451	0.050	381
	Mean	0.868	0.873	0.389	0.865	471		Mean	0.053	0.032	0.441	0.042	378
11	1	0.862	0.863	0.373	0.853	480	11	1	0.062	0.029	0.435	0.035	359
	2	0.859	0.873	0.392	0.865	458		2	0.040	0.030	0.444	0.038	390
	3	0.886	0.875	0.406	0.867	472		3	0.063	0.029	0.446	0.054	369
	Mean	0.869	0.870	0.390	0.861	470		Mean	0.055	0.029	0.442	0.043	373
12	1	0.873	0.844	0.372	0.836	496	12	1	0.039	0.030	0.435	0.037	383
	2	0.857	0.844	0.386	0.842	466		2	0.035	0.052	0.434	0.040	386
	3	0.879	0.851	0.395	0.845	479		3	0.042	0.032	0.449	0.075	394
	Mean	0.870	0.846	0.384	0.841	480		Mean	0.039	0.038	0.439	0.051	388
Mean for all assays	-	-	-	-	459	Mean for all assays	-	-	-	-	362		
SD for all assays	-	-	-	-	12	SD for all assays	-	-	-	-	16		
CV for all assays	-	-	-	-	2.6	CV for all assays	-	-	-	-	4.4		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 1
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	0.888	0.873	0.886	0.867	-4	1	1	0.041	0.032	0.041	0.036	-9
	2	0.884	0.880	0.882	0.873	-4		2	0.051	0.047	0.046	0.040	-14
	3	0.892	0.882	0.889	0.875	-4		3	0.040	0.030	0.045	0.061	-4
	Mean	0.888	0.878	0.886	0.872	-4		Mean	0.044	0.036	0.044	0.045	-9
2	1	0.895	0.880	0.893	0.869	-8	2	1	0.042	0.033	0.039	0.036	-15
	2	0.903	0.889	0.897	0.882	-4		2	0.042	0.052	0.040	0.050	-14
	3	0.900	0.879	0.897	0.871	-6		3	0.039	0.033	0.041	0.066	-10
	Mean	0.899	0.883	0.896	0.874	-6		Mean	0.041	0.039	0.040	0.051	-13
3	1	0.888	0.886	0.886	0.877	-6	3	1	0.039	0.033	0.041	0.039	-8
	2	0.892	0.891	0.885	0.885	-1		2	0.049	0.048	0.048	0.044	-10
	3	0.891	0.892	0.892	0.884	-9		3	0.040	0.032	0.040	0.057	-10
	Mean	0.890	0.890	0.888	0.882	-5		Mean	0.043	0.037	0.043	0.047	-9
4	1	0.892	0.871	0.886	0.864	-1	4	1	0.043	0.033	0.041	0.058	-21
	2	0.886	0.870	0.877	0.864	2		2	0.050	0.035	0.048	0.041	-22
	3	0.901	0.875	0.893	0.867	1		3	0.040	0.030	0.040	0.058	-20
	Mean	0.893	0.872	0.885	0.865	1		Mean	0.044	0.033	0.043	0.053	-21
5	1	0.894	0.859	0.888	0.850	-4	5	1	0.042	0.030	0.040	0.038	-13
	2	0.886	0.876	0.874	0.868	3		2	0.046	0.058	0.042	0.056	-15
	3	0.886	0.871	0.879	0.863	-2		3	0.039	0.031	0.039	0.057	-11
	Mean	0.889	0.869	0.880	0.860	-1		Mean	0.042	0.039	0.040	0.050	-13
6	1	0.881	0.868	0.874	0.861	-1	6	1	0.040	0.029	0.039	0.036	-10
	2	0.872	0.865	0.864	0.858	0		2	0.045	0.045	0.040	0.042	-14
	3	0.884	0.865	0.875	0.857	0		3	0.039	0.031	0.039	0.055	-8
	Mean	0.879	0.866	0.871	0.858	0		Mean	0.041	0.035	0.039	0.044	-11

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 1
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results *1	Assay	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.882	0.871	0.857	0.862	16	7	1	0.040	0.028	0.042	0.037	-9
	2	0.883	0.877	0.865	0.868	8		2	0.046	0.046	0.043	0.042	-14
	3	0.896	0.880	0.871	0.871	16		3	0.038	0.031	0.040	0.061	-10
	Mean	0.887	0.876	0.864	0.867	13		Mean	0.041	0.035	0.042	0.046	-11
8	1	0.874	0.853	0.849	0.849	18	8	1	0.039	0.033	0.040	0.037	-11
	2	0.873	0.859	0.854	0.852	12		2	0.045	0.048	0.042	0.039	-16
	3	0.876	0.861	0.852	0.853	17		3	0.039	0.032	0.040	0.074	-11
	Mean	0.874	0.858	0.852	0.851	16		Mean	0.041	0.038	0.041	0.050	-13
9	1	0.869	0.884	0.859	0.878	4	9	1	0.038	0.032	0.039	0.037	-9
	2	0.868	0.886	0.857	0.879	5		2	0.044	0.043	0.041	0.037	-13
	3	0.876	0.880	0.866	0.873	4		3	0.039	0.030	0.039	0.061	-11
	Mean	0.871	0.883	0.861	0.877	4		Mean	0.040	0.035	0.040	0.045	-11
10	1	0.862	0.865	0.871	0.856	-17	10	1	0.039	0.033	0.041	0.036	-7
	2	0.877	0.880	0.875	0.874	-6		2	0.039	0.033	0.041	0.039	-9
	3	0.876	0.873	0.887	0.865	-19		3	0.037	0.029	0.038	0.050	-9
	Mean	0.872	0.873	0.878	0.865	-14		Mean	0.038	0.032	0.040	0.042	-8
11	1	0.868	0.863	0.857	0.853	2	11	1	0.039	0.029	0.039	0.035	-14
	2	0.875	0.873	0.863	0.865	3		2	0.039	0.030	0.040	0.038	-13
	3	0.873	0.875	0.862	0.867	2		3	0.039	0.029	0.038	0.054	-15
	Mean	0.872	0.870	0.861	0.861	2		Mean	0.039	0.029	0.039	0.043	-14
12	1	0.863	0.844	0.872	0.836	-14	12	1	0.039	0.030	0.038	0.037	-14
	2	0.866	0.844	0.864	0.842	-3		2	0.041	0.052	0.039	0.040	-15
	3	0.867	0.851	0.878	0.845	-16		3	0.038	0.032	0.038	0.075	-13
	Mean	0.865	0.846	0.871	0.841	-11		Mean	0.039	0.038	0.038	0.051	-14
Mean for all assays	-	-	-	-	0	Mean for all assays	-	-	-	-	-	-12	
SD for all assays	-	-	-	-	9	SD for all assays	-	-	-	-	-	3	
CV for all assays	-	-	-	-	-	CV for all assays	-	-	-	-	-	-	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 2
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	0.996	1.003	0.597	0.992	390	1	1	0.034	0.038	0.380	0.042	335
	2	0.993	1.002	0.609	0.994	375		2	0.032	0.036	0.374	0.059	331
	3	0.990	1.005	0.606	0.995	375		3	0.037	0.034	0.376	0.040	328
	Mean	0.993	1.003	0.604	0.994	380		Mean	0.034	0.036	0.377	0.047	331
3	1	1.000	1.005	0.573	1.004	421	2	1	0.032	0.033	0.341	0.037	304
	2	0.995	1.005	0.582	0.998	407		2	0.034	0.035	0.339	0.040	300
	3	0.993	1.009	0.584	0.998	403		3	0.033	0.034	0.357	0.039	319
	Mean	0.996	1.006	0.580	1.000	410		Mean	0.033	0.034	0.346	0.039	308
2	1	0.994	1.007	0.570	0.999	412	3	1	0.033	0.032	0.437	0.041	396
	2	1.001	1.013	0.586	0.997	403		2	0.032	0.032	0.413	0.039	373
	3	1.001	1.009	0.589	0.998	400		3	0.032	0.034	0.384	0.042	344
	Mean	0.999	1.010	0.582	0.998	405		Mean	0.032	0.033	0.411	0.041	371
4	1	0.985	1.001	0.559	0.996	417	4	1	0.033	0.033	0.412	0.041	371
	2	1.000	1.003	0.581	0.993	410		2	0.033	0.033	0.404	0.041	363
	3	0.994	1.004	0.575	0.992	410		3	0.033	0.033	0.389	0.042	348
	Mean	0.993	1.003	0.572	0.994	412		Mean	0.033	0.033	0.402	0.041	361
5	1	0.996	1.000	0.558	0.991	428	5	1	0.032	0.032	0.358	0.038	319
	2	0.990	0.995	0.572	0.988	408		2	0.032	0.033	0.352	0.040	313
	3	0.992	1.011	0.575	0.996	407		3	0.032	0.033	0.365	0.042	326
	Mean	0.993	1.002	0.568	0.992	414		Mean	0.032	0.033	0.358	0.040	319
6	1	1.001	1.004	0.570	1.000	424	6	1	0.032	0.033	0.345	0.038	307
	2	0.991	1.000	0.584	0.994	400		2	0.032	0.032	0.344	0.038	306
	3	0.992	1.008	0.586	0.998	399		3	0.032	0.032	0.352	0.038	314
	Mean	0.995	1.004	0.580	0.997	408		Mean	0.032	0.032	0.347	0.038	309

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 2
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.988	1.002	0.548	0.986	428	7	1	0.032	0.032	0.443	0.038	404
	2	0.990	1.002	0.570	0.993	408		2	0.032	0.032	0.444	0.040	405
	3	0.997	1.004	0.578	0.995	407		3	0.032	0.033	0.464	0.040	425
	Mean	0.992	1.003	0.565	0.991	414		Mean	0.032	0.032	0.450	0.039	411
8	1	0.994	1.000	0.554	0.991	432	8	1	0.032	0.032	0.489	0.038	449
	2	0.989	0.996	0.567	0.990	414		2	0.032	0.033	0.475	0.045	435
	3	0.991	1.003	0.570	0.995	413		3	0.032	0.034	0.438	0.040	398
	Mean	0.991	1.000	0.564	0.992	420		Mean	0.032	0.033	0.467	0.041	427
9	1	0.987	0.995	0.554	0.986	424	9	1	0.033	0.032	0.401	0.038	357
	2	0.983	0.994	0.575	0.986	399		2	0.033	0.034	0.377	0.051	333
	3	0.994	1.001	0.579	0.993	406		3	0.033	0.032	0.415	0.044	371
	Mean	0.988	0.997	0.569	0.988	410		Mean	0.033	0.033	0.398	0.044	354
11	1	0.988	0.994	0.545	0.965	427	10	1	0.034	0.033	0.423	0.038	381
	2	0.988	0.997	0.561	0.987	411		2	0.032	0.032	0.405	0.045	365
	3	0.994	0.997	0.569	0.989	409		3	0.033	0.033	0.398	0.040	357
	Mean	0.990	0.996	0.558	0.980	416		Mean	0.033	0.033	0.409	0.041	368
10	1	0.993	1.002	0.545	0.990	438	11	1	0.032	0.033	0.395	0.038	357
	2	0.995	1.004	0.561	0.992	424		2	0.036	0.033	0.382	0.038	340
	3	0.991	1.008	0.571	1.003	410		3	0.032	0.033	0.417	0.040	379
	Mean	0.993	1.005	0.559	0.995	424		Mean	0.033	0.033	0.398	0.039	359
12	1	0.996	1.009	0.540	0.999	439	12	1	0.033	0.035	0.395	0.040	356
	2	1.003	1.007	0.560	0.977	426		2	0.032	0.034	0.397	0.041	359
	3	1.000	1.010	0.566	1.000	417		3	0.032	0.033	0.416	0.039	378
	Mean	1.000	1.009	0.555	0.992	427		Mean	0.032	0.034	0.403	0.040	364
Mean for all assays		-	-	-	-	412	Mean for all assays		-	-	-	-	357
SD for all assays		-	-	-	-	12	SD for all assays		-	-	-	-	37
CV for all assays		-	-	-	-	2.9	CV for all assays		-	-	-	-	10.4

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 2
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	1.004	1.003	0.996	0.992	-1	1	1	0.038	0.038	0.039	0.042	-10
	2	1.009	1.002	1.003	0.994	-3		2	0.037	0.036	0.038	0.059	-10
	3	1.008	1.005	0.999	0.995	0		3	0.037	0.034	0.038	0.040	-10
	Mean	1.007	1.003	0.999	0.994	-1		Mean	0.037	0.036	0.038	0.047	-10
2	1	1.006	1.005	0.997	1.004	3	2	1	0.038	0.033	0.039	0.037	-4
	2	1.011	1.005	1.003	0.998	2		2	0.039	0.035	0.040	0.040	-4
	3	1.013	1.009	1.001	0.998	6		3	0.039	0.034	0.041	0.039	-3
	Mean	1.010	1.006	1.000	1.000	4		Mean	0.039	0.034	0.040	0.039	-4
3	1	1.007	1.007	0.999	0.999	-4	3	1	0.038	0.032	0.038	0.041	-8
	2	1.012	1.013	0.999	0.997	1		2	0.037	0.032	0.039	0.039	-6
	3	1.011	1.009	0.999	0.998	0		3	0.037	0.034	0.038	0.042	-7
	Mean	1.010	1.010	0.999	0.998	-1		Mean	0.037	0.033	0.038	0.041	-7
4	1	1.002	1.001	0.997	0.996	-4	4	1	0.038	0.033	0.039	0.041	-7
	2	0.990	1.003	0.978	0.993	3		2	0.037	0.033	0.039	0.041	-6
	3	1.009	1.004	0.998	0.992	2		3	0.039	0.033	0.040	0.042	-7
	Mean	1.000	1.003	0.991	0.994	0		Mean	0.038	0.033	0.039	0.041	-7
5	1	1.002	1.000	0.991	0.991	1	5	1	0.038	0.032	0.038	0.038	-7
	2	1.002	0.995	0.993	0.988	-1		2	0.038	0.033	0.051	0.040	6
	3	1.008	1.011	0.999	0.996	-1		3	0.042	0.033	0.045	0.042	-4
	Mean	1.004	1.002	0.994	0.992	0		Mean	0.039	0.033	0.045	0.040	-2
6	1	1.004	1.004	1.002	1.000	-5	6	1	0.038	0.033	0.039	0.038	-5
	2	1.005	1.000	0.997	0.994	1		2	0.037	0.032	0.039	0.038	-4
	3	1.007	1.008	1.001	0.998	-1		3	0.037	0.032	0.038	0.038	-5
	Mean	1.005	1.004	1.000	0.997	-2		Mean	0.037	0.032	0.039	0.038	-5

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 2
 Chemical Name : Sulisobenzone

Singlet oxygen						Superoxide anion							
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.998	1.002	0.989	0.986	-3	7	1	0.037	0.032	0.039	0.038	-5
	2	1.005	1.002	0.995	0.993	-2		2	0.038	0.032	0.038	0.040	-7
	3	1.007	1.004	0.999	0.995	-4		3	0.037	0.033	0.040	0.040	-4
	Mean	1.003	1.003	0.994	0.991	-3		Mean	0.037	0.032	0.039	0.039	-5
8	1	1.000	1.000	0.987	0.991	5	8	1	0.037	0.032	0.037	0.038	-8
	2	1.003	0.996	0.998	0.990	-3		2	0.037	0.033	0.038	0.045	-7
	3	1.007	1.003	1.000	0.995	-1		3	0.039	0.034	0.039	0.040	-8
	Mean	1.003	1.000	0.995	0.992	0		Mean	0.038	0.033	0.038	0.041	-8
9	1	0.995	0.995	0.990	0.986	-4	9	1	0.037	0.032	0.038	0.038	-10
	2	1.001	0.994	0.992	0.986	0		2	0.037	0.034	0.038	0.051	-10
	3	1.004	1.001	0.998	0.993	-3		3	0.037	0.032	0.039	0.044	-9
	Mean	1.000	0.997	0.993	0.988	-2		Mean	0.037	0.033	0.038	0.044	-10
10	1	1.000	0.994	0.992	0.965	-8	10	1	0.037	0.033	0.039	0.038	-6
	2	1.000	0.997	0.993	0.987	-9		2	0.037	0.032	0.038	0.045	-7
	3	1.003	0.997	0.995	0.989	-8		3	0.038	0.033	0.038	0.040	-8
	Mean	1.001	0.996	0.993	0.980	-8		Mean	0.037	0.033	0.038	0.041	-7
11	1	1.000	1.002	0.990	0.990	0	11	1	0.040	0.033	0.039	0.038	-7
	2	1.007	1.004	0.999	0.992	-2		2	0.038	0.033	0.038	0.038	-6
	3	1.007	1.008	1.004	1.003	-7		3	0.037	0.033	0.038	0.040	-5
	Mean	1.005	1.005	0.998	0.995	-3		Mean	0.038	0.033	0.038	0.039	-6
12	1	1.008	1.009	0.998	0.999	-7	12	1	0.038	0.035	0.039	0.040	-5
	2	1.008	1.007	0.990	0.977	1		2	0.038	0.034	0.039	0.041	-5
	3	1.013	1.010	1.008	1.000	-12		3	0.038	0.033	0.040	0.039	-4
	Mean	1.010	1.009	0.999	0.992	-6		Mean	0.038	0.034	0.039	0.040	-5
Mean for all assays	-	-	-	-	-2	Mean for all assays	-	-	-	-	-	-6	
SD for all assays	-	-	-	-	3	SD for all assays	-	-	-	-	-	2	
CV for all assays	-	-	-	-	-	CV for all assays	-	-	-	-	-	-	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 3
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	0.988	0.996	0.606	0.982	366	1	1	0.037	0.038	0.245	0.040	205
	2	0.991	1.001	0.614	0.987	361		2	0.039	0.039	0.248	0.041	206
	3	0.984	1.000	0.614	0.983	355		3	0.038	0.038	0.269	0.040	228
	Mean	0.987	0.999	0.612	0.984	361		Mean	0.038	0.038	0.254	0.041	213
2	1	0.978	0.980	0.593	0.965	371	2	1	0.036	0.037	0.261	0.040	223
	2	0.975	0.989	0.602	0.977	359		2	0.038	0.039	0.254	0.041	213
	3	0.974	0.988	0.598	0.974	363		3	0.038	0.037	0.287	0.040	247
	Mean	0.976	0.986	0.598	0.972	364		Mean	0.038	0.038	0.267	0.040	228
3	1	0.967	0.982	0.598	0.967	357	3	1	0.037	0.038	0.236	0.040	197
	2	0.966	0.980	0.606	0.967	347		2	0.039	0.039	0.229	0.041	188
	3	0.962	0.976	0.608	0.964	342		3	0.038	0.038	0.254	0.040	213
	Mean	0.965	0.979	0.604	0.966	349		Mean	0.038	0.038	0.240	0.040	199
4	1	0.984	0.990	0.605	0.979	368	4	1	0.037	0.039	0.268	0.041	228
	2	0.986	0.997	0.618	0.985	357		2	0.039	0.039	0.262	0.041	220
	3	0.982	0.995	0.607	0.985	364		3	0.039	0.038	0.280	0.040	238
	Mean	0.984	0.994	0.610	0.983	363		Mean	0.038	0.038	0.270	0.041	229
5	1	0.979	0.988	0.610	0.975	356	5	1	0.037	0.038	0.258	0.041	218
	2	0.980	0.993	0.621	0.981	346		2	0.039	0.039	0.259	0.042	218
	3	0.974	0.992	0.609	0.979	352		3	0.038	0.038	0.270	0.040	229
	Mean	0.978	0.991	0.613	0.978	351		Mean	0.038	0.038	0.262	0.041	222
6	1	0.968	0.987	0.590	0.976	368	6	1	0.037	0.038	0.248	0.040	209
	2	0.966	0.981	0.594	0.971	362		2	0.039	0.039	0.250	0.041	209
	3	0.960	0.978	0.594	0.968	356		3	0.039	0.038	0.267	0.040	226
	Mean	0.964	0.982	0.593	0.972	362		Mean	0.038	0.038	0.255	0.040	215

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 3
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.969	0.984	0.593	0.972	365	7	1	0.037	0.038	0.233	0.041	194
	2	0.966	0.984	0.602	0.974	353		2	0.039	0.040	0.262	0.041	221
	3	0.963	0.982	0.600	0.970	351		3	0.038	0.038	0.280	0.040	240
	Mean	0.966	0.984	0.598	0.972	356		Mean	0.038	0.039	0.258	0.041	218
8	1	0.957	0.994	0.584	0.985	364	8	1	0.037	0.038	0.239	0.040	200
	2	0.954	0.978	0.593	0.969	352		2	0.039	0.039	0.235	0.041	194
	3	0.949	0.965	0.596	0.957	344		3	0.039	0.038	0.247	0.040	207
	Mean	0.954	0.979	0.591	0.970	353		Mean	0.038	0.038	0.240	0.040	200
9	1	0.970	0.982	0.589	0.970	371	9	1	0.037	0.038	0.274	0.041	235
	2	0.971	0.987	0.595	0.980	366		2	0.038	0.039	0.271	0.041	230
	3	0.968	0.994	0.596	0.984	362		3	0.042	0.037	0.278	0.040	233
	Mean	0.970	0.988	0.593	0.978	366		Mean	0.039	0.038	0.275	0.041	233
10	1	0.962	0.987	0.594	0.978	359	10	1	0.037	0.038	0.255	0.040	216
	2	0.965	0.976	0.598	0.965	357		2	0.038	0.039	0.247	0.041	207
	3	0.953	0.974	0.590	0.966	354		3	0.038	0.039	0.260	0.041	221
	Mean	0.960	0.979	0.594	0.970	357		Mean	0.038	0.039	0.254	0.041	215
11	1	0.962	0.986	0.583	0.978	372	11	1	0.037	0.038	0.255	0.040	216
	2	0.964	0.976	0.593	0.968	364		2	0.038	0.039	0.253	0.041	213
	3	0.950	0.973	0.588	0.967	355		3	0.038	0.039	0.258	0.041	218
	Mean	0.958	0.978	0.588	0.971	364		Mean	0.038	0.039	0.255	0.041	216
12	1	0.981	1.000	0.604	0.991	367	12	1	0.037	0.038	0.246	0.041	206
	2	0.969	0.976	0.597	0.965	362		2	0.038	0.040	0.246	0.041	206
	3	0.954	0.969	0.592	0.959	352		3	0.038	0.039	0.252	0.041	212
	Mean	0.968	0.982	0.597	0.972	360		Mean	0.038	0.039	0.248	0.041	208
Mean for all assays		-	-	-	-	359	Mean for all assays		-	-	-	-	216
SD for all assays		-	-	-	-	6	SD for all assays		-	-	-	-	11
CV for all assays		-	-	-	-	1.7	CV for all assays		-	-	-	-	5.1

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 3
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
1	1	0.996	0.996	0.989	0.982	-8	1	1	0.039	0.038	0.040	0.040	-3
	2	0.997	1.001	0.992	0.987	-9		2	0.040	0.039	0.041	0.041	-2
	3	1.001	1.000	0.993	0.983	-7		3	0.039	0.038	0.040	0.040	-2
	Mean	0.998	0.999	0.991	0.984	-8		Mean	0.039	0.038	0.040	0.041	-2
2	1	0.983	0.980	0.978	0.965	-8	2	1	0.039	0.038	0.039	0.040	-2
	2	0.990	0.989	0.981	0.977	-6		2	0.040	0.039	0.040	0.041	-2
	3	0.989	0.988	0.982	0.974	-7		3	0.039	0.037	0.039	0.040	-2
	Mean	0.987	0.986	0.980	0.972	-7		Mean	0.039	0.038	0.039	0.040	-2
3	1	0.987	0.982	0.981	0.967	-7	3	1	0.040	0.038	0.040	0.040	-2
	2	0.979	0.980	0.975	0.967	-9		2	0.041	0.039	0.041	0.041	-2
	3	0.979	0.976	0.974	0.964	-8		3	0.039	0.038	0.040	0.040	-1
	Mean	0.982	0.979	0.976	0.966	-8		Mean	0.040	0.038	0.040	0.040	-2
4	1	0.994	0.990	0.989	0.979	-6	4	1	0.039	0.039	0.039	0.041	-3
	2	0.993	0.997	0.989	0.985	-7		2	0.040	0.039	0.040	0.041	-3
	3	0.997	0.995	0.993	0.985	-6		3	0.039	0.038	0.040	0.040	-2
	Mean	0.995	0.994	0.990	0.983	-6		Mean	0.039	0.038	0.040	0.041	-3
5	1	0.988	0.988	0.983	0.975	-7	5	1	0.039	0.038	0.039	0.041	-3
	2	0.989	0.993	0.980	0.981	-4		2	0.043	0.039	0.042	0.042	-3
	3	0.992	0.992	0.982	0.979	-3		3	0.039	0.038	0.039	0.040	-3
	Mean	0.990	0.991	0.982	0.978	-5		Mean	0.040	0.038	0.040	0.041	-3
6	1	0.989	0.987	0.982	0.976	-2	6	1	0.039	0.038	0.039	0.040	-2
	2	0.982	0.981	0.973	0.971	-1		2	0.040	0.039	0.040	0.041	-2
	3	0.978	0.978	0.970	0.968	-2		3	0.039	0.038	0.039	0.040	-2
	Mean	0.983	0.982	0.975	0.972	-2		Mean	0.039	0.038	0.039	0.040	-2

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 3 Positive control and negative control data of phase 1 study

Laboratory : 3
 Chemical Name : Sulisobenzone

Singlet oxygen						Superoxide anion							
Assay	Run#	A440(-)		A440(+)		Results*1	Assay	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
7	1	0.993	0.984	0.985	0.972	-4	7	1	0.039	0.038	0.040	0.041	-1
	2	0.984	0.984	0.975	0.974	-4		2	0.041	0.040	0.041	0.041	-1
	3	0.982	0.982	0.976	0.970	-5		3	0.039	0.038	0.041	0.040	-1
	Mean	0.986	0.984	0.979	0.972	-4		Mean	0.040	0.039	0.041	0.041	-1
8	1	0.995	0.994	0.988	0.985	-2	8	1	0.039	0.038	0.039	0.040	-2
	2	0.975	0.978	0.968	0.969	-2		2	0.040	0.039	0.040	0.041	-2
	3	0.969	0.965	0.961	0.957	-1		3	0.039	0.038	0.040	0.040	-2
	Mean	0.980	0.979	0.972	0.970	-2		Mean	0.039	0.038	0.040	0.040	-2
9	1	0.984	0.982	0.976	0.970	-2	9	1	0.039	0.038	0.039	0.041	-3
	2	0.986	0.987	0.977	0.980	-1		2	0.040	0.039	0.040	0.041	-2
	3	0.987	0.994	0.979	0.984	-2		3	0.038	0.037	0.039	0.040	-2
	Mean	0.985	0.988	0.977	0.978	-2		Mean	0.039	0.038	0.040	0.041	-2
10	1	0.980	0.987	0.974	0.978	-2	10	1	0.040	0.038	0.040	0.040	-2
	2	0.982	0.976	0.975	0.965	-2		2	0.039	0.039	0.039	0.041	-2
	3	0.976	0.974	0.969	0.966	-2		3	0.039	0.039	0.039	0.041	-1
	Mean	0.980	0.979	0.973	0.970	-2		Mean	0.039	0.039	0.039	0.041	-2
11	1	0.981	0.986	0.973	0.978	1	11	1	0.039	0.038	0.040	0.040	-2
	2	0.981	0.976	0.974	0.968	1		2	0.039	0.039	0.039	0.041	-2
	3	0.976	0.973	0.970	0.967	-1		3	0.039	0.039	0.040	0.041	-1
	Mean	0.979	0.978	0.972	0.971	0		Mean	0.039	0.039	0.040	0.041	-2
12	1	0.993	1.000	0.987	0.991	-4	12	1	0.039	0.038	0.040	0.041	-2
	2	0.980	0.976	0.973	0.965	-3		2	0.039	0.040	0.039	0.041	-2
	3	0.973	0.969	0.966	0.959	-3		3	0.042	0.039	0.039	0.041	-4
	Mean	0.982	0.982	0.975	0.972	-3		Mean	0.040	0.039	0.039	0.041	-3
Mean for all assays	-	-	-	-	-4	Mean for all assays	-	-	-	-	-2		
SD for all assays	-	-	-	-	3	SD for all assays	-	-	-	-	1		
CV for all assays	-	-	-	-	-	CV for all assays	-	-	-	-	-		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$
 A440(-) : Absorbance before light exposure at 440 nm
 A440(+) : Absorbance after light exposure at 440 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$
 A560(-) : Absorbance before light exposure at 560 nm
 A560(+) : Absorbance after light exposure at 560 nm
 A : Mean (Blank before light exposure)
 B : Mean (Blank after exposure)

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : 5-FU

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.888	0.871	0.879	0.864	3	1	1	0.045	0.033	0.042	0.058	-24	
	2	0.887	0.870	0.878	0.864	2		2	0.031	0.035	0.040	0.041	-11	
	3	0.852	0.875	0.844	0.867	1		3	0.039	0.030	0.040	0.058	-19	
	Mean	0.876	0.872	0.867	0.865	2		Mean	0.038	0.033	0.041	0.053	-18	
2	1	0.874	0.859	0.866	0.850	-1	2	1	0.045	0.030	0.044	0.038	-12	
	2	0.873	0.876	0.865	0.868	0		2	0.033	0.058	0.039	0.056	-5	
	3	0.872	0.871	0.864	0.863	-1		3	0.051	0.031	0.039	0.057	-23	
	Mean	0.873	0.869	0.865	0.860	-1		Mean	0.043	0.039	0.041	0.050	-13	
3	1	0.868	0.868	0.859	0.861	1	3	1	0.048	0.029	0.044	0.036	-13	
	2	0.869	0.865	0.863	0.858	-3		2	0.032	0.045	0.046	0.042	-5	
	3	0.869	0.865	0.861	0.857	0		3	0.052	0.031	0.042	0.055	-20	
	Mean	0.869	0.866	0.861	0.858	-1		Mean	0.044	0.035	0.044	0.044	-9	
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	-	-13	Negative*3	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.879	0.873	0.872	0.867	1	1	1	0.035	0.032	0.046	0.036	2	
	2	0.889	0.880	0.882	0.873	0		2	0.030	0.047	0.038	0.040	0	
	3	0.887	0.882	0.881	0.875	0		3	0.059	0.030	0.042	0.061	-26	
	Mean	0.885	0.878	0.878	0.872	0		Mean	0.041	0.036	0.042	0.045	-8	
2	1	0.882	0.880	0.875	0.869	-2	2	1	0.041	0.033	0.048	0.036	-6	
	2	0.888	0.889	0.881	0.882	-2		2	0.031	0.052	0.040	0.050	-3	
	3	0.893	0.879	0.885	0.871	-1		3	0.049	0.033	0.045	0.066	-17	
	Mean	0.888	0.883	0.880	0.874	-2		Mean	0.040	0.039	0.044	0.051	-9	
3	1	0.881	0.886	0.873	0.877	0	3	1	0.039	0.033	0.040	0.039	-10	
	2	0.890	0.891	0.881	0.885	2		2	0.032	0.048	0.039	0.044	-3	
	3	0.890	0.892	0.883	0.884	0		3	0.041	0.032	0.044	0.057	-7	
	Mean	0.887	0.890	0.879	0.882	1		Mean	0.037	0.037	0.041	0.047	-7	
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	-	-8	Negative*3	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 25

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

The optical absorbance values were rounded to the third decimal place. If the calculated "results" are different from the numerical results in the raw data sheets by the number of significant digits in the data sheet and the appendices are different, the data in the raw data sheet is used in the appendices.

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.903	0.871	0.887	0.864	8	1	1	0.065	0.033	0.048	0.058	-37	
	2	0.882	0.870	0.863	0.864	12		2	0.051	0.035	0.057	0.041	-14	
	3	0.898	0.875	0.885	0.867	6		3	0.077	0.030	0.047	0.058	-50	
	Mean	0.894	0.872	0.878	0.865	9		Mean	0.064	0.033	0.051	0.053	-34	
2	1	0.879	0.859	0.866	0.850	4	2	1	0.070	0.030	0.047	0.038	-34	
	2	0.870	0.876	0.852	0.868	9		2	0.057	0.058	0.047	0.056	-21	
	3	0.881	0.871	0.865	0.863	7		3	0.077	0.031	0.048	0.057	-40	
	Mean	0.877	0.869	0.861	0.860	7		Mean	0.068	0.039	0.047	0.050	-32	
3	1	0.878	0.868	0.862	0.861	8	3	1	0.080	0.029	0.048	0.036	-41	
	2	0.882	0.865	0.861	0.858	13		2	0.050	0.045	0.044	0.042	-15	
	3	0.885	0.865	0.870	0.857	7		3	0.091	0.031	0.046	0.055	-54	
	Mean	0.882	0.866	0.864	0.858	9		Mean	0.074	0.035	0.046	0.044	-37	
Mean for 3 assays	-	-	-	-	8	Mean for 3 assays	-	-	-	-	-34	Negative*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.887	0.873	0.758	0.867	124	1	1	0.073	0.032	0.075	0.036	-7	
	2	0.875	0.880	0.758	0.873	111		2	0.056	0.047	0.072	0.040	8	
	3	0.894	0.882	0.760	0.875	128		3	0.073	0.030	0.074	0.061	-7	
	Mean	0.885	0.878	0.759	0.872	121		Mean	0.067	0.036	0.074	0.045	-2	
2	1	0.884	0.880	0.745	0.869	130	2	1	0.068	0.033	0.072	0.036	-8	
	2	0.879	0.889	0.755	0.882	115		2	0.045	0.052	0.071	0.050	15	
	3	0.891	0.879	0.759	0.871	123		3	0.054	0.033	0.072	0.066	7	
	Mean	0.885	0.883	0.753	0.874	123		Mean	0.056	0.039	0.072	0.051	5	
3	1	0.889	0.886	0.755	0.877	126	3	1	0.074	0.033	0.073	0.039	-11	
	2	0.880	0.891	0.758	0.885	114		2	0.043	0.048	0.070	0.044	17	
	3	0.895	0.892	0.760	0.884	127		3	0.056	0.032	0.073	0.057	7	
	Mean	0.888	0.890	0.758	0.882	122		Mean	0.058	0.037	0.072	0.047	4	
Mean for 3 assays	-	-	-	-	122	Mean for 3 assays	-	-	-	-	2	Positive*3		

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Amiodarone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.946	0.871	0.801	0.864	138	1	1	0.096	0.033	0.089	0.058	-27	
	2	0.941	0.870	0.802	0.864	132		2	0.093	0.035	0.087	0.041	-27	
	3	0.955	0.875	0.813	0.867	135		3	0.095	0.030	0.088	0.058	-27	
	Mean	0.947	0.872	0.805	0.865	135		Mean	0.095	0.033	0.088	0.053	-27	
2	1	0.951	0.859	0.812	0.850	131	2	1	0.088	0.030	0.084	0.038	-15	
	2	0.934	0.876	0.802	0.868	123		2	0.086	0.058	0.083	0.056	-14	
	3	0.946	0.871	0.804	0.863	133		3	0.097	0.031	0.084	0.057	-24	
	Mean	0.944	0.869	0.806	0.860	129		Mean	0.090	0.039	0.084	0.050	-18	
3	1	0.941	0.868	0.812	0.861	122	3	1	0.091	0.029	0.083	0.036	-17	
	2	0.935	0.865	0.804	0.858	124		2	0.089	0.045	0.083	0.042	-15	
	3	0.943	0.865	0.804	0.857	131		3	0.092	0.031	0.084	0.055	-17	
	Mean	0.940	0.866	0.807	0.858	126		Mean	0.091	0.035	0.083	0.044	-16	
Mean for 3 assays						-	Mean for 3 assays						-	Positive

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.579	0.873	1.296	0.867	278	1	1	0.733	0.032	0.502	0.036	-240	
	2	1.566	0.880	1.303	0.873	256		2	0.733	0.047	0.526	0.040	-216	
	3	1.582	0.882	1.332	0.875	243		3	0.732	0.030	0.506	0.061	-234	
	Mean	1.576	0.878	1.310	0.872	259		Mean	0.733	0.036	0.511	0.045	-230	
2	1	1.515	0.880	1.295	0.869	211	2	1	0.681	0.033	0.491	0.036	-203	
	2	1.496	0.889	1.304	0.882	183		2	0.684	0.052	0.505	0.050	-191	
	3	1.495	0.879	1.304	0.871	182		3	0.687	0.033	0.505	0.066	-194	
	Mean	1.502	0.883	1.301	0.874	192		Mean	0.684	0.039	0.500	0.051	-196	
3	1	1.553	0.886	1.285	0.877	260	3	1	0.721	0.033	0.492	0.039	-239	
	2	1.535	0.891	1.294	0.885	234		2	0.717	0.048	0.503	0.044	-224	
	3	1.555	0.892	1.298	0.884	248		3	0.719	0.032	0.496	0.057	-233	
	Mean	1.548	0.890	1.292	0.882	247		Mean	0.719	0.037	0.497	0.047	-232	
Mean for 3 assays						-	Mean for 3 assays						-	Positive

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Chlorpromazine

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.893	0.871	0.785	0.864	101	1	1	0.067	0.033	0.057	0.058	-30	
	2	0.870	0.870	0.787	0.864	75		2	0.037	0.035	0.056	0.041	-1	
	3	0.902	0.875	0.789	0.867	106		3	0.068	0.030	0.056	0.058	-32	
	Mean	0.888	0.872	0.787	0.865	94		Mean	0.057	0.033	0.056	0.053	-21	
2	1	0.884	0.859	0.777	0.850	98	2	1	0.062	0.030	0.059	0.038	-14	
	2	0.848	0.876	0.766	0.868	73		2	0.052	0.058	0.056	0.056	-7	
	3	0.901	0.871	0.796	0.863	96		3	0.066	0.031	0.055	0.057	-22	
	Mean	0.878	0.869	0.780	0.860	89		Mean	0.060	0.039	0.057	0.050	-14	
3	1	0.896	0.868	0.786	0.861	102	3	1	0.076	0.029	0.056	0.036	-30	
	2	0.860	0.865	0.788	0.858	65		2	0.040	0.045	0.057	0.042	9	
	3	0.897	0.865	0.789	0.857	100		3	0.067	0.031	0.056	0.055	-20	
	Mean	0.884	0.866	0.788	0.858	89		Mean	0.061	0.035	0.056	0.044	-14	
Mean for 3 assays	-	-	-	-	91	Mean for 3 assays	-	-	-	-	-	-16	Positive*3	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.867	0.873	0.862	0.867	-1	1	1	0.047	0.032	0.133	0.036	76	
	2	0.861	0.880	0.865	0.873	-10		2	0.038	0.047	0.134	0.040	87	
	3	0.877	0.882	0.870	0.875	1		3	0.050	0.030	0.135	0.061	76	
	Mean	0.868	0.878	0.866	0.872	-3		Mean	0.045	0.036	0.134	0.045	80	
2	1	0.846	0.880	0.852	0.869	-16	2	1	0.043	0.033	0.130	0.036	75	
	2	0.860	0.889	0.867	0.882	-16		2	0.038	0.052	0.133	0.050	82	
	3	0.870	0.879	0.865	0.871	-4		3	0.045	0.033	0.133	0.066	76	
	Mean	0.859	0.883	0.861	0.874	-12		Mean	0.042	0.039	0.132	0.051	78	
3	1	0.862	0.886	0.856	0.877	-2	3	1	0.045	0.033	0.130	0.039	75	
	2	0.861	0.891	0.871	0.885	-18		2	0.037	0.048	0.132	0.044	85	
	3	0.882	0.892	0.873	0.884	0		3	0.047	0.032	0.132	0.057	75	
	Mean	0.868	0.890	0.867	0.882	-7		Mean	0.043	0.037	0.131	0.047	78	
Mean for 3 assays	-	-	-	-	-7	Mean for 3 assays	-	-	-	-	-	79	Positive*3	

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.873	0.871	0.704	0.864	162	1	1	0.043	0.033	0.072	0.058	9	
	2	0.871	0.870	0.700	0.864	164		2	0.046	0.035	0.069	0.041	3	
	3	0.874	0.875	0.704	0.867	162		3	0.040	0.030	0.072	0.058	12	
	Mean	0.873	0.872	0.703	0.865	163		Mean	0.043	0.033	0.071	0.053	8	
2	1	0.880	0.859	0.710	0.850	161	2	1	0.039	0.030	0.068	0.038	18	
	2	0.880	0.876	0.712	0.868	159		2	0.052	0.058	0.072	0.056	9	
	3	0.885	0.871	0.715	0.863	161		3	0.045	0.031	0.070	0.057	14	
	Mean	0.882	0.869	0.712	0.860	160		Mean	0.045	0.039	0.070	0.050	14	
3	1	0.859	0.868	0.690	0.861	162	3	1	0.044	0.029	0.069	0.036	16	
	2	0.873	0.865	0.703	0.858	162		2	0.046	0.045	0.067	0.042	11	
	3	0.873	0.865	0.705	0.857	160		3	0.043	0.031	0.068	0.055	16	
	Mean	0.868	0.866	0.699	0.858	161		Mean	0.044	0.035	0.068	0.044	14	
Mean for 3 assays	-	-	-	-	161	Mean for 3 assays	-	-	-	-	12	Positive*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.866	0.873	0.548	0.867	312	1	1	0.050	0.032	0.410	0.036	351	
	2	0.887	0.880	0.570	0.873	311		2	0.045	0.047	0.427	0.040	372	
	3	0.886	0.882	0.567	0.875	313		3	0.050	0.030	0.428	0.061	369	
	Mean	0.880	0.878	0.562	0.872	312		Mean	0.048	0.036	0.422	0.045	364	
2	1	0.880	0.880	0.551	0.869	319	2	1	0.047	0.033	0.411	0.036	352	
	2	0.890	0.889	0.565	0.882	316		2	0.043	0.052	0.431	0.050	376	
	3	0.888	0.879	0.562	0.871	318		3	0.050	0.033	0.421	0.066	359	
	Mean	0.886	0.883	0.559	0.874	318		Mean	0.047	0.039	0.421	0.051	362	
3	1	0.899	0.886	0.566	0.877	325	3	1	0.048	0.033	0.411	0.039	353	
	2	0.894	0.891	0.566	0.885	321		2	0.048	0.048	0.430	0.044	372	
	3	0.886	0.892	0.559	0.884	318		3	0.050	0.032	0.431	0.057	371	
	Mean	0.893	0.890	0.564	0.882	321		Mean	0.049	0.037	0.424	0.047	365	
Mean for 3 assays	-	-	-	-	317	Mean for 3 assays	-	-	-	-	364	Positive*3		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Doxycycline

Test concentration 20 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.869	0.871	0.770	0.864	92	1	1	0.056	0.033	0.102	0.058	26	Positive	
	2	0.876	0.870	0.791	0.864	78		2	0.071	0.035	0.105	0.041	14		
	3	0.881	0.875	0.788	0.867	86		3	0.060	0.030	0.100	0.058	20		
	Mean	0.875	0.872	0.783	0.865	85		Mean	0.062	0.033	0.102	0.053	20		
2	1	0.874	0.859	0.790	0.850	75	2	1	0.053	0.030	0.092	0.038	28	Positive	
	2	0.840	0.876	0.776	0.868	55		2	0.051	0.058	0.095	0.056	32		
	3	0.863	0.871	0.783	0.863	72		3	0.055	0.031	0.092	0.057	26		
	Mean	0.859	0.869	0.783	0.860	67		Mean	0.053	0.039	0.093	0.050	29		
3	1	0.876	0.868	0.757	0.861	110	3	1	0.054	0.029	0.094	0.036	30	Positive	
	2	0.837	0.865	0.757	0.858	72		2	0.054	0.045	0.097	0.042	33		
	3	0.882	0.865	0.775	0.857	100		3	0.057	0.031	0.095	0.055	29		
	Mean	0.865	0.866	0.763	0.858	94		Mean	0.055	0.035	0.095	0.044	31		
Mean for 3 assays						-	Mean for 3 assays						-	27	Positive*3

Test concentration 200 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.801	0.873	0.670	0.867	125	1	1	0.048	0.032	0.373	0.036	316	Positive	
	2	0.810	0.880	0.699	0.873	105		2	0.055	0.047	0.384	0.040	319		
	3	0.814	0.882	0.700	0.875	108		3	0.050	0.030	0.388	0.061	329		
	Mean	0.808	0.878	0.690	0.872	113		Mean	0.051	0.036	0.382	0.045	321		
2	1	0.810	0.880	0.674	0.869	127	2	1	0.047	0.033	0.371	0.036	312	Positive	
	2	0.813	0.889	0.703	0.882	100		2	0.052	0.052	0.387	0.050	323		
	3	0.826	0.879	0.712	0.871	106		3	0.048	0.033	0.388	0.066	328		
	Mean	0.816	0.883	0.696	0.874	111		Mean	0.049	0.039	0.382	0.051	321		
3	1	0.811	0.886	0.672	0.877	131	3	1	0.049	0.033	0.373	0.039	315	Positive	
	2	0.814	0.891	0.698	0.885	107		2	0.047	0.048	0.384	0.044	327		
	3	0.834	0.892	0.713	0.884	113		3	0.052	0.032	0.389	0.057	327		
	Mean	0.820	0.890	0.694	0.882	117		Mean	0.049	0.037	0.382	0.047	323		
Mean for 3 assays						-	Mean for 3 assays						-	322	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank						
1	1	0.878	0.871	0.843	0.864	28	1	1	0.048	0.033	0.067	0.058	-1			
	2	0.878	0.870	0.823	0.864	48		2	0.093	0.035	0.076	0.041	-38			
	3	0.884	0.875	0.849	0.867	28		3	0.051	0.030	0.064	0.058	-7			
	Mean	0.880	0.872	0.838	0.865	35		Mean	0.064	0.033	0.069	0.053	-15			
2	1	0.887	0.859	0.850	0.850	28	2	1	0.061	0.030	0.063	0.038	-9			
	2	0.881	0.876	0.826	0.868	46		2	0.087	0.058	0.075	0.056	-22			
	3	0.898	0.871	0.859	0.863	30		3	0.066	0.031	0.065	0.057	-12			
	Mean	0.889	0.869	0.845	0.860	35		Mean	0.071	0.039	0.068	0.050	-14			
3	1	0.883	0.868	0.835	0.861	41	3	1	0.073	0.029	0.071	0.036	-11			
	2	0.879	0.865	0.822	0.858	50		2	0.085	0.045	0.079	0.042	-15			
	3	0.890	0.865	0.847	0.857	35		3	0.077	0.031	0.067	0.055	-19			
	Mean	0.884	0.866	0.835	0.858	42		Mean	0.078	0.035	0.072	0.044	-15			
Mean for 3 assays						-	Mean for 3 assays						-	-	-15	Positive

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank						
1	1	0.894	0.873	0.696	0.867	192	1	1	0.047	0.032	0.153	0.036	97			
	2	0.915	0.880	0.748	0.873	161		2	0.058	0.047	0.169	0.040	102			
	3	0.903	0.882	0.681	0.875	216		3	0.047	0.030	0.164	0.061	107			
	Mean	0.904	0.878	0.708	0.872	190		Mean	0.051	0.036	0.162	0.045	102			
2	1	0.893	0.880	0.669	0.869	215	2	1	0.042	0.033	0.149	0.036	95			
	2	0.886	0.889	0.706	0.882	171		2	0.052	0.052	0.164	0.050	100			
	3	0.903	0.879	0.692	0.871	202		3	0.048	0.033	0.163	0.066	103			
	Mean	0.894	0.883	0.689	0.874	196		Mean	0.047	0.039	0.159	0.051	99			
3	1	0.892	0.886	0.677	0.877	207	3	1	0.054	0.033	0.151	0.039	87			
	2	0.896	0.891	0.706	0.885	182		2	0.050	0.048	0.158	0.044	98			
	3	0.909	0.892	0.695	0.884	205		3	0.049	0.032	0.155	0.057	96			
	Mean	0.899	0.890	0.693	0.882	198		Mean	0.051	0.037	0.155	0.047	94			
Mean for 3 assays						-	Mean for 3 assays						-	-	98	Positive

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.864	0.865	0.822	0.856	34	1	1	0.064	0.033	0.049	0.036	-25	
	2	0.869	0.880	0.807	0.874	53		2	0.059	0.033	0.042	0.039	-27	
	3	0.874	0.873	0.829	0.865	37		3	0.073	0.029	0.047	0.050	-36	
	Mean	0.869	0.873	0.819	0.865	41		Mean	0.065	0.032	0.046	0.042	-29	
2	1	0.877	0.863	0.843	0.853	25	2	1	0.058	0.029	0.047	0.035	-25	
	2	0.876	0.873	0.823	0.865	43		2	0.057	0.030	0.045	0.038	-26	
	3	0.876	0.875	0.846	0.867	21		3	0.079	0.029	0.051	0.054	-42	
	Mean	0.876	0.870	0.837	0.861	30		Mean	0.065	0.029	0.048	0.043	-31	
3	1	0.864	0.844	0.816	0.836	42	3	1	0.047	0.030	0.050	0.037	-9	
	2	0.864	0.844	0.799	0.842	60		2	0.033	0.052	0.044	0.040	-3	
	3	0.862	0.851	0.814	0.845	42		3	0.046	0.032	0.051	0.075	-8	
	Mean	0.863	0.846	0.810	0.841	48		Mean	0.042	0.038	0.048	0.051	-7	
Mean for 3 assays						-	Mean for 3 assays						-	Positive

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.878	0.871	0.612	0.862	258	1	1	0.034	0.028	0.132	0.037	87	
	2	0.878	0.877	0.617	0.868	252		2	0.033	0.046	0.131	0.042	87	
	3	0.882	0.880	0.626	0.871	247		3	0.034	0.031	0.141	0.061	96	
	Mean	0.879	0.876	0.618	0.867	252		Mean	0.034	0.035	0.135	0.046	90	
2	1	0.857	0.853	0.601	0.849	249	2	1	0.034	0.033	0.136	0.037	91	
	2	0.863	0.859	0.606	0.852	249		2	0.032	0.048	0.137	0.039	93	
	3	0.866	0.861	0.613	0.853	247		3	0.035	0.032	0.145	0.074	98	
	Mean	0.862	0.858	0.607	0.851	248		Mean	0.034	0.038	0.139	0.050	94	
3	1	0.868	0.884	0.605	0.878	256	3	1	0.034	0.032	0.142	0.037	98	
	2	0.872	0.886	0.607	0.879	259		2	0.033	0.043	0.132	0.037	89	
	3	0.873	0.880	0.618	0.873	249		3	0.034	0.030	0.140	0.061	95	
	Mean	0.871	0.883	0.610	0.877	255		Mean	0.034	0.035	0.138	0.045	94	
Mean for 3 assays						-	Mean for 3 assays						-	Positive

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.866	0.865	0.829	0.856	29	1	1	0.037	0.033	0.224	0.036	177	
	2	0.875	0.880	0.825	0.874	42		2	0.030	0.033	0.208	0.039	168	
	3	0.872	0.873	0.838	0.865	27		3	0.033	0.029	0.214	0.050	171	
	Mean	0.871	0.873	0.831	0.865	33		Mean	0.033	0.032	0.215	0.042	172	
2	1	0.868	0.863	0.812	0.853	47	2	1	0.030	0.029	0.211	0.035	166	
	2	0.876	0.873	0.822	0.865	46		2	0.044	0.030	0.209	0.038	152	
	3	0.876	0.875	0.823	0.867	44		3	0.033	0.029	0.213	0.054	166	
	Mean	0.873	0.870	0.819	0.861	46		Mean	0.036	0.029	0.211	0.043	161	
3	1	0.861	0.844	0.822	0.836	34	3	1	0.060	0.030	0.217	0.037	144	
	2	0.864	0.844	0.814	0.842	45		2	0.051	0.052	0.227	0.040	163	
	3	0.865	0.851	0.832	0.845	28		3	0.067	0.032	0.224	0.075	144	
	Mean	0.863	0.846	0.823	0.841	36		Mean	0.059	0.038	0.223	0.051	150	
Mean for 3 assays	-	-	-	-	38	Mean for 3 assays	-	-	-	-	161	Positive*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.866	0.871	0.632	0.862	225	1	1	0.076	0.028	0.449	0.037	362	
	2	0.872	0.877	0.675	0.868	188		2	0.054	0.046	0.456	0.042	391	
	3	0.887	0.880	0.679	0.871	199		3	0.082	0.031	0.453	0.061	360	
	Mean	0.875	0.876	0.662	0.867	204		Mean	0.071	0.035	0.453	0.046	371	
2	1	0.874	0.853	0.656	0.849	211	2	1	0.069	0.033	0.455	0.037	374	
	2	0.863	0.859	0.684	0.852	173		2	0.046	0.048	0.479	0.039	422	
	3	0.875	0.861	0.689	0.853	179		3	0.064	0.032	0.450	0.074	374	
	Mean	0.871	0.858	0.676	0.851	188		Mean	0.060	0.038	0.461	0.050	390	
3	1	0.865	0.884	0.668	0.878	191	3	1	0.074	0.032	0.447	0.037	363	
	2	0.873	0.886	0.681	0.879	186		2	0.048	0.043	0.458	0.037	400	
	3	0.905	0.880	0.701	0.873	197		3	0.073	0.030	0.457	0.061	375	
	Mean	0.881	0.883	0.683	0.877	191		Mean	0.065	0.035	0.454	0.045	379	
Mean for 3 assays	-	-	-	-	194	Mean for 3 assays	-	-	-	-	380	Positive*3		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.873	0.865	0.786	0.856	79	1	1	0.053	0.033	0.061	0.036	-2	
	2	0.870	0.880	0.795	0.874	66		2	0.036	0.033	0.063	0.039	17	
	3	0.875	0.873	0.765	0.865	102		3	0.054	0.029	0.061	0.050	-3	
	Mean	0.873	0.873	0.782	0.865	82		Mean	0.048	0.032	0.062	0.042	4	
2	1	0.867	0.863	0.779	0.853	79	2	1	0.051	0.029	0.062	0.035	-3	
	2	0.869	0.873	0.795	0.865	65		2	0.035	0.030	0.061	0.038	13	
	3	0.876	0.875	0.781	0.867	86		3	0.049	0.029	0.062	0.054	-2	
	Mean	0.871	0.870	0.785	0.861	77		Mean	0.045	0.029	0.062	0.043	3	
3	1	0.866	0.844	0.750	0.836	111	3	1	0.044	0.030	0.060	0.037	2	
	2	0.865	0.844	0.733	0.842	127		2	0.033	0.052	0.060	0.040	14	
	3	0.867	0.851	0.734	0.845	129		3	0.044	0.032	0.061	0.075	4	
	Mean	0.866	0.846	0.739	0.841	122		Mean	0.040	0.038	0.060	0.051	7	
Mean for 3 assays	-	-	-	-	94	Mean for 3 assays	-	-	-	-	-	5	Positive*3	

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.870	0.871	0.643	0.862	218	1	1	0.037	0.028	0.162	0.037	114	
	2	0.870	0.877	0.657	0.868	205		2	0.035	0.046	0.165	0.042	120	
	3	0.871	0.880	0.654	0.871	209		3	0.043	0.031	0.168	0.061	114	
	Mean	0.870	0.876	0.651	0.867	211		Mean	0.038	0.035	0.165	0.046	116	
2	1	0.861	0.853	0.641	0.849	214	2	1	0.036	0.033	0.165	0.037	117	
	2	0.862	0.859	0.650	0.852	205		2	0.036	0.048	0.168	0.039	120	
	3	0.862	0.861	0.649	0.853	206		3	0.040	0.032	0.172	0.074	120	
	Mean	0.862	0.858	0.647	0.851	208		Mean	0.037	0.038	0.168	0.050	119	
3	1	0.859	0.884	0.633	0.878	219	3	1	0.037	0.032	0.159	0.037	112	
	2	0.857	0.886	0.642	0.879	208		2	0.036	0.043	0.162	0.037	116	
	3	0.870	0.880	0.650	0.873	213		3	0.039	0.030	0.167	0.061	118	
	Mean	0.862	0.883	0.642	0.877	213		Mean	0.037	0.035	0.163	0.045	115	
Mean for 3 assays	-	-	-	-	211	Mean for 3 assays	-	-	-	-	-	117	Positive*3	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.879	0.865	0.802	0.856	68	1	1	0.044	0.033	0.068	0.036	15		
	2	0.885	0.880	0.812	0.874	65		2	0.039	0.033	0.066	0.039	17		
	3	0.877	0.873	0.804	0.865	65		3	0.052	0.029	0.065	0.050	3		
	Mean	0.880	0.873	0.806	0.865	66		Mean	0.045	0.032	0.066	0.042	12		
2	1	0.865	0.863	0.785	0.853	72	2	1	0.041	0.029	0.063	0.035	8		
	2	0.872	0.873	0.796	0.865	67		2	0.036	0.030	0.062	0.038	12		
	3	0.878	0.875	0.795	0.867	74		3	0.045	0.029	0.062	0.054	3		
	Mean	0.872	0.870	0.792	0.861	71		Mean	0.041	0.029	0.062	0.043	8		
3	1	0.876	0.844	0.803	0.836	68	3	1	0.045	0.030	0.064	0.037	6		
	2	0.875	0.844	0.808	0.842	62		2	0.041	0.052	0.066	0.040	12		
	3	0.874	0.851	0.795	0.845	74		3	0.045	0.032	0.064	0.075	6		
	Mean	0.875	0.846	0.802	0.841	68		Mean	0.044	0.038	0.065	0.051	8		
Mean for 3 assays						-	Mean for 3 assays						-	9	Positive*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.845	0.871	0.861	0.862	-24	1	1	0.045	0.028	0.173	0.037	117		
	2	0.829	0.877	0.857	0.868	-37		2	0.046	0.046	0.179	0.042	122		
	3	0.849	0.880	0.867	0.871	-27		3	0.045	0.031	0.171	0.061	115		
	Mean	0.841	0.876	0.862	0.867	-29		Mean	0.045	0.035	0.174	0.046	118		
2	1	0.836	0.853	0.859	0.849	-29	2	1	0.057	0.033	0.175	0.037	107		
	2	0.829	0.859	0.856	0.852	-35		2	0.047	0.048	0.174	0.039	116		
	3	0.830	0.861	0.853	0.853	-30		3	0.055	0.032	0.172	0.074	105		
	Mean	0.832	0.858	0.856	0.851	-31		Mean	0.053	0.038	0.174	0.050	109		
3	1	0.838	0.884	0.844	0.878	-12	3	1	0.044	0.032	0.169	0.037	116		
	2	0.833	0.886	0.854	0.879	-27		2	0.045	0.043	0.173	0.037	117		
	3	0.839	0.880	0.867	0.873	-33		3	0.047	0.030	0.171	0.061	114		
	Mean	0.837	0.883	0.855	0.877	-24		Mean	0.045	0.035	0.171	0.045	116		
Mean for 3 assays						-	Mean for 3 assays						-	114	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Quinine

Test concentration 20 µM

Singlet oxygen						Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.867	0.865	0.733	0.856	126	1	1	0.064	0.033	0.081	0.036	8	
	2	0.866	0.880	0.741	0.874	116		2	0.048	0.033	0.078	0.039	20	
	3	0.873	0.873	0.735	0.865	131		3	0.067	0.029	0.080	0.050	3	
	Mean	0.869	0.873	0.736	0.865	124		Mean	0.060	0.032	0.080	0.042	10	
2	1	0.868	0.863	0.741	0.853	118	2	1	0.055	0.029	0.075	0.035	6	
	2	0.865	0.873	0.737	0.865	119		2	0.044	0.030	0.079	0.038	21	
	3	0.876	0.875	0.741	0.867	126		3	0.062	0.029	0.074	0.054	-2	
	Mean	0.870	0.870	0.740	0.861	121		Mean	0.054	0.029	0.076	0.043	8	
3	1	0.863	0.844	0.778	0.836	80	3	1	0.049	0.030	0.081	0.037	19	
	2	0.867	0.844	0.736	0.842	126		2	0.033	0.052	0.079	0.040	33	
	3	0.870	0.851	0.730	0.845	134		3	0.054	0.032	0.079	0.075	12	
	Mean	0.867	0.846	0.748	0.841	113		Mean	0.045	0.038	0.080	0.051	21	
Mean for 3 assays	-	-	-	-	119	Mean for 3 assays	-	-	-	-	13	Positive*3		

Test concentration 200 µM

Singlet oxygen						Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.853	0.871	0.396	0.862	448	1	1	0.034	0.028	0.429	0.037	384	
	2	0.854	0.877	0.419	0.868	427		2	0.034	0.046	0.424	0.042	380	
	3	0.863	0.880	0.430	0.871	424		3	0.037	0.031	0.446	0.061	398	
	Mean	0.857	0.876	0.415	0.867	433		Mean	0.035	0.035	0.433	0.046	387	
2	1	0.855	0.853	0.400	0.849	448	2	1	0.034	0.033	0.434	0.037	389	
	2	0.844	0.859	0.417	0.852	420		2	0.035	0.048	0.438	0.039	391	
	3	0.856	0.861	0.421	0.853	428		3	0.035	0.032	0.448	0.074	401	
	Mean	0.852	0.858	0.413	0.851	432		Mean	0.035	0.038	0.440	0.050	394	
3	1	0.883	0.884	0.398	0.878	479	3	1	0.060	0.032	0.424	0.037	354	
	2	0.861	0.886	0.421	0.879	434		2	0.035	0.043	0.433	0.037	388	
	3	0.885	0.880	0.425	0.873	455		3	0.035	0.030	0.441	0.061	396	
	Mean	0.876	0.883	0.415	0.877	456		Mean	0.043	0.035	0.433	0.045	379	
Mean for 3 assays	-	-	-	-	440	Mean for 3 assays	-	-	-	-	387	Positive*3		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 1
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen						Superoxide anion					Positive / Negative				
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)			A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.865	0.865	0.869	0.856	-11	1	1	0.040	0.033	0.047	0.036	-3	Negative	
	2	0.877	0.880	0.875	0.874	-6		2	0.039	0.033	0.042	0.039	-7		
	3	0.885	0.873	0.893	0.865	-16		3	0.044	0.029	0.047	0.050	-8		
	Mean	0.876	0.873	0.879	0.865	-11		Mean	0.041	0.032	0.045	0.042	-6		
2	1	0.862	0.863	0.854	0.853	-1	2	1	0.043	0.029	0.051	0.035	-6	Negative	
	2	0.864	0.873	0.857	0.865	-2		2	0.038	0.030	0.042	0.038	-10		
	3	0.870	0.875	0.864	0.867	-3		3	0.039	0.029	0.050	0.054	-4		
	Mean	0.865	0.870	0.858	0.861	-2		Mean	0.040	0.029	0.048	0.043	-7		
3	1	0.851	0.844	0.862	0.836	-16	3	1	0.055	0.030	0.047	0.037	-21	Negative	
	2	0.854	0.844	0.852	0.842	-3		2	0.045	0.052	0.040	0.040	-18		
	3	0.860	0.851	0.867	0.845	-12		3	0.054	0.032	0.040	0.075	-27		
	Mean	0.855	0.846	0.860	0.841	-10		Mean	0.051	0.038	0.042	0.051	-22		
Mean for 3 assays						-	Mean for 3 assays					-	-	-12	Negative

Test concentration 200 µM

Singlet oxygen						Superoxide anion					Positive / Negative				
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)			A560(+)		Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.879	0.871	0.864	0.862	6	1	1	0.048	0.028	0.045	0.037	-13	Negative	
	2	0.876	0.877	0.871	0.868	-4		2	0.048	0.046	0.046	0.042	-13		
	3	0.887	0.880	0.867	0.871	11		3	0.047	0.031	0.047	0.061	-12		
	Mean	0.881	0.876	0.867	0.867	4		Mean	0.048	0.035	0.046	0.046	-13		
2	1	0.876	0.853	0.860	0.849	9	2	1	0.043	0.033	0.048	0.037	-7	Negative	
	2	0.868	0.859	0.859	0.852	2		2	0.047	0.048	0.050	0.039	-10		
	3	0.880	0.861	0.862	0.853	11		3	0.046	0.032	0.050	0.074	-9		
	Mean	0.875	0.858	0.860	0.851	7		Mean	0.045	0.038	0.049	0.050	-9		
3	1	0.880	0.884	0.872	0.878	2	3	1	0.046	0.032	0.041	0.037	-15	Negative	
	2	0.882	0.886	0.873	0.879	3		2	0.047	0.043	0.041	0.037	-16		
	3	0.884	0.880	0.876	0.873	2		3	0.048	0.030	0.042	0.061	-16		
	Mean	0.882	0.883	0.874	0.877	2		Mean	0.047	0.035	0.041	0.045	-16		
Mean for 3 assays						-	Mean for 3 assays					-	-	-13	Negative

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : 5-FU

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.005	1.005	0.998	1.004	1	1	1	0.034	0.033	0.037	0.037	-2	
	2	1.007	1.005	0.999	0.998	2		2	0.034	0.035	0.038	0.040	-1	
	3	1.015	1.009	1.000	0.998	9		3	0.033	0.034	0.038	0.039	0	
	Mean	1.009	1.006	0.999	1.000	4		Mean	0.034	0.034	0.038	0.039	-1	
2	1	0.999	1.004	0.995	1.000	-3	2	1	0.033	0.033	0.038	0.038	-1	
	2	1.001	1.000	0.993	0.994	1		2	0.032	0.032	0.036	0.038	-2	
	3	1.003	1.008	0.997	0.998	-1		3	0.034	0.032	0.038	0.038	-2	
	Mean	1.001	1.004	0.995	0.997	-1		Mean	0.033	0.032	0.037	0.038	-2	
3	1	0.991	0.994	0.985	0.965	-10	3	1	0.032	0.033	0.037	0.038	-3	
	2	0.998	0.997	0.992	0.987	-10		2	0.032	0.032	0.038	0.045	-2	
	3	0.999	0.997	0.990	0.989	-7		3	0.032	0.033	0.037	0.040	-3	
	Mean	0.996	0.996	0.989	0.980	-9		Mean	0.032	0.033	0.037	0.041	-3	
Mean for 3 assays						-	Mean for 3 assays						-	Negative

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.001	1.003	0.991	0.992	1	1	1	0.032	0.038	0.039	0.042	-4	
	2	1.003	1.002	0.994	0.994	0		2	0.032	0.036	0.041	0.059	-2	
	3	1.005	1.005	0.995	0.995	1		3	0.034	0.034	0.041	0.040	-4	
	Mean	1.003	1.003	0.993	0.994	1		Mean	0.033	0.036	0.040	0.047	-3	
2	1	1.002	1.000	0.993	0.991	-1	2	1	0.031	0.032	0.038	0.038	0	
	2	0.997	0.995	0.990	0.988	-3		2	0.032	0.033	0.040	0.040	1	
	3	1.007	1.011	0.998	0.996	-1		3	0.034	0.033	0.041	0.042	0	
	Mean	1.002	1.002	0.994	0.992	-2		Mean	0.032	0.033	0.040	0.040	0	
3	1	0.993	0.995	0.983	0.986	1	3	1	0.031	0.032	0.038	0.038	-4	
	2	0.996	0.994	0.987	0.986	0		2	0.033	0.034	0.039	0.051	-5	
	3	1.001	1.001	0.992	0.993	0		3	0.033	0.032	0.039	0.044	-5	
	Mean	0.997	0.997	0.987	0.988	0		Mean	0.032	0.033	0.039	0.044	-5	
Mean for 3 assays						-	Mean for 3 assays						-	Negative

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.004	1.005	0.985	1.004	13	1	1	0.032	0.033	0.044	0.037	7	
	2	1.006	1.005	0.996	0.998	4		2	0.035	0.035	0.046	0.040	6	
	3	1.006	1.009	0.990	0.998	10		3	0.032	0.034	0.044	0.039	7	
	Mean	1.005	1.006	0.990	1.000	9		Mean	0.033	0.034	0.045	0.039	7	
2	1	1.001	1.004	0.987	1.000	7	2	1	0.032	0.033	0.048	0.038	10	
	2	1.001	1.000	0.985	0.994	9		2	0.031	0.032	0.042	0.038	5	
	3	0.995	1.008	0.986	0.998	2		3	0.033	0.032	0.042	0.038	3	
	Mean	0.999	1.004	0.986	0.997	6		Mean	0.032	0.032	0.044	0.038	6	
3	1	0.994	0.994	0.960	0.965	18	3	1	0.033	0.033	0.042	0.038	1	
	2	1.000	0.997	0.982	0.987	2		2	0.032	0.032	0.042	0.045	2	
	3	1.003	0.997	0.968	0.989	19		3	0.033	0.033	0.042	0.040	1	
	Mean	0.999	0.996	0.970	0.980	13		Mean	0.033	0.033	0.042	0.041	1	
Mean for 3 assays						-	Mean for 3 assays						-	Negative

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.993	1.003	0.922	0.992	62	1	1	0.032	0.038	0.107	0.042	64	
	2	0.993	1.002	0.924	0.994	60		2	0.035	0.036	0.106	0.059	60	
	3	0.993	1.005	0.927	0.995	57		3	0.033	0.034	0.112	0.040	68	
	Mean	0.993	1.003	0.924	0.994	60		Mean	0.033	0.036	0.108	0.047	64	
2	1	0.993	1.000	0.916	0.991	67	2	1	0.031	0.032	0.113	0.038	75	
	2	0.988	0.995	0.914	0.988	64		2	0.032	0.033	0.112	0.040	73	
	3	0.996	1.011	0.922	0.996	64		3	0.032	0.033	0.119	0.042	80	
	Mean	0.992	1.002	0.917	0.992	65		Mean	0.032	0.033	0.115	0.040	76	
3	1	0.987	0.995	0.917	0.986	61	3	1	0.032	0.032	0.093	0.038	50	
	2	0.992	0.994	0.931	0.986	52		2	0.033	0.034	0.091	0.051	47	
	3	0.991	1.001	0.920	0.993	62		3	0.033	0.032	0.097	0.044	53	
	Mean	0.990	0.997	0.923	0.988	58		Mean	0.033	0.033	0.094	0.044	50	
Mean for 3 assays						-	Mean for 3 assays						-	Positive

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Amiodarone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.096	1.005	0.994	1.004	96	1	1	0.102	0.033	0.103	0.037	-4	
	2	1.103	1.005	1.007	0.998	90		2	0.104	0.035	0.107	0.040	-2	
	3	1.099	1.009	1.000	0.998	93		3	0.102	0.034	0.100	0.039	-7	
	Mean	1.099	1.006	1.000	1.000	93		Mean	0.103	0.034	0.103	0.039	-4	
2	1	1.019	1.004	0.937	1.000	75	2	1	0.050	0.033	0.060	0.038	4	
	2	1.021	1.000	0.950	0.994	64		2	0.049	0.032	0.058	0.038	3	
	3	1.018	1.008	0.955	0.998	56		3	0.049	0.032	0.057	0.038	2	
	Mean	1.019	1.004	0.947	0.997	65		Mean	0.049	0.032	0.058	0.038	3	
3	1	1.102	0.994	0.995	0.965	91	3	1	0.091	0.033	0.093	0.038	-6	
	2	1.095	0.997	0.994	0.987	85		2	0.090	0.032	0.094	0.045	-4	
	3	1.097	0.997	1.000	0.989	81		3	0.089	0.033	0.092	0.040	-5	
	Mean	1.098	0.996	0.996	0.980	86		Mean	0.090	0.033	0.093	0.041	-5	
Mean for 3 assays	-	-	-	-	81	Mean for 3 assays	-	-	-	-	-2	Positive*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.904	1.003	1.514	0.992	381	1	1	0.847	0.038	0.822	0.042	-36	
	2	1.907	1.002	1.528	0.994	370		2	0.849	0.036	0.793	0.059	-67	
	3	1.894	1.005	1.498	0.995	387		3	0.838	0.034	0.775	0.040	-74	
	Mean	1.902	1.003	1.513	0.994	379		Mean	0.845	0.036	0.797	0.047	-59	
2	1	1.797	1.000	1.485	0.991	302	2	1	0.813	0.032	0.641	0.038	-179	
	2	1.804	0.995	1.467	0.988	327		2	0.813	0.033	0.633	0.040	-187	
	3	1.793	1.011	1.478	0.996	305		3	0.806	0.033	0.597	0.042	-216	
	Mean	1.798	1.002	1.477	0.992	311		Mean	0.811	0.033	0.624	0.040	-194	
3	1	1.955	0.995	1.485	0.986	461	3	1	0.760	0.032	0.621	0.038	-150	
	2	1.950	0.994	1.516	0.986	425		2	0.765	0.034	0.622	0.051	-154	
	3	1.940	1.001	1.489	0.993	442		3	0.758	0.032	0.597	0.044	-172	
	Mean	1.948	0.997	1.497	0.988	443		Mean	0.761	0.033	0.613	0.044	-159	
Mean for 3 assays	-	-	-	-	378	Mean for 3 assays	-	-	-	-	-137	Positive*3		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Chlorpromazine

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.994	1.005	0.919	1.004	69	1	1	0.033	0.033	0.059	0.037	21		
	2	1.002	1.005	0.918	0.998	78		2	0.035	0.035	0.060	0.040	20		
	3	1.001	1.009	0.916	0.998	79		3	0.034	0.034	0.060	0.039	21		
	Mean	0.999	1.006	0.918	1.000	75		Mean	0.034	0.034	0.060	0.039	21		
2	1	0.994	1.004	0.897	1.000	90	2	1	0.033	0.033	0.059	0.038	20		
	2	0.999	1.000	0.910	0.994	82		2	0.033	0.032	0.061	0.038	22		
	3	1.000	1.008	0.939	0.998	54		3	0.035	0.032	0.061	0.038	20		
	Mean	0.998	1.004	0.915	0.997	75		Mean	0.034	0.032	0.060	0.038	21		
3	1	0.999	0.994	0.929	0.965	54	3	1	0.034	0.033	0.060	0.038	18		
	2	0.993	0.997	0.910	0.987	67		2	0.033	0.032	0.061	0.045	20		
	3	1.001	0.997	0.925	0.989	60		3	0.033	0.033	0.059	0.040	18		
	Mean	0.998	0.996	0.921	0.980	60		Mean	0.033	0.033	0.060	0.041	19		
Mean for 3 assays						-	Mean for 3 assays						-	20	Positive*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.953	1.003	0.980	0.992	-36	1	1	0.035	0.038	0.137	0.042	91		
	2	0.983	1.002	1.012	0.994	-38		2	0.042	0.036	0.137	0.059	84		
	3	0.990	1.005	1.014	0.995	-33		3	0.035	0.034	0.140	0.040	94		
	Mean	0.975	1.003	1.002	0.994	-36		Mean	0.037	0.036	0.138	0.047	90		
2	1	0.967	1.000	0.995	0.991	-38	2	1	0.042	0.032	0.155	0.038	106		
	2	1.003	0.995	1.025	0.988	-32		2	0.036	0.033	0.159	0.040	116		
	3	0.982	1.011	1.006	0.996	-34		3	0.035	0.033	0.160	0.042	118		
	Mean	0.984	1.002	1.009	0.992	-35		Mean	0.038	0.033	0.158	0.040	113		
3	1	0.972	0.995	0.996	0.986	-33	3	1	0.034	0.032	0.142	0.038	97		
	2	0.999	0.994	1.029	0.986	-39		2	0.035	0.034	0.142	0.051	96		
	3	0.994	1.001	1.024	0.993	-39		3	0.036	0.032	0.148	0.044	101		
	Mean	0.988	0.997	1.016	0.988	-37		Mean	0.035	0.033	0.144	0.044	98		
Mean for 3 assays						-	Mean for 3 assays						-	100	Positive*3

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	1.006	1.005	0.887	1.004	113	1	1	0.041	0.033	0.055	0.037	9		
	2	1.006	1.005	0.906	0.998	94		2	0.039	0.035	0.053	0.040	9		
	3	1.006	1.009	0.903	0.998	97		3	0.039	0.034	0.054	0.039	10		
	Mean	1.006	1.006	0.899	1.000	101		Mean	0.040	0.034	0.054	0.039	9		
2	1	1.004	1.004	0.870	1.000	127	2	1	0.038	0.033	0.054	0.038	10		
	2	1.000	1.000	0.884	0.994	109		2	0.039	0.032	0.051	0.038	6		
	3	1.003	1.008	0.892	0.998	104		3	0.037	0.032	0.052	0.038	9		
	Mean	1.002	1.004	0.882	0.997	113		Mean	0.038	0.032	0.052	0.038	8		
3	1	1.000	0.994	0.884	0.965	100	3	1	0.046	0.033	0.055	0.038	1		
	2	1.006	0.997	0.911	0.987	79		2	0.039	0.032	0.053	0.045	6		
	3	1.008	0.997	0.901	0.989	91		3	0.039	0.033	0.054	0.040	7		
	Mean	1.005	0.996	0.899	0.980	90		Mean	0.041	0.033	0.054	0.041	5		
Mean for 3 assays						-	Mean for 3 assays						-	7	Positive*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.996	1.003	0.740	0.992	247	1	1	0.040	0.038	0.310	0.042	259		
	2	0.997	1.002	0.751	0.994	237		2	0.038	0.036	0.312	0.059	263		
	3	1.001	1.005	0.752	0.995	240		3	0.039	0.034	0.317	0.040	267		
	Mean	0.998	1.003	0.748	0.994	241		Mean	0.039	0.036	0.313	0.047	263		
2	1	1.005	1.000	0.721	0.991	274	2	1	0.040	0.032	0.347	0.038	300		
	2	0.996	0.995	0.729	0.988	257		2	0.038	0.033	0.354	0.040	309		
	3	1.001	1.011	0.733	0.996	258		3	0.038	0.033	0.352	0.042	307		
	Mean	1.001	1.002	0.728	0.992	263		Mean	0.039	0.033	0.351	0.040	305		
3	1	0.998	0.995	0.748	0.986	241	3	1	0.041	0.032	0.344	0.038	292		
	2	0.998	0.994	0.766	0.986	223		2	0.037	0.034	0.349	0.051	301		
	3	1.000	1.001	0.752	0.993	239		3	0.037	0.032	0.360	0.044	312		
	Mean	0.999	0.997	0.755	0.988	234		Mean	0.038	0.033	0.351	0.044	302		
Mean for 3 assays						-	Mean for 3 assays						-	290	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Doxycycline

Test concentration 20 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank	
1	1	1.006	1.005	0.913	1.004	87	1	1	0.035	0.033	0.109	0.037	69	Positive	
	2	1.009	1.005	0.927	0.998	76		2	0.035	0.035	0.105	0.040	65		
	3	1.006	1.009	0.927	0.998	73		3	0.035	0.034	0.106	0.039	66		
	Mean	1.007	1.006	0.922	1.000	79		Mean	0.035	0.034	0.107	0.039	67		
2	1	1.007	1.004	0.935	1.000	65	2	1	0.034	0.033	0.075	0.038	35	Positive	
	2	1.008	1.000	0.944	0.994	57		2	0.035	0.032	0.076	0.038	35		
	3	1.003	1.008	0.946	0.998	50		3	0.033	0.032	0.075	0.038	36		
	Mean	1.006	1.004	0.942	0.997	57		Mean	0.034	0.032	0.075	0.038	35		
3	1	1.004	0.994	0.925	0.965	63	3	1	0.034	0.033	0.095	0.038	53	Positive	
	2	1.006	0.997	0.922	0.987	68		2	0.035	0.032	0.098	0.045	55		
	3	0.998	0.997	0.916	0.989	66		3	0.035	0.033	0.104	0.040	61		
	Mean	1.003	0.996	0.921	0.980	66		Mean	0.035	0.033	0.099	0.041	56		
Mean for 3 assays						-	Mean for 3 assays						-	53	Positive*3

Test concentration 200 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank	
1	1	0.974	1.003	0.762	0.992	203	1	1	0.041	0.038	0.453	0.042	401	Positive	
	2	0.990	1.002	0.788	0.994	193		2	0.039	0.036	0.450	0.059	400		
	3	0.989	1.005	0.782	0.995	198		3	0.040	0.034	0.433	0.040	382		
	Mean	0.984	1.003	0.777	0.994	198		Mean	0.040	0.036	0.445	0.047	394		
2	1	0.985	1.000	0.733	0.991	242	2	1	0.040	0.032	0.458	0.038	411	Positive	
	2	0.992	0.995	0.759	0.988	223		2	0.040	0.033	0.461	0.040	414		
	3	0.992	1.011	0.766	0.996	216		3	0.040	0.033	0.455	0.042	408		
	Mean	0.990	1.002	0.753	0.992	227		Mean	0.040	0.033	0.458	0.040	411		
3	1	0.981	0.995	0.774	0.986	198	3	1	0.039	0.032	0.426	0.038	376	Positive	
	2	0.987	0.994	0.810	0.986	168		2	0.040	0.034	0.419	0.051	368		
	3	0.992	1.001	0.792	0.993	191		3	0.039	0.032	0.411	0.044	361		
	Mean	0.987	0.997	0.792	0.988	186		Mean	0.039	0.033	0.419	0.044	368		
Mean for 3 assays						-	Mean for 3 assays						-	391	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	1.008	1.005	0.973	1.004	29	1	1	0.037	0.033	0.053	0.037	11		
	2	1.015	1.005	0.982	0.998	27		2	0.045	0.035	0.049	0.040	-1		
	3	1.010	1.009	0.971	0.998	33		3	0.038	0.034	0.051	0.039	8		
	Mean	1.011	1.006	0.975	1.000	30		Mean	0.040	0.034	0.051	0.039	6		
2	1	1.004	1.004	0.963	1.000	34	2	1	0.038	0.033	0.056	0.038	12		
	2	1.009	1.000	0.970	0.994	32		2	0.044	0.032	0.052	0.038	2		
	3	1.006	1.008	0.971	0.998	28		3	0.036	0.032	0.051	0.038	9		
	Mean	1.006	1.004	0.968	0.997	31		Mean	0.039	0.032	0.053	0.038	8		
3	1	1.003	0.994	0.967	0.965	20	3	1	0.046	0.033	0.050	0.038	-4		
	2	1.006	0.997	0.970	0.987	20		2	0.045	0.032	0.050	0.045	-3		
	3	1.004	0.997	0.971	0.989	17		3	0.042	0.033	0.050	0.040	0		
	Mean	1.004	0.996	0.969	0.980	19		Mean	0.044	0.033	0.050	0.041	-2		
Mean for 3 assays						-	Mean for 3 assays						-	4	Positive*3

Test concentration 200 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	1.003	1.003	0.862	0.992	132	1	1	0.038	0.038	0.106	0.042	57		
	2	1.005	1.002	0.863	0.994	133		2	0.041	0.036	0.110	0.059	58		
	3	1.000	1.005	0.870	0.995	121		3	0.038	0.034	0.113	0.040	64		
	Mean	1.003	1.003	0.865	0.994	129		Mean	0.039	0.036	0.110	0.047	60		
2	1	1.000	1.000	0.848	0.991	142	2	1	0.039	0.032	0.118	0.038	72		
	2	1.005	0.995	0.847	0.988	148		2	0.041	0.033	0.126	0.040	78		
	3	1.003	1.011	0.868	0.996	125		3	0.042	0.033	0.127	0.042	78		
	Mean	1.003	1.002	0.854	0.992	138		Mean	0.041	0.033	0.124	0.040	76		
3	1	0.999	0.995	0.856	0.986	134	3	1	0.038	0.032	0.114	0.038	65		
	2	1.009	0.994	0.866	0.986	134		2	0.037	0.034	0.112	0.051	64		
	3	1.001	1.001	0.867	0.993	125		3	0.038	0.032	0.115	0.044	66		
	Mean	1.003	0.997	0.863	0.988	131		Mean	0.038	0.033	0.114	0.044	65		
Mean for 3 assays						-	Mean for 3 assays						-	67	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank	
1	1	0.994	1.001	0.956	0.996	29	1	1	0.034	0.033	0.045	0.041	3		
	2	1.000	1.003	0.961	0.993	30		2	0.034	0.033	0.046	0.041	4		
	3	1.003	1.004	0.963	0.992	31		3	0.033	0.033	0.047	0.042	6		
	Mean	0.999	1.003	0.960	0.994	30		Mean	0.034	0.033	0.046	0.041	4		
2	1	0.998	1.000	0.952	0.991	38	2	1	0.032	0.032	0.043	0.038	3		
	2	0.996	0.996	0.960	0.990	28		2	0.032	0.033	0.044	0.045	4		
	3	1.000	1.003	0.965	0.995	27		3	0.032	0.034	0.043	0.040	3		
	Mean	0.998	1.000	0.959	0.992	31		Mean	0.032	0.033	0.043	0.041	3		
3	1	1.013	1.009	0.968	0.999	28	3	1	0.032	0.035	0.042	0.040	4		
	2	1.011	1.007	0.945	0.977	49		2	0.033	0.034	0.047	0.041	8		
	3	1.010	1.010	0.971	1.000	22		3	0.036	0.033	0.047	0.039	5		
	Mean	1.011	1.009	0.961	0.992	33		Mean	0.034	0.034	0.045	0.040	6		
Mean for 3 assays						-	Mean for 3 assays						-	4	Positive*3

Test concentration 200 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank	
1	1	0.999	1.007	0.774	0.999	213	1	1	0.033	0.032	0.150	0.041	109		
	2	1.007	1.013	0.787	0.997	208		2	0.033	0.032	0.151	0.039	110		
	3	1.005	1.009	0.785	0.998	208		3	0.033	0.034	0.142	0.042	101		
	Mean	1.004	1.010	0.782	0.998	210		Mean	0.033	0.033	0.148	0.041	107		
2	1	0.992	1.002	0.771	0.986	209	2	1	0.033	0.032	0.134	0.038	94		
	2	0.999	1.002	0.787	0.993	200		2	0.032	0.032	0.133	0.040	94		
	3	1.000	1.004	0.792	0.995	196		3	0.033	0.033	0.137	0.040	97		
	Mean	0.997	1.003	0.783	0.991	202		Mean	0.033	0.032	0.135	0.039	95		
3	1	1.001	1.002	0.742	0.990	249	3	1	0.032	0.033	0.160	0.038	122		
	2	1.004	1.004	0.756	0.992	238		2	0.035	0.033	0.142	0.038	101		
	3	1.006	1.008	0.757	1.003	239		3	0.034	0.033	0.144	0.040	104		
	Mean	1.004	1.005	0.752	0.995	242		Mean	0.034	0.033	0.149	0.039	109		
Mean for 3 assays						-	Mean for 3 assays						-	104	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.000	1.001	0.961	0.996	30	1	1	0.033	0.033	0.228	0.041	187	
	2	1.005	1.003	0.969	0.993	27		2	0.033	0.033	0.231	0.041	190	
	3	1.008	1.004	0.971	0.992	28		3	0.034	0.033	0.237	0.042	195	
	Mean	1.004	1.003	0.967	0.994	28		Mean	0.033	0.033	0.232	0.041	191	
2	1	1.000	1.000	0.957	0.991	35	2	1	0.031	0.032	0.255	0.038	216	
	2	1.001	0.996	0.965	0.990	28		2	0.031	0.033	0.252	0.045	213	
	3	1.003	1.003	0.968	0.995	27		3	0.032	0.034	0.239	0.040	199	
	Mean	1.001	1.000	0.963	0.992	30		Mean	0.031	0.033	0.249	0.041	209	
3	1	1.004	1.009	0.965	0.999	22	3	1	0.033	0.035	0.219	0.040	180	
	2	1.009	1.007	0.970	0.977	22		2	0.032	0.034	0.217	0.041	179	
	3	1.005	1.010	0.971	1.000	17		3	0.033	0.033	0.222	0.039	183	
	Mean	1.006	1.009	0.969	0.992	20		Mean	0.033	0.034	0.219	0.040	181	
Mean for 3 assays	-	-	-	-	26	Mean for 3 assays	-	-	-	-	194	Positive*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.998	1.007	0.859	0.999	127	1	1	0.033	0.032	0.487	0.041	446	
	2	1.005	1.013	0.867	0.997	126		2	0.032	0.032	0.496	0.039	456	
	3	1.003	1.009	0.869	0.998	122		3	0.034	0.034	0.505	0.042	463	
	Mean	1.002	1.010	0.865	0.998	125		Mean	0.033	0.033	0.496	0.041	455	
2	1	0.994	1.002	0.850	0.986	132	2	1	0.032	0.032	0.507	0.038	468	
	2	1.000	1.002	0.861	0.993	127		2	0.032	0.032	0.511	0.040	472	
	3	1.000	1.004	0.867	0.995	121		3	0.032	0.033	0.515	0.040	476	
	Mean	0.998	1.003	0.859	0.991	127		Mean	0.032	0.032	0.511	0.039	472	
3	1	0.996	1.002	0.846	0.990	140	3	1	0.036	0.033	0.477	0.038	435	
	2	1.003	1.004	0.845	0.992	148		2	0.033	0.033	0.482	0.038	443	
	3	1.003	1.008	0.878	1.003	115		3	0.032	0.033	0.484	0.040	446	
	Mean	1.001	1.005	0.856	0.995	134		Mean	0.034	0.033	0.481	0.039	441	
Mean for 3 assays	-	-	-	-	129	Mean for 3 assays	-	-	-	-	456	Positive*3		

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.996	1.001	0.918	0.996	69	1	1	0.032	0.033	0.061	0.041	21	
	2	0.996	1.003	0.930	0.993	57		2	0.032	0.033	0.061	0.041	21	
	3	1.001	1.004	0.937	0.992	55		3	0.032	0.033	0.062	0.042	22	
	Mean	0.998	1.003	0.928	0.994	60		Mean	0.032	0.033	0.061	0.041	21	
2	1	0.993	1.000	0.920	0.991	65	2	1	0.031	0.032	0.066	0.038	27	
	2	0.995	0.996	0.933	0.990	54		2	0.031	0.033	0.067	0.045	28	
	3	0.993	1.003	0.928	0.995	57		3	0.030	0.034	0.068	0.040	30	
	Mean	0.994	1.000	0.927	0.992	59		Mean	0.031	0.033	0.067	0.041	28	
3	1	1.001	1.009	0.928	0.999	56	3	1	0.031	0.035	0.061	0.040	24	
	2	1.007	1.007	0.930	0.977	60		2	0.030	0.034	0.059	0.041	23	
	3	1.002	1.010	0.936	1.000	49		3	0.032	0.033	0.061	0.039	23	
	Mean	1.003	1.009	0.931	0.992	55		Mean	0.031	0.034	0.060	0.040	23	
Mean for 3 assays	-	-	-	-	58	Mean for 3 assays	-	-	-	-	24	Positive*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.995	1.007	0.821	0.999	162	1	1	0.031	0.032	0.190	0.041	151	
	2	0.998	1.013	0.834	0.997	152		2	0.031	0.032	0.185	0.039	146	
	3	0.998	1.009	0.837	0.998	149		3	0.031	0.034	0.186	0.042	147	
	Mean	0.997	1.010	0.831	0.998	154		Mean	0.031	0.033	0.187	0.041	148	
2	1	0.992	1.002	0.825	0.986	155	2	1	0.031	0.032	0.207	0.038	169	
	2	0.994	1.002	0.838	0.993	144		2	0.031	0.032	0.200	0.040	162	
	3	0.996	1.004	0.838	0.995	146		3	0.032	0.033	0.201	0.040	162	
	Mean	0.994	1.003	0.834	0.991	148		Mean	0.031	0.032	0.203	0.039	164	
3	1	0.989	1.002	0.818	0.990	161	3	1	0.031	0.033	0.188	0.038	151	
	2	1.000	1.004	0.827	0.992	163		2	0.032	0.033	0.185	0.038	147	
	3	0.999	1.008	0.837	1.003	152		3	0.031	0.033	0.193	0.040	156	
	Mean	0.996	1.005	0.827	0.995	159		Mean	0.031	0.033	0.189	0.039	151	
Mean for 3 assays	-	-	-	-	154	Mean for 3 assays	-	-	-	-	154	Positive*3		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.997	1.001	0.930	0.996	58	1	1	0.034	0.033	0.078	0.041	36		
	2	1.003	1.003	0.940	0.993	54		2	0.034	0.033	0.078	0.041	36		
	3	1.013	1.004	0.946	0.992	58		3	0.034	0.033	0.079	0.042	37		
	Mean	1.004	1.003	0.939	0.994	57		Mean	0.034	0.033	0.078	0.041	36		
2	1	1.001	1.000	0.913	0.991	80	2	1	0.032	0.032	0.076	0.038	36		
	2	1.006	0.996	0.943	0.990	55		2	0.033	0.033	0.075	0.045	34		
	3	1.006	1.003	0.938	0.995	60		3	0.033	0.034	0.075	0.040	34		
	Mean	1.004	1.000	0.931	0.992	65		Mean	0.033	0.033	0.075	0.041	35		
3	1	1.008	1.009	0.930	0.999	61	3	1	0.037	0.035	0.078	0.040	35		
	2	1.016	1.007	0.927	0.977	72		2	0.033	0.034	0.074	0.041	35		
	3	1.012	1.010	0.952	1.000	43		3	0.037	0.033	0.080	0.039	37		
	Mean	1.012	1.009	0.936	0.992	59		Mean	0.036	0.034	0.077	0.040	36		
Mean for 3 assays						-	Mean for 3 assays						-	36	Positive*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.993	1.007	1.036	0.999	-55	1	1	0.036	0.032	0.187	0.041	143		
	2	0.998	1.013	1.033	0.997	-47		2	0.037	0.032	0.187	0.039	142		
	3	1.003	1.009	1.027	0.998	-36		3	0.035	0.034	0.180	0.042	137		
	Mean	0.998	1.010	1.032	0.998	-46		Mean	0.036	0.033	0.185	0.041	141		
2	1	0.990	1.002	1.041	0.986	-63	2	1	0.035	0.032	0.187	0.038	145		
	2	0.993	1.002	1.042	0.993	-61		2	0.036	0.032	0.190	0.040	147		
	3	1.002	1.004	1.035	0.995	-45		3	0.036	0.033	0.192	0.040	149		
	Mean	0.995	1.003	1.039	0.991	-56		Mean	0.036	0.032	0.190	0.039	147		
3	1	0.990	1.002	1.033	0.990	-53	3	1	0.036	0.033	0.190	0.038	148		
	2	1.006	1.004	1.041	0.992	-45		2	0.041	0.033	0.191	0.038	144		
	3	0.998	1.008	1.037	1.003	-49		3	0.037	0.033	0.197	0.040	154		
	Mean	0.998	1.005	1.037	0.995	-49		Mean	0.038	0.033	0.193	0.039	149		
Mean for 3 assays						-	Mean for 3 assays						-	146	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Quinine

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.989	1.001	0.861	0.996	119	1	1	0.035	0.033	0.109	0.041	66	
	2	1.003	1.003	0.881	0.993	113		2	0.033	0.033	0.105	0.041	64	
	3	1.007	1.004	0.880	0.992	118		3	0.034	0.033	0.107	0.042	65	
	Mean	1.000	1.003	0.874	0.994	117		Mean	0.034	0.033	0.107	0.041	65	
2	1	0.997	1.000	0.860	0.991	129	2	1	0.033	0.032	0.107	0.038	66	
	2	0.998	0.996	0.881	0.990	109		2	0.032	0.033	0.102	0.045	62	
	3	1.001	1.003	0.885	0.995	108		3	0.034	0.034	0.108	0.040	66	
	Mean	0.999	1.000	0.875	0.992	115		Mean	0.033	0.033	0.106	0.041	65	
3	1	1.003	1.009	0.873	0.999	113	3	1	0.033	0.035	0.099	0.040	60	
	2	1.005	1.007	0.877	0.977	111		2	0.033	0.034	0.096	0.041	57	
	3	1.006	1.010	0.885	1.000	104		3	0.034	0.033	0.099	0.039	59	
	Mean	1.005	1.009	0.878	0.992	109		Mean	0.033	0.034	0.098	0.040	59	
Mean for 3 assays	-	-	-	-	114	Mean for 3 assays	-	-	-	-	63	Positive*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.993	1.007	0.568	0.999	413	1	1	0.033	0.032	0.443	0.041	402	
	2	1.000	1.013	0.583	0.997	405		2	0.033	0.032	0.436	0.039	395	
	3	0.999	1.009	0.587	0.998	400		3	0.033	0.034	0.428	0.042	387	
	Mean	0.997	1.010	0.579	0.998	406		Mean	0.033	0.033	0.436	0.041	395	
2	1	0.991	1.002	0.557	0.986	422	2	1	0.033	0.032	0.430	0.038	390	
	2	0.994	1.002	0.583	0.993	399		2	0.032	0.032	0.427	0.040	388	
	3	0.999	1.004	0.592	0.995	395		3	0.033	0.033	0.449	0.040	409	
	Mean	0.995	1.003	0.577	0.991	405		Mean	0.033	0.032	0.435	0.039	396	
3	1	0.996	1.002	0.556	0.990	430	3	1	0.034	0.033	0.408	0.038	368	
	2	0.998	1.004	0.575	0.992	413		2	0.033	0.033	0.400	0.038	361	
	3	0.999	1.008	0.588	1.003	401		3	0.033	0.033	0.427	0.040	388	
	Mean	0.998	1.005	0.573	0.995	415		Mean	0.033	0.033	0.412	0.039	372	
Mean for 3 assays	-	-	-	-	409	Mean for 3 assays	-	-	-	-	388	Positive*3		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 2
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.006	1.001	0.998	0.996	-1	1	1	0.038	0.033	0.042	0.041	-4	
	2	1.012	1.003	1.003	0.993	0		2	0.038	0.033	0.041	0.041	-5	
	3	1.010	1.004	1.003	0.992	-2		3	0.036	0.033	0.044	0.042	0	
	Mean	1.009	1.003	1.001	0.994	-1		Mean	0.037	0.033	0.042	0.041	-3	
2	1	1.008	1.000	0.993	0.991	7	2	1	0.035	0.032	0.040	0.038	-3	
	2	1.011	0.996	1.002	0.990	1		2	0.036	0.033	0.046	0.045	2	
	3	1.007	1.003	0.999	0.995	0		3	0.038	0.034	0.041	0.040	-5	
	Mean	1.009	1.000	0.998	0.992	3		Mean	0.036	0.033	0.041	0.041	-2	
3	1	1.011	1.009	1.002	0.999	-8	3	1	0.039	0.035	0.042	0.040	-3	
	2	1.016	1.007	1.006	0.977	-7		2	0.040	0.034	0.040	0.041	-6	
	3	1.010	1.010	1.004	1.000	-11		3	0.038	0.033	0.042	0.039	-2	
	Mean	1.012	1.009	1.004	0.992	-9		Mean	0.039	0.034	0.041	0.040	-4	
Mean for 3 assays						-	Mean for 3 assays						-	Negative*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	1.005	1.007	0.994	0.999	-1	1	1	0.037	0.032	0.039	0.041	-6	
	2	1.013	1.013	1.000	0.997	1		2	0.037	0.032	0.040	0.039	-5	
	3	1.006	1.009	0.996	0.998	-2		3	0.039	0.034	0.041	0.042	-6	
	Mean	1.008	1.010	0.997	0.998	-1		Mean	0.038	0.033	0.040	0.041	-6	
2	1	1.002	1.002	0.989	0.986	1	2	1	0.040	0.032	0.040	0.038	-7	
	2	1.008	1.002	0.997	0.993	-1		2	0.038	0.032	0.040	0.040	-5	
	3	1.006	1.004	0.997	0.995	-3		3	0.038	0.033	0.040	0.040	-5	
	Mean	1.005	1.003	0.994	0.991	-1		Mean	0.039	0.032	0.040	0.039	-6	
3	1	1.006	1.002	0.995	0.990	1	3	1	0.041	0.033	0.041	0.038	-6	
	2	1.016	1.004	0.988	0.992	18		2	0.040	0.033	0.039	0.038	-7	
	3	1.009	1.008	1.006	1.003	-7		3	0.041	0.033	0.042	0.040	-5	
	Mean	1.010	1.005	0.996	0.995	4		Mean	0.041	0.033	0.041	0.039	-6	
Mean for 3 assays						-	Mean for 3 assays						-	Negative*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : 5-FU

Test concentration 20 µM

Singlet oxygen						Superoxide anion						Positive / Negative				
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2			
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank		
1	1	0.984	0.982	0.975	0.967	-4	1	1	0.037	0.038	0.039	0.040	0			
	2	0.980	0.980	0.969	0.967	-3		2	0.039	0.039	0.041	0.041	0			
	3	0.983	0.976	0.974	0.964	-4		3	0.038	0.038	0.040	0.040	0			
	Mean	0.982	0.979	0.973	0.966	-4		Mean	0.038	0.038	0.040	0.040	0			
2	1	0.989	0.984	0.982	0.972	-4	2	1	0.037	0.038	0.040	0.041	1			
	2	0.983	0.984	0.975	0.974	-4		2	0.038	0.040	0.041	0.041	0			
	3	0.989	0.982	0.980	0.970	-3		3	0.038	0.038	0.041	0.040	1			
	Mean	0.987	0.984	0.979	0.972	-4		Mean	0.038	0.039	0.040	0.041	1			
3	1	0.982	0.987	0.974	0.978	-1	3	1	0.038	0.038	0.040	0.040	0			
	2	0.982	0.976	0.972	0.965	1		2	0.037	0.039	0.039	0.041	0			
	3	0.980	0.974	0.974	0.966	-3		3	0.038	0.039	0.065	0.041	25			
	Mean	0.981	0.979	0.974	0.970	-1		Mean	0.038	0.039	0.048	0.041	8			
Mean for 3 assays						-	Mean for 3 assays						-	-	3	Negative

Test concentration 200 µM

Singlet oxygen						Superoxide anion						Positive / Negative				
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2			
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical			Blank		
1	1	0.996	0.996	0.987	0.982	-7	1	1	0.037	0.038	0.040	0.040	0			
	2	0.999	1.001	0.991	0.987	-6		2	0.038	0.039	0.041	0.041	0			
	3	1.004	1.000	0.993	0.983	-5		3	0.037	0.038	0.040	0.040	0			
	Mean	1.000	0.999	0.991	0.984	-6		Mean	0.037	0.038	0.040	0.041	0			
2	1	0.989	0.987	0.981	0.976	-2	2	1	0.037	0.038	0.039	0.040	0			
	2	0.982	0.981	0.973	0.971	-1		2	0.038	0.039	0.040	0.041	0			
	3	0.985	0.978	0.977	0.968	-2		3	0.038	0.038	0.040	0.040	1			
	Mean	0.985	0.982	0.977	0.972	-2		Mean	0.038	0.038	0.040	0.040	0			
3	1	0.982	0.982	0.973	0.970	-1	3	1	0.037	0.038	0.039	0.041	0			
	2	0.984	0.987	0.975	0.980	-1		2	0.038	0.039	0.041	0.041	0			
	3	0.991	0.994	0.982	0.984	-1		3	0.037	0.037	0.040	0.040	0			
	Mean	0.986	0.988	0.977	0.978	-1		Mean	0.037	0.038	0.040	0.041	0			
Mean for 3 assays						-	Mean for 3 assays						-	-	0	Negative

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : 8-MOP

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank						
1	1	0.982	0.982	0.965	0.967	5	1	1	0.037	0.038	0.042	0.040	3			
	2	0.985	0.980	0.964	0.967	8		2	0.038	0.039	0.043	0.041	3			
	3	0.977	0.976	0.957	0.964	8		3	0.038	0.038	0.042	0.040	2			
	Mean	0.981	0.979	0.962	0.966	7		Mean	0.038	0.038	0.042	0.040	3			
2	1	0.989	0.984	0.973	0.972	4	2	1	0.037	0.038	0.042	0.041	4			
	2	0.991	0.984	0.973	0.974	6		2	0.038	0.040	0.044	0.041	4			
	3	0.983	0.982	0.965	0.970	7		3	0.038	0.038	0.043	0.040	3			
	Mean	0.988	0.984	0.970	0.972	6		Mean	0.038	0.039	0.043	0.041	4			
3	1	0.988	0.987	0.969	0.978	10	3	1	0.037	0.038	0.043	0.040	3			
	2	0.981	0.976	0.962	0.965	9		2	0.038	0.039	0.042	0.041	1			
	3	0.978	0.974	0.960	0.966	9		3	0.038	0.039	0.043	0.041	3			
	Mean	0.982	0.979	0.964	0.970	9		Mean	0.038	0.039	0.042	0.041	2			
Mean for 3 assays						-	Mean for 3 assays						-	-	3	Negative

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank						
1	1	0.980	0.996	0.925	0.982	40	1	1	0.037	0.038	0.055	0.040	15			
	2	0.989	1.001	0.930	0.987	43		2	0.039	0.039	0.057	0.041	15			
	3	0.988	1.000	0.931	0.983	42		3	0.038	0.038	0.056	0.040	15			
	Mean	0.985	0.999	0.929	0.984	42		Mean	0.038	0.038	0.056	0.041	15			
2	1	0.975	0.987	0.911	0.976	54	2	1	0.037	0.038	0.055	0.040	16			
	2	0.977	0.981	0.911	0.971	56		2	0.039	0.039	0.058	0.041	17			
	3	0.970	0.978	0.905	0.968	55		3	0.038	0.038	0.060	0.040	20			
	Mean	0.974	0.982	0.909	0.972	55		Mean	0.038	0.038	0.057	0.040	18			
3	1	0.955	0.982	0.894	0.970	50	3	1	0.037	0.038	0.055	0.041	15			
	2	0.980	0.987	0.918	0.980	52		2	0.039	0.039	0.056	0.041	14			
	3	0.979	0.994	0.916	0.984	52		3	0.038	0.037	0.056	0.040	16			
	Mean	0.971	0.988	0.909	0.978	51		Mean	0.038	0.038	0.056	0.041	15			
Mean for 3 assays						-	Mean for 3 assays						-	-	16	Positive

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Amiodarone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	1.061	0.982	0.966	0.967	82	1	1	0.089	0.038	0.095	0.040	4		
	2	1.059	0.980	0.962	0.967	84		2	0.091	0.039	0.097	0.041	4		
	3	1.058	0.976	0.966	0.964	79		3	0.089	0.038	0.098	0.040	7		
	Mean	1.060	0.979	0.965	0.966	82		Mean	0.090	0.038	0.097	0.040	5		
2	1	1.058	0.984	0.967	0.972	79	2	1	0.079	0.038	0.088	0.041	7		
	2	1.057	0.984	0.957	0.974	88		2	0.084	0.040	0.095	0.041	8		
	3	1.062	0.982	0.972	0.970	78		3	0.085	0.038	0.097	0.040	10		
	Mean	1.059	0.984	0.965	0.972	82		Mean	0.083	0.039	0.093	0.041	8		
3	1	1.062	0.987	0.968	0.978	85	3	1	0.080	0.038	0.088	0.040	6		
	2	1.053	0.976	0.957	0.965	87		2	0.084	0.039	0.093	0.041	7		
	3	1.055	0.974	0.964	0.966	82		3	0.087	0.039	0.096	0.041	7		
	Mean	1.057	0.979	0.963	0.970	85		Mean	0.084	0.039	0.092	0.041	7		
Mean for 3 assays						-	Mean for 3 assays						-	7	Positive

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	1.772	0.996	1.416	0.982	341	1	1	0.660	0.038	0.543	0.040	-119		
	2	1.778	1.001	1.456	0.987	307		2	0.664	0.039	0.548	0.041	-119		
	3	1.784	1.000	1.459	0.983	310		3	0.661	0.038	0.559	0.040	-105		
	Mean	1.778	0.999	1.444	0.984	319		Mean	0.662	0.038	0.550	0.041	-114		
2	1	1.830	0.987	1.403	0.976	416	2	1	0.504	0.038	0.523	0.040	17		
	2	1.818	0.981	1.417	0.971	391		2	0.513	0.039	0.534	0.041	19		
	3	1.820	0.978	1.439	0.968	371		3	0.534	0.038	0.559	0.040	24		
	Mean	1.823	0.982	1.420	0.972	393		Mean	0.517	0.038	0.539	0.040	20		
3	1	1.806	0.982	1.410	0.970	386	3	1	0.641	0.038	0.566	0.041	-79		
	2	1.809	0.987	1.442	0.980	357		2	0.653	0.039	0.580	0.041	-76		
	3	1.823	0.994	1.469	0.984	344		3	0.644	0.037	0.557	0.040	-90		
	Mean	1.813	0.988	1.440	0.978	362		Mean	0.646	0.038	0.567	0.041	-82		
Mean for 3 assays						-	Mean for 3 assays						-	-59	Positive

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Chlorpromazine

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.971	0.982	0.862	0.967	96	1	1	0.038	0.038	0.052	0.040	12	
	2	0.968	0.980	0.873	0.967	82		2	0.038	0.039	0.051	0.041	11	
	3	0.965	0.976	0.855	0.964	97		3	0.037	0.038	0.052	0.040	12	
	Mean	0.968	0.979	0.863	0.966	92		Mean	0.038	0.038	0.051	0.040	12	
2	1	0.980	0.984	0.867	0.972	101	2	1	0.038	0.038	0.052	0.041	12	
	2	0.972	0.984	0.859	0.974	102		2	0.038	0.040	0.054	0.041	13	
	3	0.969	0.982	0.860	0.970	97		3	0.038	0.038	0.053	0.040	13	
	Mean	0.974	0.984	0.862	0.972	100		Mean	0.038	0.039	0.053	0.041	13	
3	1	0.976	0.987	0.861	0.978	105	3	1	0.038	0.038	0.051	0.040	11	
	2	0.968	0.976	0.857	0.965	102		2	0.037	0.039	0.051	0.041	12	
	3	0.966	0.974	0.860	0.966	96		3	0.038	0.039	0.053	0.041	13	
	Mean	0.970	0.979	0.860	0.970	101		Mean	0.037	0.039	0.052	0.041	12	
Mean for 3 assays	-	-	-	-	98	Mean for 3 assays	-	-	-	-	12	Positive*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.963	0.996	0.937	0.982	11	1	1	0.038	0.038	0.117	0.040	76	
	2	0.992	1.001	0.945	0.987	32		2	0.039	0.039	0.120	0.041	78	
	3	0.963	1.000	0.943	0.983	5		3	0.037	0.038	0.119	0.040	79	
	Mean	0.973	0.999	0.941	0.984	16		Mean	0.038	0.038	0.119	0.041	78	
2	1	0.960	0.987	0.926	0.976	24	2	1	0.038	0.038	0.112	0.040	72	
	2	0.956	0.981	0.926	0.971	21		2	0.038	0.039	0.109	0.041	69	
	3	0.951	0.978	0.930	0.968	12		3	0.037	0.038	0.120	0.040	81	
	Mean	0.956	0.982	0.927	0.972	19		Mean	0.038	0.038	0.114	0.040	74	
3	1	0.955	0.982	0.929	0.970	16	3	1	0.038	0.038	0.114	0.041	73	
	2	0.956	0.987	0.935	0.980	11		2	0.040	0.039	0.117	0.041	75	
	3	0.951	0.994	0.941	0.984	0		3	0.037	0.037	0.121	0.040	80	
	Mean	0.954	0.988	0.935	0.978	9		Mean	0.038	0.038	0.117	0.041	76	
Mean for 3 assays	-	-	-	-	15	Mean for 3 assays	-	-	-	-	76	Positive*3		

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Diclofenac

Test concentration 20 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.982	0.982	0.808	0.967	160	1	1	0.039	0.038	0.049	0.040	8		
	2	0.987	0.980	0.815	0.967	159		2	0.041	0.039	0.051	0.041	8		
	3	0.978	0.976	0.818	0.964	146		3	0.040	0.038	0.051	0.040	9		
	Mean	0.982	0.979	0.814	0.966	155		Mean	0.040	0.038	0.050	0.040	8		
2	1	0.986	0.984	0.832	0.972	142	2	1	0.038	0.038	0.049	0.041	9		
	2	0.995	0.984	0.829	0.974	154		2	0.040	0.040	0.051	0.041	9		
	3	0.982	0.982	0.825	0.970	145		3	0.039	0.038	0.052	0.040	11		
	Mean	0.988	0.984	0.829	0.972	147		Mean	0.039	0.039	0.050	0.041	10		
3	1	0.989	0.987	0.837	0.978	143	3	1	0.039	0.038	0.049	0.040	8		
	2	0.977	0.976	0.823	0.965	145		2	0.039	0.039	0.049	0.041	8		
	3	0.980	0.974	0.824	0.966	147		3	0.039	0.039	0.051	0.041	10		
	Mean	0.982	0.979	0.828	0.970	145		Mean	0.039	0.039	0.050	0.041	9		
Mean for 3 assays						-	Mean for 3 assays						-	9	Positive*3

Test concentration 200 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.990	0.996	0.644	0.982	331	1	1	0.039	0.038	0.329	0.040	287		
	2	1.002	1.001	0.659	0.987	328		2	0.040	0.039	0.346	0.041	303		
	3	0.998	1.000	0.653	0.983	330		3	0.039	0.038	0.361	0.040	319		
	Mean	0.996	0.999	0.652	0.984	330		Mean	0.039	0.038	0.345	0.041	303		
2	1	0.985	0.987	0.640	0.976	335	2	1	0.039	0.038	0.303	0.040	263		
	2	0.988	0.981	0.651	0.971	327		2	0.040	0.039	0.331	0.041	289		
	3	0.978	0.978	0.641	0.968	327		3	0.039	0.038	0.346	0.040	306		
	Mean	0.984	0.982	0.644	0.972	330		Mean	0.039	0.038	0.327	0.040	286		
3	1	0.980	0.982	0.638	0.970	331	3	1	0.038	0.038	0.321	0.041	280		
	2	0.992	0.987	0.652	0.980	330		2	0.040	0.039	0.341	0.041	299		
	3	0.983	0.994	0.651	0.984	322		3	0.039	0.037	0.362	0.040	320		
	Mean	0.985	0.988	0.647	0.978	328		Mean	0.039	0.038	0.341	0.041	300		
Mean for 3 assays						-	Mean for 3 assays						-	296	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Doxycycline

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.973	0.982	0.913	0.967	47	1	1	0.039	0.038	0.084	0.040	43		
	2	0.970	0.980	0.906	0.967	51		2	0.039	0.039	0.086	0.041	45		
	3	0.969	0.976	0.904	0.964	52		3	0.039	0.038	0.087	0.040	46		
	Mean	0.971	0.979	0.908	0.966	50		Mean	0.039	0.038	0.085	0.040	45		
2	1	0.978	0.984	0.918	0.972	49	2	1	0.038	0.038	0.082	0.041	42		
	2	0.977	0.984	0.913	0.974	52		2	0.039	0.040	0.086	0.041	46		
	3	0.971	0.982	0.908	0.970	52		3	0.038	0.038	0.086	0.040	46		
	Mean	0.976	0.984	0.913	0.972	51		Mean	0.038	0.039	0.085	0.041	45		
3	1	0.981	0.987	0.917	0.978	55	3	1	0.038	0.038	0.082	0.040	42		
	2	0.975	0.976	0.908	0.965	58		2	0.038	0.039	0.084	0.041	44		
	3	0.970	0.974	0.906	0.966	55		3	0.039	0.039	0.087	0.041	46		
	Mean	0.975	0.979	0.910	0.970	56		Mean	0.038	0.039	0.084	0.041	44		
Mean for 3 assays						-	Mean for 3 assays						-	45	Positive*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.959	0.996	0.767	0.982	177	1	1	0.041	0.038	0.348	0.040	304		
	2	0.965	1.001	0.777	0.987	173		2	0.041	0.039	0.348	0.041	304		
	3	0.965	1.000	0.775	0.983	175		3	0.041	0.038	0.359	0.040	315		
	Mean	0.963	0.999	0.773	0.984	175		Mean	0.041	0.038	0.352	0.041	308		
2	1	0.955	0.987	0.779	0.976	166	2	1	0.040	0.038	0.345	0.040	303		
	2	0.949	0.981	0.784	0.971	156		2	0.041	0.039	0.355	0.041	312		
	3	0.948	0.978	0.778	0.968	160		3	0.040	0.038	0.373	0.040	331		
	Mean	0.951	0.982	0.780	0.972	161		Mean	0.040	0.038	0.357	0.040	315		
3	1	0.950	0.982	0.766	0.970	174	3	1	0.040	0.038	0.348	0.041	305		
	2	0.949	0.987	0.771	0.980	168		2	0.041	0.039	0.359	0.041	315		
	3	0.953	0.994	0.775	0.984	167		3	0.040	0.037	0.364	0.040	321		
	Mean	0.950	0.988	0.771	0.978	170		Mean	0.040	0.038	0.357	0.041	314		
Mean for 3 assays						-	Mean for 3 assays						-	312	Positive*3

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Furosemide

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.983	0.982	0.946	0.967	23	1	1	0.040	0.038	0.047	0.040	5	
	2	0.980	0.980	0.943	0.967	23		2	0.041	0.039	0.047	0.041	5	
	3	0.973	0.976	0.936	0.964	25		3	0.041	0.038	0.048	0.040	5	
	Mean	0.979	0.979	0.942	0.966	24		Mean	0.040	0.038	0.047	0.040	5	
2	1	0.993	0.984	0.956	0.972	25	2	1	0.039	0.038	0.047	0.041	6	
	2	0.985	0.984	0.949	0.974	24		2	0.040	0.040	0.048	0.041	6	
	3	0.982	0.982	0.946	0.970	24		3	0.040	0.038	0.047	0.040	5	
	Mean	0.987	0.984	0.950	0.972	24		Mean	0.040	0.039	0.047	0.041	6	
3	1	0.982	0.987	0.942	0.978	31	3	1	0.039	0.038	0.046	0.040	5	
	2	0.979	0.976	0.939	0.965	31		2	0.039	0.039	0.046	0.041	5	
	3	0.981	0.974	0.942	0.966	30		3	0.040	0.039	0.047	0.041	5	
	Mean	0.981	0.979	0.941	0.970	31		Mean	0.039	0.039	0.046	0.041	5	
Mean for 3 assays	-	-	-	-	26	Mean for 3 assays	-	-	-	-	5	Positive*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.992	0.996	0.864	0.982	113	1	1	0.040	0.038	0.083	0.040	40	
	2	0.993	1.001	0.869	0.987	109		2	0.041	0.039	0.085	0.041	41	
	3	0.992	1.000	0.869	0.983	108		3	0.040	0.038	0.087	0.040	44	
	Mean	0.992	0.999	0.867	0.984	110		Mean	0.040	0.038	0.085	0.041	42	
2	1	0.984	0.987	0.854	0.976	120	2	1	0.040	0.038	0.084	0.040	42	
	2	0.980	0.981	0.853	0.971	117		2	0.041	0.039	0.086	0.041	43	
	3	0.974	0.978	0.850	0.968	114		3	0.040	0.038	0.088	0.040	47	
	Mean	0.979	0.982	0.852	0.972	117		Mean	0.040	0.038	0.086	0.040	44	
3	1	0.980	0.982	0.850	0.970	120	3	1	0.039	0.038	0.084	0.041	42	
	2	0.982	0.987	0.856	0.980	116		2	0.040	0.039	0.085	0.041	42	
	3	0.980	0.994	0.855	0.984	115		3	0.040	0.037	0.087	0.040	44	
	Mean	0.981	0.988	0.854	0.978	117		Mean	0.040	0.038	0.085	0.041	43	
Mean for 3 assays	-	-	-	-	115	Mean for 3 assays	-	-	-	-	43	Positive*3		

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Ketoprofen

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.990	0.990	0.936	0.979	43	1	1	0.037	0.039	0.042	0.041	1	
	2	0.996	0.997	0.946	0.985	40		2	0.039	0.039	0.043	0.041	1	
	3	1.001	0.995	0.950	0.985	40		3	0.038	0.038	0.043	0.040	2	
	Mean	0.996	0.994	0.944	0.983	41		Mean	0.038	0.038	0.042	0.041	1	
2	1	0.993	0.994	0.942	0.985	42	2	1	0.037	0.038	0.041	0.040	2	
	2	0.976	0.978	0.926	0.969	41		2	0.038	0.039	0.043	0.041	2	
	3	0.976	0.965	0.930	0.957	37		3	0.038	0.038	0.042	0.040	2	
	Mean	0.982	0.979	0.933	0.970	40		Mean	0.038	0.038	0.042	0.040	2	
3	1	0.981	0.986	0.928	0.978	46	3	1	0.037	0.038	0.042	0.040	3	
	2	0.980	0.976	0.930	0.968	44		2	0.038	0.039	0.041	0.041	2	
	3	0.980	0.973	0.930	0.967	43		3	0.038	0.039	0.042	0.041	2	
	Mean	0.980	0.978	0.929	0.971	44		Mean	0.038	0.039	0.042	0.041	2	
Mean for 3 assays	-	-	-	-	42	Mean for 3 assays	-	-	-	-	2	Positive*3		

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.984	0.980	0.764	0.965	207	1	1	0.037	0.038	0.098	0.040	59	
	2	0.991	0.989	0.770	0.977	207		2	0.038	0.039	0.094	0.041	54	
	3	0.996	0.988	0.785	0.974	197		3	0.038	0.037	0.101	0.040	61	
	Mean	0.991	0.986	0.773	0.972	204		Mean	0.038	0.038	0.098	0.040	58	
2	1	0.984	0.988	0.770	0.975	201	2	1	0.037	0.038	0.090	0.041	50	
	2	0.989	0.993	0.780	0.981	196		2	0.039	0.039	0.094	0.042	52	
	3	0.994	0.992	0.787	0.979	194		3	0.038	0.038	0.096	0.040	55	
	Mean	0.989	0.991	0.779	0.978	197		Mean	0.038	0.038	0.093	0.041	52	
3	1	0.994	1.000	0.770	0.991	214	3	1	0.038	0.038	0.089	0.041	50	
	2	0.980	0.976	0.764	0.965	206		2	0.038	0.040	0.091	0.041	51	
	3	0.979	0.969	0.763	0.959	206		3	0.038	0.039	0.093	0.041	53	
	Mean	0.984	0.982	0.766	0.972	209		Mean	0.038	0.039	0.091	0.041	51	
Mean for 3 assays	-	-	-	-	203	Mean for 3 assays	-	-	-	-	54	Positive*3		

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Levofloxacin

Test concentration 20 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.989	0.990	0.943	0.979	35	1	1	0.037	0.039	0.202	0.041	162		
	2	0.998	0.997	0.951	0.985	36		2	0.038	0.039	0.203	0.041	162		
	3	0.997	0.995	0.953	0.985	33		3	0.038	0.038	0.204	0.040	163		
	Mean	0.995	0.994	0.949	0.983	35		Mean	0.038	0.038	0.203	0.041	162		
2	1	0.992	0.994	0.948	0.985	35	2	1	0.037	0.038	0.179	0.040	140		
	2	0.979	0.978	0.936	0.969	34		2	0.039	0.039	0.169	0.041	129		
	3	0.970	0.965	0.928	0.957	33		3	0.038	0.038	0.174	0.040	134		
	Mean	0.980	0.979	0.938	0.970	34		Mean	0.038	0.038	0.174	0.040	134		
3	1	0.987	0.986	0.941	0.978	39	3	1	0.037	0.038	0.178	0.040	139		
	2	0.979	0.976	0.934	0.968	39		2	0.037	0.039	0.178	0.041	139		
	3	0.979	0.973	0.935	0.967	38		3	0.038	0.039	0.186	0.041	146		
	Mean	0.982	0.978	0.936	0.971	39		Mean	0.037	0.039	0.181	0.041	141		
Mean for 3 assays						-	Mean for 3 assays						-	146	Positive*3

Test concentration 200 µM

Singlet oxygen						Superoxide anion						Positive / Negative			
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)		Results*2		
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.979	0.980	0.855	0.965	109	1	1	0.037	0.038	0.483	0.040	444		
	2	0.989	0.989	0.853	0.977	123		2	0.038	0.039	0.485	0.041	445		
	3	0.986	0.988	0.855	0.974	117		3	0.038	0.037	0.470	0.040	430		
	Mean	0.985	0.986	0.854	0.972	116		Mean	0.037	0.038	0.479	0.040	440		
2	1	0.980	0.988	0.846	0.975	121	2	1	0.037	0.038	0.485	0.041	445		
	2	0.988	0.993	0.860	0.981	115		2	0.040	0.039	0.501	0.042	458		
	3	0.986	0.992	0.856	0.979	117		3	0.038	0.038	0.492	0.040	451		
	Mean	0.985	0.991	0.854	0.978	118		Mean	0.038	0.038	0.492	0.041	451		
3	1	0.994	1.000	0.854	0.991	129	3	1	0.038	0.038	0.484	0.041	444		
	2	0.975	0.976	0.842	0.965	123		2	0.037	0.040	0.494	0.041	455		
	3	0.969	0.969	0.838	0.959	122		3	0.039	0.039	0.472	0.041	431		
	Mean	0.979	0.982	0.844	0.972	125		Mean	0.038	0.039	0.483	0.041	443		
Mean for 3 assays						-	Mean for 3 assays						-	445	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 25

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Norfloxacin

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.991	0.990	0.922	0.979	57	1	1	0.037	0.039	0.058	0.041	18		
	2	0.996	0.997	0.926	0.985	59		2	0.039	0.039	0.060	0.041	18		
	3	0.996	0.995	0.927	0.985	58		3	0.038	0.038	0.059	0.040	18		
	Mean	0.994	0.994	0.925	0.983	58		Mean	0.038	0.038	0.059	0.041	18		
2	1	0.994	0.994	0.932	0.985	53	2	1	0.037	0.038	0.054	0.040	15		
	2	0.976	0.978	0.913	0.969	54		2	0.039	0.039	0.055	0.041	15		
	3	0.974	0.965	0.912	0.957	52		3	0.038	0.038	0.055	0.040	15		
	Mean	0.981	0.979	0.919	0.970	53		Mean	0.038	0.038	0.055	0.040	15		
3	1	0.982	0.986	0.920	0.978	55	3	1	0.037	0.038	0.055	0.040	16		
	2	0.977	0.976	0.912	0.968	57		2	0.037	0.039	0.055	0.041	16		
	3	0.974	0.973	0.912	0.967	55		3	0.038	0.039	0.057	0.041	16		
	Mean	0.978	0.978	0.915	0.971	56		Mean	0.038	0.039	0.056	0.041	16		
Mean for 3 assays						-	Mean for 3 assays						-	16	Positive*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.982	0.980	0.807	0.965	161	1	1	0.039	0.038	0.158	0.040	117		
	2	0.985	0.989	0.817	0.977	154		2	0.038	0.039	0.161	0.041	120		
	3	0.988	0.988	0.823	0.974	150		3	0.037	0.037	0.164	0.040	125		
	Mean	0.985	0.986	0.816	0.972	155		Mean	0.038	0.038	0.161	0.040	121		
2	1	0.980	0.988	0.818	0.975	149	2	1	0.037	0.038	0.154	0.041	114		
	2	0.983	0.993	0.829	0.981	141		2	0.039	0.039	0.158	0.042	117		
	3	0.987	0.992	0.836	0.979	138		3	0.038	0.038	0.161	0.040	120		
	Mean	0.983	0.991	0.828	0.978	143		Mean	0.038	0.038	0.158	0.041	117		
3	1	0.991	1.000	0.818	0.991	163	3	1	0.038	0.038	0.140	0.041	101		
	2	0.973	0.976	0.809	0.965	154		2	0.037	0.040	0.140	0.041	100		
	3	0.969	0.969	0.809	0.959	149		3	0.039	0.039	0.146	0.041	105		
	Mean	0.978	0.982	0.812	0.972	155		Mean	0.038	0.039	0.142	0.041	102		
Mean for 3 assays						-	Mean for 3 assays						-	113	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Omeprazole

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.989	0.990	0.930	0.979	48	1	1	0.038	0.039	0.064	0.041	22		
	2	0.982	0.997	0.924	0.985	48		2	0.039	0.039	0.063	0.041	21		
	3	0.987	0.995	0.924	0.985	52		3	0.037	0.038	0.063	0.040	23		
	Mean	0.986	0.994	0.926	0.983	49		Mean	0.038	0.038	0.063	0.041	22		
2	1	0.976	0.994	0.918	0.985	49	2	1	0.038	0.038	0.056	0.040	16		
	2	0.966	0.978	0.909	0.969	48		2	0.038	0.039	0.054	0.041	14		
	3	0.962	0.965	0.908	0.957	45		3	0.038	0.038	0.053	0.040	13		
	Mean	0.968	0.979	0.912	0.970	47		Mean	0.038	0.038	0.054	0.040	14		
3	1	0.981	0.986	0.916	0.978	58	3	1	0.038	0.038	0.059	0.040	20		
	2	0.974	0.976	0.912	0.968	55		2	0.038	0.039	0.058	0.041	18		
	3	0.972	0.973	0.913	0.967	52		3	0.038	0.039	0.059	0.041	19		
	Mean	0.975	0.978	0.914	0.971	55		Mean	0.038	0.039	0.059	0.041	19		
Mean for 3 assays						-	Mean for 3 assays						-	18	Positive*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.966	0.980	0.990	0.965	-38	1	1	0.038	0.038	0.178	0.040	138		
	2	0.969	0.989	0.989	0.977	-34		2	0.038	0.039	0.181	0.041	140		
	3	0.965	0.988	0.988	0.974	-37		3	0.038	0.037	0.185	0.040	145		
	Mean	0.967	0.986	0.989	0.972	-36		Mean	0.038	0.038	0.181	0.040	141		
2	1	0.968	0.988	0.979	0.975	-24	2	1	0.039	0.038	0.170	0.041	128		
	2	0.969	0.993	0.988	0.981	-33		2	0.041	0.039	0.174	0.042	130		
	3	0.966	0.992	0.987	0.979	-34		3	0.038	0.038	0.177	0.040	136		
	Mean	0.967	0.991	0.985	0.978	-30		Mean	0.039	0.038	0.174	0.041	131		
3	1	0.975	1.000	1.025	0.991	-60	3	1	0.039	0.038	0.183	0.041	143		
	2	0.958	0.976	1.005	0.965	-58		2	0.039	0.040	0.184	0.041	144		
	3	0.954	0.969	1.003	0.959	-60		3	0.039	0.039	0.185	0.041	144		
	Mean	0.962	0.982	1.011	0.972	-59		Mean	0.039	0.039	0.184	0.041	144		
Mean for 3 assays						-	Mean for 3 assays						-	139	Positive*3

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Quinine

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.987	0.990	0.904	0.979	72	1	1	0.038	0.039	0.075	0.041	34		
	2	0.999	0.997	0.910	0.985	78		2	0.038	0.039	0.075	0.041	34		
	3	0.993	0.995	0.909	0.985	73		3	0.038	0.038	0.075	0.040	34		
	Mean	0.993	0.994	0.908	0.983	74		Mean	0.038	0.038	0.075	0.041	34		
2	1	0.985	0.994	0.890	0.985	86	2	1	0.037	0.038	0.067	0.040	28		
	2	0.976	0.978	0.882	0.969	84		2	0.039	0.039	0.066	0.041	26		
	3	0.965	0.965	0.883	0.957	74		3	0.038	0.038	0.065	0.040	25		
	Mean	0.975	0.979	0.885	0.970	81		Mean	0.038	0.038	0.066	0.040	26		
3	1	0.977	0.986	0.885	0.978	85	3	1	0.037	0.038	0.071	0.040	32		
	2	0.968	0.976	0.878	0.968	84		2	0.037	0.039	0.070	0.041	31		
	3	0.971	0.973	0.882	0.967	82		3	0.038	0.039	0.071	0.041	31		
	Mean	0.972	0.978	0.881	0.971	84		Mean	0.038	0.039	0.071	0.041	31		
Mean for 3 assays						-	Mean for 3 assays						-	30	Positive*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative		
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2	
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank					
1	1	0.968	0.980	0.605	0.965	349	1	1	0.037	0.038	0.247	0.040	209		
	2	0.982	0.989	0.621	0.977	347		2	0.038	0.039	0.253	0.041	213		
	3	0.976	0.988	0.619	0.974	343		3	0.037	0.037	0.262	0.040	223		
	Mean	0.975	0.986	0.615	0.972	346		Mean	0.037	0.038	0.254	0.040	215		
2	1	0.976	0.988	0.632	0.975	331	2	1	0.037	0.038	0.246	0.041	206		
	2	0.985	0.993	0.638	0.981	334		2	0.039	0.039	0.250	0.042	208		
	3	0.975	0.992	0.616	0.979	346		3	0.038	0.038	0.255	0.040	214		
	Mean	0.978	0.991	0.628	0.978	337		Mean	0.038	0.038	0.250	0.041	209		
3	1	0.982	1.000	0.616	0.991	355	3	1	0.037	0.038	0.232	0.041	192		
	2	0.967	0.976	0.612	0.965	345		2	0.038	0.040	0.232	0.041	192		
	3	0.965	0.969	0.620	0.959	335		3	0.038	0.039	0.242	0.041	202		
	Mean	0.971	0.982	0.616	0.972	345		Mean	0.038	0.039	0.235	0.041	195		
Mean for 3 assays						-	Mean for 3 assays						-	206	Positive*3

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 4 Individual data of phase 1 study

Laboratory : 3
 Chemical Name : Sulisobenzone

Test concentration 20 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.990	0.990	0.984	0.979	-5	1	1	0.039	0.039	0.041	0.041	-2	Negative
	2	0.995	0.997	0.984	0.985	0		2	0.039	0.039	0.041	0.041	-2	
	3	0.993	0.995	0.985	0.985	-3		3	0.039	0.038	0.040	0.040	-2	
	Mean	0.993	0.994	0.984	0.983	-3		Mean	0.039	0.038	0.040	0.041	-2	
2	1	0.991	0.994	0.985	0.985	-3	2	1	0.039	0.038	0.040	0.040	-1	Negative
	2	0.978	0.978	0.971	0.969	-1		2	0.040	0.039	0.040	0.041	-1	
	3	0.969	0.965	0.960	0.957	-1		3	0.039	0.038	0.040	0.040	-1	
	Mean	0.979	0.979	0.972	0.970	-2		Mean	0.039	0.038	0.040	0.040	-1	
3	1	0.988	0.986	0.979	0.978	2	3	1	0.038	0.038	0.040	0.040	-1	Negative
	2	0.983	0.976	0.974	0.968	2		2	0.038	0.039	0.040	0.041	-1	
	3	0.976	0.973	0.966	0.967	3		3	0.040	0.039	0.042	0.041	0	
	Mean	0.982	0.978	0.973	0.971	2		Mean	0.039	0.039	0.040	0.041	-1	
Mean for 3 assays						-	Mean for 3 assays						-	Negative*3

Test concentration 200 µM

Singlet oxygen							Superoxide anion						Positive / Negative	
Run	Run#	A440(-)		A440(+)		Results*1	Run	Run#	A560(-)		A560(+)			Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank				
1	1	0.982	0.980	0.977	0.965	-9	1	1	0.040	0.038	0.040	0.040	-1	Negative
	2	0.984	0.989	0.979	0.977	-9		2	0.040	0.039	0.041	0.041	-1	
	3	0.988	0.988	0.983	0.974	-9		3	0.039	0.037	0.040	0.040	-1	
	Mean	0.985	0.986	0.980	0.972	-9		Mean	0.039	0.038	0.040	0.040	-1	
2	1	0.983	0.988	0.974	0.975	-4	2	1	0.040	0.038	0.040	0.041	-3	Negative
	2	0.988	0.993	0.978	0.981	-3		2	0.040	0.039	0.040	0.042	-3	
	3	0.986	0.992	0.978	0.979	-4		3	0.040	0.038	0.040	0.040	-3	
	Mean	0.986	0.991	0.977	0.978	-4		Mean	0.040	0.038	0.040	0.041	-3	
3	1	1.000	1.000	0.995	0.991	-4	3	1	0.039	0.038	0.039	0.041	-2	Negative
	2	0.985	0.976	0.979	0.965	-4		2	0.041	0.040	0.039	0.041	-4	
	3	0.976	0.969	0.969	0.959	-4		3	0.041	0.039	0.040	0.041	-2	
	Mean	0.987	0.982	0.981	0.972	-4		Mean	0.040	0.039	0.040	0.041	-3	
Mean for 3 assays						-	Mean for 3 assays						-	Negative*3

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*3 : Final decision

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20

Negative : Singlet oxygen results < 25 and Superoxide anion results < 25

Appendix 5 Irradiance and temperature during the irradiation in the phase 2 study

Laboratory		1															
Experimental No. (US-)		001	002	003	004	005	006	007	023	024	025	026	027	028	029	030	
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	
		B	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	
	End of Irradiation	A	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	
		B	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	
Temperature (°C)	Beginning of Irradiation	29	27	28	29	27	27	27	24	26	26	27	27	26	27	25	
	End of Irradiation	27	28	28	28	28	29	28	25	26	26	25	26	26	26	25	

Experimental No. (US-)		031	032	033	034	035	036
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	1.8	1.8	1.8	1.8	1.8
		B	2.1	2.1	2.1	2.1	2.1
	End of Irradiation	A	1.8	1.8	1.8	1.8	1.8
		B	2.1	2.1	2.1	2.1	2.1
Temperature (°C)	Beginning of Irradiation	25	25	27	24	24	26
	End of Irradiation	24	25	26	24	24	24

A : Irradiances which were measured with each test facility's UVA detector.

B : Standardized irradiances which were calculated as the irradiances by the calibrated UVA detector Dr.Hönle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the value from the UVA detectors.

B=A × f f: correlation factor on the values of the UVA detectors (= 1.18)

Thermometer : Suntest CPS+ (Atlas)

Appendix 5 Irradiance and temperature during the irradiation in the phase 2 study

Laboratory		2															
Experimental No. (FDSC-)		001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	1.435	1.439	1.437	1.439	1.437	1.437	1.436	1.441	1.442	1.437	1.424	1.428	1.420	1.422	1.426
		B	1.9	1.9	1.9	1.9	1.9	1.9	1.9	2.0	2.0	1.9	1.8	1.9	1.8	1.8	1.9
	End of Irradiation	A	1.437	1.427	1.431	1.450	1.440	1.431	1.423	1.434	1.436	1.434	1.429	1.433	1.433	1.426	1.424
		B	1.9	1.9	1.9	2.0	1.9	1.9	1.8	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.8
Temperature (°C)	Beginning of Irradiation		22.8	24.9	24.1	24.1	24.8	24.6	23.6	24.7	23.7	24.4	24.6	24.0	25.0	24.9	24.9
	End of Irradiation		25.0	25.0	24.9	24.9	25.0	25.2	24.9	24.9	25.0	24.9	25.2	25.1	25.1	25.0	25.2

Experimental No. (FDSC-)		016	017	018	019	020	021	022	023	024	025	026	
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	1.426	1.422	1.434	1.432	1.435	1.419	1.424	1.426	1.439	1.434	1.440
		B	1.9	1.8	1.9	1.9	1.9	1.8	1.8	1.9	1.9	1.9	1.9
	End of Irradiation	A	1.423	1.424	1.446	1.437	1.438	1.426	1.436	1.424	1.423	1.436	1.442
		B	1.8	1.8	2.0	1.9	1.9	1.9	1.9	1.8	1.8	1.9	2.0
Temperature (°C)	Beginning of Irradiation		24.9	25.4	24.4	24.8	24.9	24.5	24.8	24.9	24.8	24.8	24.3
	End of Irradiation		25.0	25.2	24.8	24.8	24.9	24.9	24.9	25.1	25.1	25.0	24.9

A : Irradiances which were measured with each test facility's UVA detector.

B : Standardized irradiances which were calculated as the irradiances by the calibrated UVA detector Dr.Hönle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the value from the UVA detectors.

$B=A \times f - 7.3943$ f : correlation factor on the values of the UVA detectors (=6.4855)

Thermometer : Thermo Recorder RTR-52 (TANDD)

Appendix 5 Irradiance and temperature during the irradiation in the phase 2 study

Laboratory		3															
Experimental No. (MT-)		001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	1.0	1.1	1.1	1.2	1.1	1.2	1.1	1.2	1.1	1.1	1.2	1.2	1.2	1.1	1.2
		B	1.8	2.0	2.0	2.2	2.0	2.2	2.0	2.2	2.0	2.0	2.2	2.2	2.2	2.0	2.2
	End of Irradiation	A	1.2	1.1	1.1	1.1	1.2	1.2	1.2	1.1	1.1	1.1	1.2	1.2	1.2	1.2	1.1
		B	2.2	2.0	2.0	2.0	2.2	2.2	2.2	2.0	2.0	2.0	2.2	2.2	2.2	2.2	2.0
Temperature (°C)	Beginning of Irradiation		25.4	24.6	23.8	25.3	24.6	24.0	25.5	27.6	21.9	24.7	23.3	23.5	23.0	24.5	23.6
	End of Irradiation		28,3	23.8	23,7	24.6	24.0	24.2	28.3	26.4	26.6	23.6	23.5	23.0	23.3	23.8	24.6

Experimental No. (MT-)		016	018	019	020	021	022	023	
UVA intensity (mW/cm ²)	Beginning of Irradiation	A	1.2	1.2	1.2	1.2	1.1	1.1	
		B	2.2	2.2	2.2	2.2	2.0	2.0	
	End of Irradiation	A	1.2	1.2	1.2	1.2	1.1	1.1	
		B	2.2	2.2	2.2	2.2	2.0	2.0	
Temperature (°C)	Beginning of Irradiation		25.0	25.5	24.3	25.1	26.5	24.4	24.6
	End of Irradiation		26.2	26.3	25.1	26.5	25.9	26.0	25.8

A : Irradiances which were measured with each test facility's UVA detector.

B : Standardized irradiances which were calculated as the irradiances by the calibrated UVA detector Dr.Hönle was transferred to each facility and the conversion factor for the standardized irradiances was prepared based on the value from the UVA detectors.

$B=A \times f$: correlation factor on the values of the UVA detectors (= 1.8)

Thermometer : ondotori Jr. TR-51i (TANDD)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 1
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
US-001	1	1.023	1.014	0.481	1.006	531	US-001	1	0.049	0.033	0.463	0.046	400
	2	1.029	1.028	0.506	1.014	512		2	0.042	0.046	0.461	0.053	405
	3	1.036	1.029	0.525	1.020	501		3	0.047	0.034	0.465	0.056	405
	Mean	1.029	1.024	0.504	1.013	515		Mean	0.046	0.038	0.463	0.052	403
US-002	1	1.013	1.017	0.470	1.007	534	US-002	1	0.114	0.031	0.457	0.038	336
	2	1.040	1.028	0.504	1.021	527		2	0.110	0.043	0.453	0.041	336
	3	1.037	1.031	0.516	1.020	512		3	0.115	0.030	0.457	0.048	335
	Mean	1.030	1.025	0.497	1.016	524		Mean	0.113	0.035	0.455	0.042	336
US-003	1	1.018	1.011	0.459	1.001	549	US-003	1	0.046	0.029	0.454	0.049	385
	2	1.032	1.028	0.487	1.017	535		2	0.042	0.044	0.448	0.052	383
	3	1.035	1.024	0.493	1.016	532		3	0.046	0.030	0.471	0.070	402
	Mean	1.028	1.021	0.480	1.011	539		Mean	0.044	0.034	0.457	0.057	390
US-004	1	0.996	0.998	0.441	0.987	545	US-004	1	0.052	0.029	0.370	0.046	293
	2	1.028	1.027	0.481	1.019	537		2	0.045	0.029	0.381	0.059	313
	3	1.029	1.021	0.491	1.010	528		3	0.066	0.029	0.378	0.054	288
	Mean	1.018	1.015	0.471	1.005	537		Mean	0.054	0.029	0.376	0.053	298
US-005	1	1.006	1.015	0.454	1.004	541	US-005	1	0.040	0.029	0.470	0.043	419
	2	1.011	1.012	0.477	1.002	523		2	0.036	0.043	0.470	0.044	423
	3	1.015	1.016	0.492	1.007	511		3	0.041	0.029	0.464	0.046	412
	Mean	1.011	1.015	0.474	1.004	525		Mean	0.039	0.033	0.468	0.044	418
US-006	1	1.002	1.020	0.438	1.010	553	US-006	1	0.063	0.031	0.462	0.044	376
	2	1.016	1.026	0.465	1.011	540		2	0.047	0.031	0.466	0.048	396
	3	1.017	1.017	0.477	1.008	529		3	0.066	0.031	0.461	0.069	373
	Mean	1.012	1.021	0.460	1.010	541		Mean	0.058	0.031	0.463	0.054	382

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 1
 Chemical Name : Quinine HCl

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
US-007	1	1.017	1.006	0.439	0.991	567	US-007	1	0.062	0.035	0.467	0.046	390
	2	1.015	1.015	0.462	1.005	541		2	0.040	0.034	0.458	0.054	403
	3	1.031	1.010	0.478	1.002	542		3	0.062	0.033	0.455	0.049	378
	Mean	1.021	1.010	0.460	0.999	550		Mean	0.055	0.034	0.460	0.049	390
US-023	1	0.998	1.008	0.430	0.991	554	US-023	1	0.037	0.033	0.458	0.039	414
	2	1.005	1.024	0.458	1.013	533		2	0.033	0.049	0.451	0.044	411
	3	1.003	1.022	0.472	1.008	517		3	0.039	0.029	0.463	0.051	418
	Mean	1.002	1.018	0.453	1.004	535		Mean	0.036	0.037	0.457	0.044	414
US-024	1	1.001	1.014	0.411	1.003	576	US-024	1	0.038	0.030	0.464	0.045	410
	2	1.011	1.023	0.439	1.009	558		2	0.033	0.040	0.477	0.052	428
	3	1.013	1.023	0.452	1.006	546		3	0.038	0.029	0.466	0.051	412
	Mean	1.008	1.020	0.434	1.006	560		Mean	0.036	0.033	0.469	0.049	417
US-025	1	0.992	0.990	0.411	0.969	559	US-025	1	0.039	0.029	0.469	0.046	409
	2	1.012	1.013	0.439	0.988	551		2	0.034	0.047	0.457	0.047	402
	3	1.006	1.011	0.455	0.993	530		3	0.037	0.030	0.473	0.073	415
	Mean	1.003	1.005	0.435	0.983	547		Mean	0.037	0.035	0.466	0.056	409
US-026	1	0.988	0.973	0.410	0.965	570	US-026	1	0.036	0.032	0.470	0.040	422
	2	0.987	0.982	0.433	0.977	546		2	0.033	0.038	0.473	0.049	428
	3	0.994	0.986	0.440	0.976	546		3	0.039	0.029	0.630	0.046	579
	Mean	0.990	0.981	0.427	0.973	554		Mean	0.036	0.033	0.524	0.045	476
US-027	1	0.967	0.970	0.375	0.965	584	US-027	1	0.058	0.033	0.466	0.040	395
	2	0.997	0.979	0.417	0.970	572		2	0.041	0.030	0.468	0.047	413
	3	1.008	0.978	0.431	0.969	569		3	0.061	0.029	0.465	0.046	391
	Mean	0.991	0.976	0.408	0.968	575		Mean	0.053	0.030	0.466	0.044	400

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 1
 Chemical Name : Quinine HCl

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
US-028	1	0.962	0.980	0.397	0.971	554	US-028	1	0.052	0.029	0.469	0.051	391
	2	0.977	0.988	0.428	0.977	538		2	0.041	0.030	0.465	0.053	399
	3	0.974	0.995	0.448	0.983	515		3	0.063	0.028	0.477	0.061	389
	Mean	0.971	0.988	0.424	0.977	536		Mean	0.052	0.029	0.471	0.055	393
US-029	1	0.947	0.973	0.370	0.960	567	US-029	1	0.040	0.030	0.497	0.038	449
	2	0.988	0.986	0.413	0.979	565		2	0.036	0.044	0.506	0.044	461
	3	0.990	0.987	0.429	0.978	551		3	0.041	0.029	0.511	0.047	462
	Mean	0.975	0.982	0.404	0.972	561		Mean	0.039	0.034	0.505	0.043	457
US-030	1	0.978	0.997	0.422	1.002	556	US-030	1	0.045	0.029	0.474	0.047	408
	2	0.985	1.003	0.444	0.996	542		2	0.034	0.043	0.462	0.051	407
	3	0.982	1.001	0.455	1.005	528		3	0.039	0.030	0.463	0.066	403
	Mean	0.982	1.000	0.441	1.001	542		Mean	0.039	0.034	0.466	0.055	406
US-031	1	0.976	0.986	0.416	0.973	551	US-031	1	0.042	0.029	0.449	0.042	394
	2	0.987	0.985	0.442	0.979	535		2	0.035	0.042	0.462	0.046	414
	3	0.991	0.995	0.455	0.985	525		3	0.041	0.029	0.459	0.049	405
	Mean	0.984	0.989	0.438	0.979	537		Mean	0.039	0.033	0.457	0.046	404
US-032	1	0.964	0.987	0.413	0.976	539	US-032	1	0.037	0.028	0.468	0.045	411
	2	0.987	0.988	0.442	0.975	533		2	0.035	0.045	0.465	0.045	410
	3	0.994	1.007	0.462	0.995	520		3	0.038	0.028	0.467	0.072	409
	Mean	0.982	0.994	0.439	0.982	531		Mean	0.037	0.034	0.467	0.054	410
US-033	1	0.971	0.982	0.423	0.972	536	US-033	1	0.061	0.033	0.459	0.041	379
	2	0.979	0.989	0.447	0.975	520		2	0.041	0.034	0.466	0.051	405
	3	0.993	0.987	0.457	0.975	524		3	0.064	0.028	0.482	0.064	398
	Mean	0.981	0.986	0.442	0.974	527		Mean	0.055	0.032	0.469	0.052	394

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 1
 Chemical Name : Quinine HCl

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
US-034	1	0.964	0.974	0.399	0.965	556	US-034	1	0.037	0.036	0.468	0.049	407
	2	0.979	1.001	0.422	0.992	548		2	0.033	0.042	0.449	0.045	392
	3	0.985	0.994	0.451	0.985	525		3	0.038	0.029	0.454	0.085	392
	Mean	0.976	0.990	0.424	0.981	543		Mean	0.036	0.036	0.457	0.060	397
US-035	1	0.984	0.982	0.424	0.975	552	US-035	1	0.058	0.034	0.461	0.045	380
	2	0.985	0.985	0.449	0.974	527		2	0.039	0.030	0.485	0.048	424
	3	0.993	0.985	0.459	0.976	525		3	0.059	0.028	0.459	0.066	378
	Mean	0.987	0.984	0.444	0.975	535		Mean	0.052	0.031	0.468	0.053	394
US-036	1	0.984	0.977	0.414	0.965	560	US-036	1	0.058	0.028	0.436	0.043	354
	2	0.978	0.990	0.441	0.984	527		2	0.038	0.032	0.460	0.048	398
	3	0.986	0.988	0.450	0.977	526		3	0.069	0.029	0.479	0.072	386
	Mean	0.983	0.985	0.435	0.975	538		Mean	0.055	0.030	0.459	0.054	379
Mean for all assays		-	-	-	-	541	Mean for all assays		-	-	-	-	398
SD for all assays		-	-	-	-	14	SD for all assays		-	-	-	-	36
CV for all assays		-	-	-	-	2.6	CV for all assays		-	-	-	-	9.0

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 1
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
US-001	1	1.015	1.014	1.006	1.006	-2	US-001	1	0.044	0.033	0.044	0.046	-14
	2	1.020	1.028	1.014	1.014	-5		2	0.043	0.046	0.045	0.053	-12
	3	1.009	1.029	1.002	1.020	-4		3	0.041	0.034	0.044	0.056	-12
	Mean	1.015	1.024	1.007	1.013	-4		Mean	0.043	0.038	0.044	0.052	-13
US-002	1	1.069	1.017	1.058	1.007	2	US-002	1	0.044	0.031	0.045	0.038	-6
	2	1.051	1.028	1.037	1.021	5		2	0.042	0.043	0.045	0.041	-4
	3	1.062	1.031	1.048	1.020	5		3	0.044	0.030	0.045	0.048	-6
	Mean	1.061	1.025	1.048	1.016	4		Mean	0.043	0.035	0.045	0.042	-5
US-003	1	1.037	1.011	1.012	1.001	15	US-003	1	0.045	0.029	0.044	0.049	-24
	2	1.044	1.028	1.021	1.017	14		2	0.042	0.044	0.046	0.052	-19
	3	1.049	1.024	1.021	1.016	18		3	0.046	0.030	0.045	0.070	-24
	Mean	1.043	1.021	1.018	1.011	16		Mean	0.044	0.034	0.045	0.057	-22
US-004	1	1.016	0.998	1.005	0.987	1	US-004	1	0.038	0.029	0.056	0.046	-5
	2	1.042	1.027	1.027	1.019	5		2	0.039	0.029	0.059	0.059	-4
	3	1.050	1.021	1.036	1.010	4		3	0.039	0.029	0.058	0.054	-5
	Mean	1.036	1.015	1.023	1.005	3		Mean	0.039	0.029	0.058	0.053	-5
US-005	1	1.029	1.015	1.015	1.004	3	US-005	1	0.048	0.029	0.047	0.043	-11
	2	1.026	1.012	1.012	1.002	3		2	0.042	0.043	0.044	0.044	-8
	3	1.041	1.016	1.028	1.007	2		3	0.045	0.029	0.046	0.046	-10
	Mean	1.032	1.015	1.018	1.004	3		Mean	0.045	0.033	0.046	0.044	-10
US-006	1	1.027	1.020	1.001	1.010	15	US-006	1	0.042	0.031	0.043	0.044	-22
	2	1.035	1.026	1.013	1.011	11		2	0.041	0.031	0.044	0.048	-21
	3	1.042	1.017	1.015	1.008	16		3	0.043	0.031	0.044	0.069	-22
	Mean	1.035	1.021	1.010	1.010	14		Mean	0.042	0.031	0.043	0.054	-22

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 1
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
US-007	1	1.039	1.006	1.026	0.991	2	US-007	1	0.041	0.035	0.045	0.046	-11
	2	1.027	1.015	1.013	1.005	3		2	0.040	0.034	0.046	0.054	-10
	3	1.030	1.010	1.017	1.002	2		3	0.041	0.033	0.045	0.049	-11
	Mean	1.032	1.010	1.019	0.999	2		Mean	0.041	0.034	0.045	0.049	-11
US-023	1	1.021	1.008	1.003	0.991	4	US-023	1	0.042	0.033	0.048	0.039	-1
	2	1.028	1.024	1.012	1.013	2		2	0.041	0.049	0.043	0.044	-5
	3	1.030	1.022	1.012	1.008	4		3	0.041	0.029	0.043	0.051	-6
	Mean	1.026	1.018	1.009	1.004	3		Mean	0.041	0.037	0.045	0.044	-4
US-024	1	1.024	1.014	1.003	1.003	8	US-024	1	0.045	0.030	0.046	0.045	-15
	2	1.024	1.023	1.008	1.009	2		2	0.042	0.040	0.046	0.052	-12
	3	1.038	1.023	1.004	1.006	21		3	0.047	0.029	0.047	0.051	-15
	Mean	1.029	1.020	1.005	1.006	10		Mean	0.044	0.033	0.046	0.049	-14
US-025	1	1.018	0.990	0.987	0.969	9	US-025	1	0.041	0.029	0.042	0.046	-20
	2	1.022	1.013	0.996	0.988	4		2	0.040	0.047	0.043	0.047	-18
	3	1.022	1.011	0.993	0.993	6		3	0.043	0.030	0.043	0.073	-21
	Mean	1.020	1.005	0.992	0.983	6		Mean	0.041	0.035	0.043	0.056	-20
US-026	1	0.988	0.973	0.998	0.965	-18	US-026	1	0.041	0.032	0.042	0.040	-11
	2	1.003	0.982	1.001	0.977	-6		2	0.041	0.038	0.043	0.049	-10
	3	1.005	0.986	1.018	0.976	-20		3	0.044	0.029	0.041	0.046	-15
	Mean	0.999	0.981	1.005	0.973	-15		Mean	0.042	0.033	0.042	0.045	-12
US-027	1	0.973	0.970	0.963	0.965	3	US-027	1	0.037	0.033	0.044	0.040	-7
	2	0.992	0.979	0.973	0.970	11		2	0.040	0.030	0.043	0.047	-10
	3	0.996	0.978	0.985	0.969	3		3	0.038	0.029	0.044	0.046	-8
	Mean	0.987	0.976	0.973	0.968	6		Mean	0.038	0.030	0.044	0.044	-8

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 1
 Chemical Name : Sulisobenzone

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
US-028	1	0.994	0.980	0.968	0.971	15	US-028	1	0.040	0.029	0.042	0.051	-24
	2	1.000	0.988	0.980	0.977	10		2	0.040	0.030	0.042	0.053	-24
	3	1.003	0.995	0.976	0.983	16		3	0.037	0.028	0.042	0.061	-22
	Mean	0.999	0.988	0.975	0.977	14		Mean	0.039	0.029	0.042	0.055	-23
US-029	1	0.984	0.973	0.961	0.960	13	US-029	1	0.044	0.030	0.040	0.038	-12
	2	0.986	0.986	0.971	0.979	5		2	0.043	0.044	0.042	0.044	-9
	3	1.003	0.987	0.977	0.978	16		3	0.043	0.029	0.041	0.047	-11
	Mean	0.991	0.982	0.970	0.972	11		Mean	0.043	0.034	0.041	0.043	-11
US-030	1	0.990	0.997	0.983	1.002	8	US-030	1	0.039	0.029	0.042	0.047	-18
	2	0.998	1.003	0.986	0.996	13		2	0.040	0.043	0.043	0.051	-18
	3	1.001	1.001	0.994	1.005	7		3	0.042	0.030	0.043	0.066	-20
	Mean	0.996	1.000	0.988	1.001	9		Mean	0.040	0.034	0.043	0.055	-19
US-031	1	1.012	0.986	1.006	0.973	-4	US-031	1	0.045	0.029	0.042	0.042	-16
	2	1.008	0.985	0.999	0.979	-1		2	0.039	0.042	0.043	0.046	-9
	3	1.017	0.995	1.005	0.985	2		3	0.045	0.029	0.044	0.049	-14
	Mean	1.012	0.989	1.003	0.979	-1		Mean	0.043	0.033	0.043	0.046	-13
US-032	1	0.978	0.987	0.952	0.976	14	US-032	1	0.041	0.028	0.043	0.045	-17
	2	0.992	0.988	0.973	0.975	7		2	0.040	0.045	0.043	0.045	-17
	3	1.002	1.007	0.975	0.995	15		3	0.042	0.028	0.044	0.072	-18
	Mean	0.990	0.994	0.967	0.982	12		Mean	0.041	0.034	0.043	0.054	-17
US-033	1	0.977	0.982	0.962	0.972	3	US-033	1	0.040	0.033	0.041	0.041	-18
	2	0.995	0.989	0.977	0.975	6		2	0.039	0.034	0.041	0.051	-18
	3	0.998	0.987	0.983	0.975	3		3	0.041	0.028	0.041	0.064	-20
	Mean	0.990	0.986	0.974	0.974	4		Mean	0.040	0.032	0.041	0.052	-19

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 1
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
US-034	1	0.991	0.974	0.982	0.965	0	US-034	1	0.041	0.036	0.041	0.049	-24
	2	1.001	1.001	0.992	0.992	1		2	0.041	0.042	0.042	0.045	-23
	3	1.001	0.994	0.993	0.985	-2		3	0.042	0.029	0.041	0.085	-25
	Mean	0.998	0.990	0.989	0.981	0		Mean	0.041	0.036	0.041	0.060	-24
US-035	1	0.982	0.982	0.974	0.975	-1	US-035	1	0.043	0.034	0.041	0.045	-24
	2	0.988	0.985	0.979	0.974	0		2	0.042	0.030	0.043	0.048	-21
	3	0.996	0.985	0.985	0.976	2		3	0.044	0.028	0.042	0.066	-24
	Mean	0.989	0.984	0.979	0.975	0		Mean	0.043	0.031	0.042	0.053	-23
US-036	1	0.984	0.977	0.975	0.965	-1	US-036	1	0.041	0.028	0.042	0.043	-23
	2	0.995	0.990	0.987	0.984	-2		2	0.040	0.032	0.043	0.048	-22
	3	0.997	0.988	0.989	0.977	-3		3	0.040	0.029	0.042	0.072	-22
	Mean	0.992	0.985	0.984	0.975	-2		Mean	0.040	0.030	0.042	0.054	-22
Mean for all assays		-	-	-	-	5	Mean for all assays		-	-	-	-	-15
SD for all assays		-	-	-	-	7	SD for all assays		-	-	-	-	7
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-01	1	0.940	0.955	0.509	0.948	424	FDSC-01	1	0.031	0.033	0.320	0.043	278
	2	0.959	0.963	0.530	0.956	422		2	0.032	0.037	0.344	0.050	301
	3	0.963	0.967	0.539	0.961	417		3	0.033	0.033	0.352	0.042	308
	Mean	0.954	0.962	0.526	0.955	421		Mean	0.032	0.034	0.339	0.045	296
FDSC-02	1	0.991	0.964	0.479	0.953	502	FDSC-02	1	0.033	0.032	0.282	0.041	230
	2	0.996	0.971	0.507	0.964	479		2	0.032	0.034	0.300	0.041	249
	3	0.999	0.978	0.510	0.965	479		3	0.033	0.038	0.305	0.081	253
	Mean	0.995	0.971	0.499	0.961	487		Mean	0.033	0.035	0.296	0.054	244
FDSC-03	1	0.954	0.966	0.516	0.962	435	FDSC-03	1	0.032	0.033	0.351	0.050	302
	2	0.954	0.970	0.527	0.968	424		2	0.032	0.033	0.340	0.050	291
	3	0.965	0.974	0.537	0.970	425		3	0.034	0.035	0.363	0.053	312
	Mean	0.958	0.970	0.527	0.967	428		Mean	0.033	0.034	0.351	0.051	302
FDSC-04	1	0.960	0.972	0.511	0.963	439	FDSC-04	1	0.032	0.032	0.346	0.050	297
	2	0.970	0.970	0.526	0.959	434		2	0.033	0.032	0.346	0.049	296
	3	0.961	0.975	0.526	0.963	425		3	0.034	0.033	0.343	0.049	292
	Mean	0.964	0.972	0.521	0.962	433		Mean	0.033	0.032	0.345	0.049	295
FDSC-05	1	0.966	0.970	0.533	0.966	432	FDSC-05	1	0.032	0.032	0.321	0.048	273
	2	0.963	0.966	0.538	0.973	424		2	0.033	0.032	0.309	0.051	260
	3	0.961	0.975	0.539	0.968	421		3	0.034	0.032	0.329	0.046	279
	Mean	0.963	0.970	0.537	0.969	426		Mean	0.033	0.032	0.320	0.048	271
FDSC-06	1	0.975	0.982	0.530	0.972	436	FDSC-06	1	0.032	0.033	0.357	0.052	307
	2	0.981	0.977	0.544	0.965	428		2	0.033	0.034	0.355	0.047	304
	3	0.975	0.982	0.547	0.976	419		3	0.032	0.033	0.357	0.055	307
	Mean	0.977	0.980	0.540	0.971	428		Mean	0.032	0.033	0.356	0.051	306

*1 : decrease of $A440 \times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of $A560 \times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Quinine HCl

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-07	1	0.963	0.973	0.520	0.964	438	FDSC-07	1	0.032	0.032	0.296	0.048	248
	2	0.984	0.971	0.545	0.967	434		2	0.032	0.032	0.283	0.049	235
	3	0.970	0.978	0.538	0.976	427		3	0.032	0.033	0.295	0.047	247
	Mean	0.972	0.974	0.534	0.969	433		Mean	0.032	0.032	0.291	0.048	243
FDSC-08	1	0.977	0.987	0.517	0.980	453	FDSC-08	1	0.031	0.032	0.358	0.046	314
	2	0.992	0.989	0.541	0.982	444		2	0.032	0.032	0.325	0.048	280
	3	0.989	0.996	0.541	0.990	441		3	0.033	0.033	0.333	0.042	287
	Mean	0.986	0.991	0.533	0.984	446		Mean	0.032	0.032	0.339	0.045	294
FDSC-09	1	0.981	0.984	0.532	0.975	443	FDSC-09	1	0.032	0.032	0.329	0.052	274
	2	0.982	0.984	0.536	0.980	440		2	0.032	0.032	0.318	0.057	263
	3	0.986	0.994	0.545	0.987	435		3	0.032	0.032	0.342	0.055	287
	Mean	0.983	0.987	0.538	0.981	439		Mean	0.032	0.032	0.330	0.055	275
FDSC-10	1	0.976	0.982	0.519	0.976	448	FDSC-10	1	0.032	0.032	0.334	0.047	283
	2	0.985	0.992	0.534	0.975	442		2	0.032	0.032	0.329	0.053	278
	3	0.982	0.987	0.537	0.984	436		3	0.032	0.034	0.351	0.055	300
	Mean	0.981	0.987	0.530	0.978	442		Mean	0.032	0.033	0.338	0.052	287
FDSC-11	1	0.976	0.977	0.516	0.965	452	FDSC-11	1	0.037	0.031	0.312	0.049	256
	2	0.977	0.972	0.533	0.967	436		2	0.032	0.032	0.313	0.055	262
	3	0.978	0.979	0.531	0.971	439		3	0.033	0.034	0.325	0.050	273
	Mean	0.977	0.976	0.527	0.968	442		Mean	0.034	0.032	0.317	0.051	264
FDSC-12	1	0.959	0.967	0.513	0.961	439	FDSC-12	1	0.033	0.033	0.340	0.049	292
	2	0.969	0.962	0.530	0.954	432		2	0.033	0.035	0.337	0.050	289
	3	0.957	0.967	0.528	0.959	422		3	0.034	0.033	0.338	0.047	289
	Mean	0.962	0.965	0.524	0.958	431		Mean	0.033	0.034	0.338	0.049	290

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Quinine HCl

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-13	1	0.966	0.970	0.542	0.970	419	FDSC-13	1	0.031	0.034	0.349	0.043	309
	2	0.975	0.970	0.539	0.962	431		2	0.033	0.034	0.333	0.043	291
	3	0.963	0.972	0.529	0.965	429		3	0.033	0.033	0.369	0.044	327
	Mean	0.968	0.971	0.537	0.966	426		Mean	0.032	0.034	0.350	0.043	309
FDSC-14	1	0.958	0.971	0.511	0.960	437	FDSC-14	1	0.032	0.032	0.367	0.044	322
	2	0.967	0.968	0.526	0.959	431		2	0.033	0.033	0.362	0.050	316
	3	0.969	0.974	0.530	0.963	429		3	0.032	0.033	0.371	0.045	326
	Mean	0.965	0.971	0.522	0.961	432		Mean	0.032	0.033	0.367	0.046	321
FDSC-15	1	0.975	0.974	0.527	0.967	443	FDSC-15	1	0.031	0.033	0.351	0.054	305
	2	0.979	0.975	0.541	0.971	433		2	0.032	0.032	0.327	0.048	280
	3	0.981	0.982	0.543	0.977	433		3	0.034	0.035	0.340	0.043	291
	Mean	0.978	0.977	0.537	0.972	436		Mean	0.032	0.033	0.339	0.048	292
FDSC-16	1	0.971	0.986	0.512	0.996	459	FDSC-16	1	0.031	0.031	0.358	0.041	316
	2	0.978	0.982	0.530	0.980	448		2	0.032	0.032	0.334	0.044	291
	3	0.984	0.993	0.539	0.986	445		3	0.033	0.033	0.364	0.043	320
	Mean	0.978	0.987	0.527	0.987	451		Mean	0.032	0.032	0.352	0.043	309
FDSC-17	1	0.981	0.981	0.519	0.977	459	FDSC-17	1	0.032	0.032	0.344	0.053	294
	2	0.990	0.989	0.538	0.984	449		2	0.032	0.033	0.356	0.052	306
	3	0.990	0.995	0.541	0.993	446		3	0.032	0.032	0.372	0.046	322
	Mean	0.987	0.988	0.533	0.985	451		Mean	0.032	0.032	0.357	0.050	307
FDSC-18	1	0.948	0.960	0.503	0.955	440	FDSC-18	1	0.032	0.032	0.324	0.043	279
	2	0.953	0.955	0.520	0.951	428		2	0.032	0.032	0.335	0.047	290
	3	0.953	0.966	0.520	0.958	428		3	0.036	0.033	0.341	0.045	292
	Mean	0.951	0.960	0.514	0.955	432		Mean	0.033	0.032	0.333	0.045	287

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Quinine HCl

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-19	1	0.979	0.986	0.527	0.990	448	FDSC-19	1	0.031	0.033	0.301	0.049	252
	2	0.976	0.981	0.538	0.975	434		2	0.032	0.032	0.293	0.054	243
	3	0.976	0.991	0.542	0.982	430		3	0.033	0.033	0.319	0.050	268
	Mean	0.977	0.986	0.536	0.982	437		Mean	0.032	0.033	0.304	0.051	254
FDSC-20	1	0.965	0.974	0.515	0.949	431	FDSC-20	1	0.032	0.032	0.343	0.046	299
	2	0.976	0.972	0.534	0.954	423		2	0.032	0.032	0.348	0.045	304
	3	0.972	0.980	0.536	0.966	417		3	0.034	0.034	0.354	0.045	308
	Mean	0.971	0.975	0.528	0.956	424		Mean	0.033	0.033	0.348	0.045	304
FDSC-21	1	0.955	0.959	0.509	0.967	444	FDSC-21	1	0.031	0.031	0.279	0.043	239
	2	0.959	0.958	0.524	0.953	433		2	0.033	0.032	0.267	0.041	225
	3	0.957	0.963	0.528	0.955	427		3	0.032	0.035	0.286	0.042	245
	Mean	0.957	0.960	0.520	0.958	435		Mean	0.032	0.033	0.277	0.042	236
FDSC-22	1	0.968	0.980	0.519	0.974	443	FDSC-22	1	0.032	0.031	0.314	0.040	268
	2	0.973	0.982	0.538	0.974	429		2	0.034	0.033	0.275	0.057	227
	3	0.977	0.991	0.544	0.986	427		3	0.033	0.032	0.307	0.042	260
	Mean	0.973	0.984	0.534	0.978	433		Mean	0.033	0.032	0.299	0.046	252
FDSC-23	1	0.988	0.957	0.536	0.946	442	FDSC-23	1	0.031	0.032	0.317	0.059	268
	2	0.995	0.960	0.552	0.947	433		2	0.032	0.032	0.312	0.045	262
	3	0.995	0.965	0.557	0.959	428		3	0.033	0.033	0.344	0.045	293
	Mean	0.993	0.961	0.548	0.951	434		Mean	0.032	0.032	0.324	0.050	274
FDSC-24	1	0.967	0.972	0.518	0.963	442	FDSC-24	1	0.032	0.031	0.258	0.045	212
	2	0.968	0.970	0.528	0.963	433		2	0.034	0.032	0.258	0.048	210
	3	0.965	0.977	0.530	0.973	428		3	0.033	0.032	0.270	0.046	223
	Mean	0.967	0.973	0.525	0.966	434		Mean	0.033	0.032	0.262	0.046	215

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-25	1	0.938	0.958	0.502	0.948	424	FDSC-25	1	0.032	0.032	0.304	0.040	264
	2	0.945	0.952	0.514	0.949	419		2	0.032	0.034	0.303	0.041	263
	3	0.943	0.951	0.517	0.928	414		3	0.033	0.033	0.316	0.042	275
	Mean	0.942	0.954	0.511	0.942	419		Mean	0.032	0.033	0.308	0.041	267
FDSC-26	1	0.946	0.959	0.502	0.954	438	FDSC-26	1	0.033	0.037	0.329	0.042	285
	2	0.960	0.955	0.519	0.949	435		2	0.034	0.033	0.328	0.050	283
	3	0.955	0.964	0.523	0.956	426		3	0.033	0.033	0.351	0.043	307
	Mean	0.954	0.959	0.515	0.953	433		Mean	0.033	0.034	0.336	0.045	292
Mean for all assays		-	-	-	-	436	Mean for all assays		-	-	-	-	280
SD for all assays		-	-	-	-	13	SD for all assays		-	-	-	-	27
CV for all assays		-	-	-	-	3.0	CV for all assays		-	-	-	-	9.6

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-01	1	0.959	0.955	0.949	0.948	3	FDSC-01	1	0.038	0.033	0.039	0.043	-10
	2	0.974	0.963	0.966	0.956	1		2	0.038	0.037	0.038	0.050	-11
	3	0.976	0.967	0.971	0.961	-2		3	0.037	0.033	0.043	0.042	-5
	Mean	0.970	0.962	0.962	0.955	1		Mean	0.038	0.034	0.040	0.045	-9
FDSC-02	1	0.984	0.964	0.941	0.953	33	FDSC-02	1	0.033	0.032	0.040	0.041	-12
	2	0.990	0.971	0.963	0.964	17		2	0.033	0.034	0.040	0.041	-12
	3	0.998	0.978	0.966	0.965	22		3	0.032	0.038	0.040	0.081	-11
	Mean	0.991	0.971	0.957	0.961	24		Mean	0.033	0.035	0.040	0.054	-12
FDSC-03	1	0.968	0.966	0.962	0.962	3	FDSC-03	1	0.038	0.033	0.039	0.050	-16
	2	0.977	0.970	0.976	0.968	-2		2	0.037	0.033	0.039	0.050	-15
	3	0.976	0.974	0.972	0.970	1		3	0.041	0.035	0.039	0.053	-19
	Mean	0.974	0.970	0.970	0.967	1		Mean	0.039	0.034	0.039	0.051	-17
FDSC-04	1	0.974	0.972	0.962	0.963	2	FDSC-04	1	0.038	0.032	0.038	0.050	-17
	2	0.976	0.970	0.960	0.959	6		2	0.037	0.032	0.038	0.049	-16
	3	0.975	0.975	0.963	0.963	2		3	0.039	0.033	0.040	0.049	-16
	Mean	0.975	0.972	0.962	0.962	3		Mean	0.038	0.032	0.039	0.049	-16
FDSC-05	1	0.967	0.970	0.966	0.966	0	FDSC-05	1	0.038	0.032	0.040	0.048	-14
	2	0.969	0.966	0.968	0.973	0		2	0.037	0.032	0.039	0.051	-14
	3	0.968	0.975	0.963	0.968	4		3	0.037	0.032	0.038	0.046	-15
	Mean	0.968	0.970	0.966	0.969	1		Mean	0.037	0.032	0.039	0.048	-14
FDSC-06	1	0.983	0.982	0.978	0.972	-4	FDSC-06	1	0.038	0.033	0.039	0.052	-17
	2	0.982	0.977	0.972	0.965	1		2	0.038	0.034	0.042	0.047	-14
	3	0.987	0.982	0.981	0.976	-3		3	0.038	0.033	0.040	0.055	-16
	Mean	0.984	0.980	0.977	0.971	-2		Mean	0.038	0.033	0.040	0.051	-16

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-07	1	0.974	0.973	0.974	0.964	-5	FDSC-07	1	0.038	0.032	0.039	0.048	-15
	2	0.979	0.971	0.972	0.967	2		2	0.038	0.032	0.043	0.049	-11
	3	0.980	0.978	0.977	0.976	-2		3	0.038	0.033	0.041	0.047	-13
	Mean	0.978	0.974	0.974	0.969	-2		Mean	0.038	0.032	0.041	0.048	-13
FDSC-08	1	0.991	0.987	0.985	0.980	-1	FDSC-08	1	0.037	0.032	0.043	0.046	-7
	2	0.995	0.989	0.987	0.982	1		2	0.038	0.032	0.041	0.048	-10
	3	1.003	0.996	0.995	0.990	1		3	0.037	0.033	0.040	0.042	-10
	Mean	0.996	0.991	0.989	0.984	0		Mean	0.037	0.032	0.041	0.045	-9
FDSC-09	1	0.994	0.984	0.982	0.975	6	FDSC-09	1	0.038	0.032	0.039	0.052	-22
	2	0.990	0.984	0.986	0.980	-2		2	0.038	0.032	0.040	0.057	-21
	3	0.996	0.994	0.991	0.987	-1		3	0.038	0.032	0.038	0.055	-23
	Mean	0.993	0.987	0.986	0.981	1		Mean	0.038	0.032	0.039	0.055	-22
FDSC-10	1	0.988	0.982	0.982	0.976	-3	FDSC-10	1	0.039	0.032	0.039	0.047	-19
	2	0.990	0.992	0.985	0.975	-4		2	0.037	0.032	0.039	0.053	-17
	3	0.995	0.987	0.994	0.984	-8		3	0.039	0.034	0.040	0.055	-18
	Mean	0.991	0.987	0.987	0.978	-5		Mean	0.038	0.033	0.039	0.052	-18
FDSC-11	1	0.984	0.977	0.977	0.965	-1	FDSC-11	1	0.037	0.031	0.039	0.049	-17
	2	0.983	0.972	0.976	0.967	-1		2	0.037	0.032	0.038	0.055	-18
	3	0.985	0.979	0.975	0.971	2		3	0.037	0.034	0.039	0.050	-17
	Mean	0.984	0.976	0.976	0.968	0		Mean	0.037	0.032	0.039	0.051	-17
FDSC-12	1	0.977	0.967	0.964	0.961	6	FDSC-12	1	0.037	0.033	0.039	0.049	-13
	2	0.972	0.962	0.965	0.954	0		2	0.037	0.035	0.039	0.050	-13
	3	0.975	0.967	0.968	0.959	0		3	0.037	0.033	0.041	0.047	-11
	Mean	0.975	0.965	0.966	0.958	2		Mean	0.037	0.034	0.040	0.049	-12

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-13	1	0.969	0.970	0.971	0.970	-7	FDSC-13	1	0.037	0.034	0.039	0.043	-7
	2	0.970	0.970	0.972	0.962	-7		2	0.037	0.034	0.040	0.043	-6
	3	0.968	0.972	0.961	0.965	2		3	0.037	0.033	0.039	0.044	-7
	Mean	0.969	0.971	0.968	0.966	-4		Mean	0.037	0.034	0.039	0.043	-7
FDSC-14	1	0.971	0.971	0.962	0.960	-1	FDSC-14	1	0.037	0.032	0.040	0.044	-10
	2	0.974	0.968	0.969	0.959	-5		2	0.037	0.033	0.039	0.050	-11
	3	0.982	0.974	0.973	0.963	-1		3	0.040	0.033	0.040	0.045	-13
	Mean	0.976	0.971	0.968	0.961	-2		Mean	0.038	0.033	0.040	0.046	-11
FDSC-15	1	0.981	0.974	0.977	0.967	-1	FDSC-15	1	0.039	0.033	0.040	0.054	-14
	2	0.985	0.975	0.981	0.971	-1		2	0.037	0.032	0.040	0.048	-12
	3	0.987	0.982	0.983	0.977	-1		3	0.038	0.035	0.039	0.043	-14
	Mean	0.984	0.977	0.980	0.972	-1		Mean	0.038	0.033	0.040	0.048	-13
FDSC-16	1	0.984	0.986	0.978	0.996	6	FDSC-16	1	0.037	0.031	0.038	0.041	-10
	2	0.985	0.982	0.981	0.980	4		2	0.037	0.032	0.038	0.044	-10
	3	0.989	0.993	0.983	0.986	6		3	0.037	0.033	0.039	0.043	-9
	Mean	0.986	0.987	0.981	0.987	5		Mean	0.037	0.032	0.038	0.043	-10
FDSC-17	1	0.982	0.981	0.978	0.977	1	FDSC-17	1	0.039	0.032	0.041	0.053	-16
	2	0.991	0.989	0.988	0.984	0		2	0.037	0.033	0.040	0.052	-15
	3	0.995	0.995	0.989	0.993	3		3	0.037	0.032	0.039	0.046	-16
	Mean	0.989	0.988	0.985	0.985	1		Mean	0.038	0.032	0.040	0.050	-16
FDSC-18	1	0.963	0.960	0.956	0.955	2	FDSC-18	1	0.038	0.032	0.040	0.043	-11
	2	0.964	0.955	0.959	0.951	0		2	0.038	0.032	0.040	0.047	-11
	3	0.968	0.966	0.961	0.958	2		3	0.040	0.033	0.039	0.045	-14
	Mean	0.965	0.960	0.959	0.955	1		Mean	0.039	0.032	0.040	0.045	-12

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-19	1	0.985	0.986	0.976	0.990	5	FDSC-19	1	0.038	0.033	0.039	0.049	-17
	2	0.984	0.981	0.973	0.975	7		2	0.038	0.032	0.039	0.054	-17
	3	0.980	0.991	0.973	0.982	3		3	0.039	0.033	0.038	0.050	-19
	Mean	0.983	0.986	0.974	0.982	5		Mean	0.038	0.033	0.039	0.051	-18
FDSC-20	1	0.978	0.974	0.962	0.949	-3	FDSC-20	1	0.038	0.032	0.040	0.046	-10
	2	0.983	0.972	0.972	0.954	-8		2	0.037	0.032	0.040	0.045	-9
	3	0.988	0.980	0.980	0.966	-11		3	0.039	0.034	0.038	0.045	-13
	Mean	0.983	0.975	0.971	0.956	-7		Mean	0.038	0.033	0.039	0.045	-11
FDSC-21	1	0.967	0.959	0.957	0.967	8	FDSC-21	1	0.038	0.031	0.039	0.043	-8
	2	0.972	0.958	0.963	0.953	7		2	0.037	0.032	0.039	0.041	-7
	3	0.966	0.963	0.959	0.955	5		3	0.037	0.035	0.039	0.042	-7
	Mean	0.968	0.960	0.960	0.958	7		Mean	0.037	0.033	0.039	0.042	-7
FDSC-22	1	0.979	0.980	0.972	0.974	1	FDSC-22	1	0.038	0.031	0.039	0.040	-13
	2	0.980	0.982	0.973	0.974	1		2	0.037	0.033	0.039	0.057	-12
	3	0.988	0.991	0.977	0.986	5		3	0.037	0.032	0.039	0.042	-12
	Mean	0.982	0.984	0.974	0.978	2		Mean	0.037	0.032	0.039	0.046	-12
FDSC-23	1	0.964	0.957	0.954	0.946	0	FDSC-23	1	0.038	0.032	0.040	0.059	-16
	2	0.963	0.960	0.951	0.947	2		2	0.038	0.032	0.039	0.045	-17
	3	0.966	0.965	0.956	0.959	0		3	0.038	0.033	0.043	0.045	-13
	Mean	0.964	0.961	0.954	0.951	1		Mean	0.038	0.032	0.041	0.050	-15
FDSC-24	1	0.975	0.972	0.965	0.963	3	FDSC-24	1	0.038	0.031	0.039	0.045	-13
	2	0.975	0.970	0.965	0.963	3		2	0.038	0.032	0.040	0.048	-12
	3	0.976	0.977	0.969	0.973	0		3	0.039	0.032	0.043	0.046	-10
	Mean	0.975	0.973	0.966	0.966	2		Mean	0.038	0.032	0.041	0.046	-12

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 2
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
FDSC-25	1	0.962	0.958	0.951	0.948	-1	FDSC-25	1	0.038	0.032	0.039	0.040	-7
	2	0.959	0.952	0.952	0.949	-5		2	0.037	0.034	0.040	0.041	-5
	3	0.963	0.951	0.956	0.928	-5		3	0.038	0.033	0.039	0.042	-7
	Mean	0.961	0.954	0.953	0.942	-4		Mean	0.038	0.033	0.039	0.041	-6
FDSC-26	1	0.963	0.959	0.959	0.954	-2	FDSC-26	1	0.040	0.037	0.039	0.042	-12
	2	0.964	0.955	0.954	0.949	4		2	0.037	0.033	0.040	0.050	-8
	3	0.966	0.964	0.961	0.956	-1		3	0.039	0.033	0.038	0.043	-12
	Mean	0.964	0.959	0.958	0.953	0		Mean	0.039	0.034	0.039	0.045	-11
Mean for all assays		-	-	-	-	1	Mean for all assays		-	-	-	-	-13
SD for all assays		-	-	-	-	6	SD for all assays		-	-	-	-	4
CV for all assays		-	-	-	-	-	CV for all assays		-	-	-	-	-

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 3
 Chemical Name : Quinine HCl

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
MT-001	1	0.986	1.005	0.596	1.000	387	MT-001	1	0.032	0.030	0.340	0.035	301
	2	0.990	1.009	0.612	1.005	376		2	0.033	0.030	0.340	0.035	301
	3	0.987	1.010	0.624	1.012	361		3	0.032	0.029	0.353	0.036	314
	Mean	0.988	1.008	0.611	1.006	375		Mean	0.032	0.029	0.344	0.036	305
MT-002	1	0.992	1.011	0.627	1.005	360	MT-002	1	0.031	0.029	0.320	0.033	284
	2	0.995	1.016	0.633	1.011	357		2	0.032	0.030	0.317	0.034	280
	3	0.989	1.018	0.639	1.013	345		3	0.031	0.028	0.334	0.033	298
	Mean	0.992	1.015	0.633	1.010	354		Mean	0.031	0.029	0.324	0.034	287
MT-003	1						MT-003	1	0.031	0.029	0.337	0.036	300
	2							2	0.031	0.029	0.325	0.034	288
	3							3	0.031	0.028	0.343	0.034	307
	Mean							Mean	0.031	0.029	0.335	0.035	298
MT-004	1	0.979	0.999	0.594	0.992	379	MT-004	1	0.032	0.029	0.366	0.034	330
	2	0.982	1.003	0.605	0.997	370		2	0.032	0.029	0.369	0.034	331
	3	0.979	1.001	0.608	0.996	364		3	0.032	0.029	0.384	0.034	347
	Mean	0.980	1.001	0.602	0.995	371		Mean	0.032	0.029	0.373	0.034	336
MT-005	1	0.982	1.000	0.608	0.997	370	MT-005	1	0.032	0.029	0.322	0.034	285
	2	0.981	1.003	0.617	1.000	360		2	0.032	0.029	0.322	0.034	284
	3	0.977	1.002	0.621	0.998	352		3	0.031	0.029	0.326	0.034	289
	Mean	0.980	1.002	0.615	0.998	361		Mean	0.032	0.029	0.323	0.034	286
MT-006	1	0.986	1.002	0.606	0.996	374	MT-006	1	0.031	0.029	0.330	0.034	293
	2	0.987	1.006	0.616	0.999	365		2	0.032	0.029	0.324	0.034	287
	3	0.982	1.007	0.617	1.001	360		3	0.031	0.028	0.340	0.033	304
	Mean	0.985	1.005	0.613	0.999	366		Mean	0.031	0.029	0.331	0.034	295

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

The optical absorbance values were rounded to the third decimal place. If the calculated "results" are different from the numerical results in the raw data sheets by the number of significant digits in the data sheet and the appendices are different, the data in the raw data sheet is used in the appendices.

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 3
 Chemical Name : Quinine HCl

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
MT-007	1	0.981	0.996	0.597	0.992	382	MT-007	1	0.031	0.029	0.343	0.036	305
	2	0.984	1.002	0.607	0.998	374		2	0.032	0.029	0.340	0.035	302
	3	0.978	1.000	0.606	0.997	369		3	0.031	0.028	0.348	0.034	311
	Mean	0.981	0.999	0.603	0.996	375		Mean	0.031	0.029	0.344	0.035	306
MT-008	1	0.994	1.010	0.597	0.995	382	MT-008	1	0.033	0.031	0.335	0.035	297
	2	0.993	1.012	0.605	0.997	372		2	0.033	0.031	0.340	0.036	301
	3	0.988	1.011	0.610	0.997	362		3	0.032	0.030	0.348	0.035	311
	Mean	0.992	1.011	0.604	0.996	372		Mean	0.033	0.031	0.341	0.036	303
MT-009	1	0.976	0.994	0.603	0.989	369	MT-009	1	0.031	0.029	0.334	0.034	298
	2	0.978	0.997	0.618	0.994	357		2	0.032	0.029	0.331	0.035	295
	3	0.975	0.999	0.619	0.995	352		3	0.031	0.028	0.345	0.034	309
	Mean	0.976	0.997	0.613	0.993	359		Mean	0.031	0.029	0.337	0.034	301
MT-010	1	0.983	0.998	0.617	0.994	362	MT-010	1	0.031	0.029	0.324	0.034	288
	2	0.983	1.002	0.622	0.997	357		2	0.031	0.029	0.324	0.035	287
	3	0.978	1.001	0.623	0.996	351		3	0.031	0.028	0.326	0.034	290
	Mean	0.981	1.000	0.621	0.996	357		Mean	0.031	0.029	0.325	0.034	288
MT-011	1	0.982	0.998	0.618	0.993	359	MT-011	1	0.032	0.030	0.318	0.034	280
	2	0.985	1.005	0.627	0.999	353		2	0.032	0.029	0.318	0.035	280
	3	0.980	1.003	0.629	1.000	347		3	0.031	0.028	0.327	0.035	290
	Mean	0.982	1.002	0.625	0.997	353		Mean	0.032	0.029	0.321	0.035	283
MT-012	1	0.974	0.992	0.607	0.989	363	MT-012	1	0.032	0.030	0.317	0.034	280
	2	0.979	0.999	0.618	0.994	357		2	0.033	0.029	0.315	0.035	277
	3	0.975	0.999	0.620	0.996	351		3	0.031	0.029	0.320	0.034	283
	Mean	0.976	0.997	0.615	0.993	357		Mean	0.032	0.029	0.317	0.034	280

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 3
 Chemical Name : Quinine HCl

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
MT-013	1	0.970	0.994	0.611	0.988	356	MT-013	1	0.031	0.029	0.317	0.034	280
	2	0.973	0.995	0.618	0.991	351		2	0.032	0.029	0.309	0.035	272
	3	0.971	0.995	0.619	0.990	348		3	0.031	0.028	0.319	0.034	283
	Mean	0.971	0.994	0.616	0.990	352		Mean	0.031	0.029	0.315	0.034	278
MT-014	1	0.988	1.007	0.622	1.003	362	MT-014	1	0.032	0.029	0.317	0.035	279
	2	0.991	1.012	0.632	1.008	355		2	0.033	0.029	0.315	0.035	276
	3	0.986	1.010	0.634	1.009	348		3	0.032	0.029	0.318	0.037	280
	Mean	0.988	1.010	0.629	1.006	355		Mean	0.032	0.029	0.317	0.035	278
MT-015	1	0.983	1.002	0.617	0.997	359	MT-015	1	0.031	0.030	0.326	0.035	290
	2	0.989	1.007	0.626	1.004	355		2	0.032	0.030	0.322	0.035	286
	3	0.985	1.006	0.629	0.995	349		3	0.031	0.029	0.326	0.034	291
	Mean	0.986	1.005	0.624	0.998	354		Mean	0.031	0.030	0.325	0.034	289
MT-016	1	0.975	0.996	0.609	0.992	362	MT-016	1	0.032	0.029	0.329	0.034	293
	2	0.981	0.988	0.619	0.985	358		2	0.032	0.029	0.326	0.033	290
	3	0.969	0.989	0.616	0.984	349		3	0.031	0.029	0.330	0.033	295
	Mean	0.975	0.991	0.615	0.987	356		Mean	0.032	0.029	0.328	0.033	293
MT-018	1	0.969	0.997	0.594	0.993	371	MT-018	1	0.032	0.029	0.335	0.033	298
	2	0.976	0.992	0.607	0.987	365		2	0.032	0.028	0.332	0.033	295
	3	0.961	0.990	0.603	0.987	354		3	0.031	0.028	0.340	0.033	304
	Mean	0.969	0.993	0.601	0.989	363		Mean	0.032	0.028	0.336	0.033	299
MT-019	1	0.985	1.009	0.626	1.001	350	MT-019	1	0.033	0.030	0.325	0.035	287
	2	0.990	1.002	0.633	0.992	348		2	0.033	0.030	0.327	0.035	289
	3	0.978	1.003	0.630	0.994	339		3	0.032	0.030	0.335	0.035	298
	Mean	0.984	1.005	0.630	0.996	346		Mean	0.033	0.030	0.329	0.035	291

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 3
 Chemical Name : Quinine HCl

Singlet oxygen						Superoxide anion							
Experimental No.	Run#	A440(-)		A440(+)		Results *1	Experimental No.	Run#	A560(-)		A560(+)		Results *2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
MT-020	1	0.981	1.003	0.622	1.003	357	MT-020	1	0.033	0.030	0.315	0.035	276
	2	0.986	1.000	0.638	0.998	346		2	0.033	0.030	0.313	0.035	275
	3	0.974	0.999	0.633	0.997	339		3	0.032	0.031	0.317	0.036	279
	Mean	0.980	1.001	0.631	0.999	347		Mean	0.033	0.030	0.315	0.036	277
MT-021	1	0.982	1.008	0.626	1.001	351	MT-021	1	0.032	0.029	0.319	0.035	280
	2	0.989	1.004	0.638	1.000	346		2	0.032	0.030	0.315	0.035	277
	3	0.979	1.004	0.633	1.001	340		3	0.032	0.029	0.322	0.035	284
	Mean	0.983	1.005	0.632	1.000	346		Mean	0.032	0.029	0.319	0.035	280
MT-022	1	0.976	1.001	0.619	0.994	351	MT-022	1	0.032	0.030	0.313	0.034	276
	2	0.981	1.000	0.627	0.992	348		2	0.032	0.030	0.310	0.035	273
	3	0.971	0.996	0.624	0.993	341		3	0.032	0.029	0.323	0.034	286
	Mean	0.976	0.999	0.623	0.993	347		Mean	0.032	0.029	0.315	0.034	278
MT-023	1	0.980	1.002	0.612	0.997	364	MT-023	1	0.032	0.029	0.324	0.036	285
	2	0.985	0.998	0.626	0.993	355		2	0.032	0.029	0.324	0.036	285
	3	0.973	0.998	0.620	0.995	349		3	0.032	0.029	0.333	0.036	294
	Mean	0.979	0.999	0.619	0.995	356		Mean	0.032	0.029	0.327	0.036	288
Mean for all assays	-	-	-	-	358	Mean for all assays	-	-	-	-	-	292	
SD for all assays	-	-	-	-	9	SD for all assays	-	-	-	-	-	14	
CV for all assays	-	-	-	-	2.5	CV for all assays	-	-	-	-	-	4.8	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+)) - A560(-) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 3
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
MT-001	1	1.008	1.005	1.005	1.000	1	MT-001	1	0.034	0.030	0.034	0.035	-7
	2	1.015	1.009	1.013	1.005	0		2	0.036	0.030	0.036	0.035	-8
	3	1.014	1.010	1.011	1.012	1		3	0.034	0.029	0.034	0.036	-7
	Mean	1.012	1.008	1.010	1.006	1		Mean	0.035	0.029	0.035	0.036	-7
MT-002	1	1.011	1.011	1.008	1.005	-2	MT-002	1	0.033	0.029	0.033	0.033	-6
	2	1.012	1.016	1.009	1.011	-2		2	0.034	0.030	0.033	0.034	-6
	3	1.015	1.018	1.013	1.013	-3		3	0.034	0.028	0.032	0.033	-6
	Mean	1.013	1.015	1.010	1.010	-2		Mean	0.034	0.029	0.033	0.034	-6
MT-003	1						MT-003	1	0.033	0.029	0.033	0.036	-6
	2							2	0.034	0.029	0.034	0.034	-6
	3							3	0.033	0.028	0.033	0.034	-6
	Mean							Mean	0.033	0.029	0.033	0.035	-6
MT-004	1	1.001	0.999	0.998	0.992	-4	MT-004	1	0.033	0.029	0.033	0.034	-5
	2	1.006	1.003	1.004	0.997	-4		2	0.033	0.029	0.033	0.034	-5
	3	1.004	1.001	1.000	0.996	-2		3	0.033	0.029	0.033	0.034	-6
	Mean	1.004	1.001	1.001	0.995	-3		Mean	0.033	0.029	0.033	0.034	-5
MT-005	1	1.002	1.000	1.000	0.997	-1	MT-005	1	0.033	0.029	0.033	0.034	-5
	2	1.001	1.003	0.999	1.000	-2		2	0.034	0.029	0.034	0.034	-5
	3	1.005	1.002	1.001	0.998	1		3	0.033	0.029	0.033	0.034	-6
	Mean	1.003	1.002	1.000	0.998	-1		Mean	0.033	0.029	0.033	0.034	-5
MT-006	1	1.003	1.002	1.001	0.996	-3	MT-006	1	0.033	0.029	0.033	0.034	-5
	2	1.006	1.006	1.005	0.999	-5		2	0.034	0.029	0.034	0.034	-5
	3	1.009	1.007	1.003	1.001	0		3	0.033	0.028	0.033	0.033	-6
	Mean	1.006	1.005	1.003	0.999	-3		Mean	0.033	0.029	0.033	0.034	-5

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 3
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
MT-007	1	0.999	0.996	0.996	0.992	0	MT-007	1	0.032	0.029	0.034	0.036	-5
	2	1.001	1.002	0.999	0.998	-1		2	0.034	0.029	0.034	0.035	-6
	3	1.004	1.000	1.002	0.997	-1		3	0.032	0.028	0.034	0.034	-5
	Mean	1.001	0.999	0.999	0.996	-1		Mean	0.033	0.029	0.034	0.035	-5
MT-008	1	1.009	1.010	0.994	0.995	0	MT-008	1	0.035	0.031	0.033	0.035	-7
	2	1.010	1.012	0.996	0.997	0		2	0.035	0.031	0.034	0.036	-6
	3	1.011	1.011	0.998	0.997	-2		3	0.035	0.030	0.033	0.035	-7
	Mean	1.010	1.011	0.996	0.996	-1		Mean	0.035	0.031	0.033	0.036	-7
MT-009	1	0.998	0.994	0.996	0.989	-2	MT-009	1	0.032	0.029	0.033	0.034	-4
	2	0.998	0.997	0.995	0.994	-1		2	0.033	0.029	0.033	0.035	-5
	3	1.001	0.999	0.995	0.995	2		3	0.033	0.028	0.032	0.034	-6
	Mean	0.999	0.997	0.995	0.993	0		Mean	0.033	0.029	0.033	0.034	-5
MT-010	1	1.001	0.998	0.997	0.994	0	MT-010	1	0.032	0.029	0.032	0.034	-5
	2	1.001	1.002	0.996	0.997	1		2	0.033	0.029	0.033	0.035	-5
	3	1.004	1.001	0.999	0.996	1		3	0.033	0.028	0.032	0.034	-5
	Mean	1.002	1.000	0.997	0.996	1		Mean	0.033	0.029	0.032	0.034	-5
MT-011	1	1.001	0.998	0.998	0.993	-2	MT-011	1	0.032	0.030	0.033	0.034	-6
	2	1.001	1.005	0.997	0.999	-1		2	0.033	0.029	0.033	0.035	-6
	3	1.005	1.003	1.000	1.000	1		3	0.034	0.028	0.033	0.035	-7
	Mean	1.002	1.002	0.998	0.997	-1		Mean	0.033	0.029	0.033	0.035	-6
MT-012	1	0.997	0.992	0.993	0.989	0	MT-012	1	0.032	0.030	0.032	0.034	-5
	2	0.997	0.999	0.992	0.994	1		2	0.033	0.029	0.033	0.035	-5
	3	0.998	0.999	0.992	0.996	2		3	0.033	0.029	0.032	0.034	-6
	Mean	0.997	0.997	0.992	0.993	1		Mean	0.033	0.029	0.032	0.034	-5

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 3
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
MT-013	1	0.994	0.994	0.991	0.988	-1	MT-013	1	0.032	0.029	0.032	0.034	-5
	2	0.993	0.995	0.989	0.991	0		2	0.033	0.029	0.033	0.035	-5
	3	0.997	0.995	0.989	0.990	3		3	0.033	0.028	0.032	0.034	-5
	Mean	0.995	0.994	0.990	0.990	1		Mean	0.033	0.029	0.032	0.034	-5
MT-014	1	1.010	1.007	1.006	1.003	0	MT-014	1	0.033	0.029	0.033	0.035	-6
	2	1.010	1.012	1.005	1.008	1		2	0.034	0.029	0.033	0.035	-7
	3	1.013	1.010	1.006	1.009	3		3	0.034	0.029	0.032	0.037	-7
	Mean	1.011	1.010	1.006	1.006	1		Mean	0.034	0.029	0.033	0.035	-7
MT-015	1	1.008	1.002	1.003	0.997	-3	MT-015	1	0.033	0.030	0.032	0.035	-4
	2	1.009	1.007	1.005	1.004	-3		2	0.034	0.030	0.033	0.035	-4
	3	1.014	1.006	1.008	0.995	-1		3	0.033	0.029	0.032	0.034	-5
	Mean	1.010	1.005	1.005	0.998	-2		Mean	0.033	0.030	0.032	0.034	-4
MT-016	1	0.989	0.996	0.984	0.992	1	MT-016	1	0.034	0.029	0.033	0.034	-4
	2	1.005	0.988	1.002	0.985	-2		2	0.034	0.029	0.033	0.033	-5
	3	0.995	0.989	0.989	0.984	2		3	0.032	0.029	0.032	0.033	-4
	Mean	0.996	0.991	0.992	0.987	0		Mean	0.033	0.029	0.033	0.033	-4
MT-018	1	0.989	0.997	0.984	0.993	0	MT-018	1	0.033	0.029	0.033	0.033	-5
	2	1.000	0.992	0.983	0.987	13		2	0.033	0.028	0.033	0.033	-5
	3	0.995	0.990	0.989	0.987	2		3	0.032	0.028	0.033	0.033	-5
	Mean	0.995	0.993	0.985	0.989	5		Mean	0.033	0.028	0.033	0.033	-5
MT-019	1	1.001	1.009	0.995	1.001	-3	MT-019	1	0.033	0.030	0.033	0.035	-5
	2	1.007	1.002	0.999	0.992	0		2	0.033	0.030	0.033	0.035	-5
	3	1.005	1.003	0.998	0.994	-2		3	0.033	0.030	0.033	0.035	-5
	Mean	1.004	1.005	0.997	0.996	-2		Mean	0.033	0.030	0.033	0.035	-5

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 6 Positive control and negative control data of phase 2 study

Laboratory : 3
 Chemical Name : Sulisobenzone

Singlet oxygen							Superoxide anion						
Experimental No.	Run#	A440(-)		A440(+)		Results*1	Experimental No.	Run#	A560(-)		A560(+)		Results*2
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank	
MT-020	1	1.000	1.003	0.996	1.003	2	MT-020	1	0.034	0.030	0.034	0.035	-6
	2	1.010	1.000	1.003	0.998	5		2	0.033	0.030	0.033	0.035	-6
	3	1.008	0.999	1.001	0.997	5		3	0.032	0.031	0.032	0.036	-5
	Mean	1.006	1.001	1.000	0.999	4		Mean	0.033	0.030	0.033	0.036	-6
MT-021	1	1.000	1.008	0.993	1.001	1	MT-021	1	0.033	0.029	0.034	0.035	-6
	2	1.011	1.004	1.006	1.000	0		2	0.032	0.030	0.033	0.035	-5
	3	1.011	1.004	1.004	1.001	2		3	0.031	0.029	0.033	0.035	-5
	Mean	1.007	1.005	1.001	1.000	1		Mean	0.032	0.029	0.033	0.035	-5
MT-022	1	0.993	1.001	0.988	0.994	0	MT-022	1	0.033	0.030	0.034	0.034	-4
	2	1.002	1.000	0.994	0.992	2		2	0.032	0.030	0.033	0.035	-4
	3	1.002	0.996	0.995	0.993	1		3	0.033	0.029	0.033	0.034	-5
	Mean	0.999	0.999	0.992	0.993	1		Mean	0.033	0.029	0.033	0.034	-4
MT-023	1	0.996	1.002	0.990	0.997	2	MT-023	1	0.034	0.029	0.033	0.036	-8
	2	1.006	0.998	0.999	0.993	4		2	0.035	0.029	0.033	0.036	-8
	3	1.004	0.998	0.998	0.995	2		3	0.033	0.029	0.033	0.036	-7
	Mean	1.002	0.999	0.996	0.995	3		Mean	0.034	0.029	0.033	0.036	-8
Mean for all assays	-	-	-	-	0	Mean for all assays	-	-	-	-	-	-5	
SD for all assays	-	-	-	-	2	SD for all assays	-	-	-	-	-	1	
CV for all assays	-	-	-	-	-	CV for all assays	-	-	-	-	-	-	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-) - (B-A)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after exposure)

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Acridine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-005	1	1.028	1.015	0.784	1.004	233	US-005	1	0.055	0.029	0.283	0.043	217	Positive	Photoreactive
	2	1.027	1.012	0.793	1.002	224		2	0.033	0.043	0.293	0.044	249		
	3	1.035	1.016	0.806	1.007	219		3	0.053	0.029	0.282	0.046	218		
	Mean	1.030	1.015	0.794	1.004	225		Mean	0.047	0.033	0.286	0.044	228		
US-025	1	0.980	0.990	0.728	0.969	231	US-025	1	0.053	0.029	0.298	0.046	224	Positive	Photoreactive
	2	1.007	1.013	0.759	0.988	226		2	0.033	0.047	0.305	0.047	251		
	3	1.019	1.011	0.755	0.993	242		3	0.054	0.030	0.292	0.073	217		
	Mean	1.002	1.005	0.747	0.983	233		Mean	0.047	0.035	0.298	0.056	231		
US-032	1	0.983	0.987	0.736	0.976	235	US-032	1	0.053	0.028	0.286	0.045	214	Positive	Photoreactive
	2	0.981	0.988	0.751	0.975	218		2	0.042	0.045	0.279	0.045	216		
	3	0.999	1.007	0.773	0.995	214		3	0.053	0.028	0.287	0.072	215		
	Mean	0.988	0.994	0.753	0.982	222		Mean	0.049	0.034	0.284	0.054	215		
Mean for 3 assays		-	-	-	-	227	Mean for 3 assays		-	-	-	-	225	Positive*3	Photoreactive*3

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Acridine HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
US-005	1	1.012	1.015	0.764	1.004	236	US-005	1	0.036	0.029	0.258	0.043	211	Positive	Photoreactive
	2	1.015	1.012	0.779	1.002	224		2	0.034	0.043	0.259	0.044	214		
	3	1.019	1.016	0.796	1.007	212		3	0.038	0.029	0.257	0.046	208		
	Mean	1.015	1.015	0.780	1.004	224		Mean	0.036	0.033	0.258	0.044	211		
US-025	1	0.996	0.990	0.752	0.969	222	US-025	1	0.034	0.029	0.272	0.046	217	Positive	Photoreactive
	2	1.005	1.013	0.764	0.988	219		2	0.031	0.047	0.266	0.047	214		
	3	1.003	1.011	0.780	0.993	200		3	0.036	0.030	0.271	0.073	214		
	Mean	1.001	1.005	0.765	0.983	214		Mean	0.034	0.035	0.269	0.056	215		
US-031	1	0.998	0.986	0.758	0.973	229	US-031	1	0.037	0.029	0.267	0.042	217	Positive	Photoreactive
	2	1.003	0.985	0.769	0.979	224		2	0.033	0.042	0.264	0.046	218		
	3	0.998	0.995	0.773	0.985	215		3	0.037	0.029	0.260	0.049	211		
	Mean	0.999	0.989	0.767	0.979	223		Mean	0.035	0.033	0.264	0.046	215		
Mean for 3 assays	-	-	-	-	220	Mean for 3 assays	-	-	-	-	214	Positive	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) × 1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) × 1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Amiodarone HCl

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
US-025	1	1.028	0.990	0.968	0.969	38	US-025	1	0.077	0.029	0.068	0.046	-30	Positive	(photoreactive)
	2	1.024	1.013	0.972	0.988	30		2	0.044	0.047	0.062	0.047	-3		
	3	1.035	1.011	0.982	0.993	31		3	0.063	0.030	0.070	0.073	-14		
	Mean	1.029	1.005	0.974	0.983	33		Mean	0.061	0.035	0.067	0.056	-16		
US-031	1	1.021	0.986	0.995	0.973	17	US-031	1	0.074	0.029	0.062	0.042	-26	Inconclusive	(Non-photoreactive)
	2	0.998	0.985	0.970	0.979	18		2	0.042	0.042	0.062	0.046	7		
	3	1.005	0.995	1.001	0.985	-7		3	0.077	0.029	0.071	0.049	-19		
	Mean	1.008	0.989	0.989	0.979	9		Mean	0.065	0.033	0.065	0.046	-13		
US-033	1	0.977	0.982	0.939	0.972	26	US-033	1	0.036	0.033	0.047	0.041	-9	Inconclusive	(Non-photoreactive)
	2	0.990	0.989	0.954	0.975	24		2	0.034	0.034	0.054	0.051	0		
	3	1.001	0.987	0.973	0.975	16		3	0.038	0.028	0.052	0.064	-6		
	Mean	0.989	0.986	0.955	0.974	22		Mean	0.036	0.032	0.051	0.052	-5		
Mean for 3 assays	-	-	-	-	21	Mean for 3 assays	-	-	-	-	-11	Inconclusive*3	(Non-photoreactive)*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Chlorpromazine HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-001	1	1.020	1.014	1.028	1.006	-18	US-001	1	0.048	0.033	0.143	0.046	81	Positive	Photoreactive
	2	1.002	1.028	1.009	1.014	-17		2	0.051	0.046	0.146	0.053	82		
	3	1.017	1.029	1.024	1.020	-18		3	0.044	0.034	0.146	0.056	88		
	Mean	1.013	1.024	1.020	1.013	-18		Mean	0.048	0.038	0.145	0.052	84		
US-023	1	0.988	1.008	1.012	0.991	-38	US-023	1	0.042	0.033	0.149	0.039	101	Positive	Photoreactive
	2	0.989	1.024	1.016	1.013	-41		2	0.038	0.049	0.154	0.044	109		
	3	0.998	1.022	1.027	1.008	-43		3	0.040	0.029	0.154	0.051	107		
	Mean	0.992	1.018	1.018	1.004	-41		Mean	0.040	0.037	0.152	0.044	106		
US-029	1	0.956	0.973	0.960	0.960	-13	US-029	1	0.039	0.030	0.144	0.038	96	Positive	Photoreactive
	2	0.968	0.986	0.974	0.979	-16		2	0.040	0.044	0.146	0.044	97		
	3	0.976	0.987	0.981	0.978	-15		3	0.039	0.029	0.147	0.047	99		
	Mean	0.967	0.982	0.971	0.972	-15		Mean	0.039	0.034	0.146	0.043	97		
Mean for 3 assays	-	-	-	-	-25	Mean for 3 assays	-	-	-	-	96	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Doxycycline HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-002	1	1.041	1.017	0.743	1.007	289	US-002	1	0.062	0.031	0.412	0.038	344	Positive	Photoreactive
	2	1.043	1.028	0.785	1.021	249		2	0.067	0.043	0.436	0.041	362		
	3	1.061	1.031	0.777	1.020	275		3	0.063	0.030	0.424	0.048	354		
	Mean	1.048	1.025	0.768	1.016	271		Mean	0.064	0.035	0.424	0.042	353		
US-004	1	1.039	0.998	0.734	0.987	295	US-004	1	0.050	0.029	0.550	0.046	476	Positive	Photoreactive
	2	1.016	1.027	0.764	1.019	243		2	0.079	0.029	0.587	0.059	484		
	3	1.049	1.021	0.769	1.010	270		3	0.048	0.029	0.571	0.054	499		
	Mean	1.035	1.015	0.756	1.005	269		Mean	0.059	0.029	0.569	0.053	486		
US-028	1	0.999	0.980	0.707	0.971	281	US-028	1	0.054	0.029	0.415	0.051	335	Positive	Photoreactive
	2	0.991	0.988	0.738	0.977	241		2	0.063	0.030	0.433	0.053	344		
	3	1.016	0.995	0.740	0.983	265		3	0.054	0.028	0.428	0.061	347		
	Mean	1.002	0.988	0.728	0.977	262		Mean	0.057	0.029	0.425	0.055	342		
Mean for 3 assays	-	-	-	-	267	Mean for 3 assays	-	-	-	-	394	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Fenofibrate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
US-027	1	0.961	0.970	0.827	0.965	126	US-027	1	0.042	0.033	0.058	0.040	2	Positive	Photoreactive
	2	0.975	0.979	0.844	0.970	123		2	0.041	0.030	0.058	0.047	3		
	3	0.979	0.978	0.847	0.969	124		3	0.043	0.029	0.053	0.046	-4		
	Mean	0.972	0.976	0.840	0.968	124		Mean	0.042	0.030	0.057	0.044	0		
US-034	1	1.021	0.974	0.810	0.965	202	US-034	1	0.069	0.036	0.049	0.049	-44	Positive	Photoreactive
	2	1.014	1.001	0.802	0.992	203		2	0.057	0.042	0.049	0.045	-33		
	3	1.029	0.994	0.819	0.985	201		3	0.067	0.029	0.050	0.085	-41		
	Mean	1.022	0.990	0.810	0.981	202		Mean	0.064	0.036	0.049	0.060	-39		
US-036	1	0.995	0.977	0.807	0.965	178	US-036	1	0.066	0.028	0.059	0.043	-31	Positive	Photoreactive
	2	0.997	0.990	0.817	0.984	170		2	0.063	0.032	0.062	0.048	-25		
	3	1.001	0.988	0.819	0.977	172		3	0.066	0.029	0.053	0.072	-38		
	Mean	0.998	0.985	0.814	0.975	173		Mean	0.065	0.030	0.058	0.054	-31		
Mean for 3 assays		-	-	-	-	166	Mean for 3 assays		-	-	-	-	-23	Positive*3	Photoreactive*3

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Furosemide

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement			
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol		
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank					
US-007	1	1.008	1.006	0.773	0.991	224	US-007	1	0.042	0.035	0.166	0.046	109	Positive	Photoreactive	
	2	1.024	1.015	0.784	1.005	229		2	0.043	0.034	0.173	0.054				116
	3	1.028	1.010	0.791	1.002	227		3	0.044	0.033	0.179	0.049				120
	Mean	1.020	1.010	0.783	0.999	227		Mean	0.043	0.034	0.173	0.049				115
US-027	1	0.980	0.970	0.728	0.965	244	US-027	1	0.041	0.033	0.172	0.040	118	Positive	Photoreactive	
	2	0.988	0.979	0.742	0.970	238		2	0.043	0.030	0.178	0.047				122
	3	0.998	0.978	0.759	0.969	231		3	0.045	0.029	0.181	0.046				122
	Mean	0.989	0.976	0.743	0.968	238		Mean	0.043	0.030	0.177	0.044				121
US-034	1	0.993	0.974	0.744	0.965	240	US-034	1	0.044	0.036	0.164	0.049	97	Positive	Photoreactive	
	2	0.988	1.001	0.760	0.992	219		2	0.044	0.042	0.169	0.045				101
	3	0.997	0.994	0.776	0.985	212		3	0.044	0.029	0.175	0.085				107
	Mean	0.993	0.990	0.760	0.981	224		Mean	0.044	0.036	0.169	0.060				102
Mean for 3 assays	-	-	-	-	230	Mean for 3 assays	-	-	-	-	113	Positive	Photoreactive ^{*3}			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Ketoprofen

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-005	1	1.012	1.015	0.640	1.004	361	US-005	1	0.048	0.029	0.185	0.043	126	Positive	Photoreactive
	2	1.024	1.012	0.652	1.002	362		2	0.037	0.043	0.177	0.044	129		
	3	1.027	1.016	0.664	1.007	351		3	0.044	0.029	0.190	0.046	135		
	Mean	1.021	1.015	0.652	1.004	358		Mean	0.043	0.033	0.184	0.044	130		
US-025	1	1.014	0.990	0.627	0.969	365	US-025	1	0.047	0.029	0.184	0.046	116	Positive	Photoreactive
	2	1.017	1.013	0.629	0.988	366		2	0.036	0.047	0.183	0.047	126		
	3	1.025	1.011	0.648	0.993	355		3	0.043	0.030	0.190	0.073	125		
	Mean	1.019	1.005	0.635	0.983	362		Mean	0.042	0.035	0.185	0.056	122		
US-031	1	1.004	0.986	0.624	0.973	371	US-031	1	0.064	0.029	0.195	0.042	119	Positive	Photoreactive
	2	1.005	0.985	0.623	0.979	372		2	0.040	0.042	0.216	0.046	163		
	3	1.008	0.995	0.638	0.985	360		3	0.058	0.029	0.199	0.049	129		
	Mean	1.006	0.989	0.628	0.979	368		Mean	0.054	0.033	0.204	0.046	137		
Mean for 3 assays	-	-	-	-	363	Mean for 3 assays	-	-	-	-	130	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : 6-methylcoumarine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
US-002	1	1.018	1.017	0.894	1.007	115	US-002	1	0.073	0.031	0.174	0.038	94	Positive	Photoreactive
	2	1.036	1.028	0.918	1.021	109		2	0.040	0.043	0.173	0.041	126		
	3	1.048	1.031	0.919	1.020	119		3	0.069	0.030	0.181	0.048	106		
	Mean	1.034	1.025	0.910	1.016	114		Mean	0.061	0.035	0.176	0.042	109		
US-004	1	1.039	0.998	0.907	0.987	123	US-004	1	0.037	0.029	0.187	0.046	126	Positive	Photoreactive
	2	1.038	1.027	0.922	1.019	106		2	0.033	0.029	0.186	0.059	129		
	3	1.038	1.021	0.923	1.010	105		3	0.038	0.029	0.190	0.054	128		
	Mean	1.038	1.015	0.917	1.005	111		Mean	0.036	0.029	0.188	0.053	128		
US-028	1	1.000	0.980	0.859	0.971	130	US-028	1	0.040	0.029	0.197	0.051	131	Positive	Photoreactive
	2	1.001	0.988	0.874	0.977	116		2	0.038	0.030	0.192	0.053	128		
	3	1.009	0.995	0.885	0.983	114		3	0.040	0.028	0.197	0.061	131		
	Mean	1.003	0.988	0.872	0.977	120		Mean	0.039	0.029	0.195	0.055	130		
Mean for 3 assays	-	-	-	-	115	Mean for 3 assays	-	-	-	-	122	Positive	Photoreactive ^{*3}		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : 8-MOP

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-006	1	1.022	1.020	0.924	1.010	87	US-006	1	0.073	0.031	0.166	0.044	69	Positive	Photoreactive
	2	1.023	1.026	0.929	1.011	83		2	0.047	0.031	0.160	0.048	90		
	3	1.021	1.017	0.930	1.008	80		3	0.077	0.031	0.168	0.069	68		
	Mean	1.022	1.021	0.928	1.010	83		Mean	0.066	0.031	0.164	0.054	76		
US-026	1	0.978	0.973	0.869	0.965	101	US-026	1	0.037	0.032	0.191	0.040	142	Positive	Photoreactive
	2	0.986	0.982	0.879	0.977	98		2	0.034	0.038	0.186	0.049	140		
	3	0.991	0.986	0.880	0.976	103		3	0.043	0.029	0.187	0.046	132		
	Mean	0.985	0.981	0.876	0.973	101		Mean	0.038	0.033	0.188	0.045	138		
US-032	1	0.974	0.987	0.883	0.976	79	US-032	1	0.041	0.028	0.174	0.045	113	Positive	Photoreactive
	2	0.987	0.988	0.890	0.975	85		2	0.034	0.045	0.167	0.045	113		
	3	1.000	1.007	0.917	0.995	71		3	0.043	0.028	0.175	0.072	112		
	Mean	0.987	0.994	0.897	0.982	78		Mean	0.040	0.034	0.172	0.054	113		
Mean for 3 assays	-	-	-	-	87	Mean for 3 assays	-	-	-	-	109	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Nalidixic acid

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement			
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol		
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank					
US-006	1	0.997	1.020	0.622	1.010	364	US-006	1	0.047	0.031	0.376	0.044	306	Positive	Photoreactive	
	2	0.996	1.026	0.645	1.011	340		2	0.041	0.031	0.383	0.048				319
	3	1.008	1.017	0.656	1.008	341		3	0.046	0.031	0.510	0.069				441
	Mean	1.000	1.021	0.641	1.010	348		Mean	0.045	0.031	0.423	0.054				355
US-026	1	0.992	0.973	0.799	0.965	185	US-026	1	0.062	0.032	0.327	0.040	254	Positive	Photoreactive	
	2	0.992	0.982	0.791	0.977	193		2	0.033	0.038	0.319	0.049				274
	3	0.999	0.986	0.814	0.976	177		3	0.061	0.029	0.357	0.046				284
	Mean	0.994	0.981	0.801	0.973	185		Mean	0.052	0.033	0.335	0.045				271
US-034	1	0.985	0.974	0.784	0.965	192	US-034	1	0.054	0.036	0.360	0.049	282	Positive	Photoreactive	
	2	0.990	1.001	0.798	0.992	183		2	0.044	0.042	0.351	0.045				283
	3	1.000	0.994	0.821	0.985	170		3	0.058	0.029	0.309	0.085				227
	Mean	0.991	0.990	0.801	0.981	182		Mean	0.052	0.036	0.340	0.060				264
Mean for 3 assays	-	-	-	-	238	Mean for 3 assays	-	-	-	-	297	Positive	Photoreactive ^{*3}			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Nalidixic acid (Na salt)

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-006	1	1.016	1.020	0.812	1.010	193	US-006	1	0.047	0.031	0.319	0.044	249	Positive	Photoreactive
	2	1.022	1.026	0.836	1.011	175		2	0.035	0.031	0.309	0.048	251		
	3	1.047	1.017	0.854	1.008	182		3	0.043	0.031	0.427	0.069	361		
	Mean	1.028	1.021	0.834	1.010	183		Mean	0.042	0.031	0.352	0.054	287		
US-026	1	0.993	0.973	0.823	0.965	162	US-026	1	0.058	0.032	0.288	0.040	219	Positive	Photoreactive
	2	1.005	0.982	0.823	0.977	175		2	0.039	0.038	0.300	0.049	249		
	3	1.009	0.986	0.843	0.976	159		3	0.071	0.029	0.314	0.046	231		
	Mean	1.003	0.981	0.829	0.973	165		Mean	0.056	0.033	0.301	0.045	233		
US-032	1	0.993	0.987	0.790	0.976	191	US-032	1	0.068	0.028	0.284	0.045	196	Positive	Photoreactive
	2	0.992	0.988	0.804	0.975	176		2	0.040	0.045	0.266	0.045	206		
	3	1.008	1.007	0.814	0.995	181		3	0.068	0.028	0.296	0.072	208		
	Mean	0.998	0.994	0.803	0.982	183		Mean	0.058	0.034	0.282	0.054	203		
Mean for 3 assays	-	-	-	-	177	Mean for 3 assays	-	-	-	-	241	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Norfloxacin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-002	1	1.022	1.017	0.791	1.007	222	US-002	1	0.042	0.031	0.197	0.038	147	Positive	Photoreactive
	2	1.040	1.028	0.812	1.021	219		2	0.037	0.043	0.192	0.041	148		
	3	1.039	1.031	0.824	1.020	205		3	0.042	0.030	0.189	0.048	140		
	Mean	1.034	1.025	0.809	1.016	215		Mean	0.040	0.035	0.192	0.042	145		
US-004	1	1.000	0.998	0.766	0.987	224	US-004	1	0.053	0.029	0.216	0.046	139	Positive	Photoreactive
	2	1.036	1.027	0.807	1.019	220		2	0.044	0.029	0.214	0.059	147		
	3	1.038	1.021	0.815	1.010	214		3	0.058	0.029	0.212	0.054	131		
	Mean	1.025	1.015	0.796	1.005	219		Mean	0.051	0.029	0.214	0.053	139		
US-028	1	0.984	0.980	0.754	0.971	220	US-028	1	0.056	0.029	0.199	0.051	117	Positive	Photoreactive
	2	0.992	0.988	0.765	0.977	216		2	0.045	0.030	0.192	0.053	120		
	3	0.997	0.995	0.781	0.983	205		3	0.060	0.028	0.188	0.061	102		
	Mean	0.991	0.988	0.767	0.977	214		Mean	0.054	0.029	0.193	0.055	113		
Mean for 3 assays	-	-	-	-	216	Mean for 3 assays	-	-	-	-	132	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Ofloxacin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
US-002	1	1.026	1.017	0.813	1.007	204	1	0.046	0.031	0.388	0.038	335	Positive	Photoreactive
	2	1.015	1.028	0.814	1.021	192	2	0.044	0.043	0.389	0.041	338		
	3	1.043	1.031	0.852	1.020	182	3	0.048	0.030	0.434	0.048	379		
	Mean	1.028	1.025	0.826	1.016	193	Mean	0.046	0.035	0.403	0.042	351		
US-004	1	1.002	0.998	0.776	0.987	216	1	0.060	0.029	0.296	0.046	212	Positive	Photoreactive
	2	1.027	1.027	0.814	1.019	203	2	0.041	0.029	0.303	0.059	238		
	3	1.031	1.021	0.830	1.010	191	3	0.059	0.029	0.316	0.054	233		
	Mean	1.020	1.015	0.807	1.005	203	Mean	0.053	0.029	0.305	0.053	228		
US-028	1	0.986	0.980	0.769	0.971	206	1	0.062	0.029	0.359	0.051	271	Positive	Photoreactive
	2	0.989	0.988	0.783	0.977	194	2	0.044	0.030	0.359	0.053	289		
	3	0.993	0.995	0.805	0.983	177	3	0.056	0.028	0.345	0.061	262		
	Mean	0.989	0.988	0.786	0.977	192	Mean	0.054	0.029	0.354	0.055	274		
Mean for 3 assays	-	-	-	-	196	Mean for 3 assays	-	-	-	-	284	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Piroxicam

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 20 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
US-006	1	1.010	1.020	0.793	1.010	206	US-0026	1	0.045	0.032	0.088	0.040	32	Positive	Photoreactive
	2	1.027	1.026	0.824	1.011	191		2	0.063	0.038	0.101	0.049	26		
	3	1.033	1.017	0.846	1.008	177		3	0.058	0.029	0.097	0.046	26		
	Mean	1.023	1.021	0.821	1.010	191		Mean	0.055	0.033	0.095	0.045	28		
US-026	1	0.995	0.973	0.761	0.965	226	US-0032	1	0.057	0.028	0.113	0.045	35	Positive	Photoreactive
	2	0.999	0.982	0.792	0.977	200		2	0.070	0.045	0.120	0.045	30		
	3	1.004	0.986	0.793	0.976	203		3	0.050	0.028	0.118	0.072	48		
	Mean	0.999	0.981	0.782	0.973	210		Mean	0.059	0.034	0.117	0.054	38		
US-032	1	1.000	0.987	0.736	0.976	252	US-033	1	0.039	0.033	0.088	0.041	29	Positive	Photoreactive
	2	0.981	0.988	0.758	0.975	211		2	0.051	0.034	0.092	0.051	21		
	3	1.010	1.007	0.764	0.995	234		3	0.047	0.028	0.094	0.064	27		
	Mean	0.997	0.994	0.753	0.982	232		Mean	0.045	0.032	0.091	0.052	26		
Mean for 3 assays	-	-	-	-	211	Mean for 3 assays	-	-	-	-	31	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Promethazine HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
US-001	1	1.002	1.014	0.911	1.006	80	US-001	1	0.041	0.033	0.121	0.046	66	Positive	Photoreactive
	2	1.017	1.028	0.934	1.014	72		2	0.037	0.046	0.119	0.053	68		
	3	1.008	1.029	0.939	1.020	59		3	0.043	0.034	0.123	0.056	67		
	Mean	1.009	1.024	0.928	1.013	70		Mean	0.040	0.038	0.121	0.052	67		
US-023	1	1.001	1.008	0.921	0.991	67	US-023	1	0.040	0.033	0.129	0.039	82	Positive	Photoreactive
	2	1.005	1.024	0.933	1.013	59		2	0.034	0.049	0.127	0.044	86		
	3	1.005	1.022	0.933	1.008	59		3	0.041	0.029	0.136	0.051	89		
	Mean	1.004	1.018	0.929	1.004	62		Mean	0.038	0.037	0.131	0.044	86		
US-029	1	0.971	0.973	0.872	0.960	89	US-029	1	0.038	0.030	0.129	0.038	81	Positive	Photoreactive
	2	0.984	0.986	0.894	0.979	80		2	0.033	0.044	0.131	0.044	89		
	3	0.994	0.987	0.900	0.978	84		3	0.038	0.029	0.136	0.047	89		
	Mean	0.983	0.982	0.889	0.972	84		Mean	0.036	0.034	0.132	0.043	86		
Mean for 3 assays	-	-	-	-	72	Mean for 3 assays	-	-	-	-	80	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Rosiglitazone

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Test concentration
 Singlet oxygen 200 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-003	1	1.000	1.011	0.861	1.001	129	US-0024	1	0.043	0.030	0.099	0.045	Positive	Photoreactive	
	2	0.991	1.028	0.866	1.017	115		2	0.037	0.040	0.098	0.052			45
	3	1.003	1.024	0.883	1.016	110		3	0.044	0.029	0.096	0.051			37
	Mean	0.998	1.021	0.870	1.011	118		Mean	0.041	0.033	0.098	0.049			41
US-024	1	0.966	1.014	0.846	1.003	107	US-030	1	0.044	0.029	0.100	0.047	Positive	Photoreactive	
	2	0.995	1.023	0.876	1.009	105		2	0.040	0.043	0.100	0.051			39
	3	0.994	1.023	0.880	1.006	100		3	0.045	0.030	0.100	0.066			34
	Mean	0.985	1.020	0.868	1.006	104		Mean	0.043	0.034	0.100	0.055			36
US-030	1	0.979	0.997	0.844	1.002	137	US-035	1	0.041	0.034	0.092	0.045	Positive	Photoreactive	
	2	0.965	1.003	0.841	0.996	125		2	0.041	0.030	0.092	0.048			29
	3	0.978	1.001	0.847	1.005	132		3	0.042	0.028	0.092	0.066			28
	Mean	0.974	1.000	0.844	1.001	131		Mean	0.041	0.031	0.092	0.053			29
Mean for 3 assays	-	-	-	-	118	Mean for 3 assays	-	-	-	-	35	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Tetracycline

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-001	1	1.039	1.014	0.810	1.006	217	US-001	1	0.060	0.033	0.271	0.046	197	Positive	Photoreactive
	2	1.037	1.028	0.840	1.014	186		2	0.058	0.046	0.266	0.053	194		
	3	1.063	1.029	0.853	1.020	198		3	0.056	0.034	0.269	0.056	200		
	Mean	1.046	1.024	0.835	1.013	200		Mean	0.058	0.038	0.269	0.052	197		
US-023	1	1.026	1.008	0.807	0.991	205	US-023	1	0.050	0.033	0.268	0.039	211	Positive	Photoreactive
	2	1.011	1.024	0.812	1.013	185		2	0.047	0.049	0.267	0.044	214		
	3	1.039	1.022	0.833	1.008	192		3	0.051	0.029	0.280	0.051	222		
	Mean	1.025	1.018	0.817	1.004	194		Mean	0.049	0.037	0.272	0.044	216		
US-029	1	1.029	0.973	0.781	0.960	237	US-029	1	0.054	0.030	0.274	0.038	212	Positive	Photoreactive
	2	1.038	0.986	0.834	0.979	194		2	0.048	0.044	0.280	0.044	224		
	3	1.052	0.987	0.874	0.978	168		3	0.052	0.029	0.279	0.047	218		
	Mean	1.039	0.982	0.830	0.972	200		Mean	0.051	0.034	0.278	0.043	218		
Mean for 3 assays	-	-	-	-	198	Mean for 3 assays	-	-	-	-	210	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Anthracene

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement			
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol		
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank					
US-024	1	1.088	1.014	0.790	1.003	284	US-024	1	0.040	0.030	0.102	0.045	46	Positive	Photoreactive	
	2	1.094	1.023	0.819	1.009	261		2	0.033	0.040	0.090	0.052				41
	3	1.099	1.023	0.849	1.006	237		3	0.040	0.029	0.087	0.051				31
	Mean	1.094	1.020	0.819	1.006	261		Mean	0.038	0.033	0.093	0.049				39
US-030	1	1.068	0.997	0.717	1.002	353	US-030	1	0.059	0.029	0.106	0.047	26	Positive	Photoreactive	
	2	1.065	1.003	0.715	0.996	352		2	0.033	0.043	0.146	0.051				92
	3	1.073	1.001	0.758	1.005	316		3	0.061	0.030	0.107	0.066				25
	Mean	1.069	1.000	0.730	1.001	340		Mean	0.051	0.034	0.120	0.055				48
US-033	1	1.056	0.982	0.755	0.972	289	US-033	1	0.048	0.033	0.067	0.041	0	Positive	Photoreactive	
	2	1.078	0.989	0.786	0.975	280		2	0.038	0.034	0.060	0.051				2
	3	1.074	0.987	0.814	0.975	248		3	0.050	0.028	0.073	0.064				2
	Mean	1.069	0.986	0.785	0.974	272		Mean	0.045	0.032	0.067	0.052				1
Mean for 3 assays	-	-	-	-	291	Mean for 3 assays	-	-	-	-	29	Positive	Photoreactive ^{*3}			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Avobenzone

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-029	1	1.079	0.973	0.923	0.960	146	US-029	1	0.059	0.030	0.100	0.038	Positive	Photoreactive	
	2	1.091	0.986	0.943	0.979	138		2	0.034	0.044	0.135	0.044			92
	3	1.076	0.987	0.925	0.978	142		3	0.065	0.029	0.107	0.047			33
	Mean	1.082	0.982	0.930	0.972	142		Mean	0.053	0.034	0.114	0.043			52
US-035	1	1.074	0.982	0.951	0.975	114	US-035	1	0.063	0.034	0.101	0.045	Positive	Photoreactive	
	2	1.077	0.985	0.938	0.974	130		2	0.039	0.030	0.101	0.048			40
	3	1.089	0.985	0.962	0.976	118		3	0.075	0.028	0.098	0.066			1
	Mean	1.080	0.984	0.950	0.975	121		Mean	0.059	0.031	0.100	0.053			19
US-036	1	1.068	0.977	0.933	0.965	125	US-036	1	0.053	0.028	0.121	0.043	Positive	Photoreactive	
	2	1.063	0.990	0.938	0.984	115		2	0.044	0.032	0.102	0.048			34
	3	1.072	0.988	0.951	0.977	111		3	0.062	0.029	0.104	0.072			18
	Mean	1.067	0.985	0.941	0.975	117		Mean	0.053	0.030	0.109	0.054			32
Mean for 3 assays	-	-	-	-	127	Mean for 3 assays	-	-	-	-	34	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Bithionol

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-002	1	0.979	1.017	0.886	1.007	84	US-028	1	0.061	0.029	0.106	0.051	Positive	Photoreactive	
	2	0.997	1.028	0.907	1.021	81		2	0.058	0.030	0.109	0.053			25
	3	0.994	1.031	0.906	1.020	79		3	0.063	0.028	0.107	0.061			18
	Mean	0.990	1.025	0.900	1.016	81		Mean	0.060	0.029	0.107	0.055			21
US-004	1	0.989	0.998	0.855	0.987	125	US-030	1	0.070	0.029	0.115	0.047	Positive	Photoreactive	
	2	0.996	1.027	0.880	1.019	106		2	0.060	0.043	0.120	0.051			39
	3	1.003	1.021	0.884	1.010	109		3	0.073	0.030	0.115	0.066			21
	Mean	0.996	1.015	0.873	1.005	113		Mean	0.068	0.034	0.116	0.055			28
US-028	1	0.976	0.980	0.847	0.971	118	US-035	1	0.074	0.034	0.114	0.045	Positive	Photoreactive	
	2	0.968	0.988	0.856	0.977	102		2	0.063	0.030	0.114	0.048			29
	3	0.986	0.995	0.855	0.983	121		3	0.064	0.028	0.112	0.066			26
	Mean	0.977	0.988	0.852	0.977	114		Mean	0.067	0.031	0.113	0.053			24
Mean for 3 assays	-	-	-	-	103	Mean for 3 assays	-	-	-	-	24	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Hexachlorophene

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-001	1	0.958	1.014	0.622	1.006	325	US-001	1	0.085	0.033	0.095	0.046	-3	Positive	Photoreactive
	2	0.968	1.028	0.642	1.014	315		2	0.063	0.046	0.096	0.053	19		
	3	0.975	1.029	0.651	1.020	313		3	0.084	0.034	0.099	0.056	1		
	Mean	0.967	1.024	0.638	1.013	318		Mean	0.077	0.038	0.097	0.052	6		
US-023	1	0.973	1.008	0.615	0.991	344	US-023	1	0.068	0.033	0.096	0.039	21	Positive	Photoreactive
	2	0.955	1.024	0.619	1.013	322		2	0.055	0.049	0.086	0.044	24		
	3	0.963	1.022	0.616	1.008	332		3	0.071	0.029	0.098	0.051	20		
	Mean	0.964	1.018	0.617	1.004	333		Mean	0.065	0.037	0.093	0.044	22		
US-029	1	0.912	0.973	0.559	0.960	343	US-029	1	0.069	0.030	0.074	0.038	-3	Positive	Photoreactive
	2	0.940	0.986	0.577	0.979	352		2	0.045	0.044	0.078	0.044	24		
	3	0.963	0.987	0.582	0.978	370		3	0.068	0.029	0.077	0.047	0		
	Mean	0.938	0.982	0.573	0.972	355		Mean	0.061	0.034	0.077	0.043	7		
Mean for 3 assays	-	-	-	-	335	Mean for 3 assays	-	-	-	-	12	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Rose bengal

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-001	1	1.235	1.014	0.527	1.006	697	US-001	1	3.648	0.033	3.165	0.046	No data ^{*4}	Positive	Photoreactive
	2	1.227	1.028	0.550	1.014	666		2	3.650	0.046	3.200	0.053	No data ^{*4}		
	3	1.249	1.029	0.556	1.020	682		3	3.634	0.034	3.160	0.056	No data ^{*4}		
	Mean	1.237	1.024	0.544	1.013	682		Mean	3.644	0.038	3.175	0.052	No data ^{*4}		
US-023	1	1.255	1.008	0.544	0.991	697	US-023	1	3.607	0.033	3.262	0.039	No data ^{*4}	Positive	Photoreactive
	2	1.260	1.024	0.571	1.013	675		2	3.630	0.049	3.287	0.044	No data ^{*4}		
	3	1.270	1.022	0.574	1.008	682		3	3.589	0.029	3.211	0.051	No data ^{*4}		
	Mean	1.262	1.018	0.563	1.004	685		Mean	3.609	0.037	3.253	0.044	No data ^{*4}		
US-029	1	1.210	0.973	0.511	0.960	689	US-029	1	3.667	0.030	3.171	0.038	No data ^{*4}	Positive	Photoreactive
	2	1.219	0.986	0.547	0.979	662		2	3.669	0.044	3.266	0.044	No data ^{*4}		
	3	1.246	0.987	0.550	0.978	686		3	3.645	0.029	3.172	0.047	No data ^{*4}		
	Mean	1.225	0.982	0.536	0.972	679		Mean	3.660	0.034	3.203	0.043	No data ^{*4}		
Mean for 3 assays	-	-	-	-	682	Mean for 3 assays	-	-	-	-	-	No data ^{*4}	Positive ^{*3}	Photoreactive ^{*3}	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*4 : Over the OD criteria

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Aspirin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-007	1	1.022	1.006	1.012	0.991	-1	US-007	1	0.055	0.035	0.054	0.046	-16	Negative	Non-photoreactive
	2	1.023	1.015	1.010	1.005	3		2	0.039	0.034	0.045	0.054	-9		
	3	1.031	1.010	1.020	1.002	0		3	0.052	0.033	0.056	0.049	-12		
	Mean	1.026	1.010	1.014	0.999	1		Mean	0.049	0.034	0.052	0.049	-12		
US-027	1	0.977	0.970	0.968	0.965	1	US-027	1	0.051	0.033	0.047	0.040	-18	Negative	Non-photoreactive
	2	0.988	0.979	0.975	0.970	5		2	0.040	0.030	0.044	0.047	-9		
	3	0.996	0.978	0.987	0.969	0		3	0.053	0.029	0.050	0.046	-17		
	Mean	0.987	0.976	0.977	0.968	2		Mean	0.048	0.030	0.047	0.044	-15		
US-034	1	0.975	0.974	0.962	0.965	4	US-034	1	0.038	0.036	0.081	0.049	19	Negative	Non-photoreactive
	2	0.996	1.001	0.982	0.992	5		2	0.033	0.042	0.062	0.045	5		
	3	0.995	0.994	0.983	0.985	3		3	0.037	0.029	0.067	0.085	6		
	Mean	0.989	0.990	0.976	0.981	4		Mean	0.036	0.036	0.070	0.060	10		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	-6	Negative*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Benzocaine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
US-002	1	1.029	1.017	1.016	1.007	4	1	0.040	0.031	0.055	0.038	7	Negative	Non-photoreactive
	2	1.039	1.028	1.020	1.021	10	2	0.035	0.043	0.050	0.041	8		
	3	1.040	1.031	1.025	1.020	5	3	0.042	0.030	0.061	0.048	12		
	Mean	1.036	1.025	1.020	1.016	6	Mean	0.039	0.035	0.055	0.042	9		
US-004	1	1.000	0.998	0.988	0.987	2	1	0.053	0.029	0.095	0.046	18	Positive	Weakly photoreactive
	2	1.024	1.027	1.008	1.019	6	2	0.041	0.029	0.091	0.059	26		
	3	1.034	1.021	1.022	1.010	2	3	0.056	0.029	0.096	0.054	15		
	Mean	1.019	1.015	1.006	1.005	3	Mean	0.050	0.029	0.094	0.053	20		
US-028	1	0.995	0.980	0.987	0.971	-3	1	0.053	0.029	0.076	0.051	-3	Negative	Non-photoreactive
	2	0.997	0.988	0.984	0.977	2	2	0.041	0.030	0.067	0.053	-1		
	3	1.001	0.995	0.996	0.983	-5	3	0.052	0.028	0.081	0.061	3		
	Mean	0.998	0.988	0.989	0.977	-2	Mean	0.049	0.029	0.075	0.055	0		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	10	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Erythromycin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen					Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)		A560(-)				A560(+)		Judged by Original Criteria	Judged by criteria for Proposed Protocol			
		Test Chemical	Blank	Test Chemical	Blank	Test Chemical				Blank	Test Chemical				Blank	
US-003	1	1.021	1.011	1.024	1.001	-13	US-003	1	0.058	0.029	0.085	0.049	4	Negative	Non-photoreactive	
	2	1.020	1.028	1.029	1.017	-18		2	0.051	0.044	0.078	0.052	4			
	3	1.035	1.024	1.040	1.016	-16		3	0.059	0.030	0.085	0.070	4			
	Mean	1.025	1.021	1.031	1.011	-16		Mean	0.056	0.034	0.082	0.057	4			
US-024	1	0.996	1.014	0.976	1.003	6	US-024	1	0.051	0.030	0.073	0.045	6	Negative	Non-photoreactive	
	2	0.999	1.023	0.980	1.009	5		2	0.041	0.040	0.069	0.052	11			
	3	1.002	1.023	0.984	1.006	4		3	0.050	0.029	0.073	0.051	7			
	Mean	0.999	1.020	0.980	1.006	5		Mean	0.047	0.033	0.071	0.049	8			
US-030	1	1.001	0.997	0.994	1.002	9	US-030	1	0.046	0.029	0.072	0.047	5	Negative	Non-photoreactive	
	2	0.995	1.003	0.991	0.996	5		2	0.041	0.043	0.071	0.051	9			
	3	1.016	1.001	1.006	1.005	10		3	0.049	0.030	0.075	0.066	5			
	Mean	1.004	1.000	0.997	1.001	8		Mean	0.045	0.034	0.073	0.055	6			
Mean for 3 assays		-	-	-	-	-1	Mean for 3 assays		-	-	-	-	6	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Penicillin G

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-003	1	1.046	1.011	1.015	1.001	21	US-003	1	0.064	0.029	0.097	0.049	10	Negative	Non-photoreactive
	2	1.036	1.028	1.017	1.017	9		2	0.049	0.044	0.096	0.052	24		
	3	1.048	1.024	1.017	1.016	21		3	0.076	0.030	0.097	0.070	-2		
	Mean	1.043	1.021	1.016	1.011	17		Mean	0.063	0.034	0.097	0.057	11		
US-024	1	1.007	1.014	0.987	1.003	6	US-024	1	0.078	0.030	0.106	0.045	13	Positive	Weakly photoreactive
	2	1.016	1.023	0.994	1.009	8		2	0.049	0.040	0.110	0.052	46		
	3	1.033	1.023	1.015	1.006	4		3	0.071	0.029	0.107	0.051	20		
	Mean	1.019	1.020	0.999	1.006	6		Mean	0.066	0.033	0.108	0.049	26		
US-030	1	1.012	0.997	1.006	1.002	6	US-030	1	0.053	0.029	0.110	0.047	36	Positive	Weakly photoreactive
	2	1.008	1.003	0.996	0.996	13		2	0.045	0.043	0.108	0.051	43		
	3	1.014	1.001	1.008	1.005	7		3	0.049	0.030	0.112	0.066	42		
	Mean	1.011	1.000	1.003	1.001	9		Mean	0.049	0.034	0.110	0.055	40		
Mean for 3 assays	-	-	-	-	11	Mean for 3 assays	-	-	-	-	26	Positive	Weakly photoreactive ^{*3}		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Phenytoin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
US-007	1	1.019	1.006	1.006	0.991	2	1	0.047	0.035	0.115	0.046	53	Positive	Weakly photoreactive
	2	1.016	1.015	1.006	1.005	-1	2	0.039	0.034	0.128	0.054	74		
	3	1.025	1.010	1.013	1.002	1	3	0.046	0.033	0.124	0.049	63		
	Mean	1.020	1.010	1.008	0.999	1	Mean	0.044	0.034	0.122	0.049	63		
US-027	1	0.999	0.970	0.970	0.965	21	1	0.045	0.033	0.112	0.040	53	Positive	Weakly photoreactive
	2	1.006	0.979	0.982	0.970	17	2	0.044	0.030	0.118	0.047	60		
	3	1.011	0.978	0.982	0.969	20	3	0.044	0.029	0.111	0.046	53		
	Mean	1.005	0.976	0.978	0.968	19	Mean	0.044	0.030	0.114	0.044	55		
US-035	1	0.985	0.982	0.975	0.975	1	1	0.040	0.034	0.095	0.045	33	Positive	Weakly photoreactive
	2	0.996	0.985	0.982	0.974	5	2	0.034	0.030	0.093	0.048	36		
	3	1.001	0.985	0.989	0.976	2	3	0.050	0.028	0.099	0.066	27		
	Mean	0.994	0.984	0.982	0.975	3	Mean	0.042	0.031	0.096	0.053	32		
Mean for 3 assays	-	-	-	-	8	Mean for 3 assays	-	-	-	-	50	Positive	Weakly photoreactive ^{*3}	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Bumetrizole

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 2 µM

Experimental No.	Run#	Singlet oxygen					Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)		A560(-)				A560(+)		Judged by Original Criteria	Judged by Criteria for Proposed Protocol			
		Test Chemical	Blank	Test Chemical	Blank	Test Chemical				Blank	Test Chemical				Blank	
US-027	1	1.041	0.970	1.041	0.965	-8	US-027	1	0.047	0.033	0.053	0.040	-9	Inconclusive	(Non-photoreactive)	
	2	1.081	0.979	1.079	0.970	-5		2	0.040	0.030	0.048	0.047	-6			
	3	1.086	0.978	1.085	0.969	-7		3	0.048	0.029	0.053	0.046	-9			
	Mean	1.069	0.976	1.068	0.968	-7		Mean	0.045	0.030	0.051	0.044	-8			
US-034	1	1.031	0.974	1.031	0.965	-9	US-034	1	0.039	0.036	0.067	0.049	4	Inconclusive	(Non-photoreactive)	
	2	1.038	1.001	1.040	0.992	-11		2	0.036	0.042	0.072	0.045	12			
	3	1.038	0.994	1.041	0.985	-12		3	0.040	0.029	0.076	0.085	12			
	Mean	1.036	0.990	1.037	0.981	-11		Mean	0.038	0.036	0.072	0.060	9			
US-036	1	1.041	0.977	1.040	0.965	-8	US-036	1	0.037	0.028	0.053	0.043	-8	Inconclusive	(Non-photoreactive)	
	2	1.050	0.990	1.054	0.984	-14		2	0.035	0.032	0.056	0.048	-3			
	3	1.051	0.988	1.052	0.977	-11		3	0.037	0.029	0.053	0.072	-7			
	Mean	1.048	0.985	1.049	0.975	-11		Mean	0.036	0.030	0.054	0.054	-6			
Mean for 3 assays		-	-	-	-	-10	Mean for 3 assays		-	-	-	-	-2	Inconclusive*3 (Non-photoreactive)*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Camphor sulfonic acid

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
US-006	1	1.038	1.020	1.017	1.010	10	1	0.039	0.031	0.057	0.044	-4	Negative	Non-photoreactive
	2	1.039	1.026	1.030	1.011	-2	2	0.034	0.031	0.056	0.048	-1		
	3	1.054	1.017	1.031	1.008	12	3	0.041	0.031	0.057	0.069	-7		
	Mean	1.044	1.021	1.026	1.010	7	Mean	0.038	0.031	0.057	0.054	-4		
US-026	1	0.976	0.973	0.977	0.965	-9	1	0.060	0.032	0.050	0.040	-22	Negative	Non-photoreactive
	2	0.994	0.982	0.979	0.977	7	2	0.043	0.038	0.051	0.049	-4		
	3	0.993	0.986	0.988	0.976	-3	3	0.061	0.029	0.062	0.046	-11		
	Mean	0.988	0.981	0.981	0.973	-2	Mean	0.055	0.033	0.054	0.045	-12		
US-032	1	1.001	0.987	0.977	0.976	12	1	0.075	0.028	0.068	0.045	-27	Negative	Non-photoreactive
	2	1.004	0.988	0.990	0.975	2	2	0.043	0.045	0.057	0.045	-6		
	3	1.014	1.007	0.993	0.995	9	3	0.073	0.028	0.061	0.072	-32		
	Mean	1.006	0.994	0.987	0.982	8	Mean	0.064	0.034	0.062	0.054	-22		
Mean for 3 assays	-	-	-	-	4	Mean for 3 assays	-	-	-	-	-13	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Chlorhexidine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-007	1	0.911	1.006	0.906	0.991	-6	US-007	1	0.044	0.035	0.086	0.046	26	Positive	Weakly photoreactive
	2	0.964	1.015	0.958	1.005	-5		2	0.046	0.034	0.096	0.054	35		
	3	0.983	1.010	0.975	1.002	-3		3	0.046	0.033	0.084	0.049	23		
	Mean	0.953	1.010	0.947	0.999	-5		Mean	0.045	0.034	0.088	0.049	28		
US-027	1	0.880	0.970	0.865	0.965	7	US-027	1	0.051	0.033	0.103	0.040	38	Positive	Weakly photoreactive
	2	0.925	0.979	0.909	0.970	8		2	0.049	0.030	0.099	0.047	36		
	3	0.937	0.978	0.921	0.969	8		3	0.052	0.029	0.098	0.046	32		
	Mean	0.914	0.976	0.898	0.968	8		Mean	0.051	0.030	0.100	0.044	35		
US-035	1	0.882	0.982	0.882	0.975	-9	US-035	1	0.049	0.034	0.079	0.045	8	Negative	Non-photoreactive
	2	0.917	0.985	0.922	0.974	-13		2	0.048	0.030	0.076	0.048	6		
	3	0.932	0.985	0.937	0.976	-14		3	0.048	0.028	0.075	0.066	5		
	Mean	0.910	0.984	0.913	0.975	-12		Mean	0.048	0.031	0.077	0.053	6		
Mean for 3 assays	-	-	-	-	-3	Mean for 3 assays	-	-	-	-	23	Positive*3	Weakly photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Cinnamic acid

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-003	1	1.022	1.011	1.001	1.001	11	US-003	1	0.056	0.029	0.135	0.049	56	Positive	Weakly photoreactive
	2	1.030	1.028	1.025	1.017	-5		2	0.033	0.044	0.141	0.052	84		
	3	1.054	1.024	1.033	1.016	11		3	0.059	0.030	0.124	0.070	42		
	Mean	1.036	1.021	1.020	1.011	6		Mean	0.049	0.034	0.133	0.057	61		
US-024	1	1.034	1.014	1.020	1.003	0	US-024	1	0.062	0.030	0.129	0.045	51	Positive	Photoreactive
	2	1.026	1.023	1.013	1.009	-1		2	0.031	0.040	0.151	0.052	104		
	3	1.039	1.023	1.025	1.006	0		3	0.062	0.029	0.131	0.051	54		
	Mean	1.033	1.020	1.020	1.006	0		Mean	0.052	0.033	0.137	0.049	70		
US-031	1	1.012	0.986	1.002	0.973	0	US-031	1	0.062	0.029	0.102	0.042	27	Positive	Weakly photoreactive
	2	1.008	0.985	0.997	0.979	1		2	0.040	0.042	0.098	0.046	46		
	3	1.015	0.995	1.006	0.985	-1		3	0.058	0.029	0.105	0.049	34		
	Mean	1.012	0.989	1.002	0.979	0		Mean	0.053	0.033	0.102	0.046	36		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	56	Positive	Weakly photoreactive ^{*3}		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Drometrizole

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
US-025	1	1.036	0.990	1.015	0.969	0	1	0.064	0.029	0.071	0.046	-14	Inconclusive	Non-photoreactive
	2	1.028	1.013	0.999	0.988	7	2	0.045	0.047	0.057	0.047	-9		
	3	1.045	1.011	1.010	0.993	13	3	0.065	0.030	0.078	0.073	-9		
	Mean	1.036	1.005	1.008	0.983	7	Mean	0.058	0.035	0.069	0.056	-11		
US-032	1	0.984	0.987	0.979	0.976	-7	1	0.037	0.028	0.068	0.045	11	Inconclusive	Non-photoreactive
	2	0.990	0.988	0.976	0.975	2	2	0.034	0.045	0.066	0.045	12		
	3	0.991	1.007	0.986	0.995	-7	3	0.038	0.028	0.074	0.072	15		
	Mean	0.988	0.994	0.980	0.982	-4	Mean	0.037	0.034	0.069	0.054	13		
US-033	1	0.976	0.982	0.972	0.972	-8	1	0.055	0.033	0.073	0.041	-2	Inconclusive	Non-photoreactive
	2	0.996	0.989	0.991	0.975	-7	2	0.047	0.034	0.056	0.051	-10		
	3	1.005	0.987	0.998	0.975	-5	3	0.058	0.028	0.068	0.064	-11		
	Mean	0.992	0.986	0.987	0.974	-7	Mean	0.053	0.032	0.066	0.052	-8		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	-2	Inconclusive*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : L-Histidine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
US-028	1	0.979	0.980	0.952	0.971	16	1	0.031	0.029	0.116	0.051	59	Positive	Weakly photoreactive
	2	0.979	0.988	0.962	0.977	6	2	0.030	0.030	0.118	0.053	62		
	3	0.986	0.995	0.961	0.983	15	3	0.032	0.028	0.119	0.061	61		
	Mean	0.981	0.988	0.958	0.977	12	Mean	0.031	0.029	0.117	0.055	61		
US-030	1	0.986	0.997	0.972	1.002	15	1	0.033	0.029	0.129	0.047	76	Positive	Photoreactive
	2	0.994	1.003	0.978	0.996	16	2	0.031	0.043	0.119	0.051	67		
	3	0.996	1.001	0.982	1.005	15	3	0.034	0.030	0.131	0.066	76		
	Mean	0.992	1.000	0.977	1.001	15	Mean	0.032	0.034	0.126	0.055	73		
US-035	1	0.985	0.982	0.969	0.975	7	1	0.056	0.034	0.115	0.045	37	Positive	Weakly photoreactive
	2	0.993	0.985	0.977	0.974	7	2	0.029	0.030	0.111	0.048	60		
	3	0.997	0.985	0.983	0.976	6	3	0.033	0.028	0.111	0.066	56		
	Mean	0.992	0.984	0.976	0.975	7	Mean	0.039	0.031	0.112	0.053	51		
Mean for 3 assays	-	-	-	-	11	Mean for 3 assays	-	-	-	-	62	Positive	Weakly photoreactive ^{*3}	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Methylbenzylidene camphor

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
US-026	1	0.985	0.973	0.988	0.965	-11	US-026	1	0.047	0.032	0.051	0.040	-8	Inconclusive	Non-photoreactive
	2	0.994	0.982	0.987	0.977	-1		2	0.032	0.038	0.043	0.049	-1		
	3	0.995	0.986	0.997	0.976	-9		3	0.049	0.029	0.052	0.046	-9		
	Mean	0.991	0.981	0.991	0.973	-7		Mean	0.043	0.033	0.049	0.045	-6		
US-034	1	0.968	0.974	0.961	0.965	-2	US-034	1	0.031	0.036	0.061	0.049	6	Inconclusive	Non-photoreactive
	2	0.981	1.001	0.975	0.992	-3		2	0.030	0.042	0.052	0.045	-2		
	3	0.978	0.994	0.972	0.985	-3		3	0.032	0.029	0.067	0.085	11		
	Mean	0.976	0.990	0.969	0.981	-3		Mean	0.031	0.036	0.060	0.060	5		
US-036	1	0.971	0.977	0.965	0.965	-4	US-036	1	0.041	0.028	0.064	0.043	-1	Inconclusive	Non-photoreactive
	2	0.981	0.990	0.973	0.984	-2		2	0.032	0.032	0.051	0.048	-6		
	3	0.982	0.988	0.978	0.977	-6		3	0.043	0.029	0.063	0.072	-4		
	Mean	0.978	0.985	0.972	0.975	-4		Mean	0.039	0.030	0.060	0.054	-4		
Mean for 3 assays	-	-	-	-	-5	Mean for 3 assays	-	-	-	-	-2	Inconclusive*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Octrizole

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement			
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol		
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank					
US-026	1	1.003	0.973	0.989	0.965	6	US-026	1	0.039	0.032	0.105	0.040	54	Positive	(Weakly photoreactive)	
	2	0.999	0.982	0.988	0.977	3		2	0.036	0.038	0.094	0.049				46
	3	1.009	0.986	0.999	0.976	2		3	0.041	0.029	0.121	0.046				67
	Mean	1.004	0.981	0.992	0.973	4		Mean	0.039	0.033	0.106	0.045				56
US-032	1	0.977	0.987	0.978	0.976	-13	US-032	1	0.041	0.028	0.113	0.045	52	Positive	(Weakly photoreactive)	
	2	0.992	0.988	0.973	0.975	6		2	0.035	0.045	0.089	0.045				34
	3	0.992	1.007	0.992	0.995	-12		3	0.038	0.028	0.114	0.072				56
	Mean	0.987	0.994	0.981	0.982	-6		Mean	0.038	0.034	0.106	0.054				47
US-033	1	0.984	0.982	0.969	0.972	3	US-033	1	0.037	0.033	0.082	0.041	25	Positive	(Weakly photoreactive)	
	2	0.987	0.989	0.971	0.975	4		2	0.035	0.034	0.071	0.051				16
	3	0.992	0.987	0.984	0.975	-4		3	0.040	0.028	0.085	0.064				25
	Mean	0.987	0.986	0.975	0.974	1		Mean	0.037	0.032	0.079	0.052				22
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	42	Positive	(Weakly photoreactive)*3			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Octyl methacrylate

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
US-001	1	1.028	1.014	0.994	1.006	24	US-001	1	0.067	0.033	0.070	0.046	-10	Negative	Non-photoreactive
	2	1.034	1.028	1.011	1.014	12		2	0.044	0.046	0.059	0.053	1		
	3	1.049	1.029	1.022	1.020	16		3	0.062	0.034	0.077	0.056	1		
	Mean	1.037	1.024	1.009	1.013	17		Mean	0.058	0.038	0.069	0.052	-3		
US-023	1	1.053	1.008	1.014	0.991	25	US-023	1	0.086	0.033	0.066	0.039	-27	Positive	Photoreactive
	2	1.047	1.024	1.008	1.013	26		2	0.071	0.049	0.052	0.044	-27		
	3	1.053	1.022	1.012	1.008	27		3	0.083	0.029	0.070	0.051	-20		
	Mean	1.051	1.018	1.011	1.004	26		Mean	0.080	0.037	0.063	0.044	-25		
US-029	1	1.031	0.973	0.967	0.960	54	US-029	1	0.091	0.030	0.055	0.038	-45	Positive	Photoreactive
	2	1.036	0.986	0.975	0.979	51		2	0.073	0.044	0.050	0.044	-32		
	3	1.044	0.987	0.983	0.978	51		3	0.090	0.029	0.061	0.047	-38		
	Mean	1.037	0.982	0.975	0.972	52		Mean	0.085	0.034	0.055	0.043	-38		
Mean for 3 assays	-	-	-	-	32	Mean for 3 assays	-	-	-	-	-22	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Octyl methoxycinnamate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
US-027	1	0.990	0.970	0.977	0.965	5	US-027	1	0.072	0.033	0.068	0.040	-18	Inconclusive	Non-photoreactive
	2	1.001	0.979	0.989	0.970	5		2	0.067	0.030	0.067	0.047	-14		
	3	1.007	0.978	0.991	0.969	8		3	0.073	0.029	0.069	0.046	-18		
	Mean	0.999	0.976	0.985	0.968	6		Mean	0.070	0.030	0.068	0.044	-17		
US-034	1	1.008	0.974	0.998	0.965	1	US-034	1	0.081	0.036	0.079	0.049	-27	Inconclusive	Non-photoreactive
	2	1.021	1.001	1.010	0.992	3		2	0.063	0.042	0.099	0.045	12		
	3	1.032	0.994	1.021	0.985	3		3	0.082	0.029	0.076	0.085	-30		
	Mean	1.021	0.990	1.010	0.981	2		Mean	0.075	0.036	0.085	0.060	-15		
US-036	1	1.012	0.977	0.993	0.965	9	US-036	1	0.061	0.028	0.073	0.043	-12	Inconclusive	Non-photoreactive
	2	1.017	0.990	0.999	0.984	8		2	0.058	0.032	0.070	0.048	-12		
	3	1.018	0.988	1.010	0.977	-1		3	0.059	0.029	0.074	0.072	-9		
	Mean	1.016	0.985	1.000	0.975	5		Mean	0.059	0.030	0.072	0.054	-11		
Mean for 3 assays		-	-	-	-	4	Mean for 3 assays		-	-	-	-	-14	Inconclusive*3	Non-photoreactive*3

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : Octyl salicylate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
US-024	1	1.026	1.014	1.013	1.003	-1	1	0.054	0.030	0.081	0.045	11	Inconclusive	Non-photoreactive
	2	1.027	1.023	1.011	1.009	3	2	0.041	0.040	0.067	0.052	10		
	3	1.040	1.023	1.026	1.006	-1	3	0.054	0.029	0.084	0.051	14		
	Mean	1.031	1.020	1.017	1.006	0	Mean	0.050	0.033	0.077	0.049	12		
US-030	1	1.019	0.997	1.005	1.002	15	1	0.061	0.029	0.086	0.047	4	Inconclusive	Non-photoreactive
	2	1.014	1.003	1.003	0.996	13	2	0.048	0.043	0.065	0.051	-4		
	3	1.022	1.001	1.010	1.005	13	3	0.058	0.030	0.086	0.066	7		
	Mean	1.018	1.000	1.006	1.001	14	Mean	0.056	0.034	0.079	0.055	2		
US-035	1	0.996	0.982	0.985	0.975	3	1	0.042	0.034	0.067	0.045	2	Inconclusive	Non-photoreactive
	2	0.990	0.985	0.982	0.974	-1	2	0.042	0.030	0.063	0.048	-1		
	3	1.016	0.985	1.003	0.976	5	3	0.042	0.028	0.068	0.066	3		
	Mean	1.001	0.984	0.990	0.975	2	Mean	0.042	0.031	0.066	0.053	1		
Mean for 3 assays	-	-	-	-	5	Mean for 3 assays	-	-	-	-	5	Inconclusive*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : PABA

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen					Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)		A560(-)				A560(+)		Judged by Original Criteria	Judged by Criteria for Proposed Protocol			
		Test Chemical	Blank	Test Chemical	Blank	Test Chemical				Blank	Test Chemical				Blank	
US-005	1	1.021	1.015	0.994	1.004	17	US-005	1	0.038	0.029	0.057	0.043	8	Negative	Non-photoreactive	
	2	1.045	1.012	1.014	1.002	20		2	0.032	0.043	0.051	0.044	9			
	3	1.050	1.016	1.023	1.007	16		3	0.036	0.029	0.059	0.046	12			
	Mean	1.039	1.015	1.010	1.004	18		Mean	0.035	0.033	0.056	0.044	10			
US-025	1	0.997	0.990	0.985	0.969	-10	US-025	1	0.036	0.029	0.052	0.046	-4	Negative	Non-photoreactive	
	2	1.003	1.013	0.982	0.988	-1		2	0.031	0.047	0.048	0.047	-4			
	3	1.004	1.011	0.995	0.993	-13		3	0.039	0.030	0.052	0.073	-8			
	Mean	1.001	1.005	0.987	0.983	-8		Mean	0.035	0.035	0.051	0.056	-5			
US-031	1	0.998	0.986	0.985	0.973	3	US-031	1	0.038	0.029	0.057	0.042	6	Negative	Non-photoreactive	
	2	1.006	0.985	0.990	0.979	6		2	0.033	0.042	0.055	0.046	9			
	3	1.008	0.995	0.993	0.985	5		3	0.038	0.029	0.059	0.049	8			
	Mean	1.004	0.989	0.989	0.979	5		Mean	0.036	0.033	0.057	0.046	8			
Mean for 3 assays		-	-	-	-	5	Mean for 3 assays		-	-	-	-	4	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : SDS

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
US-005	1	0.975	1.015	0.950	1.004	13	US-031	1	0.057	0.029	0.089	0.042	Inconclusive	Non-photoreactive
	2	1.004	1.012	0.975	1.002	19		2	0.056	0.042	0.088	0.046		
	3	1.020	1.016	0.988	1.007	21		3	0.059	0.029	0.083	0.049		
	Mean	0.999	1.015	0.971	1.004	18		Mean	0.057	0.033	0.087	0.046		
US-025	1	0.994	0.990	0.953	0.969	20	US-033	1	0.066	0.033	0.088	0.041	Inconclusive	Non-photoreactive
	2	0.999	1.013	0.963	0.988	14		2	0.064	0.034	0.088	0.051		
	3	1.004	1.011	0.965	0.993	17		3	0.066	0.028	0.096	0.064		
	Mean	0.999	1.005	0.960	0.983	17		Mean	0.065	0.032	0.091	0.052		
US-031	1	0.969	0.986	0.944	0.973	15	US-036	1	0.063	0.028	0.091	0.043	Inconclusive	Non-photoreactive
	2	0.973	0.985	0.944	0.979	19		2	0.066	0.032	0.098	0.048		
	3	0.982	0.995	0.960	0.985	12		3	0.064	0.029	0.086	0.072		
	Mean	0.975	0.989	0.949	0.979	15		Mean	0.064	0.030	0.092	0.054		
Mean for 3 assays	-	-	-	-	17	Mean for 3 assays	-	-	-	-	8	Inconclusive*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 1
 Chemical Name : UV-571

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
US-024	1	1.064	1.014	1.069	1.003	-19	US-024	1	0.057	0.030	0.084	0.045	11	Inconclusive (Non-photoreactive)
	2	1.062	1.023	1.058	1.009	-10		2	0.040	0.040	0.070	0.052	14	
	3	1.077	1.023	1.080	1.006	-18		3	0.066	0.029	0.087	0.051	5	
	Mean	1.068	1.020	1.069	1.006	-16		Mean	0.054	0.033	0.080	0.049	10	
US-033	1	1.032	0.982	1.042	0.972	-22	US-033	1	0.037	0.033	0.065	0.041	8	Inconclusive (Non-photoreactive)
	2	1.058	0.989	1.062	0.975	-16		2	0.036	0.034	0.056	0.051	0	
	3	1.049	0.987	1.055	0.975	-18		3	0.039	0.028	0.064	0.064	5	
	Mean	1.046	0.986	1.053	0.974	-19		Mean	0.037	0.032	0.062	0.052	4	
US-036	1	1.061	0.977	1.068	0.965	-17	US-036	1	0.038	0.028	0.082	0.043	19	Inconclusive (Non-photoreactive)
	2	1.053	0.990	1.057	0.984	-14		2	0.033	0.032	0.060	0.048	3	
	3	1.052	0.988	1.057	0.977	-15		3	0.039	0.029	0.086	0.072	23	
	Mean	1.055	0.985	1.061	0.975	-15		Mean	0.037	0.030	0.076	0.054	15	
Mean for 3 assays	-	-	-	-	-17	Mean for 3 assays	-	-	-	-	10	Inconclusive*3 (Non-photoreactive)*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Acridine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
FDSC-06	1	0.959	0.982	0.719	0.972	231	FDSC-06	1	0.032	0.033	0.250	0.052	Positive	Photoreactive	
	2	0.965	0.977	0.732	0.965	224		2	0.033	0.034	0.240	0.047			189
	3	0.964	0.982	0.723	0.976	232		3	0.032	0.033	0.233	0.055			183
	Mean	0.963	0.980	0.725	0.971	229		Mean	0.032	0.033	0.241	0.051			191
FDSC-15	1	0.955	0.974	0.724	0.967	226	FDSC-15	1	0.032	0.033	0.256	0.054	Positive	Photoreactive	
	2	0.962	0.975	0.748	0.971	209		2	0.032	0.032	0.255	0.048			208
	3	0.971	0.982	0.747	0.977	219		3	0.033	0.035	0.259	0.043			211
	Mean	0.963	0.977	0.740	0.972	218		Mean	0.032	0.033	0.257	0.048			209
FDSC-22	1	0.948	0.980	0.714	0.974	228	FDSC-22	1	0.034	0.031	0.234	0.040	Positive	Photoreactive	
	2	0.956	0.982	0.755	0.974	195		2	0.035	0.033	0.242	0.057			193
	3	0.956	0.991	0.732	0.986	218		3	0.033	0.032	0.239	0.042			192
	Mean	0.953	0.984	0.734	0.978	214		Mean	0.034	0.032	0.238	0.046			190
Mean for 3 assays	-	-	-	-	220	Mean for 3 assays	-	-	-	-	197	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Acridine HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-04	1	0.951	0.972	0.707	0.963	234	1	0.030	0.032	0.233	0.050	186	Positive	Photoreactive
	2	0.958	0.970	0.728	0.959	220	2	0.034	0.032	0.256	0.049	205		
	3	0.951	0.975	0.717	0.963	224	3	0.031	0.033	0.250	0.049	202		
	Mean	0.953	0.972	0.717	0.962	226	Mean	0.032	0.032	0.246	0.049	198		
FDSC-13	1	0.946	0.970	0.722	0.970	219	1	0.032	0.034	0.259	0.043	218	Positive	Photoreactive
	2	0.964	0.970	0.743	0.962	216	2	0.034	0.034	0.249	0.043	206		
	3	0.955	0.972	0.736	0.965	214	3	0.032	0.033	0.252	0.044	211		
	Mean	0.955	0.971	0.734	0.966	216	Mean	0.033	0.034	0.253	0.043	212		
FDSC-20	1	0.959	0.974	0.684	0.949	256	1	0.032	0.032	0.204	0.046	160	Positive	Photoreactive
	2	0.972	0.972	0.745	0.954	208	2	0.032	0.032	0.218	0.045	174		
	3	0.971	0.980	0.744	0.966	208	3	0.033	0.034	0.254	0.045	209		
	Mean	0.967	0.975	0.724	0.956	224	Mean	0.032	0.033	0.225	0.045	181		
Mean for 3 assays	-	-	-	-	222	Mean for 3 assays	-	-	-	-	197	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Amiodarone HCl

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-10	1	0.985	0.982	0.950	0.976	26	1	0.034	0.032	0.055	0.047	2	Positive	(Photoreactive)
	2	0.988	0.992	0.951	0.975	28	2	0.033	0.032	0.056	0.053	4		
	3	0.990	0.987	0.961	0.984	20	3	0.033	0.034	0.055	0.055	3		
	Mean	0.988	0.987	0.954	0.978	25	Mean	0.033	0.033	0.055	0.052	3		
FDSC-16	1	0.985	0.986	0.939	0.996	46	1	0.033	0.031	0.049	0.041	5	Positive	(Photoreactive)
	2	0.988	0.982	0.953	0.980	35	2	0.034	0.032	0.051	0.044	6		
	3	0.989	0.993	0.957	0.986	32	3	0.035	0.033	0.049	0.043	3		
	Mean	0.987	0.987	0.950	0.987	38	Mean	0.034	0.032	0.050	0.043	5		
FDSC-23	1	0.965	0.957	0.928	0.946	27	1	0.033	0.032	0.046	0.059	-5	Positive	(Photoreactive)
	2	0.977	0.960	0.937	0.947	30	2	0.035	0.032	0.050	0.045	-3		
	3	0.975	0.965	0.938	0.959	27	3	0.034	0.033	0.045	0.045	-7		
	Mean	0.972	0.961	0.934	0.951	28	Mean	0.034	0.032	0.047	0.050	-5		
Mean for 3 assays	-	-	-	-	30	Mean for 3 assays	-	-	-	-	1	Positive*3	(Photoreactive)*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Chlorpromazine HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-05	1	0.954	0.970	0.961	0.966	-8	1	0.038	0.032	0.147	0.048	93	Positive	Photoreactive
	2	0.972	0.966	0.969	0.973	2	2	0.033	0.032	0.147	0.051	98		
	3	0.962	0.975	0.961	0.968	0	3	0.034	0.032	0.150	0.046	100		
	Mean	0.963	0.970	0.964	0.969	-2	Mean	0.035	0.032	0.148	0.048	97		
FDSC-14	1	0.953	0.971	0.958	0.960	-15	1	0.031	0.032	0.143	0.044	99	Positive	Photoreactive
	2	0.960	0.968	0.965	0.959	-15	2	0.033	0.033	0.143	0.050	97		
	3	0.960	0.974	0.960	0.963	-10	3	0.036	0.033	0.150	0.045	101		
	Mean	0.958	0.971	0.961	0.961	-13	Mean	0.033	0.033	0.145	0.046	99		
FDSC-21	1	0.939	0.959	0.944	0.967	-7	1	0.032	0.031	0.147	0.043	106	Positive	Photoreactive
	2	0.956	0.958	0.960	0.953	-6	2	0.032	0.032	0.145	0.041	104		
	3	0.946	0.963	0.950	0.955	-6	3	0.032	0.035	0.158	0.042	117		
	Mean	0.947	0.960	0.951	0.958	-6	Mean	0.032	0.033	0.150	0.042	109		
Mean for 3 assays	-	-	-	-	-7	Mean for 3 assays	-	-	-	-	102	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Doxycycline HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
FDSC-06	1	0.952	0.982	0.717	0.972	226	FDSC-06	1	0.038	0.033	0.463	0.052	Positive	Photoreactive	
	2	0.964	0.977	0.733	0.965	222		2	0.038	0.034	0.466	0.047			410
	3	0.964	0.982	0.738	0.976	217		3	0.039	0.033	0.480	0.055			423
	Mean	0.960	0.980	0.729	0.971	222		Mean	0.038	0.033	0.470	0.051			413
FDSC-14	1	0.940	0.971	0.688	0.960	242	FDSC-14	1	0.041	0.032	0.485	0.044	Positive	Photoreactive	
	2	0.950	0.968	0.711	0.959	229		2	0.040	0.033	0.488	0.050			435
	3	0.956	0.974	0.716	0.963	230		3	0.041	0.033	0.500	0.045			446
	Mean	0.949	0.971	0.705	0.961	234		Mean	0.041	0.033	0.491	0.046			437
FDSC-21	1	0.934	0.959	0.705	0.967	227	FDSC-21	1	0.040	0.031	0.453	0.043	Positive	Photoreactive	
	2	0.951	0.958	0.731	0.953	218		2	0.040	0.032	0.459	0.041			410
	3	0.947	0.963	0.725	0.955	220		3	0.041	0.035	0.468	0.042			418
	Mean	0.944	0.960	0.720	0.958	222		Mean	0.040	0.033	0.460	0.042			411
Mean for 3 assays	-	-	-	-	226	Mean for 3 assays	-	-	-	-	420	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Fenofibrate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Test concentration
 Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-10	1	0.975	0.982	0.960	0.976	6	1	0.033	0.032	0.044	0.047	-8	Inconclusive	(Non-photoreactive)
	2	0.989	0.992	0.971	0.975	9	2	0.032	0.032	0.046	0.053	-5		
	3	0.985	0.987	0.977	0.984	-1	3	0.032	0.034	0.045	0.055	-6		
	Mean	0.983	0.987	0.969	0.978	5	Mean	0.032	0.033	0.045	0.052	-6		
FDSC-16	1	0.972	0.986	0.954	0.996	18	1	0.032	0.031	0.046	0.041	3	Inconclusive	(Non-photoreactive)
	2	0.976	0.982	0.957	0.980	19	2	0.031	0.032	0.059	0.044	17		
	3	0.981	0.993	0.963	0.986	18	3	0.032	0.033	0.075	0.043	32		
	Mean	0.976	0.987	0.958	0.987	18	Mean	0.032	0.032	0.060	0.043	17		
FDSC-23	1	0.958	0.957	0.938	0.946	10	1	0.031	0.032	0.040	0.059	-9	Inconclusive	(Non-photoreactive)
	2	0.965	0.960	0.940	0.947	15	2	0.031	0.032	0.041	0.045	-8		
	3	0.969	0.965	0.950	0.959	9	3	0.032	0.033	0.041	0.045	-9		
	Mean	0.964	0.961	0.943	0.951	11	Mean	0.031	0.032	0.041	0.050	-9		
Mean for 3 assays	-	-	-	-	11	Mean for 3 assays	-	-	-	-	1	Inconclusive*3	(Non-photoreactive)*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Furosemide

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-01	1	0.958	0.955	0.803	0.948	148	1	0.037	0.033	0.108	0.043	60	Positive	Photoreactive
	2	0.978	0.963	0.830	0.956	141	2	0.038	0.037	0.112	0.050	63		
	3	0.972	0.967	0.839	0.961	126	3	0.043	0.033	0.117	0.042	63		
	Mean	0.969	0.962	0.824	0.955	138	Mean	0.039	0.034	0.112	0.045	62		
FDSC-12	1	0.962	0.967	0.815	0.961	140	1	0.038	0.033	0.119	0.049	66	Positive	Photoreactive
	2	0.965	0.962	0.829	0.954	129	2	0.036	0.035	0.113	0.050	62		
	3	0.963	0.967	0.832	0.959	124	3	0.040	0.033	0.127	0.047	72		
	Mean	0.963	0.965	0.825	0.958	131	Mean	0.038	0.034	0.120	0.049	67		
FDSC-19	1	0.972	0.986	0.828	0.990	140	1	0.036	0.033	0.123	0.049	69	Positive	Photoreactive
	2	0.970	0.981	0.832	0.975	134	2	0.034	0.032	0.118	0.054	66		
	3	0.969	0.991	0.841	0.982	124	3	0.035	0.033	0.118	0.050	65		
	Mean	0.970	0.986	0.834	0.982	133	Mean	0.035	0.033	0.120	0.051	67		
Mean for 3 assays	-	-	-	-	134	Mean for 3 assays	-	-	-	-	65	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Ketoprofen

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
FDSC-04	1	0.976	0.972	0.714	0.963	252	FDSC-04	1	0.036	0.032	0.161	0.050	Positive	Photoreactive	
	2	0.982	0.970	0.728	0.959	244		2	0.034	0.032	0.158	0.049			107
	3	0.975	0.975	0.725	0.963	240		3	0.035	0.033	0.159	0.049			107
	Mean	0.978	0.972	0.722	0.962	245		Mean	0.035	0.032	0.159	0.049			107
FDSC-13	1	0.972	0.970	0.696	0.970	271	FDSC-13	1	0.035	0.034	0.160	0.043	Positive	Photoreactive	
	2	0.969	0.970	0.702	0.962	262		2	0.035	0.034	0.159	0.043			115
	3	0.965	0.972	0.716	0.965	244		3	0.034	0.033	0.162	0.044			119
	Mean	0.969	0.971	0.705	0.966	259		Mean	0.035	0.034	0.160	0.043			117
FDSC-20	1	0.926	0.974	0.663	0.949	244	FDSC-20	1	0.034	0.032	0.151	0.046	Positive	Photoreactive	
	2	0.932	0.972	0.677	0.954	236		2	0.034	0.032	0.158	0.045			112
	3	0.936	0.980	0.677	0.966	240		3	0.036	0.034	0.159	0.045			111
	Mean	0.931	0.975	0.672	0.956	240		Mean	0.035	0.033	0.156	0.045			109
Mean for 3 assays	-	-	-	-	248	Mean for 3 assays	-	-	-	-	111	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : 6-methylcoumarine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
FDSC-03	1	0.961	0.966	0.835	0.962	123	FDSC-03	1	0.032	0.033	0.136	0.050	87	Positive	Photoreactive
	2	0.963	0.970	0.877	0.968	83		2	0.032	0.033	0.133	0.050	84		
	3	0.968	0.974	0.862	0.970	103		3	0.034	0.035	0.141	0.053	90		
	Mean	0.964	0.970	0.858	0.967	103		Mean	0.033	0.034	0.137	0.051	87		
FDSC-12	1	0.969	0.967	0.851	0.961	111	FDSC-12	1	0.033	0.033	0.157	0.049	109	Positive	Photoreactive
	2	0.975	0.962	0.842	0.954	126		2	0.032	0.035	0.157	0.050	110		
	3	0.974	0.967	0.839	0.959	128		3	0.033	0.033	0.160	0.047	112		
	Mean	0.973	0.965	0.844	0.958	122		Mean	0.033	0.034	0.158	0.049	110		
FDSC-19	1	0.967	0.986	0.818	0.990	145	FDSC-19	1	0.032	0.033	0.146	0.049	96	Positive	Photoreactive
	2	0.978	0.981	0.833	0.975	141		2	0.031	0.032	0.149	0.054	100		
	3	0.978	0.991	0.834	0.982	140		3	0.031	0.033	0.152	0.050	103		
	Mean	0.974	0.986	0.828	0.982	142		Mean	0.031	0.033	0.149	0.051	100		
Mean for 3 assays		-	-	-	-	122	Mean for 3 assays		-	-	-	-	99	Positive*3	Photoreactive*3

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : 8-MOP

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	A440(-)			A440(+)			A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
FDSC-04	1	0.963	0.972	0.868	0.963	85	1	0.032	0.032	0.135	0.050	86	Positive	Photoreactive
	2	0.971	0.970	0.887	0.959	74	2	0.033	0.032	0.135	0.049	85		
	3	0.968	0.975	0.874	0.963	84	3	0.033	0.033	0.141	0.049	91		
	Mean	0.967	0.972	0.876	0.962	81	Mean	0.033	0.032	0.137	0.049	87		
FDSC-13	1	0.966	0.970	0.877	0.970	84	1	0.033	0.034	0.140	0.043	98	Positive	Photoreactive
	2	0.969	0.970	0.885	0.962	79	2	0.033	0.034	0.146	0.043	104		
	3	0.963	0.972	0.885	0.965	73	3	0.033	0.033	0.149	0.044	107		
	Mean	0.966	0.971	0.882	0.966	79	Mean	0.033	0.034	0.145	0.043	103		
FDSC-20	1	0.939	0.974	0.843	0.949	77	1	0.033	0.032	0.136	0.046	91	Positive	Photoreactive
	2	0.951	0.972	0.902	0.954	30	2	0.032	0.032	0.130	0.045	86		
	3	0.954	0.980	0.863	0.966	72	3	0.033	0.034	0.145	0.045	100		
	Mean	0.948	0.975	0.869	0.956	60	Mean	0.033	0.033	0.137	0.045	92		
Mean for 3 assays	-	-	-	-	73	Mean for 3 assays	-	-	-	-	94	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Nalidixic acid

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-01	1	0.947	0.955	0.790	0.948	150	1	0.032	0.033	0.323	0.043	280	Positive	Photoreactive
	2	0.966	0.963	0.812	0.956	147	2	0.032	0.037	0.262	0.050	219		
	3	0.970	0.967	0.818	0.961	145	3	0.033	0.033	0.308	0.042	264		
	Mean	0.961	0.962	0.807	0.955	147	Mean	0.032	0.034	0.298	0.045	254		
FDSC-12	1	0.961	0.967	0.804	0.961	150	1	0.033	0.033	0.265	0.049	217	Positive	Photoreactive
	2	0.963	0.962	0.814	0.954	142	2	0.034	0.035	0.230	0.050	181		
	3	0.966	0.967	0.819	0.959	140	3	0.034	0.033	0.270	0.047	221		
	Mean	0.963	0.965	0.812	0.958	144	Mean	0.034	0.034	0.255	0.049	206		
FDSC-19	1	0.971	0.986	0.818	0.990	149	1	0.032	0.033	0.317	0.049	267	Positive	Photoreactive
	2	0.973	0.981	0.824	0.975	145	2	0.032	0.032	0.285	0.054	235		
	3	0.977	0.991	0.832	0.982	141	3	0.033	0.033	0.304	0.050	253		
	Mean	0.974	0.986	0.825	0.982	145	Mean	0.032	0.033	0.302	0.051	252		
Mean for 3 assays	-	-	-	-	145	Mean for 3 assays	-	-	-	-	237	Positive	Photoreactive ^{*3}	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Nalidixic acid (Na salt)

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-15	1	0.972	0.974	0.814	0.967	153	1	0.032	0.033	0.252	0.054	205	Positive	Photoreactive
	2	0.976	0.975	0.829	0.971	142	2	0.031	0.032	0.282	0.048	236		
	3	0.979	0.982	0.836	0.977	138	3	0.032	0.035	0.261	0.043	214		
	Mean	0.976	0.977	0.826	0.972	144	Mean	0.032	0.033	0.265	0.048	218		
FDSC-22	1	0.958	0.980	0.809	0.974	143	1	0.031	0.031	0.214	0.040	169	Positive	Photoreactive
	2	0.958	0.982	0.813	0.974	139	2	0.033	0.033	0.234	0.057	187		
	3	0.966	0.991	0.822	0.986	138	3	0.031	0.032	0.266	0.042	221		
	Mean	0.961	0.984	0.815	0.978	140	Mean	0.032	0.032	0.238	0.046	192		
FDSC-26	1	0.962	0.959	0.800	0.954	156	1	0.033	0.037	0.270	0.042	226	Positive	Photoreactive
	2	0.959	0.955	0.805	0.949	148	2	0.033	0.033	0.253	0.050	209		
	3	0.962	0.964	0.812	0.956	144	3	0.034	0.033	0.242	0.043	197		
	Mean	0.961	0.959	0.806	0.953	149	Mean	0.033	0.034	0.255	0.045	211		
Mean for 3 assays	-	-	-	-	144	Mean for 3 assays	-	-	-	-	207	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Norfloxacin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-12	1	0.985	0.967	0.755	0.961	223	1	0.033	0.033	0.169	0.049	121	Positive	Photoreactive
	2	0.986	0.962	0.804	0.954	175	2	0.033	0.035	0.182	0.050	134		
	3	0.977	0.967	0.803	0.959	167	3	0.034	0.033	0.190	0.047	141		
	Mean	0.983	0.965	0.787	0.958	188	Mean	0.033	0.034	0.180	0.049	132		
FDSC-19	1	0.976	0.986	0.745	0.990	227	1	0.032	0.033	0.153	0.049	103	Positive	Photoreactive
	2	0.977	0.981	0.756	0.975	217	2	0.032	0.032	0.172	0.054	122		
	3	0.980	0.991	0.755	0.982	221	3	0.033	0.033	0.185	0.050	134		
	Mean	0.978	0.986	0.752	0.982	222	Mean	0.032	0.033	0.170	0.051	120		
FDSC-26	1	0.956	0.959	0.751	0.954	199	1	0.033	0.037	0.159	0.042	115	Positive	Photoreactive
	2	0.956	0.955	0.747	0.949	203	2	0.032	0.033	0.162	0.050	119		
	3	0.963	0.964	0.759	0.956	198	3	0.033	0.033	0.166	0.043	122		
	Mean	0.958	0.959	0.752	0.953	200	Mean	0.033	0.034	0.162	0.045	119		
Mean for 3 assays	-	-	-	-	203	Mean for 3 assays	-	-	-	-	124	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Ofloxacin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-06	1	0.973	0.982	0.826	0.972	138	1	0.032	0.033	0.338	0.052	288	Positive	Photoreactive
	2	0.975	0.977	0.839	0.965	127	2	0.034	0.034	0.322	0.047	270		
	3	0.979	0.982	0.839	0.976	131	3	0.032	0.033	0.356	0.055	306		
	Mean	0.976	0.980	0.835	0.971	132	Mean	0.033	0.033	0.339	0.051	288		
FDSC-14	1	0.967	0.971	0.813	0.960	144	1	0.032	0.032	0.327	0.044	282	Positive	Photoreactive
	2	0.974	0.968	0.830	0.959	134	2	0.035	0.033	0.329	0.050	281		
	3	0.976	0.974	0.832	0.963	134	3	0.033	0.033	0.359	0.045	313		
	Mean	0.972	0.971	0.825	0.961	137	Mean	0.033	0.033	0.338	0.046	292		
FDSC-21	1	0.963	0.959	0.784	0.967	177	1	0.033	0.031	0.368	0.043	326	Positive	Photoreactive
	2	0.977	0.958	0.837	0.953	138	2	0.034	0.032	0.272	0.041	229		
	3	0.970	0.963	0.836	0.955	132	3	0.033	0.035	0.363	0.042	321		
	Mean	0.970	0.960	0.819	0.958	149	Mean	0.033	0.033	0.334	0.042	292		
Mean for 3 assays	-	-	-	-	139	Mean for 3 assays	-	-	-	-	291	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Piroxicam

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-05	1	0.964	0.970	0.745	0.966	218	FDSC-09	1	0.039	0.032	0.114	0.052	Positive	Photoreactive
	2	0.966	0.966	0.764	0.973	201		2	0.038	0.032	0.118	0.057		
	3	0.965	0.975	0.762	0.968	202		3	0.038	0.032	0.121	0.055		
	Mean	0.965	0.970	0.757	0.969	207		Mean	0.038	0.032	0.118	0.055		
FDSC-09	1	0.998	0.984	0.751	0.975	241	FDSC-17	1	0.038	0.032	0.096	0.053	Positive	Photoreactive
	2	0.992	0.984	0.762	0.980	224		2	0.037	0.033	0.102	0.052		
	3	0.995	0.994	0.763	0.987	226		3	0.037	0.032	0.108	0.046		
	Mean	0.995	0.987	0.759	0.981	230		Mean	0.037	0.032	0.102	0.050		
FDSC-17	1	0.989	0.981	0.752	0.977	234	FDSC-18	1	0.040	0.032	0.094	0.043	Positive	Photoreactive
	2	0.995	0.989	0.777	0.984	215		2	0.038	0.032	0.094	0.047		
	3	0.988	0.995	0.771	0.993	214		3	0.039	0.033	0.096	0.045		
	Mean	0.991	0.988	0.767	0.985	221		Mean	0.039	0.032	0.095	0.045		
Mean for 3 assays	-	-	-	-	219	Mean for 3 assays	-	-	-	-	49	Positive	Photoreactive ^{*3}	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Promethazine HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	A440(-)			A440(+)			A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
FDSC-05	1	0.959	0.970	0.869	0.966	89	1	0.033	0.032	0.092	0.048	43	Positive	Photoreactive
	2	0.966	0.966	0.873	0.973	92	2	0.033	0.032	0.090	0.051	41		
	3	0.966	0.975	0.874	0.968	91	3	0.035	0.032	0.095	0.046	44		
	Mean	0.964	0.970	0.872	0.969	91	Mean	0.034	0.032	0.092	0.048	43		
FDSC-14	1	0.950	0.971	0.851	0.960	89	1	0.031	0.032	0.094	0.044	50	Positive	Photoreactive
	2	0.974	0.968	0.878	0.959	86	2	0.034	0.033	0.094	0.050	47		
	3	0.970	0.974	0.869	0.963	91	3	0.032	0.033	0.100	0.045	55		
	Mean	0.965	0.971	0.866	0.961	89	Mean	0.032	0.033	0.096	0.046	51		
FDSC-21	1	0.948	0.959	0.845	0.967	101	1	0.031	0.031	0.085	0.043	45	Positive	Photoreactive
	2	0.964	0.958	0.858	0.953	104	2	0.032	0.032	0.088	0.041	47		
	3	0.961	0.963	0.856	0.955	103	3	0.032	0.035	0.092	0.042	51		
	Mean	0.958	0.960	0.853	0.958	103	Mean	0.032	0.033	0.088	0.042	48		
Mean for 3 assays	-	-	-	-	94	Mean for 3 assays	-	-	-	-	47	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Rosiglitazone

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-06	1	0.955	0.982	0.860	0.972	86	FDSC-09	1	0.036	0.032	0.080	0.052	Positive	Photoreactive
	2	0.936	0.977	0.837	0.965	90		2	0.036	0.032	0.096	0.057		
	3	0.943	0.982	0.840	0.976	94		3	0.037	0.032	0.083	0.055		
	Mean	0.945	0.980	0.846	0.971	90		Mean	0.036	0.032	0.086	0.055		
FDSC-09	1	0.967	0.984	0.879	0.975	82	FDSC-17	1	0.037	0.032	0.081	0.053	Positive	Photoreactive
	2	0.967	0.984	0.874	0.980	87		2	0.036	0.033	0.083	0.052		
	3	0.963	0.994	0.864	0.987	93		3	0.037	0.032	0.088	0.046		
	Mean	0.966	0.987	0.872	0.981	87		Mean	0.037	0.032	0.084	0.050		
FDSC-17	1	0.946	0.981	0.862	0.977	81	FDSC-18	1	0.037	0.032	0.086	0.043	Positive	Photoreactive
	2	0.973	0.989	0.871	0.984	99		2	0.037	0.032	0.082	0.047		
	3	0.965	0.995	0.863	0.993	99		3	0.036	0.033	0.085	0.045		
	Mean	0.961	0.988	0.865	0.985	93		Mean	0.037	0.032	0.084	0.045		
Mean for 3 assays	-	-	-	-	90	Mean for 3 assays	-	-	-	-	30	Positive*3	Photoreactive*3	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 20 or 2 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Tetracycline

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-05	1	0.968	0.970	0.792	0.966	175	1	0.039	0.032	0.289	0.048	234	Positive	Photoreactive
	2	0.995	0.966	0.829	0.973	165	2	0.042	0.032	0.299	0.051	241		
	3	0.992	0.975	0.829	0.968	162	3	0.040	0.032	0.301	0.046	245		
	Mean	0.985	0.970	0.817	0.969	167	Mean	0.040	0.032	0.296	0.048	240		
FDSC-15	1	0.964	0.974	0.793	0.967	166	1	0.040	0.033	0.305	0.054	250	Positive	Photoreactive
	2	0.971	0.975	0.809	0.971	157	2	0.042	0.032	0.314	0.048	257		
	3	0.986	0.982	0.823	0.977	158	3	0.043	0.035	0.316	0.043	258		
	Mean	0.974	0.977	0.808	0.972	160	Mean	0.042	0.033	0.312	0.048	255		
FDSC-22	1	0.961	0.980	0.783	0.974	172	1	0.040	0.031	0.291	0.040	237	Positive	Photoreactive
	2	0.973	0.982	0.806	0.974	161	2	0.046	0.033	0.297	0.057	237		
	3	0.981	0.991	0.809	0.986	166	3	0.038	0.032	0.296	0.042	244		
	Mean	0.972	0.984	0.799	0.978	166	Mean	0.041	0.032	0.295	0.046	239		
Mean for 3 assays	-	-	-	-	164	Mean for 3 assays	-	-	-	-	245	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Anthracene

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-10	1	0.982	0.982	0.977	0.976	-4	1	0.033	0.032	0.054	0.047	2	Inconclusive	(Non-photoreactive)
	2	0.989	0.992	0.979	0.975	1	2	0.036	0.032	0.059	0.053	4		
	3	0.994	0.987	0.987	0.984	-2	3	0.033	0.034	0.055	0.055	3		
	Mean	0.988	0.987	0.981	0.978	-2	Mean	0.034	0.033	0.056	0.052	3		
FDSC-16	1	0.981	0.986	0.976	0.996	5	1	0.032	0.031	0.050	0.041	7	Inconclusive	(Non-photoreactive)
	2	0.986	0.982	0.978	0.980	8	2	0.032	0.032	0.053	0.044	10		
	3	0.992	0.993	0.985	0.986	7	3	0.033	0.033	0.052	0.043	8		
	Mean	0.986	0.987	0.980	0.987	7	Mean	0.032	0.032	0.052	0.043	8		
FDSC-23	1	0.968	0.957	0.953	0.946	5	1	0.033	0.032	0.049	0.059	-2	Inconclusive	(Non-photoreactive)
	2	0.972	0.960	0.956	0.947	6	2	0.032	0.032	0.048	0.045	-2		
	3	0.978	0.965	0.962	0.959	6	3	0.033	0.033	0.047	0.045	-4		
	Mean	0.973	0.961	0.957	0.951	6	Mean	0.033	0.032	0.048	0.050	-3		
Mean for 3 assays	-	-	-	-	4	Mean for 3 assays	-	-	-	-	3	Inconclusive*3	(Non-photoreactive)*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Avobenzone

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-10	1	0.986	0.982	0.975	0.976	2	1	0.033	0.032	0.080	0.047	28	Positive	(Weakly photoreactive)
	2	0.992	0.992	0.979	0.975	4	2	0.034	0.032	0.084	0.053	31		
	3	0.989	0.987	0.985	0.984	-5	3	0.033	0.034	0.081	0.055	29		
	Mean	0.989	0.987	0.980	0.978	0	Mean	0.033	0.033	0.082	0.052	29		
FDSC-16	1	0.979	0.986	0.966	0.996	13	1	0.033	0.031	0.079	0.041	35	Positive	(Weakly photoreactive)
	2	0.980	0.982	0.972	0.980	8	2	0.033	0.032	0.081	0.044	37		
	3	0.989	0.993	0.973	0.986	16	3	0.034	0.033	0.081	0.043	36		
	Mean	0.983	0.987	0.970	0.987	12	Mean	0.033	0.032	0.080	0.043	36		
FDSC-23	1	0.970	0.957	0.951	0.946	9	1	0.034	0.032	0.080	0.059	28	Positive	(Weakly photoreactive)
	2	0.983	0.960	0.958	0.947	15	2	0.035	0.032	0.082	0.045	29		
	3	0.983	0.965	0.966	0.959	7	3	0.045	0.033	0.080	0.045	17		
	Mean	0.979	0.961	0.958	0.951	10	Mean	0.038	0.032	0.081	0.050	25		
Mean for 3 assays	-	-	-	-	7	Mean for 3 assays	-	-	-	-	30	Positive	(Weakly photoreactive) ^{*3}	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Bithionol

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
FDSC-03	1	0.935	0.966	0.795	0.962	137	FDSC-11	1	0.034	0.031	0.064	0.049	11	Positive	Photoreactive
	2	0.948	0.970	0.809	0.968	136		2	0.033	0.032	0.067	0.055	15		
	3	0.944	0.974	0.802	0.970	139		3	0.034	0.034	0.067	0.050	14		
	Mean	0.942	0.970	0.802	0.967	137		Mean	0.034	0.032	0.066	0.051	13		
FDSC-09	1	0.954	0.984	0.806	0.975	142	FDSC-17	1	0.035	0.032	0.068	0.053	15	Positive	Photoreactive
	2	0.946	0.984	0.798	0.980	142		2	0.035	0.033	0.068	0.052	15		
	3	0.966	0.994	0.815	0.987	145		3	0.035	0.032	0.067	0.046	14		
	Mean	0.955	0.987	0.806	0.981	143		Mean	0.035	0.032	0.068	0.050	15		
FDSC-11	1	0.950	0.977	0.803	0.965	139	FDSC-18	1	0.034	0.032	0.068	0.043	21	Positive	Photoreactive
	2	0.965	0.972	0.817	0.967	140		2	0.034	0.032	0.059	0.047	12		
	3	0.952	0.979	0.802	0.971	142		3	0.034	0.033	0.060	0.045	13		
	Mean	0.956	0.976	0.807	0.968	140		Mean	0.034	0.032	0.062	0.045	15		
Mean for 3 assays	-	-	-	-	140	Mean for 3 assays	-	-	-	-	14	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Hexachlorophene

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 2 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
FDSC-02	1	0.921	0.964	0.659	0.953	252	FDSC-11	1	0.033	0.031	0.046	0.049	-6	Positive	Photoreactive
	2	0.928	0.971	0.671	0.964	247		2	0.034	0.032	0.047	0.055	-6		
	3	0.918	0.978	0.668	0.965	240		3	0.035	0.034	0.048	0.050	-6		
	Mean	0.922	0.971	0.666	0.961	246		Mean	0.034	0.032	0.047	0.051	-6		
FDSC-09	1	0.929	0.984	0.669	0.975	254	FDSC-17	1	0.035	0.032	0.069	0.053	16	Positive	Photoreactive
	2	0.941	0.984	0.678	0.980	257		2	0.035	0.033	0.066	0.052	13		
	3	0.944	0.994	0.683	0.987	255		3	0.035	0.032	0.068	0.046	15		
	Mean	0.938	0.987	0.677	0.981	255		Mean	0.035	0.032	0.068	0.050	15		
FDSC-11	1	0.921	0.977	0.656	0.965	257	FDSC-18	1	0.034	0.032	0.044	0.043	-3	Positive	Photoreactive
	2	0.924	0.972	0.658	0.967	258		2	0.033	0.032	0.043	0.047	-3		
	3	0.932	0.979	0.665	0.971	259		3	0.034	0.033	0.043	0.045	-4		
	Mean	0.926	0.976	0.660	0.968	258		Mean	0.034	0.032	0.043	0.045	-3		
Mean for 3 assays		-	-	-	-	253	Mean for 3 assays		-	-	-	-	2	Positive*3	Photoreactive*3

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Rose bengal

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
FDSC-02	1	1.247	0.964	0.607	0.953	630	FDSC-02	1	3.498	0.032	3.334	0.041	No data ^{*4}	Positive	Photoreactive
	2	1.259	0.971	0.618	0.964	631		2	3.460	0.034	3.338	0.041	No data ^{*4}		
	3	1.258	0.978	0.617	0.965	631		3	3.566	0.038	3.374	0.081	No data ^{*4}		
	Mean	1.255	0.971	0.614	0.961	631		Mean	3.508	0.035	3.349	0.054	No data ^{*4}		
FDSC-12	1	1.241	0.967	0.607	0.961	627	FDSC-12	1	OVER	0.033	3.747	0.049	No data ^{*4}	Positive	Photoreactive
	2	1.267	0.962	0.626	0.954	634		2	OVER	0.035	OVER	0.050	No data ^{*4}		
	3	1.276	0.967	0.629	0.959	640		3	OVER	0.033	3.943	0.047	No data ^{*4}		
	Mean	1.261	0.965	0.621	0.958	634		Mean	#DIV/0!	0.034	3.845	0.049	No data ^{*4}		
FDSC-19	1	1.254	0.986	0.576	0.990	674	FDSC-19	1	OVER	0.033	3.513	0.049	No data ^{*4}	Positive	Photoreactive
	2	1.250	0.981	0.583	0.975	663		2	OVER	0.032	3.467	0.054	No data ^{*4}		
	3	1.253	0.991	0.585	0.982	664		3	OVER	0.033	3.424	0.050	No data ^{*4}		
	Mean	1.252	0.986	0.581	0.982	667		Mean	#DIV/0!	0.033	3.468	0.051	No data ^{*4}		
Mean for 3 assays	-	-	-	-	644	Mean for 3 assays	-	-	-	-	-	No data ^{*4}	Positive ^{*3}	Photoreactive ^{*3}	

OVER: Optical density was not measurable because it was more than 4.0.

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*4 : Over the OD criteria

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Aspirin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-01	1	0.957	0.955	0.949	0.948	1	1	0.034	0.033	0.049	0.043	4	Negative	Non-photoreactive
	2	0.979	0.963	0.969	0.956	3	2	0.035	0.037	0.045	0.050	-1		
	3	0.979	0.967	0.973	0.961	-1	3	0.035	0.033	0.043	0.042	-3		
	Mean	0.972	0.962	0.964	0.955	1	Mean	0.035	0.034	0.046	0.045	0		
FDSC-12	1	0.962	0.967	0.957	0.961	-2	1	0.033	0.033	0.041	0.049	-7	Negative	Non-photoreactive
	2	0.972	0.962	0.965	0.954	0	2	0.033	0.035	0.043	0.050	-5		
	3	0.967	0.967	0.961	0.959	-1	3	0.032	0.033	0.042	0.047	-5		
	Mean	0.967	0.965	0.961	0.958	-1	Mean	0.033	0.034	0.042	0.049	-6		
FDSC-19	1	0.970	0.986	0.965	0.990	1	1	0.031	0.033	0.045	0.049	-4	Negative	Non-photoreactive
	2	0.973	0.981	0.968	0.975	1	2	0.032	0.032	0.046	0.054	-4		
	3	0.979	0.991	0.974	0.982	1	3	0.032	0.033	0.046	0.050	-4		
	Mean	0.974	0.986	0.969	0.982	1	Mean	0.032	0.033	0.046	0.051	-4		
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	-3	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Benzocaine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
FDSC-03	1	0.961	0.966	0.952	0.962	6	FDSC-03	1	0.033	0.033	0.045	0.050	-5	Negative	Non-photoreactive
	2	0.970	0.970	0.967	0.968	0		2	0.031	0.033	0.044	0.050	-4		
	3	0.973	0.974	0.950	0.970	20		3	0.033	0.035	0.046	0.053	-4		
	Mean	0.968	0.970	0.956	0.967	9		Mean	0.032	0.034	0.045	0.051	-4		
FDSC-13	1	0.963	0.970	0.956	0.970	2	FDSC-13	1	0.031	0.034	0.054	0.043	14	Negative	Non-photoreactive
	2	0.965	0.970	0.960	0.962	0		2	0.034	0.034	0.054	0.043	11		
	3	0.962	0.972	0.955	0.965	2		3	0.032	0.033	0.051	0.044	10		
	Mean	0.963	0.971	0.957	0.966	1		Mean	0.032	0.034	0.053	0.043	12		
FDSC-20	1	0.966	0.974	0.955	0.949	-8	FDSC-20	1	0.031	0.032	0.053	0.046	10	Negative	Non-photoreactive
	2	0.973	0.972	0.967	0.954	-13		2	0.031	0.032	0.051	0.045	8		
	3	0.982	0.980	0.977	0.966	-14		3	0.033	0.034	0.045	0.045	0		
	Mean	0.974	0.975	0.966	0.956	-12		Mean	0.032	0.033	0.050	0.045	6		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	5	Negative*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Erythromycin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-06	1	0.980	0.982	0.967	0.972	4	1	0.039	0.033	0.064	0.052	7	Negative	Non-photoreactive
	2	0.958	0.977	0.946	0.965	3	2	0.037	0.034	0.069	0.047	14		
	3	0.975	0.982	0.956	0.976	10	3	0.035	0.033	0.073	0.055	20		
	Mean	0.971	0.980	0.956	0.971	6	Mean	0.037	0.033	0.069	0.051	14		
FDSC-14	1	0.960	0.971	0.950	0.960	0	1	0.034	0.032	0.052	0.044	5	Negative	Non-photoreactive
	2	0.977	0.968	0.968	0.959	-1	2	0.035	0.033	0.052	0.050	4		
	3	0.974	0.974	0.966	0.963	-2	3	0.036	0.033	0.053	0.045	4		
	Mean	0.970	0.971	0.961	0.961	-1	Mean	0.035	0.033	0.052	0.046	4		
FDSC-21	1	0.948	0.959	0.929	0.967	17	1	0.037	0.031	0.083	0.043	37	Positive	Weakly photoreactive
	2	0.963	0.958	0.950	0.953	11	2	0.036	0.032	0.073	0.041	28		
	3	0.967	0.963	0.954	0.955	11	3	0.036	0.035	0.085	0.042	40		
	Mean	0.959	0.960	0.944	0.958	13	Mean	0.036	0.033	0.080	0.042	35		
Mean for 3 assays	-	-	-	-	6	Mean for 3 assays	-	-	-	-	18	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Penicillin G

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-06	1	0.979	0.982	0.969	0.972	1	1	0.034	0.033	0.090	0.052	38	Positive	Weakly photoreactive
	2	0.981	0.977	0.970	0.965	2	2	0.035	0.034	0.091	0.047	38		
	3	0.978	0.982	0.971	0.976	-2	3	0.041	0.033	0.094	0.055	35		
	Mean	0.979	0.980	0.970	0.971	0	Mean	0.037	0.033	0.092	0.051	37		
FDSC-14	1	0.975	0.971	0.967	0.960	-2	1	0.034	0.032	0.082	0.044	35	Positive	Weakly photoreactive
	2	0.979	0.968	0.972	0.959	-3	2	0.034	0.033	0.081	0.050	34		
	3	0.985	0.974	0.974	0.963	1	3	0.034	0.033	0.087	0.045	40		
	Mean	0.980	0.971	0.971	0.961	-1	Mean	0.034	0.033	0.083	0.046	36		
FDSC-21	1	0.970	0.959	0.964	0.967	4	1	0.034	0.031	0.077	0.043	34	Positive	Weakly photoreactive
	2	0.977	0.958	0.973	0.953	2	2	0.036	0.032	0.075	0.041	30		
	3	0.976	0.963	0.969	0.955	5	3	0.034	0.035	0.081	0.042	38		
	Mean	0.974	0.960	0.969	0.958	4	Mean	0.035	0.033	0.078	0.042	34		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	36	Positive	Weakly photoreactive ^{*3}	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Phenytoin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-02	1	0.955	0.964	0.945	0.953	0	1	0.033	0.032	0.102	0.041	50	Positive	Weakly photoreactive
	2	0.962	0.971	0.952	0.964	0	2	0.036	0.034	0.104	0.041	49		
	3	0.969	0.978	0.958	0.965	1	3	0.034	0.038	0.109	0.081	56		
	Mean	0.962	0.971	0.952	0.961	0	Mean	0.034	0.035	0.105	0.054	52		
FDSC-12	1	0.970	0.967	0.967	0.961	-4	1	0.037	0.033	0.105	0.049	53	Positive	Weakly photoreactive
	2	0.971	0.962	0.967	0.954	-3	2	0.037	0.035	0.104	0.050	52		
	3	0.966	0.967	0.963	0.959	-4	3	0.040	0.033	0.109	0.047	54		
	Mean	0.969	0.965	0.966	0.958	-4	Mean	0.038	0.034	0.106	0.049	53		
FDSC-19	1	0.970	0.986	0.964	0.990	2	1	0.033	0.033	0.099	0.049	48	Positive	Weakly photoreactive
	2	0.979	0.981	0.973	0.975	2	2	0.035	0.032	0.100	0.054	47		
	3	0.977	0.991	0.970	0.982	3	3	0.038	0.033	0.104	0.050	48		
	Mean	0.975	0.986	0.969	0.982	2	Mean	0.035	0.033	0.101	0.051	48		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	51	Positive*3	Weakly photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Bumetrizole

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-10	1	0.985	0.982	0.978	0.976	-2	1	0.033	0.032	0.044	0.047	-8	Inconclusive	(Non-photoreactive)
	2	0.990	0.992	0.985	0.975	-4	2	0.035	0.032	0.049	0.053	-5		
	3	0.997	0.987	0.996	0.984	-8	3	0.034	0.034	0.046	0.055	-7		
	Mean	0.991	0.987	0.986	0.978	-5	Mean	0.034	0.033	0.046	0.052	-7		
FDSC-16	1	0.976	0.986	0.954	0.996	22	1	0.032	0.031	0.045	0.041	2	Inconclusive	(Non-photoreactive)
	2	0.983	0.982	0.979	0.980	4	2	0.033	0.032	0.044	0.044	0		
	3	0.983	0.993	0.979	0.986	4	3	0.033	0.033	0.043	0.043	-1		
	Mean	0.981	0.987	0.971	0.987	10	Mean	0.033	0.032	0.044	0.043	0		
FDSC-23	1	0.960	0.957	0.948	0.946	2	1	0.033	0.032	0.043	0.059	-8	Inconclusive	(Non-photoreactive)
	2	0.966	0.960	0.954	0.947	2	2	0.033	0.032	0.045	0.045	-6		
	3	0.968	0.965	0.961	0.959	-3	3	0.034	0.033	0.046	0.045	-6		
	Mean	0.965	0.961	0.954	0.951	0	Mean	0.033	0.032	0.045	0.050	-7		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	-5	Inconclusive*3	(Non-photoreactive)*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Camphor sulfonic acid

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-04	1	0.969	0.972	0.956	0.963	3	1	0.032	0.032	0.045	0.050	-4	Negative	Non-photoreactive
	2	0.975	0.970	0.966	0.959	-1	2	0.033	0.032	0.046	0.049	-4		
	3	0.969	0.975	0.963	0.963	-4	3	0.034	0.033	0.046	0.049	-5		
	Mean	0.971	0.972	0.962	0.962	-1	Mean	0.033	0.032	0.046	0.049	-4		
FDSC-13	1	0.967	0.970	0.960	0.970	2	1	0.032	0.034	0.046	0.043	5	Negative	Non-photoreactive
	2	0.974	0.970	0.965	0.962	4	2	0.033	0.034	0.047	0.043	5		
	3	0.970	0.972	0.963	0.965	2	3	0.032	0.033	0.046	0.044	5		
	Mean	0.970	0.971	0.963	0.966	3	Mean	0.032	0.034	0.046	0.043	5		
FDSC-26	1	0.963	0.959	0.954	0.954	3	1	0.033	0.037	0.042	0.042	-2	Negative	Non-photoreactive
	2	0.968	0.955	0.958	0.949	4	2	0.033	0.033	0.044	0.050	0		
	3	0.969	0.964	0.963	0.956	0	3	0.033	0.033	0.041	0.043	-3		
	Mean	0.967	0.959	0.958	0.953	2	Mean	0.033	0.034	0.042	0.045	-2		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	0	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Chlorhexidine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
FDSC-05	1	0.916	0.970	0.902	0.966	13	FDSC-05	1	0.039	0.032	0.068	0.048	13	Negative	Non-photoreactive
	2	0.933	0.966	0.920	0.973	12		2	0.038	0.032	0.064	0.051	10		
	3	0.918	0.975	0.910	0.968	7		3	0.038	0.032	0.069	0.046	15		
	Mean	0.922	0.970	0.911	0.969	11		Mean	0.038	0.032	0.067	0.048	13		
FDSC-14	1	0.904	0.971	0.902	0.960	-8	FDSC-14	1	0.036	0.032	0.067	0.044	18	Positive	Weakly photoreactive
	2	0.904	0.968	0.901	0.959	-7		2	0.037	0.033	0.077	0.050	27		
	3	0.905	0.974	0.907	0.963	-12		3	0.038	0.033	0.068	0.045	17		
	Mean	0.904	0.971	0.903	0.961	-9		Mean	0.037	0.033	0.071	0.046	21		
FDSC-21	1	0.892	0.959	0.880	0.967	10	FDSC-21	1	0.037	0.031	0.062	0.043	16	Negative	Non-photoreactive
	2	0.901	0.958	0.892	0.953	7		2	0.037	0.032	0.062	0.041	16		
	3	0.889	0.963	0.880	0.955	7		3	0.037	0.035	0.062	0.042	16		
	Mean	0.894	0.960	0.884	0.958	8		Mean	0.037	0.033	0.062	0.042	16		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	17	Negative*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Cinnamic acid

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-03	1	0.977	0.966	0.967	0.962	7	1	0.035	0.033	0.100	0.050	48	Positive	Weakly photoreactive
	2	0.978	0.970	0.972	0.968	3	2	0.032	0.033	0.102	0.050	53		
	3	0.969	0.974	0.962	0.970	4	3	0.033	0.035	0.104	0.053	54		
	Mean	0.975	0.970	0.967	0.967	5	Mean	0.033	0.034	0.102	0.051	52		
FDSC-13	1	0.962	0.970	0.956	0.970	1	1	0.032	0.034	0.075	0.043	34	Positive	Weakly photoreactive
	2	0.963	0.970	0.958	0.962	0	2	0.033	0.034	0.077	0.043	35		
	3	0.964	0.972	0.959	0.965	0	3	0.032	0.033	0.081	0.044	40		
	Mean	0.963	0.971	0.958	0.966	0	Mean	0.032	0.034	0.078	0.043	36		
FDSC-20	1	0.966	0.974	0.951	0.949	-4	1	0.031	0.032	0.080	0.046	37	Positive	Weakly photoreactive
	2	0.978	0.972	0.968	0.954	-9	2	0.031	0.032	0.077	0.045	34		
	3	0.979	0.980	0.972	0.966	-12	3	0.033	0.034	0.084	0.045	39		
	Mean	0.974	0.975	0.964	0.956	-8	Mean	0.032	0.033	0.080	0.045	37		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	42	Positive*3	Weakly photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Drometrizole

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-15	1	0.975	0.974	0.966	0.967	4	1	0.035	0.033	0.058	0.054	8	Inconclusive	Non-photoreactive
	2	0.988	0.975	0.981	0.971	2	2	0.034	0.032	0.058	0.048	9		
	3	0.990	0.982	0.984	0.977	1	3	0.037	0.035	0.059	0.043	7		
	Mean	0.984	0.977	0.977	0.972	2	Mean	0.035	0.033	0.058	0.048	8		
FDSC-22	1	0.967	0.980	0.957	0.974	4	1	0.034	0.031	0.050	0.040	2	Inconclusive	Non-photoreactive
	2	0.977	0.982	0.964	0.974	7	2	0.034	0.033	0.052	0.057	4		
	3	0.977	0.991	0.965	0.986	6	3	0.033	0.032	0.050	0.042	3		
	Mean	0.974	0.984	0.962	0.978	6	Mean	0.034	0.032	0.051	0.046	3		
FDSC-26	1	0.969	0.959	0.955	0.954	8	1	0.035	0.037	0.047	0.042	1	Inconclusive	Non-photoreactive
	2	0.971	0.955	0.955	0.949	10	2	0.036	0.033	0.050	0.050	3		
	3	0.970	0.964	0.958	0.956	6	3	0.035	0.033	0.049	0.043	3		
	Mean	0.970	0.959	0.956	0.953	8	Mean	0.035	0.034	0.049	0.045	2		
Mean for 3 assays	-	-	-	-	5	Mean for 3 assays	-	-	-	-	4	Inconclusive*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : L-Histidine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-18	1	0.939	0.960	0.930	0.955	4	1	0.031	0.032	0.099	0.043	55	Positive	Weakly photoreactive
	2	0.940	0.955	0.933	0.951	2	2	0.032	0.032	0.098	0.047	53		
	3	0.947	0.966	0.937	0.958	5	3	0.033	0.033	0.102	0.045	56		
	Mean	0.942	0.960	0.933	0.955	4	Mean	0.032	0.032	0.100	0.045	55		
FDSC-24	1	0.975	0.972	0.959	0.963	9	1	0.031	0.031	0.042	0.045	-3	Negative	Non-photoreactive
	2	0.980	0.970	0.955	0.963	18	2	0.034	0.032	0.042	0.048	-6		
	3	0.981	0.977	0.974	0.973	0	3	0.032	0.032	0.043	0.046	-3		
	Mean	0.979	0.973	0.963	0.966	9	Mean	0.032	0.032	0.042	0.046	-4		
FDSC-25	1	0.940	0.958	0.928	0.948	0	1	0.031	0.032	0.109	0.040	70	Positive	Weakly photoreactive
	2	0.939	0.952	0.932	0.949	-5	2	0.032	0.034	0.108	0.041	68		
	3	0.943	0.951	0.935	0.928	-4	3	0.033	0.033	0.106	0.042	65		
	Mean	0.941	0.954	0.932	0.942	-3	Mean	0.032	0.033	0.108	0.041	68		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	40	Positive*3	Weakly photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Methylbenzylidene camphor

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement			
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol		
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank					
FDSC-07	1	0.957	0.973	0.956	0.964	-4	FDSC-07	1	0.031	0.032	0.050	0.048	3	Inconclusive	Non-photoreactive	
	2	0.967	0.971	0.963	0.967	-1		2	0.033	0.032	0.053	0.049				4
	3	0.968	0.978	0.966	0.976	-3		3	0.035	0.033	0.055	0.047				4
	Mean	0.964	0.974	0.962	0.969	-3		Mean	0.033	0.032	0.053	0.048				4
FDSC-15	1	0.964	0.974	0.960	0.967	-1	FDSC-15	1	0.033	0.033	0.061	0.054	13	Inconclusive	Non-photoreactive	
	2	0.982	0.975	0.979	0.971	-2		2	0.034	0.032	0.052	0.048				3
	3	0.980	0.982	0.976	0.977	-1		3	0.032	0.035	0.051	0.043				4
	Mean	0.975	0.977	0.972	0.972	-1		Mean	0.033	0.033	0.055	0.048				7
FDSC-22	1	0.962	0.980	0.956	0.974	0	FDSC-22	1	0.033	0.031	0.043	0.040	-4	Inconclusive	Non-photoreactive	
	2	0.966	0.982	0.962	0.974	-2		2	0.034	0.033	0.043	0.057				-5
	3	0.965	0.991	0.959	0.986	0		3	0.034	0.032	0.042	0.042				-6
	Mean	0.964	0.984	0.959	0.978	-1		Mean	0.034	0.032	0.043	0.046				-5
Mean for 3 assays	-	-	-	-	-2	Mean for 3 assays	-	-	-	-	2	Inconclusive*3	Non-photoreactive*3			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Octrizole

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-11	1	0.973	0.977	0.969	0.965	-4	1	0.034	0.031	0.055	0.049	2	Inconclusive	(Non-photoreactive)
	2	0.979	0.972	0.973	0.967	-2	2	0.035	0.032	0.057	0.055	3		
	3	0.983	0.979	0.977	0.971	-2	3	0.035	0.034	0.056	0.050	2		
	Mean	0.978	0.976	0.973	0.968	-3	Mean	0.035	0.032	0.056	0.051	2		
FDSC-17	1	0.982	0.981	0.975	0.977	4	1	0.033	0.032	0.068	0.053	17	Positive	(Weakly photoreactive)
	2	0.997	0.989	0.991	0.984	3	2	0.034	0.033	0.076	0.052	24		
	3	0.997	0.995	0.993	0.993	1	3	0.033	0.032	0.069	0.046	18		
	Mean	0.992	0.988	0.986	0.985	3	Mean	0.033	0.032	0.071	0.050	20		
FDSC-18	1	0.958	0.960	0.952	0.955	1	1	0.034	0.032	0.053	0.043	6	Inconclusive	(Non-photoreactive)
	2	0.967	0.955	0.964	0.951	-2	2	0.033	0.032	0.053	0.047	7		
	3	0.967	0.966	0.970	0.958	-8	3	0.035	0.033	0.053	0.045	5		
	Mean	0.964	0.960	0.962	0.955	-3	Mean	0.034	0.032	0.053	0.045	6		
Mean for 3 assays	-	-	-	-	-1	Mean for 3 assays	-	-	-	-	9	Inconclusive*3	(Non-photoreactive)*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Octyl methacrylate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-07	1	0.975	0.973	0.973	0.964	-3	1	0.034	0.032	0.044	0.048	-6	Inconclusive	Non-photoreactive
	2	0.982	0.971	0.973	0.967	4	2	0.033	0.032	0.043	0.049	-6		
	3	0.984	0.978	0.977	0.976	2	3	0.034	0.033	0.044	0.047	-6		
	Mean	0.980	0.974	0.974	0.969	1	Mean	0.034	0.032	0.044	0.048	-6		
FDSC-15	1	0.981	0.974	0.975	0.967	1	1	0.032	0.033	0.046	0.054	-1	Inconclusive	Non-photoreactive
	2	0.981	0.975	0.979	0.971	-3	2	0.032	0.032	0.047	0.048	0		
	3	0.987	0.982	0.984	0.977	-2	3	0.033	0.035	0.046	0.043	-2		
	Mean	0.983	0.977	0.979	0.972	-1	Mean	0.032	0.033	0.046	0.048	-1		
FDSC-22	1	0.963	0.980	0.952	0.974	5	1	0.033	0.031	0.040	0.040	-7	Inconclusive	Non-photoreactive
	2	0.965	0.982	0.956	0.974	3	2	0.036	0.033	0.042	0.057	-8		
	3	0.970	0.991	0.959	0.986	5	3	0.033	0.032	0.040	0.042	-7		
	Mean	0.966	0.984	0.956	0.978	4	Mean	0.034	0.032	0.041	0.046	-7		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	-5	Inconclusive*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Octyl methoxycinnamate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-10	1	0.977	0.982	0.971	0.976	-3	1	0.032	0.032	0.045	0.047	-6	Inconclusive	(Non-photoreactive)
	2	0.982	0.992	0.977	0.975	-4	2	0.031	0.032	0.046	0.053	-4		
	3	0.985	0.987	0.982	0.984	-6	3	0.032	0.034	0.046	0.055	-5		
	Mean	0.981	0.987	0.977	0.978	-4	Mean	0.032	0.033	0.046	0.052	-5		
FDSC-16	1	0.977	0.986	0.972	0.996	5	1	0.031	0.031	0.042	0.041	0	Inconclusive	(Non-photoreactive)
	2	0.979	0.982	0.974	0.980	5	2	0.030	0.032	0.044	0.044	3		
	3	0.980	0.993	0.973	0.986	7	3	0.031	0.033	0.044	0.043	2		
	Mean	0.979	0.987	0.973	0.987	6	Mean	0.031	0.032	0.043	0.043	2		
FDSC-23	1	0.962	0.957	0.950	0.946	2	1	0.030	0.032	0.046	0.059	-2	Inconclusive	(Non-photoreactive)
	2	0.969	0.960	0.955	0.947	4	2	0.031	0.032	0.050	0.045	1		
	3	0.965	0.965	0.955	0.959	0	3	0.032	0.033	0.055	0.045	5		
	Mean	0.965	0.961	0.953	0.951	2	Mean	0.031	0.032	0.050	0.050	1		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	-1	Inconclusive*3	(Non-photoreactive)*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : Octyl salicylate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-07	1	0.983	0.973	0.979	0.964	-1	1	0.041	0.032	0.062	0.048	5	Inconclusive	Non-photoreactive
	2	0.992	0.971	0.985	0.967	2	2	0.042	0.032	0.068	0.049	10		
	3	0.996	0.978	0.988	0.976	3	3	0.041	0.033	0.063	0.047	6		
	Mean	0.990	0.974	0.984	0.969	1	Mean	0.041	0.032	0.064	0.048	7		
FDSC-15	1	0.996	0.974	0.983	0.967	8	1	0.043	0.033	0.060	0.054	2	Inconclusive	Non-photoreactive
	2	1.004	0.975	0.993	0.971	6	2	0.043	0.032	0.058	0.048	0		
	3	1.005	0.982	0.994	0.977	6	3	0.044	0.035	0.062	0.043	3		
	Mean	1.002	0.977	0.990	0.972	7	Mean	0.043	0.033	0.060	0.048	2		
FDSC-22	1	0.984	0.980	0.970	0.974	8	1	0.043	0.031	0.054	0.040	-3	Inconclusive	Non-photoreactive
	2	0.989	0.982	0.976	0.974	7	2	0.044	0.033	0.056	0.057	-2		
	3	0.993	0.991	0.980	0.986	7	3	0.046	0.032	0.054	0.042	-6		
	Mean	0.989	0.984	0.975	0.978	7	Mean	0.044	0.032	0.055	0.046	-4		
Mean for 3 assays	-	-	-	-	5	Mean for 3 assays	-	-	-	-	2	Inconclusive*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : PABA

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	A440(-)			A440(+)			A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
FDSC-04	1	0.964	0.972	0.953	0.963	1	1	0.031	0.032	0.042	0.050	-6	Negative	Non-photoreactive
	2	0.969	0.970	0.955	0.959	4	2	0.032	0.032	0.040	0.049	-9		
	3	0.973	0.975	0.962	0.963	1	3	0.033	0.033	0.039	0.049	-11		
	Mean	0.969	0.972	0.957	0.962	2	Mean	0.032	0.032	0.040	0.049	-9		
FDSC-13	1	0.965	0.970	0.957	0.970	3	1	0.032	0.034	0.041	0.043	0	Negative	Non-photoreactive
	2	0.966	0.970	0.960	0.962	1	2	0.032	0.034	0.040	0.043	-1		
	3	0.960	0.972	0.952	0.965	3	3	0.033	0.033	0.037	0.044	-5		
	Mean	0.964	0.971	0.956	0.966	2	Mean	0.032	0.034	0.039	0.043	-2		
FDSC-20	1	0.966	0.974	0.918	0.949	29	1	0.030	0.032	0.043	0.046	1	Negative	Non-photoreactive
	2	0.971	0.972	0.963	0.954	-11	2	0.030	0.032	0.042	0.045	0		
	3	0.976	0.980	0.954	0.966	3	3	0.032	0.034	0.039	0.045	-5		
	Mean	0.971	0.975	0.945	0.956	7	Mean	0.031	0.033	0.041	0.045	-1		
Mean for 3 assays	-	-	-	-	4	Mean for 3 assays	-	-	-	-	-4	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : SDS

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-04	1	0.945	0.972	0.931	0.963	4	FDSC-11	1	0.033	0.031	0.050	0.049	-2	Inconclusive (Non-photoreactive)
	2	0.960	0.970	0.941	0.959	9		2	0.035	0.032	0.059	0.055	5	
	3	0.950	0.975	0.937	0.963	3		3	0.035	0.034	0.054	0.050	0	
	Mean	0.952	0.972	0.936	0.962	5		Mean	0.034	0.032	0.054	0.051	1	
FDSC-09	1	0.977	0.984	0.959	0.975	12	FDSC-17	1	0.033	0.032	0.047	0.053	-4	Inconclusive (Non-photoreactive)
	2	0.989	0.984	0.981	0.980	2		2	0.034	0.033	0.052	0.052	0	
	3	0.985	0.994	0.969	0.987	10		3	0.035	0.032	0.046	0.046	-7	
	Mean	0.984	0.987	0.970	0.981	8		Mean	0.034	0.032	0.048	0.050	-4	
FDSC-11	1	0.961	0.977	0.919	0.965	34	FDSC-18	1	0.034	0.032	0.050	0.043	3	Inconclusive (Non-photoreactive)*3
	2	0.974	0.972	0.968	0.967	-2		2	0.035	0.032	0.048	0.047	0	
	3	0.971	0.979	0.958	0.971	5		3	0.036	0.033	0.050	0.045	1	
	Mean	0.969	0.976	0.948	0.968	12		Mean	0.035	0.032	0.049	0.045	1	
Mean for 3 assays	-	-	-	-	8	Mean for 3 assays	-	-	-	-	-1	Inconclusive*3 (Non-photoreactive)*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 2
 Chemical Name : UV-571

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
FDSC-10	1	0.997	0.982	0.994	0.976	-6	FDSC-10	1	0.034	0.032	0.054	0.047	1	Inconclusive (Non-photoreactive)
	2	0.996	0.992	0.997	0.975	-10		2	0.034	0.032	0.055	0.053	2	
	3	0.999	0.987	1.003	0.984	-13		3	0.034	0.034	0.057	0.055	4	
	Mean	0.997	0.987	0.998	0.978	-10		Mean	0.034	0.033	0.055	0.052	2	
FDSC-16	1	0.988	0.986	0.989	0.996	-1	FDSC-16	1	0.034	0.031	0.052	0.041	7	Inconclusive (Non-photoreactive)
	2	0.993	0.982	0.994	0.980	-1		2	0.033	0.032	0.053	0.044	9	
	3	0.998	0.993	0.976	0.986	22		3	0.034	0.033	0.053	0.043	8	
	Mean	0.993	0.987	0.986	0.987	7		Mean	0.034	0.032	0.053	0.043	8	
FDSC-26	1	0.956	0.959	0.954	0.954	-4	FDSC-26	1	0.033	0.037	0.045	0.042	1	Inconclusive (Non-photoreactive)
	2	0.964	0.955	0.961	0.949	-3		2	0.033	0.033	0.045	0.050	1	
	3	0.964	0.964	0.963	0.956	-5		3	0.034	0.033	0.044	0.043	-1	
	Mean	0.961	0.959	0.959	0.953	-4		Mean	0.033	0.034	0.045	0.045	0	
Mean for 3 assays	-	-	-	-	-2	Mean for 3 assays	-	-	-	-	3	Inconclusive*3 (Non-photoreactive)*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Acridine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-001	1	0.988	1.005	0.757	1.000	228	1	0.029	0.030	0.227	0.035	191	Positive	Photoreactive
	2	1.005	1.009	0.778	1.005	225	2	0.030	0.030	0.229	0.035	192		
	3	0.994	1.010	0.775	1.012	217	3	0.029	0.029	0.228	0.036	192		
	Mean	0.996	1.008	0.770	1.006	223	Mean	0.030	0.029	0.228	0.036	192		
MT-009	1	0.980	0.994	0.756	0.989	221	1	0.029	0.029	0.203	0.034	169	Positive	Photoreactive
	2	0.997	0.997	0.771	0.994	222	2	0.030	0.029	0.205	0.035	171		
	3	0.988	0.999	0.765	0.995	219	3	0.029	0.028	0.209	0.034	176		
	Mean	0.988	0.997	0.764	0.993	221	Mean	0.029	0.029	0.206	0.034	172		
MT-016	1	0.983	0.996	0.751	0.992	228	1	0.028	0.029	0.205	0.034	173	Positive	Photoreactive
	2	0.982	0.988	0.757	0.985	221	2	0.028	0.029	0.200	0.033	168		
	3	0.986	0.989	0.764	0.984	219	3	0.029	0.029	0.196	0.033	163		
	Mean	0.984	0.991	0.757	0.987	223	Mean	0.028	0.029	0.200	0.033	168		
Mean for 3 assays	-	-	-	-	222	Mean for 3 assays	-	-	-	-	177	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Acridine HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-001	1	0.995	1.005	0.774	1.000	219	1	0.031	0.030	0.212	0.035	175	Positive	Photoreactive
	2	1.001	1.009	0.785	1.005	214	2	0.030	0.030	0.212	0.035	175		
	3	1.003	1.010	0.788	1.012	213	3	0.029	0.029	0.215	0.036	179		
	Mean	0.999	1.008	0.782	1.006	215	Mean	0.030	0.029	0.213	0.036	176		
MT-009	1	0.982	0.994	0.757	0.989	221	1	0.028	0.029	0.213	0.034	180	Positive	Photoreactive
	2	0.987	0.997	0.770	0.994	213	2	0.029	0.029	0.214	0.035	180		
	3	0.996	0.999	0.780	0.995	212	3	0.028	0.028	0.217	0.034	184		
	Mean	0.988	0.997	0.769	0.993	215	Mean	0.029	0.029	0.215	0.034	181		
MT-016	1	0.981	0.996	0.755	0.992	222	1	0.029	0.029	0.199	0.034	166	Positive	Photoreactive
	2	0.990	0.988	0.770	0.985	216	2	0.029	0.029	0.196	0.033	163		
	3	0.992	0.989	0.771	0.984	217	3	0.028	0.029	0.196	0.033	164		
	Mean	0.988	0.991	0.765	0.987	218	Mean	0.029	0.029	0.197	0.033	164		
Mean for 3 assays	-	-	-	-	216	Mean for 3 assays	-	-	-	-	174	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Amiodarone HCl

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-010	1	0.979	0.998	0.938	0.994	37	MT-010	1	0.032	0.029	0.037	0.034	0	Positive (Photoreactive)
	2	1.007	1.002	0.965	0.997	38		2	0.032	0.029	0.038	0.035	1	
	3	1.017	1.001	0.981	0.996	32		3	0.032	0.028	0.038	0.034	0	
	Mean	1.001	1.000	0.961	0.996	36		Mean	0.032	0.029	0.038	0.034	0	
MT-016	1	0.990	0.996	0.936	0.992	50	MT-016	1	0.032	0.029	0.037	0.034	1	Positive (Photoreactive)
	2	0.990	0.988	0.934	0.985	53		2	0.031	0.029	0.037	0.033	1	
	3	0.991	0.989	0.938	0.984	49		3	0.031	0.029	0.039	0.033	3	
	Mean	0.990	0.991	0.936	0.987	51		Mean	0.031	0.029	0.038	0.033	2	
MT-023	1	0.991	1.002	0.936	0.997	51	MT-023	1	0.031	0.029	0.038	0.036	0	Positive (Photoreactive)
	2	0.997	0.998	0.943	0.993	50		2	0.031	0.029	0.038	0.036	1	
	3	0.996	0.998	0.949	0.995	43		3	0.030	0.029	0.038	0.036	0	
	Mean	0.995	0.999	0.943	0.995	48		Mean	0.031	0.029	0.038	0.036	0	
Mean for 3 assays	-	-	-	-	45	Mean for 3 assays	-	-	-	-	1	Positive*3 (Photoreactive)*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Chlorpromazine HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement			
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol		
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank					
MT-005	1	0.971	1.000	0.986	0.997	-20	MT-005	1	0.029	0.029	0.119	0.034	85	Positive	Photoreactive	
	2	0.973	1.003	0.992	1.000	-23		2	0.029	0.029	0.121	0.034				87
	3	0.973	1.002	0.993	0.998	-23		3	0.029	0.029	0.122	0.034				88
	Mean	0.972	1.002	0.990	0.998	-22		Mean	0.029	0.029	0.121	0.034				87
MT-013	1	0.963	0.994	0.994	0.988	-35	MT-013	1	0.028	0.029	0.117	0.034	84	Positive	Photoreactive	
	2	0.966	0.995	0.992	0.991	-30		2	0.029	0.029	0.119	0.035				84
	3	0.970	0.995	0.998	0.990	-31		3	0.029	0.028	0.119	0.034				85
	Mean	0.966	0.994	0.994	0.990	-32		Mean	0.029	0.029	0.118	0.034				84
MT-020	1	0.973	1.003	0.992	1.003	-21	MT-020	1	0.029	0.030	0.113	0.035	78	Positive	Photoreactive	
	2	0.981	1.000	1.006	0.998	-26		2	0.029	0.030	0.113	0.035				78
	3	0.977	0.999	0.997	0.997	-22		3	0.029	0.031	0.113	0.036				79
	Mean	0.977	1.001	0.998	0.999	-23		Mean	0.029	0.030	0.113	0.036				78
Mean for 3 assays	-	-	-	-	-26	Mean for 3 assays	-	-	-	-	83	Positive	Photoreactive ^{*3}			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Doxycycline HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
MT-006	1	0.978	1.002	0.805	0.996	167	MT-006	1	0.036	0.029	0.280	0.034	240	Positive	Photoreactive
	2	0.995	1.006	0.824	0.999	165		2	0.037	0.029	0.292	0.034	250		
	3	0.987	1.007	0.817	1.001	165		3	0.036	0.028	0.291	0.033	250		
	Mean	0.987	1.005	0.815	0.999	166		Mean	0.036	0.029	0.288	0.034	247		
MT-014	1	0.980	1.007	0.814	1.003	162	MT-014	1	0.035	0.029	0.282	0.035	241	Positive	Photoreactive
	2	0.999	1.012	0.837	1.008	159		2	0.036	0.029	0.293	0.035	251		
	3	0.992	1.010	0.828	1.009	160		3	0.035	0.029	0.296	0.037	255		
	Mean	0.991	1.010	0.826	1.006	160		Mean	0.035	0.029	0.290	0.035	249		
MT-021	1	0.984	1.008	0.816	1.001	163	MT-021	1	0.035	0.029	0.297	0.035	257	Positive	Photoreactive
	2	0.982	1.004	0.820	1.000	157		2	0.035	0.030	0.307	0.035	266		
	3	0.987	1.004	0.823	1.001	159		3	0.034	0.029	0.300	0.035	260		
	Mean	0.985	1.005	0.820	1.000	160		Mean	0.035	0.029	0.302	0.035	261		
Mean for 3 assays	-	-	-	-	162	Mean for 3 assays	-	-	-	-	252	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Fenofibrate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
MT-002	1	1.011	1.011	0.831	1.005	175	MT-002	1	0.038	0.029	0.033	0.033	-9	Positive	Photoreactive
	2	1.009	1.016	0.851	1.011	153		2	0.038	0.030	0.033	0.034	-9		
	3	1.009	1.018	0.850	1.013	154		3	0.037	0.028	0.034	0.033	-8		
	Mean	1.009	1.015	0.844	1.010	161		Mean	0.038	0.029	0.034	0.034	-9		
MT-011	1	1.004	0.998	0.839	0.993	160	MT-011	1	0.039	0.030	0.033	0.034	-12	Positive	Photoreactive
	2	1.007	1.005	0.839	0.999	162		2	0.039	0.029	0.033	0.035	-11		
	3	1.004	1.003	0.838	1.000	161		3	0.038	0.028	0.033	0.035	-12		
	Mean	1.005	1.002	0.839	0.997	161		Mean	0.039	0.029	0.033	0.035	-12		
MT-019	1	1.013	1.009	0.846	1.001	159	MT-019	1	0.039	0.030	0.033	0.035	-12	Positive	Photoreactive
	2	1.015	1.002	0.845	0.992	161		2	0.040	0.030	0.033	0.035	-12		
	3	1.017	1.003	0.845	0.994	163		3	0.039	0.030	0.033	0.035	-11		
	Mean	1.015	1.005	0.845	0.996	161		Mean	0.039	0.030	0.033	0.035	-12		
Mean for 3 assays	-	-	-	-	161	Mean for 3 assays	-	-	-	-	-11	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Furosemide

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-004	1	0.993	0.999	0.838	0.992	150	1	0.032	0.029	0.087	0.034	50	Positive	Photoreactive
	2	1.002	1.003	0.850	0.997	146	2	0.033	0.029	0.088	0.034	50		
	3	0.998	1.001	0.854	0.996	138	3	0.031	0.029	0.092	0.034	56		
	Mean	0.998	1.001	0.847	0.995	145	Mean	0.032	0.029	0.089	0.034	52		
MT-012	1	0.990	0.992	0.836	0.989	150	1	0.031	0.030	0.084	0.034	48	Positive	Photoreactive
	2	0.997	0.999	0.843	0.994	150	2	0.032	0.029	0.085	0.035	48		
	3	0.992	0.999	0.850	0.996	139	3	0.031	0.029	0.089	0.034	53		
	Mean	0.993	0.997	0.843	0.993	146	Mean	0.031	0.029	0.086	0.034	50		
MT-018	1	0.998	0.997	0.841	0.993	154	1	0.031	0.029	0.089	0.033	53	Positive	Photoreactive
	2	0.989	0.992	0.845	0.987	140	2	0.031	0.028	0.090	0.033	53		
	3	0.989	0.990	0.846	0.987	138	3	0.031	0.028	0.091	0.033	55		
	Mean	0.992	0.993	0.844	0.989	144	Mean	0.031	0.028	0.090	0.033	54		
Mean for 3 assays	-	-	-	-	145	Mean for 3 assays	-	-	-	-	52	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Ketoprofen

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-001	1	1.010	1.005	0.779	1.000	229	1	0.030	0.030	0.115	0.035	78	Positive	Photoreactive
	2	1.011	1.009	0.788	1.005	221	2	0.031	0.030	0.117	0.035	79		
	3	1.014	1.010	0.788	1.012	223	3	0.030	0.029	0.119	0.036	82		
	Mean	1.012	1.008	0.785	1.006	224	Mean	0.030	0.029	0.117	0.036	80		
MT-009	1	0.996	0.994	0.770	0.989	222	1	0.029	0.029	0.120	0.034	86	Positive	Photoreactive
	2	1.002	0.997	0.776	0.994	222	2	0.030	0.029	0.123	0.035	88		
	3	1.000	0.999	0.782	0.995	215	3	0.029	0.028	0.124	0.034	89		
	Mean	1.000	0.997	0.776	0.993	220	Mean	0.030	0.029	0.122	0.034	88		
MT-016	1	0.992	0.996	0.781	0.992	207	1	0.030	0.029	0.120	0.034	86	Positive	Photoreactive
	2	0.994	0.988	0.784	0.985	207	2	0.030	0.029	0.121	0.033	88		
	3	0.996	0.989	0.788	0.984	204	3	0.029	0.029	0.121	0.033	87		
	Mean	0.994	0.991	0.784	0.987	206	Mean	0.030	0.029	0.121	0.033	87		
Mean for 3 assays	-	-	-	-	217	Mean for 3 assays	-	-	-	-	85	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : 6-methylcoumarine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
MT-006	1	0.999	1.002	0.885	0.996	108	MT-006	1	0.029	0.029	0.096	0.034	62	Positive	Photoreactive
	2	1.007	1.006	0.896	0.999	105		2	0.029	0.029	0.095	0.034	61		
	3	1.008	1.007	0.898	1.001	104		3	0.029	0.028	0.098	0.033	64		
	Mean	1.005	1.005	0.893	0.999	106		Mean	0.029	0.029	0.097	0.034	62		
MT-014	1	1.004	1.007	0.905	1.003	95	MT-014	1	0.029	0.029	0.104	0.035	69	Positive	Photoreactive
	2	1.014	1.012	0.914	1.008	96		2	0.030	0.029	0.105	0.035	69		
	3	1.009	1.010	0.910	1.009	96		3	0.029	0.029	0.107	0.037	72		
	Mean	1.009	1.010	0.909	1.006	96		Mean	0.029	0.029	0.105	0.035	70		
MT-021	1	1.005	1.008	0.905	1.001	96	MT-021	1	0.030	0.029	0.101	0.035	65	Positive	Photoreactive
	2	1.005	1.004	0.902	1.000	98		2	0.030	0.030	0.102	0.035	66		
	3	1.006	1.004	0.899	1.001	102		3	0.030	0.029	0.105	0.035	70		
	Mean	1.005	1.005	0.902	1.000	99		Mean	0.030	0.029	0.103	0.035	67		
Mean for 3 assays	-	-	-	-	100	Mean for 3 assays	-	-	-	-	66	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : 8-MOP

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
MT-001	1	0.994	1.005	0.925	1.000	67	MT-001	1	0.031	0.030	0.061	0.035	23	Positive	Photoreactive
	2	1.002	1.009	0.933	1.005	68		2	0.030	0.030	0.059	0.035	22		
	3	1.001	1.010	0.938	1.012	61		3	0.030	0.029	0.060	0.036	23		
	Mean	0.999	1.008	0.932	1.006	65		Mean	0.030	0.029	0.060	0.036	23		
MT-009	1	0.990	0.994	0.909	0.989	77	MT-009	1	0.030	0.029	0.064	0.034	29	Positive	Photoreactive
	2	0.994	0.997	0.910	0.994	80		2	0.030	0.029	0.064	0.035	29		
	3	0.993	0.999	0.915	0.995	74		3	0.030	0.028	0.065	0.034	31		
	Mean	0.992	0.997	0.911	0.993	77		Mean	0.030	0.029	0.065	0.034	30		
MT-016	1	0.986	0.996	0.911	0.992	70	MT-016	1	0.030	0.029	0.068	0.034	34	Positive	Photoreactive
	2	0.989	0.988	0.912	0.985	73		2	0.029	0.029	0.064	0.033	31		
	3	0.984	0.989	0.912	0.984	68		3	0.030	0.029	0.062	0.033	29		
	Mean	0.986	0.991	0.912	0.987	70		Mean	0.030	0.029	0.065	0.033	31		
Mean for 3 assays	-	-	-	-	71	Mean for 3 assays	-	-	-	-	28	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Nalidixic acid

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-002	1	1.009	1.011	0.866	1.005	138	1	0.030	0.029	0.351	0.033	316	Positive	Photoreactive
	2	1.020	1.016	0.881	1.011	134	2	0.030	0.030	0.317	0.034	282		
	3	1.014	1.018	0.880	1.013	130	3	0.029	0.028	0.318	0.033	284		
	Mean	1.014	1.015	0.875	1.010	134	Mean	0.029	0.029	0.329	0.034	294		
MT-011	1	0.999	0.998	0.859	0.993	135	1	0.029	0.030	0.386	0.034	351	Positive	Photoreactive
	2	1.011	1.005	0.876	0.999	130	2	0.030	0.029	0.397	0.035	362		
	3	1.005	1.003	0.874	1.000	126	3	0.029	0.028	0.388	0.035	354		
	Mean	1.005	1.002	0.870	0.997	130	Mean	0.029	0.029	0.390	0.035	356		
MT-019	1	1.004	1.009	0.877	1.001	118	1	0.029	0.030	0.339	0.035	305	Positive	Photoreactive
	2	1.003	1.002	0.875	0.992	119	2	0.029	0.030	0.362	0.035	328		
	3	1.007	1.003	0.878	0.994	120	3	0.029	0.030	0.344	0.035	310		
	Mean	1.005	1.005	0.877	0.996	119	Mean	0.029	0.030	0.348	0.035	314		
Mean for 3 assays	-	-	-	-	128	Mean for 3 assays	-	-	-	-	321	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Nalidixic acid (Na salt)

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement			
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol		
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank					
MT-002	1	1.007	1.011	0.873	1.005	129	MT-002	1	0.029	0.029	0.331	0.033	297	Positive	Photoreactive	
	2	1.015	1.016	0.886	1.011	124		2	0.030	0.030	0.342	0.034				308
	3	1.012	1.018	0.886	1.013	121		3	0.029	0.028	0.327	0.033				293
	Mean	1.011	1.015	0.882	1.010	125		Mean	0.029	0.029	0.334	0.034				299
MT-011	1	0.993	0.998	0.862	0.993	126	MT-011	1	0.030	0.030	0.367	0.034	331	Positive	Photoreactive	
	2	1.006	1.005	0.874	0.999	127		2	0.030	0.029	0.389	0.035				353
	3	1.002	1.003	0.877	1.000	120		3	0.029	0.028	0.376	0.035				340
	Mean	1.000	1.002	0.871	0.997	124		Mean	0.030	0.029	0.377	0.035				341
MT-019	1	1.008	1.009	0.879	1.001	120	MT-019	1	0.031	0.030	0.327	0.035	291	Positive	Photoreactive	
	2	1.007	1.002	0.881	0.992	116		2	0.030	0.030	0.397	0.035				362
	3	1.005	1.003	0.876	0.994	120		3	0.030	0.030	0.342	0.035				308
	Mean	1.007	1.005	0.879	0.996	119		Mean	0.030	0.030	0.355	0.035				320
Mean for 3 assays	-	-	-	-	123	Mean for 3 assays	-	-	-	-	320	Positive	Photoreactive ^{*3}			

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Norfloxacin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-005	1	0.995	1.000	0.826	0.997	164	1	0.031	0.029	0.184	0.034	148	Positive	Photoreactive
	2	0.999	1.003	0.832	1.000	163	2	0.030	0.029	0.181	0.034	147		
	3	0.997	1.002	0.828	0.998	164	3	0.030	0.029	0.186	0.034	152		
	Mean	0.997	1.002	0.829	0.998	164	Mean	0.030	0.029	0.184	0.034	149		
MT-013	1	0.989	0.994	0.811	0.988	174	1	0.030	0.029	0.182	0.034	147	Positive	Photoreactive
	2	0.995	0.995	0.820	0.991	171	2	0.030	0.029	0.182	0.035	148		
	3	0.992	0.995	0.819	0.990	169	3	0.029	0.028	0.187	0.034	153		
	Mean	0.992	0.994	0.816	0.990	171	Mean	0.030	0.029	0.184	0.034	149		
MT-020	1	1.008	1.003	0.832	1.003	174	1	0.030	0.030	0.175	0.035	139	Positive	Photoreactive
	2	1.009	1.000	0.841	0.998	167	2	0.030	0.030	0.177	0.035	142		
	3	1.003	0.999	0.834	0.997	166	3	0.030	0.031	0.173	0.036	137		
	Mean	1.007	1.001	0.836	0.999	169	Mean	0.030	0.030	0.175	0.036	139		
Mean for 3 assays	-	-	-	-	168	Mean for 3 assays	-	-	-	-	146	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Ofloxacin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-006	1	0.999	1.002	0.863	0.996	130	1	0.029	0.029	0.450	0.034	416	Positive	Photoreactive
	2	1.003	1.006	0.870	0.999	127	2	0.030	0.029	0.462	0.034	427		
	3	1.007	1.007	0.880	1.001	121	3	0.029	0.028	0.450	0.033	416		
	Mean	1.003	1.005	0.871	0.999	126	Mean	0.030	0.029	0.454	0.034	420		
MT-014	1	1.002	1.007	0.870	1.003	128	1	0.030	0.029	0.468	0.035	432	Positive	Photoreactive
	2	1.003	1.012	0.875	1.008	124	2	0.031	0.029	0.489	0.035	452		
	3	1.007	1.010	0.881	1.009	122	3	0.030	0.029	0.469	0.037	433		
	Mean	1.004	1.010	0.875	1.006	125	Mean	0.030	0.029	0.475	0.035	439		
MT-021	1	0.993	1.008	0.870	1.001	118	1	0.030	0.029	0.480	0.035	445	Positive	Photoreactive
	2	1.002	1.004	0.881	1.000	116	2	0.030	0.030	0.486	0.035	450		
	3	1.007	1.004	0.886	1.001	116	3	0.029	0.029	0.479	0.035	444		
	Mean	1.001	1.005	0.879	1.000	117	Mean	0.030	0.029	0.482	0.035	446		
Mean for 3 assays	-	-	-	-	123	Mean for 3 assays	-	-	-	-	435	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Piroxicam

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
MT-002	1	1.008	1.011	0.825	1.005	178	MT-003	1	0.033	0.029	0.043	0.036	4	Positive	Photoreactive
	2	1.011	1.016	0.840	1.011	166		2	0.034	0.029	0.044	0.034	4		
	3	1.011	1.018	0.846	1.013	160		3	0.033	0.028	0.043	0.034	4		
	Mean	1.010	1.015	0.837	1.010	168		Mean	0.033	0.029	0.044	0.035	4		
MT-011	1	1.012	0.998	0.840	0.993	167	MT-011	1	0.032	0.030	0.041	0.034	3	Positive	Photoreactive
	2	1.021	1.005	0.858	0.999	158		2	0.034	0.029	0.042	0.035	3		
	3	1.026	1.003	0.866	1.000	155		3	0.033	0.028	0.041	0.035	3		
	Mean	1.020	1.002	0.855	0.997	160		Mean	0.033	0.029	0.042	0.035	3		
MT-019	1	1.001	1.009	0.835	1.001	157	MT-019	1	0.033	0.030	0.045	0.035	7	Positive	Photoreactive
	2	1.002	1.002	0.841	0.992	152		2	0.033	0.030	0.045	0.035	7		
	3	1.003	1.003	0.843	0.994	151		3	0.033	0.030	0.046	0.035	8		
	Mean	1.002	1.005	0.840	0.996	153		Mean	0.033	0.030	0.045	0.035	7		
Mean for 3 assays	-	-	-	-	160	Mean for 3 assays	-	-	-	-	5	Positive	Photoreactive ^{*3}		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Promethazine HCl

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-004	1	0.981	0.999	0.933	0.992	43	1	0.030	0.029	0.070	0.034	34	Positive	Photoreactive
	2	0.985	1.003	0.935	0.997	43	2	0.030	0.029	0.069	0.034	34		
	3	0.985	1.001	0.935	0.996	43	3	0.029	0.029	0.071	0.034	37		
	Mean	0.984	1.001	0.935	0.995	43	Mean	0.030	0.029	0.070	0.034	35		
MT-012	1	0.974	0.992	0.922	0.989	48	1	0.030	0.030	0.067	0.034	32	Positive	Photoreactive
	2	0.978	0.999	0.925	0.994	49	2	0.030	0.029	0.067	0.035	32		
	3	0.978	0.999	0.932	0.996	42	3	0.029	0.029	0.068	0.034	34		
	Mean	0.977	0.997	0.926	0.993	46	Mean	0.030	0.029	0.067	0.034	33		
MT-018	1	0.976	0.997	0.938	0.993	34	1	0.029	0.029	0.068	0.033	34	Positive	Photoreactive
	2	0.979	0.992	0.936	0.987	39	2	0.029	0.028	0.069	0.033	35		
	3	0.973	0.990	0.926	0.987	43	3	0.029	0.028	0.072	0.033	38		
	Mean	0.976	0.993	0.933	0.989	39	Mean	0.029	0.028	0.070	0.033	36		
Mean for 3 assays	-	-	-	-	43	Mean for 3 assays	-	-	-	-	35	Positive*3	Photoreactive*3	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) - (A-B) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) - (B-A) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 20 or 2 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Rosiglitazone

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Experimental No.	Superoxide anion						Judgement	
	A440(-)			A440(+)				A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1		Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
MT-006	1	0.962	1.002	0.901	0.996	54	MT-006	1	0.032	0.029	0.051	0.034	14	Positive	Photoreactive
	2	0.968	1.006	0.904	0.999	57		2	0.032	0.029	0.053	0.034	16		
	3	0.966	1.007	0.900	1.001	61		3	0.032	0.028	0.053	0.033	16		
	Mean	0.965	1.005	0.902	0.999	57		Mean	0.032	0.029	0.052	0.034	15		
MT-014	1	0.966	1.007	0.907	1.003	55	MT-014	1	0.032	0.029	0.055	0.035	17	Positive	Photoreactive
	2	0.969	1.012	0.909	1.008	56		2	0.032	0.029	0.055	0.035	17		
	3	0.968	1.010	0.912	1.009	52		3	0.031	0.029	0.053	0.037	16		
	Mean	0.968	1.010	0.910	1.006	54		Mean	0.032	0.029	0.054	0.035	17		
MT-021	1	0.965	1.008	0.909	1.001	51	MT-021	1	0.031	0.029	0.053	0.035	16	Positive	Photoreactive
	2	0.973	1.004	0.912	1.000	56		2	0.030	0.030	0.051	0.035	15		
	3	0.966	1.004	0.906	1.001	56		3	0.031	0.029	0.052	0.035	15		
	Mean	0.968	1.005	0.909	1.000	54		Mean	0.031	0.029	0.052	0.035	15		
Mean for 3 assays	-	-	-	-	55	Mean for 3 assays	-	-	-	-	16	Positive*3	Photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Tetracycline

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-004	1	0.999	0.999	0.858	0.992	135	1	0.034	0.029	0.185	0.034	146	Positive	Photoreactive
	2	1.000	1.003	0.860	0.997	134	2	0.034	0.029	0.187	0.034	148		
	3	0.995	1.001	0.857	0.996	133	3	0.034	0.029	0.182	0.034	143		
	Mean	0.998	1.001	0.858	0.995	134	Mean	0.034	0.029	0.185	0.034	146		
MT-012	1	0.989	0.992	0.857	0.989	129	1	0.036	0.030	0.144	0.034	103	Positive	Photoreactive
	2	0.998	0.999	0.864	0.994	131	2	0.033	0.029	0.141	0.035	103		
	3	0.991	0.999	0.860	0.996	127	3	0.033	0.029	0.134	0.034	97		
	Mean	0.993	0.997	0.860	0.993	129	Mean	0.034	0.029	0.140	0.034	101		
MT-018	1	0.980	0.997	0.850	0.993	127	1	0.034	0.029	0.164	0.033	125	Positive	Photoreactive
	2	0.989	0.992	0.853	0.987	131	2	0.034	0.028	0.166	0.033	127		
	3	0.986	0.990	0.854	0.987	128	3	0.034	0.028	0.157	0.033	118		
	Mean	0.985	0.993	0.852	0.989	129	Mean	0.034	0.028	0.162	0.033	123		
Mean for 3 assays	-	-	-	-	131	Mean for 3 assays	-	-	-	-	123	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Anthracene

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
MT-008	1	1.011	1.010	0.991	0.995	4	MT-008	1	0.031	0.031	0.041	0.035	Inconclusive	(Non-photoreactive)	
	2	1.007	1.012	0.986	0.997	6		2	0.032	0.031	0.041	0.036			4
	3	1.012	1.011	0.992	0.997	5		3	0.031	0.030	0.040	0.035			4
	Mean	1.010	1.011	0.990	0.996	5		Mean	0.032	0.031	0.041	0.036			4
MT-015	1	1.006	1.002	0.995	0.997	4	MT-015	1	0.028	0.030	0.037	0.035	Inconclusive	(Non-photoreactive)	
	2	1.013	1.007	1.002	1.004	4		2	0.029	0.030	0.037	0.035			4
	3	1.014	1.006	1.004	0.995	2		3	0.028	0.029	0.036	0.034			4
	Mean	1.011	1.005	1.000	0.998	3		Mean	0.029	0.030	0.037	0.034			4
MT-022	1	0.996	1.001	0.988	0.994	2	MT-022	1	0.029	0.030	0.037	0.034	Inconclusive	(Non-photoreactive)	
	2	1.000	1.000	0.988	0.992	6		2	0.028	0.030	0.037	0.035			4
	3	1.001	0.996	0.990	0.993	5		3	0.028	0.029	0.037	0.034			3
	Mean	0.999	0.999	0.988	0.993	4		Mean	0.028	0.029	0.037	0.034			4
Mean for 3 assays	-	-	-	-	4	Mean for 3 assays	-	-	-	-	4	Inconclusive*3	(Non-photoreactive)*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Avobenzone

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-005	1	1.011	1.000	0.950	0.997	56	1	0.066	0.029	0.084	0.034	13	Positive	Photoreactive
	2	1.024	1.003	0.960	1.000	61	2	0.066	0.029	0.084	0.034	13		
	3	1.016	1.002	0.955	0.998	58	3	0.065	0.029	0.083	0.034	13		
	Mean	1.017	1.002	0.955	0.998	58	Mean	0.066	0.029	0.084	0.034	13		
MT-013	1	1.014	0.994	0.955	0.988	55	1	0.071	0.029	0.085	0.034	9	Positive	Photoreactive
	2	1.026	0.995	0.960	0.991	61	2	0.072	0.029	0.086	0.035	9		
	3	1.020	0.995	0.951	0.990	65	3	0.071	0.028	0.086	0.034	10		
	Mean	1.020	0.994	0.956	0.990	60	Mean	0.071	0.029	0.086	0.034	9		
MT-020	1	1.031	1.003	0.966	1.003	63	1	0.058	0.030	0.083	0.035	18	Positive	Photoreactive
	2	1.032	1.000	0.964	0.998	66	2	0.059	0.030	0.081	0.035	17		
	3	1.038	0.999	0.969	0.997	67	3	0.058	0.031	0.083	0.036	19		
	Mean	1.034	1.001	0.966	0.999	65	Mean	0.058	0.030	0.082	0.036	18		
Mean for 3 assays	-	-	-	-	61	Mean for 3 assays	-	-	-	-	13	Positive*3	Photoreactive*3	

*1 : decrease of A440 $\times 10^3 = (A440(-) - A440(+)) \times 1000$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 $\times 10^3 = (A560(+) - A560(-)) \times 1000$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 20 at 200, 20 or 2 µM

Negative : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥ 25 or Superoxide anion results ≥ 70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results < 25 and Superoxide anion results ≥ 20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results < 25 and Superoxide anion results < 20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Bithionol

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-006	1	0.955	1.002	0.869	0.996	80	1	0.083	0.029	0.122	0.034	34	Positive	Photoreactive
	2	0.960	1.006	0.875	0.999	79	2	0.083	0.029	0.122	0.034	34		
	3	0.963	1.007	0.874	1.001	84	3	0.082	0.028	0.122	0.033	35		
	Mean	0.959	1.005	0.872	0.999	81	Mean	0.082	0.029	0.122	0.034	34		
MT-014	1	0.928	1.007	0.857	1.003	67	1	0.081	0.029	0.117	0.035	30	Positive	Photoreactive
	2	0.942	1.012	0.870	1.008	68	2	0.081	0.029	0.116	0.035	29		
	3	0.936	1.010	0.862	1.009	70	3	0.080	0.029	0.116	0.037	30		
	Mean	0.935	1.010	0.863	1.006	68	Mean	0.080	0.029	0.116	0.035	30		
MT-021	1	0.935	1.008	0.857	1.001	72	1	0.080	0.029	0.119	0.035	33	Positive	Photoreactive
	2	0.946	1.004	0.870	1.000	71	2	0.080	0.030	0.119	0.035	33		
	3	0.945	1.004	0.868	1.001	72	3	0.080	0.029	0.120	0.035	34		
	Mean	0.942	1.005	0.865	1.000	72	Mean	0.080	0.029	0.120	0.035	33		
Mean for 3 assays	-	-	-	-	74	Mean for 3 assays	-	-	-	-	32	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Hexachlorophene

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-005	1	0.911	1.000	0.682	0.997	225	1	0.056	0.029	0.072	0.034	10	Positive	Photoreactive
	2	0.927	1.003	0.696	1.000	227	2	0.056	0.029	0.072	0.034	10		
	3	0.938	1.002	0.704	0.998	230	3	0.055	0.029	0.071	0.034	11		
	Mean	0.925	1.002	0.694	0.998	227	Mean	0.056	0.029	0.072	0.034	10		
MT-013	1	0.875	0.994	0.650	0.988	221	1	0.054	0.029	0.067	0.034	8	Positive	Photoreactive
	2	0.901	0.995	0.668	0.991	229	2	0.055	0.029	0.067	0.035	8		
	3	0.909	0.995	0.677	0.990	228	3	0.054	0.028	0.067	0.034	8		
	Mean	0.895	0.994	0.665	0.990	226	Mean	0.054	0.029	0.067	0.034	8		
MT-020	1	0.911	1.003	0.694	1.003	215	1	0.056	0.030	0.068	0.035	6	Positive	Photoreactive
	2	0.926	1.000	0.705	0.998	219	2	0.055	0.030	0.068	0.035	6		
	3	0.932	0.999	0.713	0.997	217	3	0.055	0.031	0.068	0.036	7		
	Mean	0.923	1.001	0.704	0.999	217	Mean	0.055	0.030	0.068	0.036	6		
Mean for 3 assays	-	-	-	-	223	Mean for 3 assays	-	-	-	-	8	Positive*3	Photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Rose bengal

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
MT-005	1	1.201	1.000	0.581	0.997	617	MT-005	1	2.728	0.029	2.679	0.034	No data ^{*4}	Positive	Photoreactive
	2	1.207	1.003	0.599	1.000	604		2	2.759	0.029	2.689	0.034	No data ^{*4}		
	3	1.208	1.002	0.602	0.998	602		3	2.729	0.029	2.687	0.034	No data ^{*4}		
	Mean	1.205	1.002	0.594	0.998	608		Mean	2.738	0.029	2.685	0.034	No data ^{*4}		
MT-013	1	1.154	0.994	0.563	0.988	587	MT-013	1	2.720	0.029	2.672	0.034	No data ^{*4}	Positive	Photoreactive
	2	1.169	0.995	0.571	0.991	595		2	2.757	0.029	2.686	0.035	No data ^{*4}		
	3	1.164	0.995	0.574	0.990	586		3	2.736	0.028	2.682	0.034	No data ^{*4}		
	Mean	1.162	0.994	0.569	0.990	589		Mean	2.738	0.029	2.680	0.034	No data ^{*4}		
MT-020	1	1.193	1.003	0.580	1.003	611	MT-020	1	2.750	0.030	2.706	0.035	No data ^{*4}	Positive	Photoreactive
	2	1.205	1.000	0.597	0.998	606		2	2.735	0.030	2.703	0.035	No data ^{*4}		
	3	1.199	0.999	0.593	0.997	603		3	2.742	0.031	2.691	0.036	No data ^{*4}		
	Mean	1.199	1.001	0.590	0.999	607		Mean	2.742	0.030	2.700	0.036	No data ^{*4}		
Mean for 3 assays		-	-	-	-	601	Mean for 3 assays		-	-	-	-	No data ^{*4}	Positive ^{*3}	Photoreactive ^{*3}

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*4 : Over the OD criteria

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Aspirin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-004	1	1.001	0.999	0.996	0.992	-1	1	0.029	0.029	0.033	0.034	-1	Negative	Non-photoreactive
	2	1.003	1.003	0.998	0.997	-2	2	0.030	0.029	0.034	0.034	-1		
	3	1.005	1.001	1.002	0.996	-2	3	0.029	0.029	0.033	0.034	-1		
	Mean	1.003	1.001	0.999	0.995	-2	Mean	0.029	0.029	0.033	0.034	-1		
MT-012	1	0.994	0.992	0.988	0.989	2	1	0.029	0.030	0.033	0.034	-1	Negative	Non-photoreactive
	2	0.998	0.999	0.989	0.994	4	2	0.030	0.029	0.035	0.035	0		
	3	1.002	0.999	0.996	0.996	2	3	0.029	0.029	0.033	0.034	-1		
	Mean	0.998	0.997	0.991	0.993	3	Mean	0.029	0.029	0.034	0.034	-1		
MT-018	1	0.992	0.997	0.987	0.993	1	1	0.029	0.029	0.034	0.033	0	Negative	Non-photoreactive
	2	0.996	0.992	0.989	0.987	3	2	0.029	0.028	0.034	0.033	0		
	3	0.995	0.990	0.993	0.987	-2	3	0.029	0.028	0.034	0.033	0		
	Mean	0.994	0.993	0.990	0.989	1	Mean	0.029	0.028	0.034	0.033	0		
Mean for 3 assays	-	-	-	-	1	Mean for 3 assays	-	-	-	-	-1	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Benzocaine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-006	1	1.005	1.002	0.998	0.996	1	1	0.028	0.029	0.033	0.034	0	Negative	Non-photoreactive
	2	1.005	1.006	0.999	0.999	0	2	0.029	0.029	0.034	0.034	0		
	3	1.014	1.007	1.008	1.001	0	3	0.028	0.028	0.034	0.033	1		
	Mean	1.008	1.005	1.002	0.999	0	Mean	0.028	0.029	0.034	0.034	0		
MT-014	1	1.008	1.007	0.998	1.003	6	1	0.029	0.029	0.036	0.035	1	Negative	Non-photoreactive
	2	1.010	1.012	1.002	1.008	4	2	0.030	0.029	0.036	0.035	1		
	3	1.010	1.010	1.003	1.009	3	3	0.029	0.029	0.035	0.037	0		
	Mean	1.009	1.010	1.001	1.006	4	Mean	0.029	0.029	0.036	0.035	1		
MT-021	1	1.003	1.008	0.995	1.001	3	1	0.029	0.029	0.035	0.035	0	Negative	Non-photoreactive
	2	1.004	1.004	0.997	1.000	3	2	0.029	0.030	0.035	0.035	0		
	3	1.009	1.004	1.000	1.001	3	3	0.029	0.029	0.035	0.035	0		
	Mean	1.005	1.005	0.997	1.000	3	Mean	0.029	0.029	0.035	0.035	0		
Mean for 3 assays	-	-	-	-	2	Mean for 3 assays	-	-	-	-	0	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Erythromycin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
MT-007	1	0.958	0.996	0.955	0.992	0	MT-007	1	0.030	0.029	0.038	0.036	2	Negative	Non-photoreactive
	2	0.965	1.002	0.961	0.998	1		2	0.031	0.029	0.038	0.035	1		
	3	0.970	1.000	0.968	0.997	0		3	0.030	0.028	0.038	0.034	1		
	Mean	0.964	0.999	0.961	0.996	0		Mean	0.030	0.029	0.038	0.035	1		
MT-015	1	0.974	1.002	0.968	0.997	-2	MT-015	1	0.030	0.030	0.038	0.035	4	Negative	Non-photoreactive
	2	0.980	1.007	0.978	1.004	-5		2	0.031	0.030	0.038	0.035	3		
	3	0.987	1.006	0.987	0.995	-6		3	0.030	0.029	0.037	0.034	4		
	Mean	0.980	1.005	0.978	0.998	-4		Mean	0.030	0.030	0.038	0.034	4		
MT-022	1	0.955	1.001	0.953	0.994	-4	MT-022	1	0.030	0.030	0.037	0.034	2	Negative	Non-photoreactive
	2	0.971	1.000	0.968	0.992	-3		2	0.030	0.030	0.037	0.035	2		
	3	0.968	0.996	0.966	0.993	-3		3	0.030	0.029	0.037	0.034	2		
	Mean	0.965	0.999	0.962	0.993	-3		Mean	0.030	0.029	0.037	0.034	2		
Mean for 3 assays	-	-	-	-	-2	Mean for 3 assays	-	-	-	-	2	Negative*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Penicillin G

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
MT-006	1	1.004	1.002	0.998	0.996	0	MT-006	1	0.031	0.029	0.041	0.034	5	Negative	Non-photoreactive
	2	1.006	1.006	0.999	0.999	0		2	0.031	0.029	0.040	0.034	4		
	3	1.013	1.007	1.006	1.001	1		3	0.031	0.028	0.040	0.033	4		
	Mean	1.007	1.005	1.001	0.999	0		Mean	0.031	0.029	0.040	0.034	4		
MT-021	1	1.004	1.008	0.993	1.001	5	MT-021	1	0.031	0.029	0.049	0.035	11	Negative	Non-photoreactive
	2	1.007	1.004	0.997	1.000	4		2	0.032	0.030	0.049	0.035	12		
	3	1.009	1.004	0.999	1.001	6		3	0.031	0.029	0.047	0.035	10		
	Mean	1.007	1.005	0.996	1.000	5		Mean	0.031	0.029	0.048	0.035	11		
MT-023	1	1.001	1.002	0.992	0.997	5	MT-023	1	0.031	0.029	0.055	0.036	17	Negative	Non-photoreactive
	2	1.003	0.998	0.996	0.993	4		2	0.031	0.029	0.055	0.036	17		
	3	1.003	0.998	0.996	0.995	3		3	0.032	0.029	0.054	0.036	15		
	Mean	1.003	0.999	0.994	0.995	4		Mean	0.031	0.029	0.055	0.036	16		
Mean for 3 assays		-	-	-	-	3	Mean for 3 assays		-	-	-	-	10	Negative*3	Weakly photoreactive*3

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Phenytoin

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-004	1	0.994	0.999	0.985	0.992	3	1	0.031	0.029	0.070	0.034	34	Positive	Weakly photoreactive
	2	1.003	1.003	0.996	0.997	1	2	0.032	0.029	0.071	0.034	34		
	3	0.998	1.001	0.989	0.996	3	3	0.031	0.029	0.073	0.034	37		
	Mean	0.998	1.001	0.990	0.995	2	Mean	0.031	0.029	0.071	0.034	35		
MT-012	1	0.987	0.992	0.980	0.989	3	1	0.031	0.030	0.052	0.034	16	Negative	Non-photoreactive
	2	1.001	0.999	0.990	0.994	7	2	0.032	0.029	0.053	0.035	17		
	3	0.994	0.999	0.983	0.996	7	3	0.031	0.029	0.053	0.034	17		
	Mean	0.994	0.997	0.984	0.993	6	Mean	0.031	0.029	0.053	0.034	17		
MT-018	1	0.987	0.997	0.979	0.993	4	1	0.030	0.029	0.059	0.033	24	Positive	Weakly photoreactive
	2	0.979	0.992	0.972	0.987	3	2	0.031	0.028	0.061	0.033	25		
	3	0.984	0.990	0.976	0.987	4	3	0.030	0.028	0.065	0.033	29		
	Mean	0.983	0.993	0.975	0.989	4	Mean	0.030	0.028	0.062	0.033	26		
Mean for 3 assays	-	-	-	-	4	Mean for 3 assays	-	-	-	-	26	Positive	Weakly photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Bumetrizole

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-002	1	1.021	1.011	1.023	1.005	-7	1	0.067	0.029	0.070	0.033	-2	Inconclusive	Non-photoreactive
	2	1.026	1.016	1.027	1.011	-6	2	0.069	0.030	0.072	0.034	-2		
	3	1.023	1.018	1.024	1.013	-6	3	0.070	0.028	0.073	0.033	-2		
	Mean	1.023	1.015	1.025	1.010	-6	Mean	0.068	0.029	0.072	0.034	-2		
MT-011	1	1.017	0.998	1.028	0.993	-16	1	0.066	0.030	0.075	0.034	2	Inconclusive	Non-photoreactive
	2	1.022	1.005	1.033	0.999	-16	2	0.066	0.029	0.074	0.035	2		
	3	1.024	1.003	1.035	1.000	-16	3	0.066	0.028	0.074	0.035	3		
	Mean	1.021	1.002	1.032	0.997	-16	Mean	0.066	0.029	0.074	0.035	2		
MT-019	1	1.028	1.009	1.037	1.001	-18	1	0.055	0.030	0.069	0.035	9	Inconclusive	Non-photoreactive
	2	1.031	1.002	1.042	0.992	-19	2	0.056	0.030	0.070	0.035	9		
	3	1.026	1.003	1.039	0.994	-21	3	0.056	0.030	0.070	0.035	9		
	Mean	1.028	1.005	1.039	0.996	-19	Mean	0.056	0.030	0.070	0.035	9		
Mean for 3 assays	-	-	-	-	-14	Mean for 3 assays	-	-	-	-	3	Inconclusive*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Camphor sulfonic acid

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	A440(-)			A440(+)			A560(-)			A560(+)				
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
MT-001	1	1.012	1.005	1.006	1.000	4	1	0.031	0.030	0.034	0.035	-4	Negative	Non-photoreactive
	2	1.013	1.009	1.009	1.005	2	2	0.031	0.030	0.034	0.035	-3		
	3	1.010	1.010	1.004	1.012	4	3	0.031	0.029	0.034	0.036	-4		
	Mean	1.012	1.008	1.007	1.006	3	Mean	0.031	0.029	0.034	0.036	-4		
MT-009	1	1.002	0.994	0.997	0.989	1	1	0.030	0.029	0.034	0.034	-1	Negative	Non-photoreactive
	2	1.003	0.997	0.998	0.994	2	2	0.030	0.029	0.034	0.035	-1		
	3	1.001	0.999	0.996	0.995	1	3	0.030	0.028	0.034	0.034	-1		
	Mean	1.002	0.997	0.997	0.993	1	Mean	0.030	0.029	0.034	0.034	-1		
MT-016	1	0.993	0.996	0.985	0.992	3	1	0.030	0.029	0.034	0.034	0	Negative	Non-photoreactive
	2	0.997	0.988	0.988	0.985	4	2	0.031	0.029	0.035	0.033	0		
	3	0.999	0.989	0.991	0.984	4	3	0.029	0.029	0.033	0.033	-1		
	Mean	0.996	0.991	0.988	0.987	4	Mean	0.030	0.029	0.034	0.033	0		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	-2	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Chlorhexidine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
MT-004	1	0.932	0.999	0.900	0.992	25	MT-004	1	0.033	0.029	0.052	0.034	14	Negative	Non-photoreactive
	2	0.932	1.003	0.901	0.997	25		2	0.034	0.029	0.051	0.034	12		
	3	0.928	1.001	0.902	0.996	20		3	0.033	0.029	0.050	0.034	12		
	Mean	0.931	1.001	0.901	0.995	23		Mean	0.033	0.029	0.051	0.034	13		
MT-012	1	0.917	0.992	0.893	0.989	20	MT-012	1	0.033	0.030	0.046	0.034	8	Negative	Non-photoreactive
	2	0.922	0.999	0.894	0.994	24		2	0.034	0.029	0.045	0.035	7		
	3	0.925	0.999	0.898	0.996	22		3	0.033	0.029	0.044	0.034	6		
	Mean	0.921	0.997	0.895	0.993	22		Mean	0.033	0.029	0.045	0.034	7		
MT-018	1	0.911	0.997	0.890	0.993	17	MT-018	1	0.032	0.029	0.049	0.033	12	Negative	Non-photoreactive
	2	0.919	0.992	0.888	0.987	27		2	0.033	0.028	0.048	0.033	10		
	3	0.918	0.990	0.891	0.987	23		3	0.033	0.028	0.047	0.033	9		
	Mean	0.916	0.993	0.889	0.989	22		Mean	0.033	0.028	0.048	0.033	10		
Mean for 3 assays	-	-	-	-	22	Mean for 3 assays	-	-	-	-	10	Negative*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Cinnamic acid

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-007	1	0.999	0.996	0.995	0.992	1	1	0.029	0.029	0.044	0.036	9	Negative	Non-photoreactive
	2	1.012	1.002	1.010	0.998	-1	2	0.029	0.029	0.044	0.035	8		
	3	1.007	1.000	1.002	0.997	1	3	0.028	0.028	0.043	0.034	9		
	Mean	1.006	0.999	1.002	0.996	0	Mean	0.029	0.029	0.044	0.035	9		
MT-015	1	1.008	1.002	1.002	0.997	0	1	0.029	0.030	0.043	0.035	11	Negative	Non-photoreactive
	2	1.024	1.007	1.017	1.004	0	2	0.029	0.030	0.043	0.035	10		
	3	1.014	1.006	1.009	0.995	-2	3	0.029	0.029	0.043	0.034	10		
	Mean	1.016	1.005	1.009	0.998	-1	Mean	0.029	0.030	0.043	0.034	10		
MT-022	1	1.000	1.001	0.992	0.994	2	1	0.029	0.030	0.041	0.034	7	Negative	Non-photoreactive
	2	0.997	1.000	0.990	0.992	2	2	0.030	0.030	0.041	0.035	6		
	3	1.002	0.996	0.994	0.993	3	3	0.029	0.029	0.042	0.034	7		
	Mean	1.000	0.999	0.992	0.993	2	Mean	0.029	0.029	0.041	0.034	7		
Mean for 3 assays	-	-	-	-	0	Mean for 3 assays	-	-	-	-	9	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Drometrizole

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 20 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
MT-010	1	0.998	0.998	0.988	0.994	6	MT-010	1	0.030	0.029	0.041	0.034	5	Inconclusive	(Non-photoreactive)
	2	1.012	1.002	0.999	0.997	10		2	0.031	0.029	0.041	0.035	6		
	3	1.015	1.001	1.002	0.996	9		3	0.030	0.028	0.041	0.034	6		
	Mean	1.009	1.000	0.996	0.996	8		Mean	0.030	0.029	0.041	0.034	6		
MT-016	1	0.996	0.996	0.989	0.992	3	MT-016	1	0.030	0.029	0.041	0.034	8	Inconclusive	(Non-photoreactive)
	2	1.000	0.988	0.994	0.985	3		2	0.029	0.029	0.041	0.033	8		
	3	1.000	0.989	0.995	0.984	1		3	0.029	0.029	0.039	0.033	6		
	Mean	0.999	0.991	0.992	0.987	2		Mean	0.029	0.029	0.041	0.033	7		
MT-023	1	1.004	1.002	0.995	0.997	5	MT-023	1	0.030	0.029	0.043	0.036	6	Inconclusive	(Non-photoreactive)
	2	1.003	0.998	0.996	0.993	4		2	0.029	0.029	0.043	0.036	7		
	3	1.004	0.998	0.997	0.995	3		3	0.029	0.029	0.043	0.036	7		
	Mean	1.004	0.999	0.996	0.995	4		Mean	0.029	0.029	0.043	0.036	7		
Mean for 3 assays		-	-	-	-	5	Mean for 3 assays		-	-	-	-	7	Inconclusive*3 (Non-photoreactive)*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : L-Histidine

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-005	1	0.995	1.000	0.987	0.997	4	1	0.030	0.029	0.086	0.034	51	Positive	Weakly photoreactive
	2	0.997	1.003	0.990	1.000	4	2	0.030	0.029	0.085	0.034	50		
	3	0.995	1.002	0.987	0.998	4	3	0.029	0.029	0.086	0.034	52		
	Mean	0.996	1.002	0.988	0.998	4	Mean	0.029	0.029	0.085	0.034	51		
MT-013	1	1.011	0.994	1.006	0.988	1	1	0.030	0.029	0.083	0.034	48	Positive	Weakly photoreactive
	2	1.011	0.995	1.001	0.991	7	2	0.030	0.029	0.082	0.035	47		
	3	1.012	0.995	1.006	0.990	2	3	0.029	0.028	0.082	0.034	48		
	Mean	1.011	0.994	1.004	0.990	3	Mean	0.030	0.029	0.082	0.034	48		
MT-020	1	1.015	1.003	1.010	1.003	3	1	0.029	0.030	0.083	0.035	48	Positive	Weakly photoreactive
	2	1.018	1.000	1.012	0.998	4	2	0.029	0.030	0.083	0.035	48		
	3	1.021	0.999	1.014	0.997	5	3	0.029	0.031	0.083	0.036	49		
	Mean	1.018	1.001	1.012	0.999	4	Mean	0.029	0.030	0.083	0.036	48		
Mean for 3 assays	-	-	-	-	4	Mean for 3 assays	-	-	-	-	49	Positive	Weakly photoreactive ^{*3}	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Methylbenzylidene camphor

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
MT-002	1	1.011	1.011	1.010	1.005	-4	MT-002	1	0.029	0.029	0.032	0.033	-2	Inconclusive	Non-photoreactive
	2	1.008	1.016	1.008	1.011	-4		2	0.029	0.030	0.032	0.034	-2		
	3	1.005	1.018	1.004	1.013	-4		3	0.028	0.028	0.031	0.033	-2		
	Mean	1.008	1.015	1.007	1.010	-4		Mean	0.029	0.029	0.032	0.034	-2		
MT-011	1	0.998	0.998	0.996	0.993	-3	MT-011	1	0.029	0.030	0.032	0.034	-2	Inconclusive	Non-photoreactive
	2	1.002	1.005	1.001	0.999	-4		2	0.029	0.029	0.033	0.035	-3		
	3	0.999	1.003	0.997	1.000	-3		3	0.028	0.028	0.032	0.035	-3		
	Mean	1.000	1.002	0.998	0.997	-3		Mean	0.029	0.029	0.032	0.035	-3		
MT-019	1	0.999	1.009	0.997	1.001	-7	MT-019	1	0.028	0.030	0.032	0.035	-1	Inconclusive	Non-photoreactive
	2	1.003	1.002	1.001	0.992	-8		2	0.028	0.030	0.032	0.035	-1		
	3	1.001	1.003	1.000	0.994	-8		3	0.028	0.030	0.033	0.035	0		
	Mean	1.001	1.005	0.999	0.996	-8		Mean	0.028	0.030	0.033	0.035	-1		
Mean for 3 assays	-	-	-	-	-5	Mean for 3 assays	-	-	-	-	-2	Inconclusive*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Octrizole

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
MT-002	1	1.033	1.011	1.040	1.005	-12	MT-002	1	0.063	0.029	0.071	0.033	Inconclusive	Non-photoreactive	
	2	1.028	1.016	1.036	1.011	-13		2	0.066	0.030	0.075	0.034			4
	3	1.033	1.018	1.042	1.013	-14		3	0.066	0.028	0.077	0.033			5
	Mean	1.031	1.015	1.040	1.010	-13		Mean	0.065	0.029	0.074	0.034			4
MT-011	1	1.011	0.998	1.017	0.993	-11	MT-011	1	0.060	0.030	0.072	0.034	Inconclusive	Non-photoreactive	
	2	1.011	1.005	1.016	0.999	-11		2	0.062	0.029	0.073	0.035			5
	3	1.013	1.003	1.020	1.000	-12		3	0.061	0.028	0.072	0.035			6
	Mean	1.012	1.002	1.018	0.997	-11		Mean	0.061	0.029	0.072	0.035			6
MT-019	1	1.023	1.009	1.029	1.001	-14	MT-019	1	0.063	0.030	0.079	0.035	Inconclusive	Non-photoreactive	
	2	1.032	1.002	1.037	0.992	-15		2	0.063	0.030	0.079	0.035			11
	3	1.031	1.003	1.044	0.994	-22		3	0.063	0.030	0.079	0.035			11
	Mean	1.029	1.005	1.037	0.996	-17		Mean	0.063	0.030	0.079	0.035			11
Mean for 3 assays	-	-	-	-	-14	Mean for 3 assays	-	-	-	-	7	Inconclusive*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Octyl methacrylate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-013	1	0.989	0.994	0.974	0.988	11	1	0.030	0.029	0.034	0.034	0	Inconclusive	Non-photoreactive
	2	0.996	0.995	0.981	0.991	10	2	0.030	0.029	0.035	0.035	0		
	3	0.992	0.995	0.980	0.990	8	3	0.030	0.028	0.035	0.034	-1		
	Mean	0.992	0.994	0.979	0.990	10	Mean	0.030	0.029	0.035	0.034	0		
MT-020	1	1.008	1.003	1.002	1.003	4	1	0.030	0.030	0.035	0.035	-1	Inconclusive	Non-photoreactive
	2	1.005	1.000	1.001	0.998	2	2	0.029	0.030	0.036	0.035	0		
	3	1.006	0.999	0.998	0.997	7	3	0.029	0.031	0.035	0.036	0		
	Mean	1.006	1.001	1.000	0.999	4	Mean	0.029	0.030	0.035	0.036	0		
MT-023	1	1.001	1.002	0.991	0.997	7	1	0.029	0.029	0.035	0.036	-1	Inconclusive	Non-photoreactive
	2	1.001	0.998	0.991	0.993	6	2	0.028	0.029	0.035	0.036	0		
	3	1.007	0.998	0.998	0.995	4	3	0.028	0.029	0.035	0.036	-1		
	Mean	1.003	0.999	0.993	0.995	6	Mean	0.029	0.029	0.035	0.036	-1		
Mean for 3 assays	-	-	-	-	7	Mean for 3 assays	-	-	-	-	0	Inconclusive*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Octyl methoxycinnamate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	A440(-)			A440(+)			A560(-)			A560(+)					
	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*1	Run#	Test Chemical	Blank	Test Chemical	Blank	Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
MT-004	1	1.009	0.999	1.002	0.992	2	MT-004	1	0.045	0.029	0.046	0.034	-5	Inconclusive	Non-photoreactive
	2	1.012	1.003	1.003	0.997	3		2	0.046	0.029	0.046	0.034	-5		
	3	1.012	1.001	1.003	0.996	3		3	0.045	0.029	0.046	0.034	-5		
	Mean	1.011	1.001	1.003	0.995	3		Mean	0.046	0.029	0.046	0.034	-5		
MT-012	1	1.010	0.992	1.005	0.989	1	MT-012	1	0.046	0.030	0.046	0.034	-5	Inconclusive	Non-photoreactive
	2	1.017	0.999	1.010	0.994	3		2	0.046	0.029	0.047	0.035	-4		
	3	1.016	0.999	1.011	0.996	1		3	0.046	0.029	0.046	0.034	-5		
	Mean	1.014	0.997	1.009	0.993	2		Mean	0.046	0.029	0.046	0.034	-5		
MT-018	1	1.011	0.997	1.003	0.993	4	MT-018	1	0.046	0.029	0.047	0.033	-4	Inconclusive	Non-photoreactive
	2	1.009	0.992	1.001	0.987	4		2	0.046	0.028	0.046	0.033	-5		
	3	1.011	0.990	1.004	0.987	3		3	0.046	0.028	0.047	0.033	-4		
	Mean	1.010	0.993	1.003	0.989	4		Mean	0.046	0.028	0.047	0.033	-4		
Mean for 3 assays	-	-	-	-	3	Mean for 3 assays	-	-	-	-	-5	Inconclusive*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+) - A560(-)) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : Octyl salicylate

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined
 Superoxide anion 200 µM : Precipitation 20 µM : Solution 2 µM : Not determined

Test concentration

Singlet oxygen 20 µM
 Superoxide anion 20 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement		
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol	
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank				
MT-007	1	1.004	0.996	1.004	0.992	-3	MT-007	1	0.036	0.029	0.042	0.036	0	Inconclusive	Non-photoreactive
	2	1.014	1.002	1.012	0.998	-1		2	0.037	0.029	0.042	0.035	0		
	3	1.010	1.000	1.011	0.997	-4		3	0.036	0.028	0.042	0.034	0		
	Mean	1.009	0.999	1.009	0.996	-3		Mean	0.036	0.029	0.042	0.035	0		
MT-015	1	1.009	1.002	1.005	0.997	-3	MT-015	1	0.036	0.030	0.041	0.035	1	Inconclusive	Non-photoreactive
	2	1.020	1.007	1.016	1.004	-3		2	0.037	0.030	0.041	0.035	0		
	3	1.017	1.006	1.015	0.995	-4		3	0.036	0.029	0.041	0.034	0		
	Mean	1.015	1.005	1.012	0.998	-3		Mean	0.037	0.030	0.041	0.034	0		
MT-022	1	1.014	1.001	1.011	0.994	-3	MT-022	1	0.036	0.030	0.041	0.034	0	Inconclusive	Non-photoreactive
	2	1.012	1.000	1.008	0.992	-3		2	0.037	0.030	0.040	0.035	-1		
	3	1.013	0.996	1.010	0.993	-4		3	0.036	0.029	0.041	0.034	0		
	Mean	1.013	0.999	1.010	0.993	-3		Mean	0.036	0.029	0.041	0.034	0		
Mean for 3 assays	-	-	-	-	-3	Mean for 3 assays	-	-	-	-	0	Inconclusive*3	Non-photoreactive*3		

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : PABA

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-007	1	0.997	0.996	0.994	0.992	1	1	0.030	0.029	0.034	0.036	-2	Negative	Non-photoreactive
	2	1.001	1.002	1.014	0.998	-16	2	0.029	0.029	0.034	0.035	-1		
	3	1.000	1.000	0.996	0.997	1	3	0.029	0.028	0.033	0.034	-1		
	Mean	1.000	0.999	1.001	0.996	-5	Mean	0.029	0.029	0.034	0.035	-1		
MT-015	1	1.007	1.002	1.002	0.997	-2	1	0.030	0.030	0.034	0.035	0	Negative	Non-photoreactive
	2	1.013	1.007	1.009	1.004	-3	2	0.030	0.030	0.034	0.035	0		
	3	1.012	1.006	1.008	0.995	-3	3	0.029	0.029	0.033	0.034	0		
	Mean	1.011	1.005	1.006	0.998	-3	Mean	0.030	0.030	0.033	0.034	0		
MT-022	1	1.003	1.001	0.993	0.994	4	1	0.029	0.030	0.033	0.034	-1	Negative	Non-photoreactive
	2	1.005	1.000	0.996	0.992	3	2	0.030	0.030	0.033	0.035	-1		
	3	1.000	0.996	0.991	0.993	3	3	0.030	0.029	0.033	0.034	-1		
	Mean	1.003	0.999	0.993	0.993	3	Mean	0.030	0.029	0.033	0.034	-1		
Mean for 3 assays	-	-	-	-	-2	Mean for 3 assays	-	-	-	-	-1	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : SDS

Solubility

Singlet oxygen 200 µM : Solution 20 µM : Not determined 2 µM : Not determined
 Superoxide anion 200 µM : Solution 20 µM : Not determined 2 µM : Not determined

Test concentration

Singlet oxygen 200 µM
 Superoxide anion 200 µM

Experimental No.	Singlet oxygen						Superoxide anion						Judgement	
	Run#	A440(-)		A440(+)		Results*1	Run#	A560(-)		A560(+)		Results*2	Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank			Test Chemical	Blank	Test Chemical	Blank			
MT-007	1	0.982	0.996	0.975	0.992	4	1	0.132	0.029	0.145	0.036	7	Negative	Non-photoreactive
	2	0.986	1.002	0.977	0.998	5	2	0.132	0.029	0.143	0.035	5		
	3	0.983	1.000	0.973	0.997	7	3	0.132	0.028	0.141	0.034	3		
	Mean	0.984	0.999	0.975	0.996	5	Mean	0.132	0.029	0.143	0.035	5		
MT-015	1	0.993	1.002	0.981	0.997	6	1	0.115	0.030	0.125	0.035	6	Negative	Non-photoreactive
	2	0.996	1.007	0.983	1.004	6	2	0.115	0.030	0.125	0.035	6		
	3	0.991	1.006	0.980	0.995	4	3	0.115	0.029	0.124	0.034	5		
	Mean	0.994	1.005	0.981	0.998	5	Mean	0.115	0.030	0.125	0.034	6		
MT-022	1	0.978	1.001	0.966	0.994	6	1	0.097	0.030	0.117	0.034	15	Negative	Non-photoreactive
	2	0.983	1.000	0.968	0.992	9	2	0.098	0.030	0.116	0.035	14		
	3	0.985	0.996	0.970	0.993	9	3	0.097	0.029	0.114	0.034	12		
	Mean	0.982	0.999	0.968	0.993	8	Mean	0.097	0.029	0.116	0.034	14		
Mean for 3 assays	-	-	-	-	6	Mean for 3 assays	-	-	-	-	8	Negative*3	Non-photoreactive*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)) - A560(-) - (B-A) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

*3 : Final judgment based on the mean value of three assays

Appendix 7 Individual data of phase 2 study

Laboratory : 3
 Chemical Name : UV-571

Solubility

Singlet oxygen 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution
 Superoxide anion 200 µM : Precipitation 20 µM : Precipitation 2 µM : Solution

Test concentration

Singlet oxygen 2 µM
 Superoxide anion 2 µM

Experimental No.	Run#	Singlet oxygen				Results*1	Experimental No.	Run#	Superoxide anion				Results*2	Judgement	
		A440(-)		A440(+)					A560(-)		A560(+)			Judged by Original Criteria	Judged by Criteria for Proposed Protocol
		Test Chemical	Blank	Test Chemical	Blank				Test Chemical	Blank	Test Chemical	Blank			
MT-008	1	1.009	1.010	0.999	0.995	-6	MT-008	1	0.032	0.031	0.038	0.035	1	Inconclusive	(Non-photoreactive)
	2	1.015	1.012	1.004	0.997	-4		2	0.032	0.031	0.038	0.036	1		
	3	1.010	1.011	0.999	0.997	-4		3	0.032	0.030	0.038	0.035	1		
	Mean	1.011	1.011	1.001	0.996	-5		Mean	0.032	0.031	0.038	0.036	1		
MT-015	1	1.013	1.002	1.008	0.997	-2	MT-015	1	0.031	0.030	0.037	0.035	2	Inconclusive	(Non-photoreactive)
	2	1.016	1.007	1.013	1.004	-4		2	0.031	0.030	0.036	0.035	2		
	3	1.012	1.006	1.010	0.995	-5		3	0.030	0.029	0.035	0.034	1		
	Mean	1.014	1.005	1.011	0.998	-4		Mean	0.031	0.030	0.036	0.034	2		
MT-022	1	1.002	1.001	1.001	0.994	-5	MT-022	1	0.032	0.030	0.038	0.034	1	Inconclusive	(Non-photoreactive)
	2	1.004	1.000	1.003	0.992	-4		2	0.030	0.030	0.036	0.035	1		
	3	1.005	0.996	1.002	0.993	-3		3	0.030	0.029	0.036	0.034	1		
	Mean	1.004	0.999	1.002	0.993	-4		Mean	0.031	0.029	0.037	0.034	1		
Mean for 3 assays		-	-	-	-	-4	Mean for 3 assays		-	-	-	-	1	Inconclusive*3 (Non-photoreactive)*3	

*1 : decrease of A440 x10³ = (A440(-) - A440(+)) - (A-B) ×1000

A440(-) : Absorbance before light exposure at 440 nm

A440(+): Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

*2 : increase of A560 x10³ = (A560(+)-A560(-) - (B-A)) ×1000

A560(-) : Absorbance before light exposure at 560 nm

A560(+): Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank after light exposure)

Judged by Original Criteria:

Positive : Singlet oxygen results ≥25 or Superoxide anion results ≥20 at 200, 20 or 2 µM

Negative : Singlet oxygen results <25 and Superoxide anion results <20 at 200 µM

Inconclusive : The results does not meet the positive or negative criterion

Judged by Criteria for Proposed Protocol:

Photoreactive : Singlet oxygen results ≥25 or Superoxide anion results ≥70 at 200 or 20 µM

Weakly photoreactive : Singlet oxygen results <25 and Superoxide anion results ≥20 and less than 70 at 200 or 20 µM

Non-photoreactive : Singlet oxygen results <25 and Superoxide anion results <20 at 200 or 20 µM

(Photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the photoreactive criteria

(Weakly photoreactive): Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Weakly photoreactive criteria

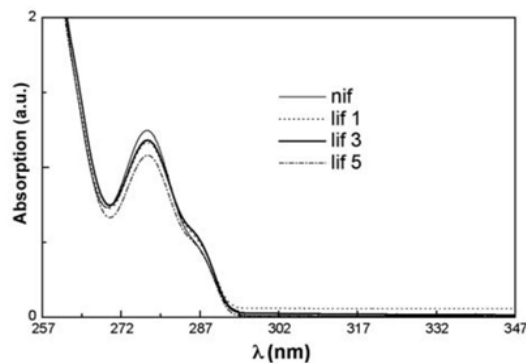
(Non-photoreactive):Ros assay was conducted at 2 µM due to precipitation at 20µM and 200µM. Although, the results met the Non-photoreactive criteria

The results shown in the parenthesis since the results at 2 µM were not used for the judgment of integrated results or data analyses.

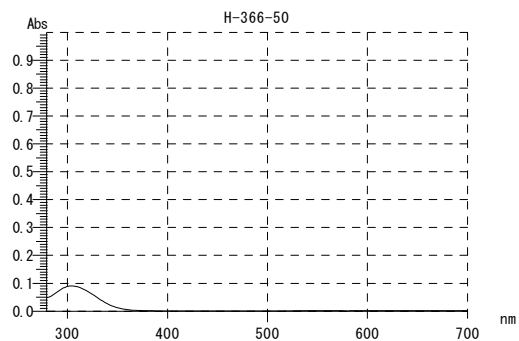
*3 : Final judgment based on the mean value of three assays

Appendix 8 UV spectrum of test chemicals

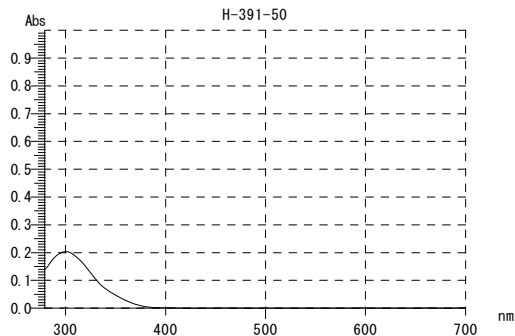
Chemical Name	: 5-FU ¹⁾
Solvent	: Saline(adjusted to pH8.4)
Test concentration	: 100 μ M



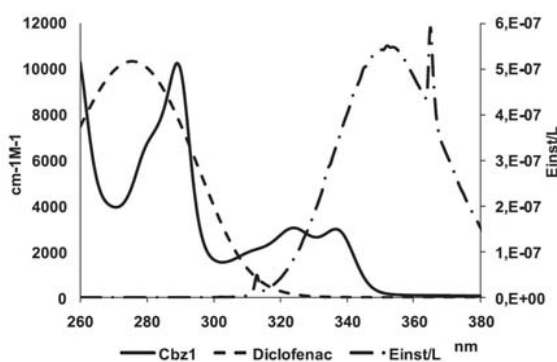
Chemical Name	: Chlorpromazine HCl
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 50 μ M



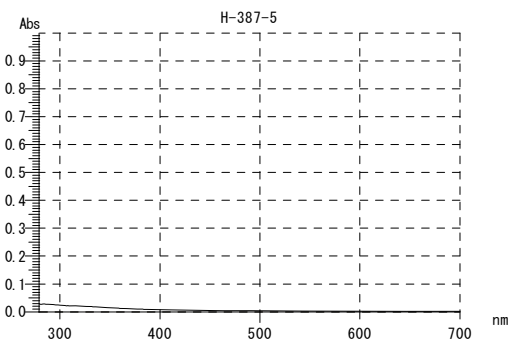
Chemical Name	: 8-MOP
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 50 μ M



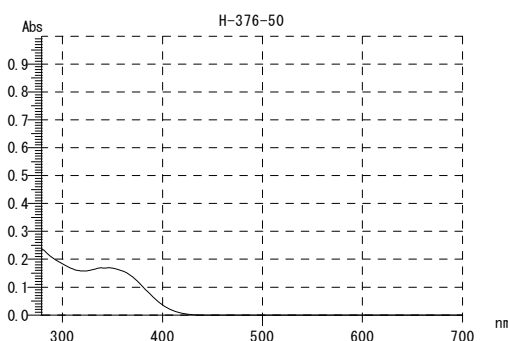
Chemical Name	: Diclofenac ²⁾
Solvent	: Phosphate buffer(pH8.5)
Sample Concentration	: <5 μ M



Chemical Name	: Amiodarone HCl
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 5 μ M



Chemical Name	: Doxycycline HCl
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 50 μ M



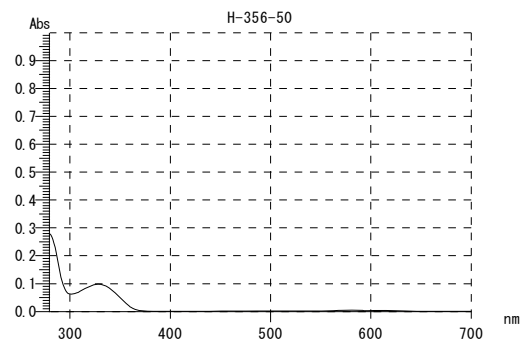
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

1)UV spectrum of 5-FU(normal line (nif)) was extracted from the article of M. L. Pascu, M. Brezeanu, L. Voicu, A. Staicu, B. Carstocea, R. A. Pascu (2005) *in vivo*, 19, 215-220

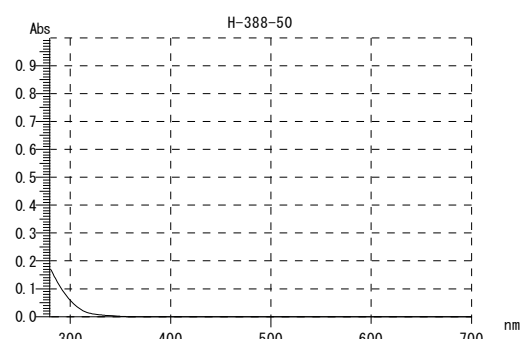
2)UV spectrum of Diclofenac (dashed line) was extracted from the article of J. Eriksson, J. Svanfelt, L. Kronberg (2010) *Photochemistry and Photobiology*, 86, 528-532

Appendix 8 UV spectrum of test chemicals

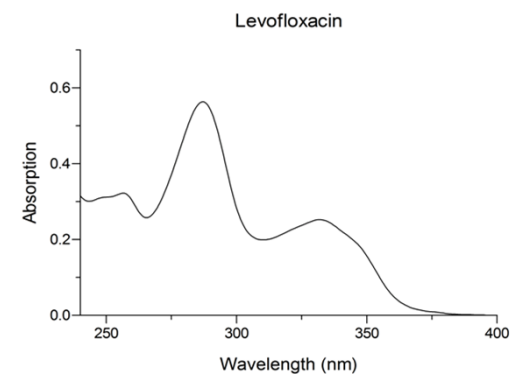
Chemical Name : Furoseimide
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



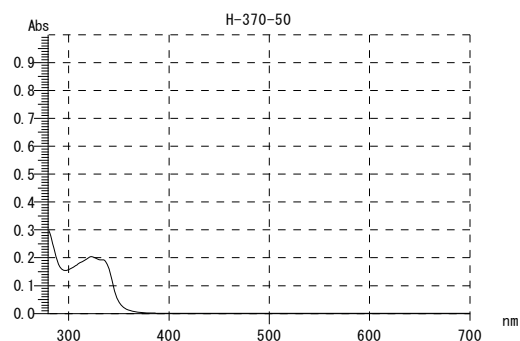
Chemical Name : Ketoprofen
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



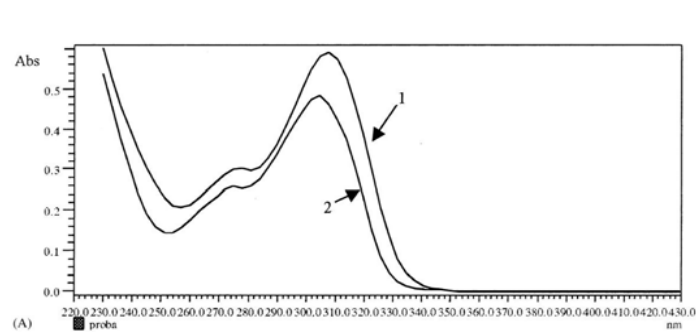
Chemical Name : Levofloxacin¹⁾
 Solvent : 20 mM NaPB(pH7.4)
 Test concentration : 20 μ M



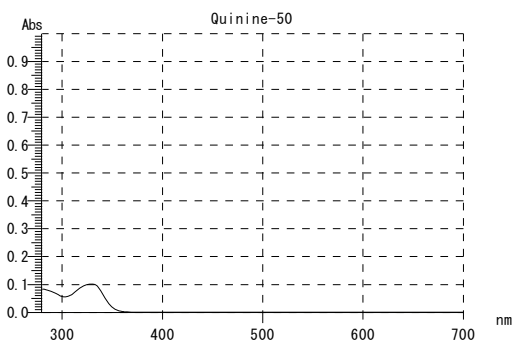
Chemical Name : Norfloxacin
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



Chemical Name : Omeprazole²⁾
 Solvent : Methanol /NH3 4.0% v/v (pH* 9.0)
 Sample Concentration : 8.64 μ g/mL (23.5 μ M)



Chemical Name : Quinine HCl
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



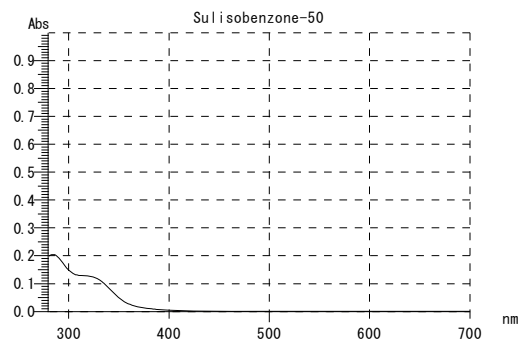
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

1) Test chemical was dissolved in 20 mM NaPB(pH7.4). UV/vis absorption spectra was recorded with a HITACHI U-2010 spectrophotometer (HITACHI, Tokyo, Japan).

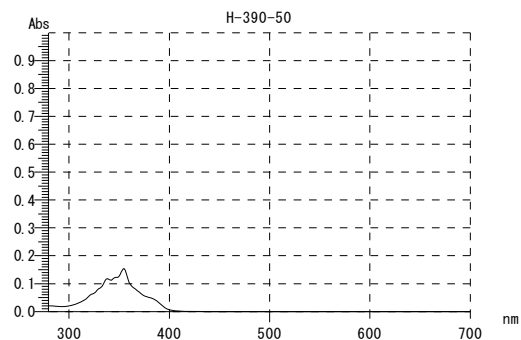
2) Omeprazole (curve 1) was extracted from the article of K. K. Rajic, D. Novovic, V. Mrinkovic, D. Agbaba (2003) *Journal of Pharmaceutical and Biomedical Analysis*, 32, 1019-1027

Appendix 8 UV spectrum of test chemicals

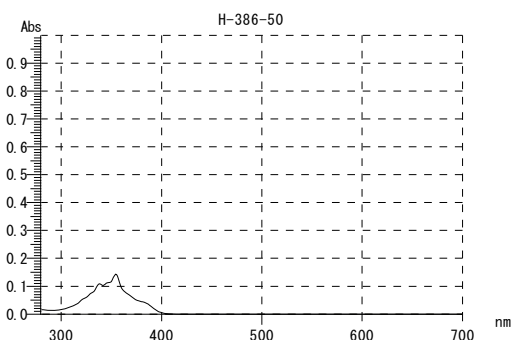
Chemical Name : Sulisobenzone
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



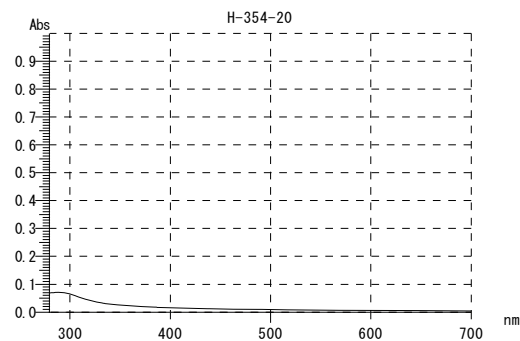
Chemical Name : Acridine
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



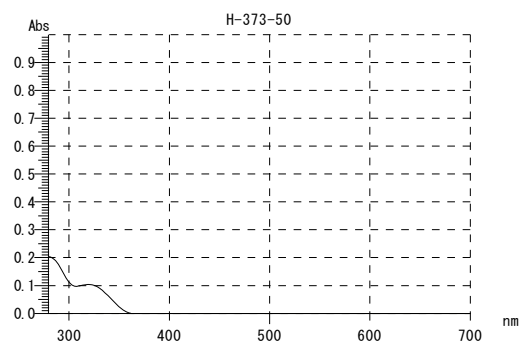
Chemical Name : Acridine HCl
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



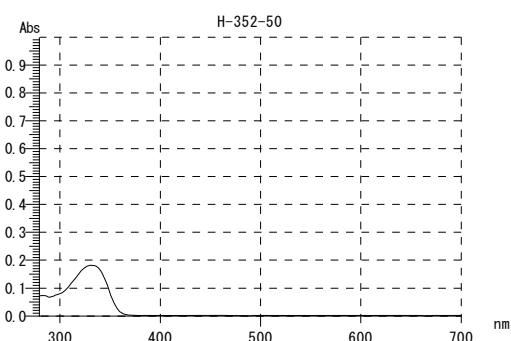
Chemical Name : Fenofibrate
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 20 μ M



Chemical Name : 6-methylcoumarine
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



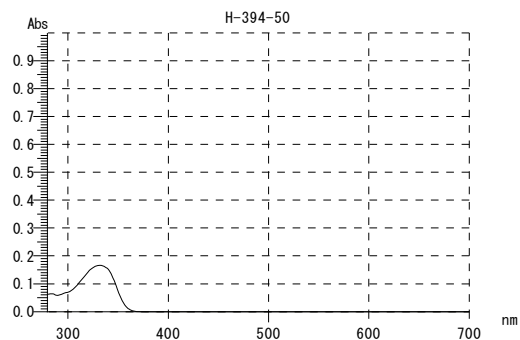
Chemical Name : Nalidixic acid
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



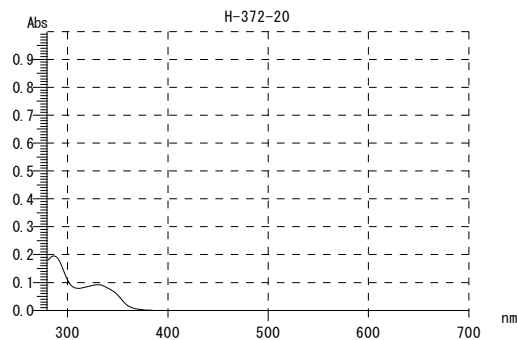
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 8 UV spectrum of test chemicals

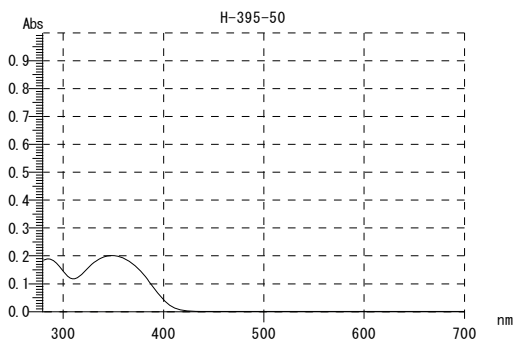
Chemical Name : Nalidixic acid (Na salt)
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



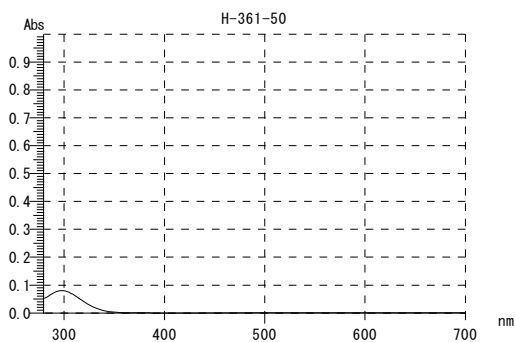
Chemical Name : Ofloxacin
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 20 μ M



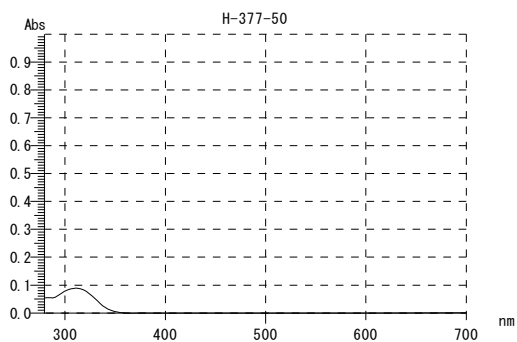
Chemical Name : Piroxicam
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Test concentration : 50 μ M



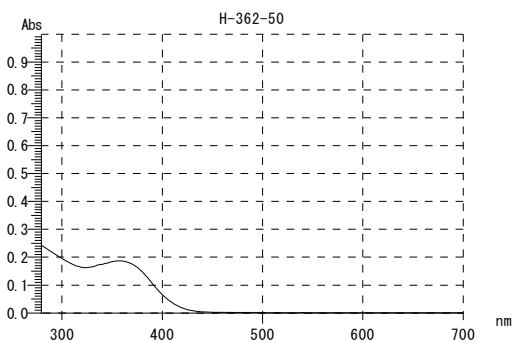
Chemical Name : Promethazine HCl
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



Chemical Name : Rosiglitazone
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



Chemical Name : Tetracycline
 Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO
 Sample Concentration : 50 μ M



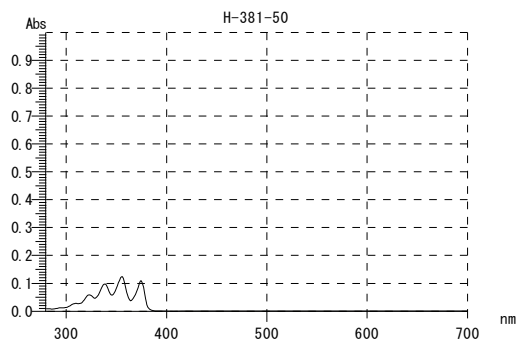
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 8 UV spectrum of test chemicals

Chemical Name : Anthracene

Solvent : Methanol, 0.5% DMSO

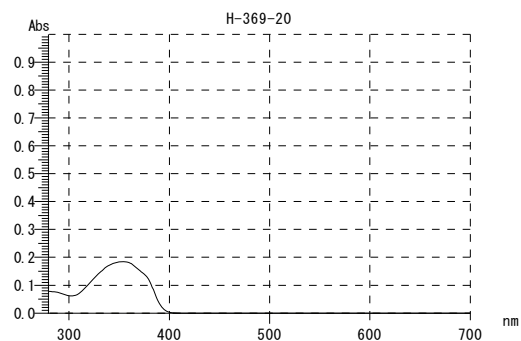
Test concentration : 50 μ M



Chemical Name : Avobenzene

Solvent : Methanol, 0.5% DMSO

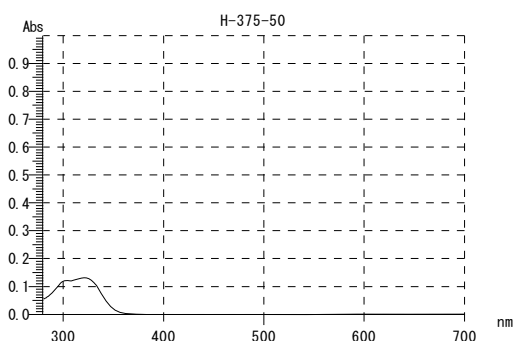
Test concentration : 20 μ M



Chemical Name : Bithionol

Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO

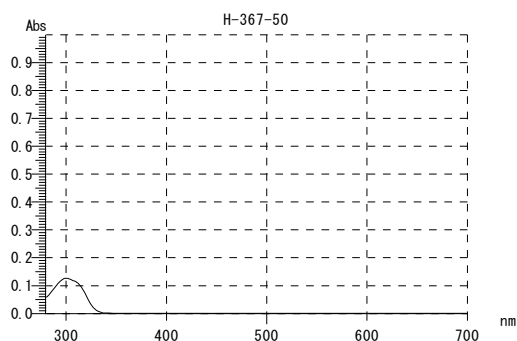
Test concentration : 50 μ M



Chemical Name : Hexachlorophene

Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO

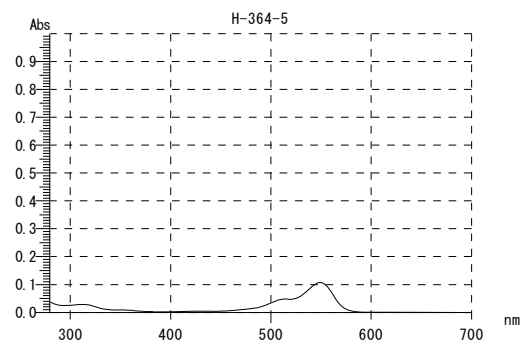
Sample Concentration : 50 μ M



Chemical Name : Rose bengal

Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO

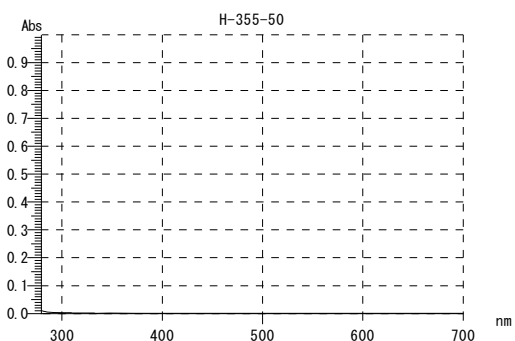
Sample Concentration : 5 μ M



Chemical Name : Aspirin

Solvent : 20 mM NaPB(pH7.4), 0.5% DMSO

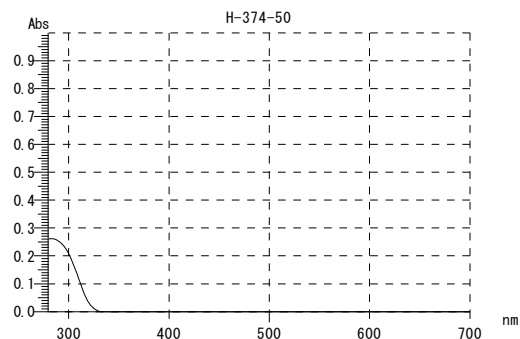
Sample Concentration : 50 μ M



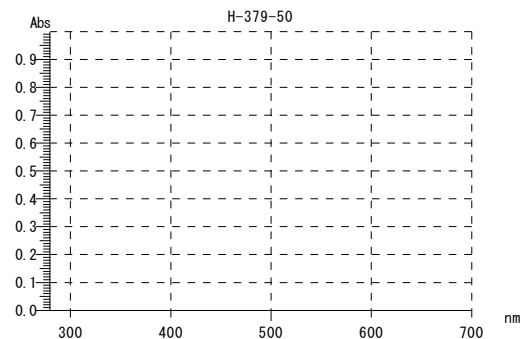
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 8 UV spectrum of test chemicals

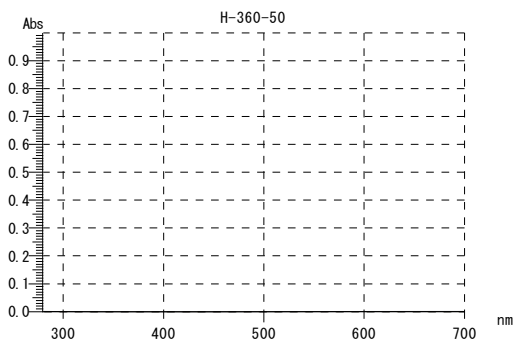
Chemical Name	: Benzocaine
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 50 μ M



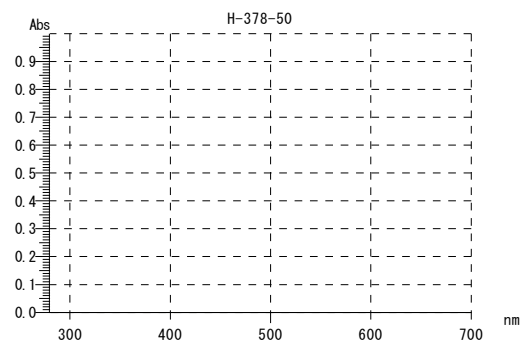
Chemical Name	: Erythromycin
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 50 μ M



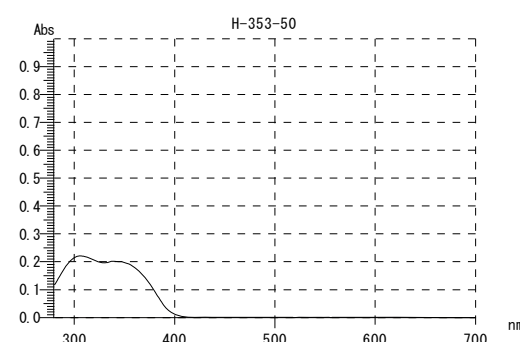
Chemical Name	: Phenytoin
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 50 μ M



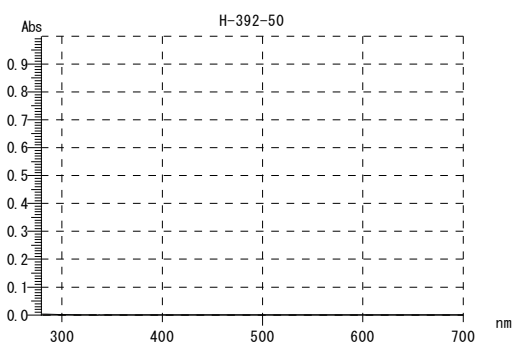
Chemical Name	: Penicillin G
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 50 μ M



Chemical Name	: Bumetizole
Solvent	: Methanol, 0.5% DMSO
Sample Concentration	: 50 μ M



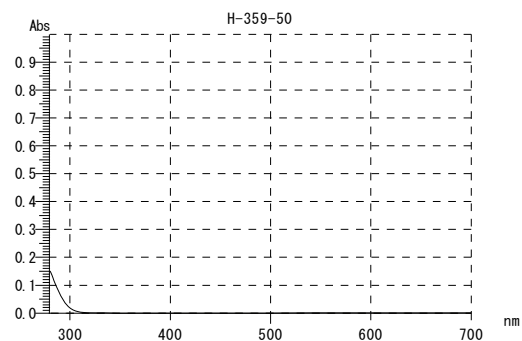
Chemical Name	: Camphor sulfonic acid
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 50 μ M



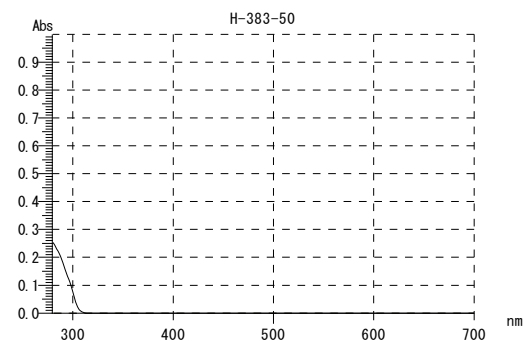
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 8 UV spectrum of test chemicals

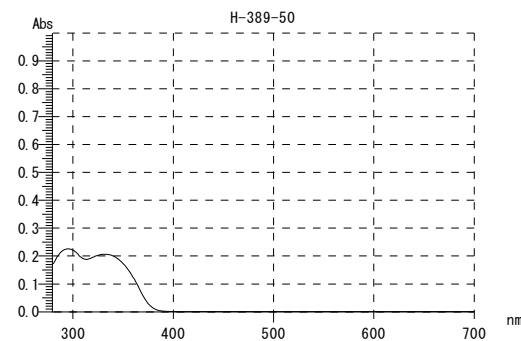
Chemical Name	: Chlorhexidine
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 50 μ M



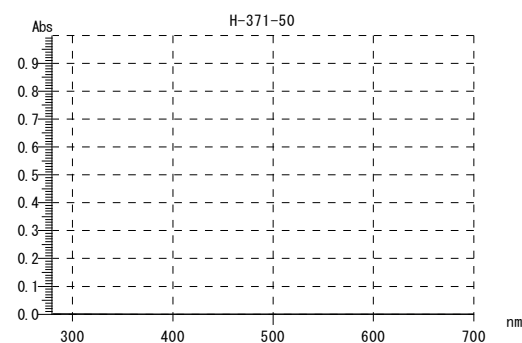
Chemical Name	: Cinnamic acid
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 50 μ M



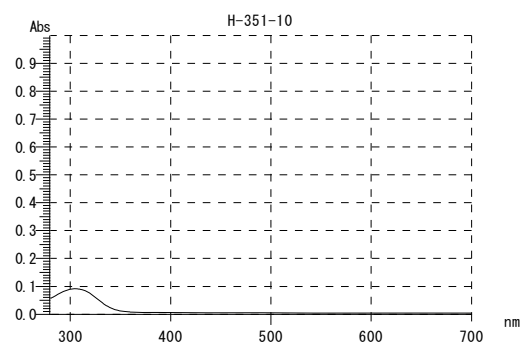
Chemical Name	: Drometrizole
Solvent	: Methanol, 0.5% DMSO
Test concentration	: 50 μ M



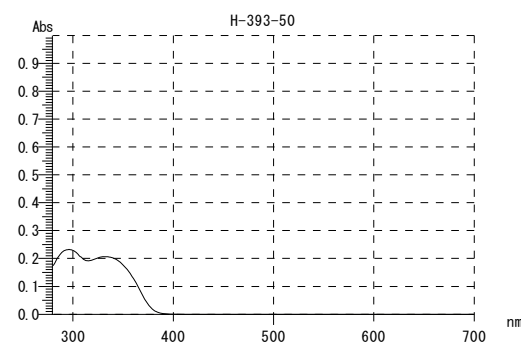
Chemical Name	: L-histidine
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 50 μ M



Chemical Name	: Methylbenzylidenecamphor
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 10 μ M



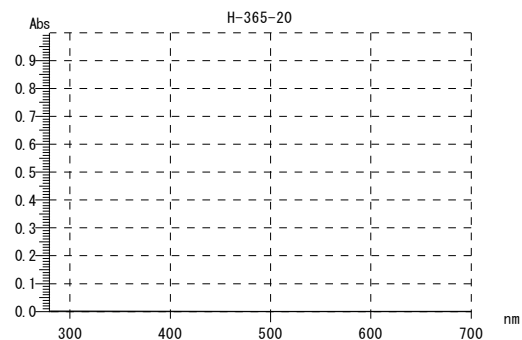
Chemical Name	: Octrizole
Solvent	: Methanol, 0.5% DMSO
Sample Concentration	: 50 μ M



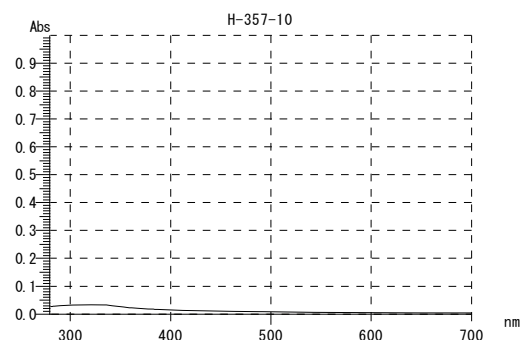
Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 8 UV spectrum of test chemicals

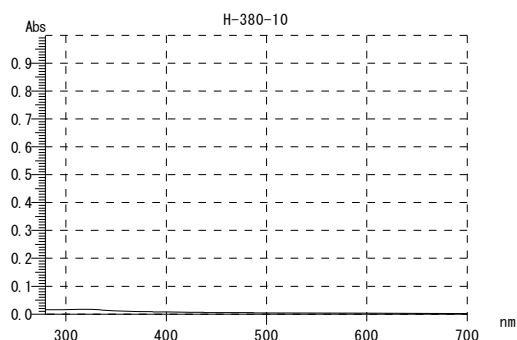
Chemical Name	: Octyl methacrylate
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 20 μ M



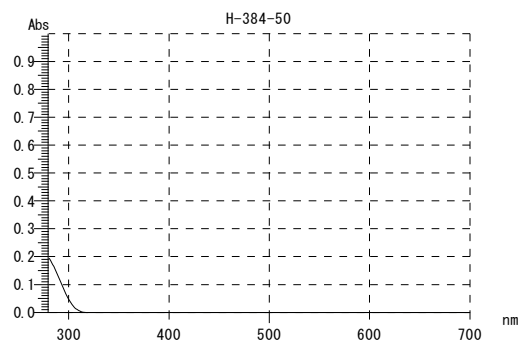
Chemical Name	: Octylmethoxycinnamate
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 10 μ M



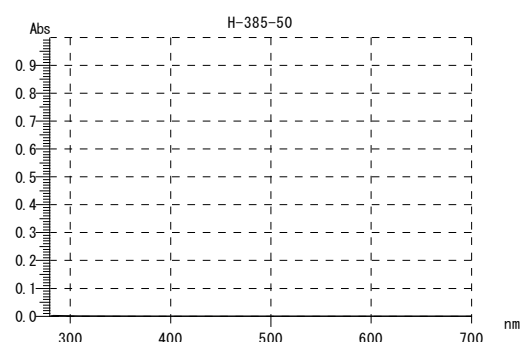
Chemical Name	: Octyl salicylate
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Test concentration	: 10 μ M



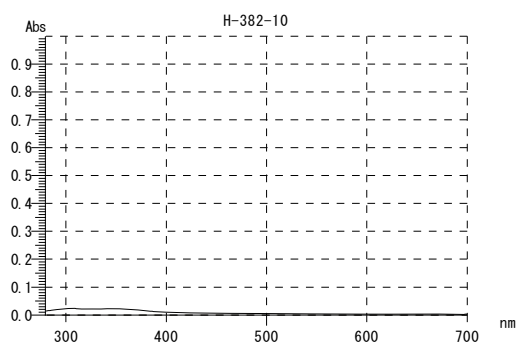
Chemical Name	: PABA
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 50 μ M



Chemical Name	: SDS
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 50 μ M



Chemical Name	: UV-571
Solvent	: 20 mM NaPB(pH7.4), 0.5% DMSO
Sample Concentration	: 10 μ M



Test chemicals were dissolved in DMSO at 10 mM and diluted with 20 mM NaPB(pH7.4) or methanol. In the each case, final concentration of DMSO was unified to 0.5%. UV/vis absorption spectra were recorded with a HITACHI U-3500 spectrophotometer (HITACHI, Tokyo, Japan). Quartz cell with a 1 cm pathlength was employed.

Appendix 9 Preparation information and appearance of the stock solutions and the reaction mixtures

		Lab 1										Lab 2						Lab 3							
Chemicals		Stock solution			Reaction mixture				Stock solution			Reaction mixture			Stock solution			Reaction mixture							
NO.	Name	1st Solvent	Concentration			Concentration ^{a)}				Concentration			Concentration ^{a)}			Concentration			Concentration ^{a)}						
			10 mM	1 mM	0.1 mM	200 µM	20 µM	2 µM	10 mM	1 mM	0.1 mM	200 µM	20 µM	2 µM	10 mM	1 mM	0.1 mM	200 µM	20 µM	2 µM					
II - 1	Acridine	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 2	Acridine HCl	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 3	Amiodarone HCl	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.
			SA	Sus.	Sus.	Sol.	SA	Sus.	Sus.					Sol.	SA	Sus.	Sus.					Sol.	SA	Sus.	Sus.
II - 4	Chlorpromazine HCl	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 5	Doxycycline HCl	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 6	Fenofibrate	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-
			SA	Sus.	Sol.	-	SA	Sus.	Sus.					Sol.	SA	Sus.	Sus.					Sol.	SA	Sus.	Sol.
II - 7	Furosemide	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 8	Ketoprofen	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 9	6-methylcoumarine	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 10	8-MOP	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 11	Nalidixic acid	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 12	Nalidixic acid (Na salt)	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 13	Norfloxacin	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 14	Ofloxacin	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 15	Piroxicam	DMSO	Sol.	Sol.	-	SO	Sol.	-	-	DMSO	Sol.	Sol.	-	SO	Sol.	-	-	DMSO	Sol.	Sol.	-	SO	Sol.	-	-
			SA	Sus.	Sol.	-	SA	Sus.	Sol.					-	SA	Sus.	Sol.					-	SA	Sus.	Sol.
II - 16	Promethazine HCl	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 17	Rosiglitazone	DMSO	Sol.	Sol.	-	SO	Sol.	-	-	DMSO	Sol.	Sol.	-	SO	Sol.	-	-	DMSO	Sol.	Sol.	-	SO	Sol.	-	-
			SA	Sus.	Sol.	-	SA	Sus.	Sol.					-	SA	Sus.	Sol.					-	SA	Sus.	Sol.
II - 18	Tetracycline	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-
II - 19	Anthracene	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.
			SA	Sus.	Sus.	Sol.	SA	Sus.	Sus.					Sol.	SA	Sus.	Sus.					Sol.	SA	Sus.	Sus.
II - 20	Avobenzone	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-
			SA	Sus.	Sus.	Sol.	SA	Sus.	Sus.					Sol.	SA	Sus.	Sus.					Sol.	SA	Sus.	Sol.
II - 21	Bithionol	DMSO	Sol.	Sol.	-	SO	Sol.	-	-	DMSO	Sol.	Sol.	Sol.	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sus.	Sol.	-	SA	Sus.	Sol.					-	SA	Sus.	Sol.					-	SA	Sol.	-
II - 22	Hexachlorophene	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	Sol.	Sol.	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sus.	Sus.					Sol.	SA	Sol.	-
II - 23	Rose bengal	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
			SA	Sol.	-	-	SA	Sol.	-					-	SA	Sol.	-					-	SA	Sol.	-

Appearance; Sol. : Solution, Sus. : Suspension - : Not prepared

SO : Singlet oxygen SA : Superoxide anion DMSO : Dimethyl sulfoxide NaPB : 2% DMSO / 20 mM sodium phosphate buffer

a) For each test chemical, 20 µL of stock solution was added to the reaction mixtures whose final volume was 1000 µL. The stock solution whose concentration was 10 mM were used to prepare 200 µM reaction mixtures.

The stock solutions whose concentrations were 1 and 0.1 mM were used to prepare 20 and 2 µM.

Appendix 9 Preparation information and appearance of the stock solutions and the reaction mixtures (continued)

N0.	Chemicals Name	Lab 1							Lab 2							Lab 3									
		Stock solution			Reaction mixture				Stock solution			Reaction mixture				Stock solution			Reaction mixture						
		1st Solvent	Concentration		Concentration ^{a)}			1st Solvent	Concentration		Concentration ^{a)}			1st Solvent	Concentration		Concentration ^{a)}								
	10 mM	1 mM	0.1 mM	200 µM	20 µM	2 µM		10 mM	1 mM	0.1 mM	200 µM	20 µM	2 µM		10 mM	1 mM	0.1 mM	200 µM	20 µM	2 µM					
II - 24 Aspirin		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 25 Benzocaine		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 26 Erythromycin		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 27 Penicillin G		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 28 Phenytoin		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 29 Bumetrizole		DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-
						SA	Sus.	Sus.	Sol.					SA	Sus.	Sus.	Sol.					SA	Sus.	Sol.	-
II - 30 Camphor sulfonic acid		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 31 Chlorhexidine		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 32 Cinnamic acid		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 33 Drometrizole		DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.
						SA	Sus.	Sol.	-					SA	Sus.	Sol.	-					SA	Sus.	Sol.	-
II - 34 L-Histidine		DMSO	Sus.	-	-	SO	-	-	-	DMSO	Sus.	-	-	SO	-	-	-	DMSO	Sus.	-	-	SO	-	-	-
						SA	-	-	-					SA	-	-	-					SA	-	-	-
		NaPB	Sol.	-	-	SO	Sol.	-	-	NaPB	Sol.	-	-	SO	Sol.	-	-	NaPB	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 35 Methylbenzylidene camphor		DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-
						SA	Sus.	Sol.	-					SA	Sus.	Sol.	-					SA	Sus.	Sol.	-
II - 36 Ocotrizole		DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-
						SA	Sus.	Sus.	Sol.					SA	Sus.	Sus.	Sol.					SA	Sus.	Sol.	-
II - 37 Octyl methacrylate		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-
						SA	Sol.	-	-					SA	Sus.	Sol.	-					SA	Sus.	Sol.	-
II - 38 Octyl methoxycinnamate		DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-
						SA	Sus.	Sol.	-					SA	Sus.	Sus.	Sol.					SA	Sus.	Sol.	-
II - 39 Octyl salicylate		DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	-	SO	Sus.	Sol.	-
						SA	Sus.	Sol.	-					SA	Sus.	Sol.	-					SA	Sus.	Sol.	-
II - 40 PABA		DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sol.	-	-					SA	Sol.	-	-					SA	Sol.	-	-
II - 41 SDS		DMSO	Sol.	Sol.	-	SO	Sol.	-	-	DMSO	Sol.	Sol.	Sol.	SO	Sol.	-	-	DMSO	Sol.	-	-	SO	Sol.	-	-
						SA	Sus.	Sol.	-					SA	Sus.	Sus.	Sol.					SA	Sol.	-	-
II - 42 UV-571		DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sol.	-	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.	DMSO	Sol.	Sol.	Sol.	SO	Sus.	Sus.	Sol.
						SA	Sus.	Sus.	Sol.					SA	Sus.	Sus.	Sol.					SA	Sus.	Sus.	Sol.

Appearance; Sol. : Solution, Sus. : Suspension - : Not prepared

SO : Singlet oxygen SA : Superoxide anion DMSO : Dimethyl sulfoxide NaPB : 2% DMSO / 20 mM sodium phosphate buffer

a): For each test chemical, 20 µL of stock solution was added to the reaction mixtures whose final volume was 1000 µL. The stock solution whose concentration was 10 mM were used to prepare 200 µM reaction mixtures.

The stock solutions whose concentrations were 1 and 0.1 mM were used to prepare 20 and 2 µM.

**INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE
OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING
PHOTOTOXIC POTENTIAL OF CHEMICALS
(VERSION 1.0)**

Issued by: the Validation Management Team (VMT)

Date: 22 April 2011.

1. Purpose of this document

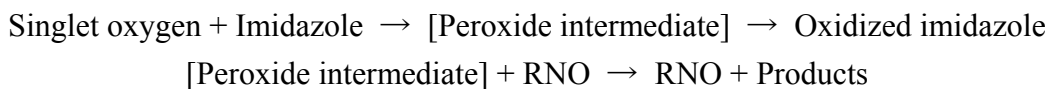
This document is provided to clarify the conduct of an international validation study to evaluate the ability of ROS (reactive oxidative species) assay as a photochemical property study to examine necessity of phototoxicity tests. This document represents the first study protocol developed as a result of the collaboration efforts of the participating testing facilities and the VMT. Each testing facility will develop a study protocol based on the information provided in this document.

2. Background of ROS assay

Drug-induced photoirritation can be defined as an inflammatory reaction of the skin after topical or systemic administration of pharmaceutical substances. Several classes of drugs including antibacterials, thiazide diuretics, non-steroidal anti-inflammatory drugs, quinolones, and tricyclic antidepressants, even though nontoxic by themselves, may become reactive under exposure to environmental light, leading to undesired side effects. The primary event in any photosensitization process is the absorption of photons of the appropriate wavelength, which allows chromophore to reach an excited state. The excitation energy is often transferred to oxygen molecules, followed by generation of ROS: superoxide through type I reaction and singlet oxygen through type II reaction by photo-excited drug molecules. These appear to be the principal intermediate species in the phototoxic response. From the standpoint of risk assessment, previous study demonstrated that determination of ROS from pharmaceutical substances irradiated with UVA/B and Vis would be of help in recognizing their phototoxic potential.

In the ROS assay, generation of singlet oxygen was detected by spectrophotometric measurement of *p*-nitrosodimethyl aniline (RNO) bleaching, followed by decreased absorbance of RNO at 440 nm. Although singlet oxygen does not react chemically

with RNO, the RNO bleaching is a consequence of singlet oxygen capture by the imidazole ring, resulting in the formation of a trans-annular peroxide intermediate capable of inducing the bleaching of RNO as follows;



The generation of superoxide could be determined by the reduction of nitroblue tetrazolium (NBT) as indicated below; NBT can be reduced by superoxide anion via a one-electron transfer reaction, yielding partially reduced ($2 e^-$) monoformazan (NBT^+) as a stable intermediate. Thus, superoxide can reduce NBT to NBT^+ , whose formation can be monitored spectrophotometrically at 560 nm.



3. Materials

3.1. Control compounds

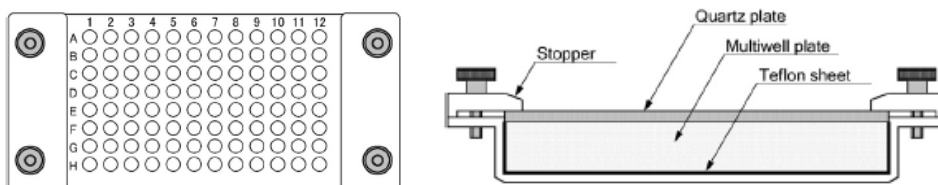
Name	CAS No.	Manufacture	Molecular weight
Quinine monohydrochloride dihydrate (Positive control)	6119-47-7	Sigma (Catalog No. 145920)	396.91
Sulisobenzone (Negative control)	4065-45-6	Tokyo Chemical Industry (Catalog No. H0466)	308.31

3.2. Solvent

Dimethylsulfoxide (DMSO): analytical grade

3.3. Instruments

Instrument	Model
Solar simulator	Suntest CPS+, quipped UV (<290 nm) cut filter (Atlas Material Technology), or its equivalent Suntest CPS, quipped UV (<290 nm) cut filter (Atlas Material Technology), or its equivalent
UVA detector	Topcon or Dr. Hönle
Microplate spectrophotometer, equipped 440 and 560 nm filters	Spectra Max M2 (Molecular Devices), Tecan Safire (Tecan), or their equivalents
Quartz reaction container	Made-to-order (Ozawa Sciences, Onoue et al. 2008, see below Figure)



4. Preparations

4.1. Reagents

All reagents should be sonicated and used within 1 month after preparation.

4.1.1. 20 mM Sodium phosphate buffer (NaPB), pH 7.4

Transfer 593 mg of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 5.8 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ to a 1L flask, add 900 mL of purified water, adjust with HCl to a pH of 7.4, dilute with purified water to volume, and mix.

Stored at refrigerator or room temperature.

4.1.2. p-Nitrosodimethylaniline (RNO)

Dissolve 3 mg of RNO in 100 mL of 20 mM NaPB at the concentration of 2×10^{-4} M.

Stored at refrigerator and keep to protect from light

4.1.3. Imidazole

Dissolve 13.6 mg of Imidazole in 10 mL of 20 mM NaPB at the concentration of 2×10^{-2} M

Dilute the 2×10^{-2} M Imidazole solution 100 times with 20 mM NaPB.

Stored at refrigerator and keep to protect from light

4.1.4. Nitroblue tetrazolium chloride (NBT)

Dissolve 32.7 mg of NBT in 100 mL of 20 mM NaPB at the concentration of 4×10^{-4} M.

Stored at refrigerator and keep to protect from light

4.2. Test compounds

Name	Concentration of preparation	Final concentration
Test compounds	1 and 10 mM ¹⁾	20 and 200 μM
Quinine (positive control)	10 mM	200 μM
Sulisobenzone (negative control)	10 mM ²⁾	200 μM
DMSO (blank)		

Notes

- 1) Compound will be weighed in a tube, and added DMSO at the concentration 10 mM as a stock solution. The tube will be mixed with vortex mixer and sonicated for 5 to 10 min under UV-cut illumination or shade. The solutions will be divided to 3 or more new tubes at volume of >50 μL each, kept to protect from light, and stored at freezer (below -20°C). Just before use, the stock solution will be thawed and diluted in DMSO at 1 mM. All preparations should be checked solubility (solution or suspension) with the naked eye.
- 2) Sulisobenzone solution will be prepared at 3.825 mg/mL (net weight) in DMSO because of dried material of 19.4% (lot Z61BE).

5. Methods

A tube (e.g. 1.5 mL of eppendorf tube) and a plastic clear flat bottomed 96-well microplate will be used. The reaction mixture should be prepared under UV-cut illumination or shade.

Experiments will be performed in triplicate wells in 3 independent runs.

【Singlet oxygen】		【Superoxide anion】	
20 mM NaPB	480 μL	20 mM NaPB	855 μL
Imidazole	250 μL	NBT	125 μL
RNO	250 μL	Compound	20 μL
Compound	20 μL		
↓		↓	
Mix (Vortex and Sonication for 5 – 10 min)		Mix (Vortex and sonication for 5-10 min)	
↓		↓	
Check solubility ¹⁾		Check solubility ¹⁾	
↓		↓	
Add 200 μL of mixture to each well (n=3)		Add 200 μL of mixture to each well (n=3)	
↓		↓	
Pre-read Abs at 440 nm after shaking for 5 sec		Pre-read Abs at 560 nm after shaking for 5 sec	
↓		↓	
Light exposure (250 W/m^2 for 1 hr at 25°C) ²⁾		Light exposure (250 W/m^2 for 1 hr at 25°C) ²⁾	
↓		↓	
Read Abs at 440 nm after shaking for 5 sec		Read Abs 560 nm after shaking for 5 sec	
↓		↓	
Check solubility ¹⁾		Check solubility ¹⁾	

Notes

- 1) The reaction mixture will be checked solubility (solution or suspension) with naked eye before or after light exposure.
- 2) The 96-well plate will be placed in to the Quartz reaction container. The container will be set quarts cover and fasten with bolts. The solar simulator will be use at least 30 min after turning on the power supply at the indicator setting value of 250 W/m² (for CPS+). After the final experiment of the day, UVA intensity will be measured using a UVA detector. Temperature and light exposure will be written the set values by light exposure equipment (for CPS+). An example of 96-well plate configuration is shown as follows;

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
C		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
D		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
E		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
F		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
G		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
H												

B :Blank (DMSO)

P :Positive control (Quinine), 200 μM

N :Negative control (Sulisobenzone), 200 μM

SP1-SP7: test compound No. 1-7, 200 μM or 20 μM

6. Data analysis

Individual data will be presented in a fixed tabular form (excel file) provided form the VMT.

6.1. Singlet oxygen

$$\text{decrease of } A_{440} \times 10^3 = (A_{440(-)} - A_{440(+)} - (A-B)) \times 1000$$

A₄₄₀₍₋₎ : Absorbance before light exposure at 440 nm

A₄₄₀₍₊₎ : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

6.2. Superoxide anion

$$\text{increase of A560} \times 10^3 = (\text{A560}(+) - \text{A560}(-) - (\text{B}-\text{A})) \times 1000$$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

7. Criteria for data acceptance

The following criteria should be satisfied in each experiment at the present. The final criteria will be decided after validation study.

7-1. Data

Without data lack in positive control, negative control, blank, and test compound.

7-2. OD values

Each net OD value of positive control, negative control, and test compound: 0.02 to 1.1

7-3. Calculation values

Positive control value at 200 μM (mean of 3 wells)

Singlet oxygen: 150 to 500

Superoxide anion: 200 to 400

Negative control value at 200 μM (mean of 3 wells)

Singlet oxygen: -20 to 24

Superoxide anion: -20 to 19

8. Criteria for judgment

The test compound will be judged as a positive response when mean value of 3 wells at 20 and/or 200 μM is over 25 for singlet oxygen and over 20 for superoxide anion. The final criteria will be decided after validation study.

9. Archives and review

The study report and all raw data from this study will be retained according to the SOP in each testing facility. All raw data will be submitted to the VMT for review if required.

10. Reference

S. Onoue, N. Igarashi, S. Yamada, Y. Tsuda, *Journal of Pharmaceutical and Biomedical Analysis*, 46 (2008) 187-193.

**INTERNATIONAL VALIDATION STUDY ON ROS (REACTIVE
OXIDATIVE SPECIES) ASSAY AS A TEST EVALUATING
PHOTOTOXIC POTENTIAL OF CHEMICALS
(ATLAS VERSION 3.02)**

Issued by: the Validation Management Team (VMT)

Date: 21 May 2012.

1. Purpose of this document

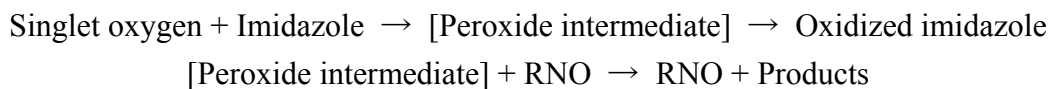
This document is provided to clarify the conduct of an international validation study to evaluate the ability of ROS (reactive oxidative species) assay as a photochemical property study to examine necessity of phototoxicity tests. This document represents the last study protocol developed as a result of the collaboration efforts of the participating testing facilities and the VMT. Each testing facility will develop a study protocol based on the information provided in this document.

2. Background of ROS assay

Drug-induced photoirritation can be defined as an inflammatory reaction of the skin after topical or systemic administration of pharmaceutical substances. Several classes of drugs including antibacterials, thiazide diuretics, non-steroidal anti-inflammatory drugs, quinolones, and tricyclic antidepressants, even though nontoxic by themselves, may become reactive under exposure to environmental light, leading to undesired side effects. The primary event in any photosensitization process is the absorption of photons of the appropriate wavelength, which allows chromophore to reach an excited state. The excitation energy is often transferred to oxygen molecules, followed by generation of ROS: superoxide through type I reaction and singlet oxygen through type II reaction by photo-excited drug molecules. These appear to be the principal intermediate species in the phototoxic response. From the standpoint of risk assessment, previous study demonstrated that determination of ROS from pharmaceutical substances irradiated with UVA/B and visible light would be of help in recognizing their phototoxic potential.

In the ROS assay, generation of singlet oxygen was detected by spectrophotometric measurement of *p*-nitrosodimethyl aniline (RNO) bleaching, followed by decreased absorbance of RNO at 440 nm. Although singlet oxygen does not react chemically

with RNO, the RNO bleaching is a consequence of singlet oxygen capture by the imidazole ring, resulting in the formation of a trans-annular peroxide intermediate capable of inducing the bleaching of RNO as follows;



The generation of superoxide could be determined by the reduction of nitroblue tetrazolium (NBT) as indicated below; NBT can be reduced by superoxide anion via a one-electron transfer reaction, yielding partially reduced ($2 e^-$) monoformazan (NBT^+) as a stable intermediate. Thus, superoxide can reduce NBT to NBT^+ , whose formation can be monitored spectrophotometrically at 560 nm.



3. Materials

3.1. Test chemicals

Coded test chemicals and essential information about the test substances (physical state, weight or volume of the test chemicals, specific density for liquids, storage instructions, molecular weight, and conversion factor) will be supplied to each testing facility by the VMT. Safety information of the test chemicals will be provided to an appropriate individual within the organization who is not involved in the study. The test chemicals should be stored according to the VMT instructions until termination of the study. Study personnel can confirm the safety information in the case of emergency. If the safety information is opened, appropriate documentation and justification will need to be provided to the VMT.

3.2. Control chemicals

Positive and negative control chemicals will be supplied to each testing facility by the VMT. Both chemicals will be stored in an air-tight container in a refrigerator and protected from light.

Name	CAS No.	Molecular weight
Quinine monohydrochloride dihydrate (Quinine, positive control)	6119-47-7	396.91
Sulisobenzone (Negative control)	4065-45-6	308.31

3.3. Solvent

Dimethylsulfoxide (DMSO, analytical grade) should be used at first. DMSO should be stored at room temperature. In the case of DMSO-insoluble chemical,

20 mM sodium phosphate buffer (NaPB, see Section 4.1.1.) should be used as a solvent. If a test chemical is insoluble in either DMSO or 20 mM NaPB, it is impossible for the chemical to evaluate in this assay.

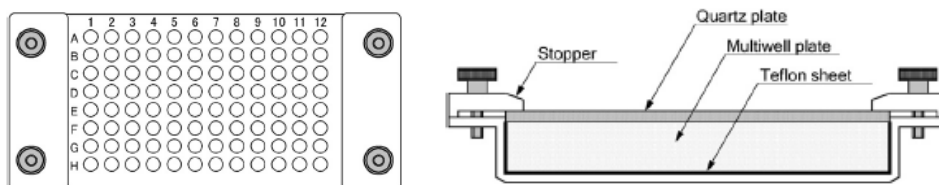
3.4. Reagents

The following reagents will be used and stored according to the instructions of manufacturers.

- $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$: e.g. Wako, catalog No. 192-02815
- $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$: e.g. Wako, catalog No. 194-02831
- p-Nitrosodimethylaniline (RNO): e.g. APOLLO, catalog No. OR30877
- Imidazole: e.g. Wako, catalog No. 099-00013
- Nitroblue tetrazolium chloride (NBT): e.g. Wako, catalog No. 144-01993
- Purified water

3.5. Technical equipments

- Solar simulator: Suntest CPS+ or CPS, equipped with a xenon arc lamp, UV (<290 nm) cut filter, and temperature control unit (Atlas Material Technology), or their equivalents
- UVA detector (as a calibrator, Dr. Hönle #0037)
- UVA detector for regular use: e.g. Topcon or Dr. Hönle
- Thermometer
- Microplate spectrophotometer, equipped 440 and 560 nm filters: Spectra Max M2 (Molecular Devices), Tecan Safire (Tecan), or equivalents
- Quartz reaction container: Made-to-order (Ozawa Sciences, Onoue et al. 2008, see below Figure), supplied from the VMT.
- Microscope
- Voltex mixer
- Plate shaker
- Sonicator
- Pipetting aid
- Pipettes, 8-channel-pipettes
- Polypropylene tubes
- Plastic 96-well plates (clear, non-treat flat-bottom)
- Plastic- and glassware



4. Preparations

4.1. Reagents

All reagents should be sonicated and used within 1 month after preparation. Representative preparation methods are shown as follows;

4.1.1. 20 mM sodium phosphate buffer (NaPB), pH 7.4

Weigh 593 mg of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 5.8 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, add 900 mL of purified water, adjust with HCl to a pH of 7.4, dilute with purified water up to 1 L, and mix.

Stored at refrigerator or room temperature.

4.1.2. p-Nitrosodimethylaniline (RNO)

Dissolve 3 mg of RNO in 100 mL of 20 mM NaPB at the concentration of 2×10^{-4} M.

Stored at refrigerator and keep to protect from light.

4.1.3. Imidazole

Dissolve 13.6 mg of imidazole in 10 mL of 20 mM NaPB at the concentration of 2×10^{-2} M.

Dilute the 2×10^{-2} M imidazole solution 100 times with 20 mM NaPB.

Stored at refrigerator and keep to protect from light.

4.1.4. Nitroblue tetrazolium chloride (NBT)

Dissolve 32.7 mg of NBT in 100 mL of 20 mM NaPB at the concentration of 4×10^{-4} M.

Stored at refrigerator and keep to protect from light.

4.2. Test chemicals

The test chemicals will be prepared using DMSO just before use.

Each test chemical will be weighed in a tube, and added DMSO at the concentration 10 mM at first. The tube will be mixed with vortex mixer and sonicated for 5 to 10 min under UV-cut illumination or shade. All preparations should be kept to protect from light. The final concentration in a reaction mixture (see section 5.2.) will be set at 200 μM . When precipitation is observed at 20 μM in the reaction mixture

under a microscope, 1 mM of the test chemical solution should be prepared using DMSO. Furthermore, 0.1 mM of the test chemical solution should be prepared using DMSO when precipitation is observed at 2 μ M in the reaction mixture.

In the case of DMSO-insoluble chemical, the final concentration in the reaction mixture including 20 μ L of DMSO (see Section 5.2.) will be used at the maximum concentration without precipitation (2, 20, or 200 μ M).

4.3. Positive and negative control chemicals

Stock solutions of quinine and sulisobenzone will be prepared at 10 mM each in DMSO (the final concentration of 200 μ M) according to the procedure of section 4.2., divided into some tubes, and stored in a freezer (generally below -20°C) for up to 1 month. The stock solution will be thawed just before the experiment and used within the day.

5. Methods

5.1. Calibration of the UVA irradiance

The UVA irradiance should be calibrated as described below prior to the study.

UVA intensity will be set at an appropriate value (e.g. the indicator setting value of 250 W/m^2 for CPS+). The solar simulator and the temperature control unit (or its equivalent) will be turned on the power supply. UVA intensity on the plate position will be measured under a stable condition using a UVA detector of the testing facility and the calibrated UVA detector (Dr. Hönle #0037) delivered by the VMT. The measurement will be performed at some different intensity using the two UVA detectors. Correlation curve on the values of the UVA detectors will be confirmed.

5.2. Calibration of the solar simulator

On the day of assays, 1.8-2.2 mW/cm^2 of UVA intensity for the calibrated UVA detector will be set.

When the solar simulator has a temperature control unit, the temperature will be adjusted at 25°C . As for the solar simulator without a temperature control unit, a range of 20 - 29°C after light exposure is acceptable.

5.3. Test procedure

A tube (e.g. 1.5 mL of eppendorf tube) and a plastic clear flat bottomed 96-well microplate will be used. The reaction mixture should be prepared under UV-cut

illumination or shade. DMSO will be used in a blank.

Experiments will be performed in triplicate wells in three independent runs.

As the final concentrations, 200 μM of test chemical solutions will be used generally. When precipitation is observed at 200 μM , additional experiments should be performed at 20 μM . Further experiments should be performed at 2 μM when precipitation is observed at 20 μM . When precipitation is observed at 2 μM in the reaction mixture, further experiment is not needed. When questionable data (e.g. technical error) is obtained, each testing facility can perform an additional experiment using the questionable chemical(s) and the positive/negative chemicals. If the values of the positive/negative chemicals would not be met the criteria (see section 7), the additional experiment using the all chemicals of the plate should be needed. The reason of the additional experiment should be described in the raw data. The adoption of the triplicate data will be decided by the VMT.

【Singlet oxygen】		【Superoxide anion】	
20 mM NaPB	480 μL	20 mM NaPB	855 μL
Imidazole	250 μL	NBT	125 μL
RNO	250 μL	Chemical	20 μL
Chemical	20 μL		
↓		↓	
Mix (Vortex and Sonication for 5 – 10 min)		Mix (Vortex and sonication for 5-10 min)	
↓		↓	
Add 200 μL of mixture to each well (n=3) ¹⁾		Add 200 μL of mixture to each well (n=3) ¹⁾	
↓		↓	
Check solubility ²⁾		Check solubility ²⁾	
↓		↓	
Pre-read Abs at 440 nm after shaking for 5 sec		Pre-read Abs at 560 nm after shaking for 5 sec	
↓		↓	
Light exposure for 1 hr ³⁾		Light exposure for 1 hr ³⁾	
↓		↓	
Read Abs at 440 nm after shaking for 1 min		Read Abs 560 nm after shaking for 1 min	

Notes

1) An example of 96-well plate configuration is shown as follows;

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
C		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
D		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
E		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
F		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
G		B	P	N	SP1	SP2	SP3	SP4	SP5	SP6	SP7	
H												

B: Blank (DMSO)

P: Positive control (Quinine), 200 µM

N: Negative control (Sulisobenzone), 200 µM

SP1-SP7: test chemical No. 1-7

B2-B11, C2-C11, and D2-D11 wells: singlet oxygen

E2-E11, F2-F11, and G2-G11 wells: superoxide anion

- The reaction mixture will be checked solubility (solution or suspension) using a microscope (×100) before light exposure.
- The 96-well plate will be placed in to the Quartz reaction container. The container will be set quarts cover and fasten with bolts. The solar simulator and the temperature control unit (or its equivalent) will be use under a stable condition. After the experiment, UVA intensity and temperature on the plate position will be measured using the UVA detector of the testing facility and thermometer. Acceptable ranges of temperature and UVA intensity after light exposure are shown in section 5.1.

6. Data analysis

Individual data will be presented in a fixed tabular form (excel file) provided by the VMT. The data of 3 wells in each chemical will be calculated as mean and standard division in each experiment.

6.1. Singlet oxygen

$$\text{decrease of A440} \times 10^3 = (\text{A440(-)} - \text{A440(+)} - (\text{A-B})) \times 1000$$

A440(-) : Absorbance before light exposure at 440 nm

A440(+) : Absorbance after light exposure at 440 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

6.2. Superoxide anion

$$\text{increase of A560} \times 10^3 = (\text{A560}(+) - \text{A560}(-) - (\text{B}-\text{A})) \times 1000$$

A560(-) : Absorbance before light exposure at 560 nm

A560(+) : Absorbance after light exposure at 560 nm

A : Mean (Blank before light exposure)

B : Mean (Blank before after exposure)

7. Criteria for data acceptance

The following criteria should be satisfied in each experiment at the present. The criteria will be decided after validation study.

7.1. Precipitation

Without precipitation of test chemical in the reaction mixture before light exposure.

7.2. Data

Without data lack in positive control, negative control, blank, and test chemical.

7.3. OD values

Each net OD value of positive control, negative control, and test chemical: 0.02 to 1.5.

7.4. Calculation values

Positive control value at 200 μM (mean of 3 wells)

Singlet oxygen: 150 or more

Superoxide anion: 200 or more

Negative control value at 200 μM (mean of 3 wells)

Singlet oxygen: less than 25

Superoxide anion: less than 20

8. Criteria for judgment

The final criteria including obligatory endpoint(s) for ROS assay, singlet oxygen and/or superoxide, will be decided after validation study. At present, each test chemical will be judged in each experiment as follows;

Positive (mean of 3 wells)

Singlet oxygen: 25 or more at 200, 100, 50 or 20 μM , or

Superoxide anion: 20 or more at 200, 100, 50 or 20 μM

Negative (mean of 3 wells)

Singlet oxygen: less than 25 at 200 μM and

Superoxide anion: less than 20 at 200 μM

Inconclusive (mean of 3 wells)

The results does not meet the positive or negative criterion.

The final judgment will be estimated on the results of triplicate experiments in each testing facility as follows;

Positive (mean of 3 assays)

Singlet oxygen: 25 or more at 200, 100, 50 or 20 μM , or

Superoxide anion: 20 or more at 200, 100, 50 or 20 μM

Negative (mean of 3 assays)

Singlet oxygen: less than 25 at 200 μM and

Superoxide anion: less than 20 at 200 μM and

Inconclusive (mean of 3 wells)

The results does not meet the positive or negative criterion.

9. Archives and review

The study report and all raw data from this study will be retained according to the SOP in each testing facility. All raw data (pdf files) and the results (excel files) will be submitted to the VMT for review.

10. Reference

S. Onoue, N. Igarashi, S. Yamada, Y. Tsuda, *Journal of Pharmaceutical and Biomedical Analysis*, 46 (2008) 187-193.

Appendix 1: Amendment of Protocol

1. Version 3.01

Date: 29 August 2011

Items: 8. Criteria for Judgment (Page 8)

Positive (mean of 3 wells)

	Version 3.0	Version 3.01
Singlet oxygen:	<u>150</u> or more	---> <u>25</u> or more
Superoxide anion:	<u>200</u> or more	---> <u>20</u> or more

Reason: Mistake of description

1. Version 3.02

Date: 21 May 2012

Items: 8. Criteria for judgment (Page 8-9)

Positive (mean of 3 wells)

Singlet oxygen: 25 or more at 200, 100, 50 or 20 μ M, or

Superoxide anion: 20 or more at 200, 100, 50 or 20 μ M

Negative (mean of 3 wells)

Singlet oxygen: less than 25 at 200 μ M and

Superoxide anion: less than 20 at 200 μ M

Inconclusive (mean of 3 wells)

The results does not meet the positive or negative criterion.

The final judgment will be estimated on the results of triplicate experiments in each testing facility as follows;

Positive (mean of 3 assays)

Singlet oxygen: 25 or more at 200, 100, 50 or 20 μ M, or

Superoxide anion: 20 or more at 200, 100, 50 or 20 μ M

Negative (mean of 3 assays)

Singlet oxygen: less than 25 at 200 μ M and

Superoxide anion: less than 20 at 200 μ M and

Inconclusive (mean of 3 wells)

The results does not meet the positive or negative criterion.

Reason: Information on the test concentration was added to the positive and negative criterion. The negative results should be judged from the results at 200 μ M only. The positive results can be judged from the results at any concentration tested. And,

when the test chemical is not judged either "Positive" or "Negative" from the assay results, the assay results should be judged as "Inconclusive". For the final judgment, the same criterion for the 3 assay results should be adopted.