Photochemistry and Photobiology, 2023, 99: 672–679

Special Issue Research Article

Singlet Oxygen Quenching by Resveratrol Derivatives[†]

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Received 6 June 2022, accepted 24 August 2022, DOI: 10.1111/php.13704

ABSTRACT

We investigated the singlet oxygen quenching ability of several derivatives of *trans*-resveratrol which have been reported to have significant antioxidant ability, including photoprotective activity. We measured the total rate constants of singlet oxygen removal (k_T) by the methylated resveratrol derivative 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)ethenyl]benzene, and the partially methylated resveratrol derivatives 4-((E)-2-(3,5-dimethoxyphenyl)ethenyl)phenol (pterostilbene), 5-[(E)-2-(4-methoxyphenyl)ethenyl]benzene-1,3-diol and (2*R*,3*R*)-3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-2,3-dihydrochromen-4-one (dihydromyricetin). A protic solvent system results in higher k_T values, except for the completely methylated derivative. We also investigated the ability of *trans*-resveratrol to directly act as a photosensitizer (rather than *via* secondary photoproducts resulting from other primary photochemical reactions) for the production of singlet oxygen but found that neither resveratrol nor any of its derivatives are able to do so. We then studied the chemical reactions of the methylated derivative with singlet oxygen. The main pathway consists of a [4 + 2] cycloaddition reaction involving the *trans*-double bond and the *para*-substituted benzene ring similar to what has been observed for *trans*-resveratrol. Unlike *trans*-resveratrol, the primary singlet oxygen product undergoes a second [4 + 2] cycloaddition with singlet oxygen leading to the formation of diendoperoxides. A second reactivity pathway for both *trans*-resveratrol and the methylated derivative leads to the formation of aldehydes *via* cleavage of a transient dioxetane.

INTRODUCTION

Singlet oxygen is a reactive oxygen species generated by the excitation of ground state (triplet) oxygen. It is most commonly generated through the transfer of energy from excited photosensitizer to ground-state (triplet) oxygen, although it can also be generated intracellularly by neutrophils using NADPH oxidase and myeloperoxidase (1–4). Once generated, singlet oxygen can react with fatty acids, amino acids and the DNA base guanine (5–9). Unlike many other reactive oxygen species (ROS), singlet oxygen is not a radical; instead, it is strongly electrophilic due to its low-lying LUMO. The intracellular lifetime of singlet oxygen is roughly 3 μs, and it has a spherical spatial domain in the cell of about 100 nm (10–12).

It is one of the most common ROS involved in metastasis (13,14). It is also implicated in Alzheimer's disease, Parkinson's disease and some cancers because of the damage it causes to DNA bases (15,16).

Due to singlet oxygen's reactivity with biomolecules in the cellular environment, the ability of antioxidants to remove (*i.e.* quench) singlet oxygen has been a topic of considerable interest. The health benefits of *trans*-resveratrol (**1**, 5-[(*E*)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol), a compound found in red wine grapes, cranberries, blueberries and peanuts, have been extensively discussed in the literature (17–24). Resveratrol has been said to ameliorate diabetes, protect folate from UV-mediated damage and act as an antioxidant, potentially through synergistically assisting β-carotene (25–28). However, recent work has called into question resveratrol's ability to act as an antioxidant against free radical oxidations, and it has even been shown to be cytotoxic and genotoxic under certain conditions (17,28,29). There is, however, evidence of resveratrol's potential activity as an antioxidant against singlet oxygen in its potential ability to prevent photooxidative damage to the retina. Fluorophore A2E, a molecule found in the retina, may act as a sensitizer for singlet oxygen, which can subsequently cause damage to the epithelial cells. Several studies have reported that resveratrol may prevent epithelial cell damage in the eyes by quenching singlet oxygen before it interacts with fluorophore A2E (30–33).

Fotiou *et al*. (19) found that upon irradiation with UVC light, resveratrol undergoes isomerization from its *trans* to its *cis* form. During this process, resveratrol reportedly transfers its excitation energy to triplet oxygen, generating singlet oxygen. However, the generation of singlet oxygen was measured indirectly by detecting changes in chemiluminescence resulting from oxidative damage to DNA. While other ROS may cause oxidative damage to DNA, the authors noted that the addition of mannitol (a hydroxyl radical scavenger) and superoxide dismutase (which removes superoxide from the cell) did not affect the chemiluminescence measurements, providing evidence that singlet oxygen

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[†]This article is part of a Special Issue celebrating the 50th Anniversary of the American Society for Photobiology.

may indeed have been the oxidant. Trigos *et al*. (34) also reported the generation of singlet oxygen by resveratrol after irradiation. In this case, resveratrol was used as a photosensitizer for the photooxidation of ergosterol *via* a [4 + 2] cycloaddition. In contrast to the above studies, Liang *et al*. did not find evidence that resveratrol was able to directly act as a photosensitizer for the production of singlet oxygen. Based on steady-state irradiation of *trans*-resveratrol with UVB light (which is at the maximum absorption of trans-resveratrol), the sole primary photoproduct was observed to be the *cis*-isomer. None of the photoproducts obtained upon the reaction of trans-resveratrol with externally generated singlet oxygen (*i.e. via* the photosensitizer Methylene Blue [MB]) were observed when *trans*-resveratrol was directly irradiated without an external photosensitizer until isomerization to the *cis*-isomer was complete. Liang *et al*. (18) proposed that after the isomerization of *trans*-resveratrol to *cis*-resveratrol under UVB irradiation, the *cis*-resveratrol would undergo a photocyclization reaction to form 2,4,6-phenanthrenetriol, which is able to act as a photosensitizer. A benzofuran compound, 2-(4-hydroxyphenyl)-5,6-benzofurandione was also formed upon UVB irradiation of *trans*-resveratrol. The authors postulated that the latter compound formed through a reaction of resveratrol with singlet oxygen which would have been generated by the phenanthrene compound. This reaction would initially form an endoperoxide that would undergo further rearrangement to the observed benzofuran by a mechanism similar to the Moracin M synthesis reported by the Selke group in 2011. Thus, it remains uncertain if resveratrol can produce singlet oxygen either directly or indirectly from secondary photoproducts. Furthermore, no quantum yield measurements to determine how much singlet oxygen (if any) is produced from *trans*-resveratrol have been reported (19,29).

Singlet oxygen can be removed from a system in two ways: physical quenching, which includes several processes that result in the regeneration of triplet oxygen without changing the structure of the quenching compound and chemical quenching, in which singlet oxygen reacts with the quenching molecule to generate new product(s). The sum of the physical (k_q) and chemical (k_r) quenching rate constants is the total rate of singlet oxygen removal (k_T). The effectiveness of a molecule as a singlet oxygen quencher depends on several factors. One important factor is the relative reaction rate of the quencher with singlet oxygen compared to those of relevant biomolecules with singlet oxygen. More effective quenchers will have quenching rates that are significantly higher than the rates of reaction of biomolecules with singlet oxygen. For example, the k_T value for quenching by the carbon–carbon double bond in fatty acids in CD_3OD is roughly 1–5 $\times$ 10^4 M^{-1} s^{-1}, and in CD_3OD resveratrol has a k_T value of 1.5 $\times$ 10^6 M^{-1} s^{-1} (17,35). Comparing these values, we can see that resveratrol could potentially protect against fatty acid degradation by singlet oxygen. However, compared with other well-known singlet oxygen quenchers such as α-tocopherol and β-carotene, which have (*in vitro*) k_T values of 6.7 $\times$ 10^8 and 1.0 $\times$ 10^{10} M^{-1} s^{-1}, respectively, resveratrol does not appear to be a particularly good quencher (17,28,36).

Another important factor to be considered when determining the efficacy of a potential antioxidant is the nature of any reaction products that may form during reactions with ROS. In the case of resveratrol, we have previously reported that chemical reaction with singlet oxygen, which accounts for roughly 25% of the total quenching, proceeds through either [4 + 2] or [2 + 2] cycloadditions. The [4 + 2] product, which accounts for 60% of chemical reaction products, forms an endoperoxide, whereas the [2 + 2] reaction produces a transient dioxetane that cleaves to form aldehydes (17). Aldehydes have been shown to be implicated in cytotoxicity and genotoxicity and are also involved in modulating signaling pathways essential to cell survival (37–40). If the reaction between resveratrol and singlet oxygen produces potentially toxic aldehydes (and that chemical reaction accounts for a significant proportion of resveratrol's reactivity), the use of resveratrol as an antioxidant against singlet oxygen would be of questionable benefit.

The [4 + 2] addition reaction between singlet oxygen and resveratrol involves the ring bearing the *para*-hydroxy group. Other groups have also noted that the *para*-hydroxy group is more reactive than the *meta*-hydroxy groups (41,42) but quantitative data of how substitution at the various hydroxy groups affects singlet oxygen quenching and reactivity is unknown. We therefore determined the singlet oxygen quenching ability of various methylated derivatives of resveratrol, namely the completely methylated derivative 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl) ethenyl]benzene (**2**), pterostilbene which is methylated at the resorcinol ring (**3**, 4-((E)-2-(3,5-dimethoxyphenyl)ethenyl)phenol) and 5-[(E)-2-(4-methoxyphenyl)ethenyl]benzene-1,3-diol (**4**) which is methylated at the *para*-position. Determination of the k_T values for compounds **2**–**4** allows us to determine the effects of placing hydroxy groups at the *para- vs meta*-positions on the stilbenoid. We also investigated singlet oxygen quenching by the related antioxidant (+)-dihydromyricetin (**5**, (2*R*,3*R*)-3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-2,3-dihydrochromen-4-one) which has several additional hydroxy groups on the ring containing the reactive *para*-hydroxy group. The structures of the resveratrol derivatives investigated in this study are depicted in Fig. 1 below.

Compound **3** (pterostilbene) has been reported to exhibit antioxidant activity through reducing ROS production (including that of singlet oxygen) and oxidative damage, as well as reducing inflammation (43–46). The fully methylated compound **2**, while less genotoxic than resveratrol, has been shown to be an ineffective antioxidant against free radicals (21,43,47,48). Methylation of the *para*-hydroxy group has been reported to enhance the ability of **2** and **4** to inhibit cytochrome P450 (49,50). Compound **5** has also been reported to exhibit antioxidant activity as a free radical scavenger and in its ability to reduce lipid peroxidation (51–54). None of these reports have reported quantitative data for singlet oxygen removal.

MATERIALS AND METHODS

Trans-resveratrol (**1**) and the methylated derivatives **2**–**4** were purchased from Sigma Aldrich. (+)-Dihydromyricetin (**5**) was purchased from Arctom Scientific. All deuterated solvents were purchased from Cambridge Isotope Laboratories. Compounds **1**–**5** were used as received after their purity was verified by ^{1}H NMR (400 MHz Bruker Avance).

Singlet oxygen luminescence quenching experiments. The singlet oxygen ($^1O_2$) luminescence quenching experiments involve preparation of photosensitizer and quencher stock solutions. Either Methylene Blue (MB) or Rose Bengal (RB) were used as external photosensitizers for singlet oxygen production. In preparing the photosensitizer stock solution, 6 mL of the desired solvent system was added to a securely capped vial. A small amount of photosensitizer (MB or RB) was added to that solvent solution so that an absorbance of 0.2–0.3 was obtained at λ_{ex} = 532 nm. A 2 mL sample of the photosensitizer solution was then added to a fluorescence-grade quartz cuvette. Stock solutions of the quencher (resveratrol and its derivatives, *i.e*. compounds **1**–**5**) were

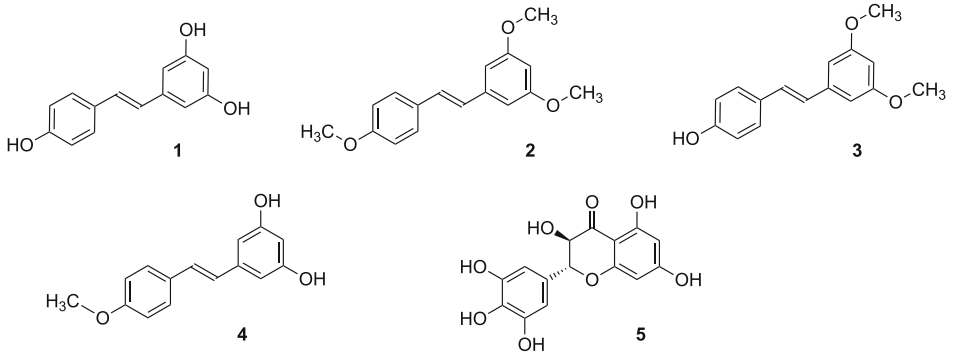

Figure 1. Chemical structures of resveratrol (**1**), methylated and partially methylated derivatives **2**–**4** and (+)-dihydromyricetin (**5**).

prepared in either d_3-acetonitrile, or a 1:1 mixture of d_3-acetonitrile: d_4-methanol. Between 0.3 and 0.57 mg of the quencher was added to 1 mL of solvent in a 1 mL volumetric flask, resulting in a starting concentration range of 2.0–2.5 × 10^{-6} M.

A 400 V Nd: YAG laser (New Wave Research Mini-Lase II) was used for the pulsed irradiation (2 ns) of each cuvette. We employed a thermoelectric cooled near-infrared photomultiplier tube (NIR-PMT, Hamamatsu H10330B-45) to detect the singlet oxygen NIR emission signal. The initial measurement was taken upon excitation of the 2 mL photosensitizer solution which yields the decay of singlet oxygen without an added quencher. A 20 µL sample of the quencher stock solution was then added for every subsequent data point collection. All measurements were taken in air-saturated solutions. The luminescence signals from each run were analyzed *via* OriginPro software. Using $I = I_o e^{-kt}$, the observed rate constant of the decay k_{obs} of $^1O_2$ for each data point was calculated. The observed rate constant k_{obs} was plotted against the quencher concentration; the slope of this plot yields the rate constant k_T, that is, the total rate constant of $^1O_2$ quenching.

*Photooxidation of 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)ethenyl] benzene (**2**).* 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)ethenyl]benzene (**2**, 1.85 mM to 21.9 mM) was dissolved in 1 mL of CD_3CN containing MB. The mixture was placed into an NMR tube where a slow stream of oxygen gas was bubbled through the solution during the photooxidation studies. A 200 W tungsten-halogen lamp was used as a light source. The time frame of irradiation of the samples ranged from 0–10 h. Wavelengths below 493 nm were blocked using a filter for the light source to prevent trans to cis isomerization. The reaction was monitored by ^{1}H-NMR on a Bruker 400 MHz instrument. The new aldehyde peaks appearing at 9.86 and 9.89 ppm were compared with the spectra of authentic samples of 4-methoxybenzaldehyde and 3,5-dimethoxybenzaldehyde (Sigma–Aldrich).

*Analyses of photooxidation products of 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl) ethenyl]benzene (**2**) by electrospray ionization mass spectrometry.* Electrospray ionization mass spectrometry (ESI–MS) was used to characterize the photooxidation product of **2** with singlet oxygen. The mass spectra were recorded on a linear ion trap mass spectrometer (Thermo Fisher, San Jose, CA) equipped with an ESI source. Samples were diluted 100-fold with 50/50 water/acetonitrile with 0.1% formic acid. Samples were introduced into the mass spectrometer at a flow rate of 20 µL min^{-1}. ESI–MS was conducted in the positive ion mode at a spray voltage of 4 kV and the capillary temperature was 250°C. The sheath and auxiliary gases were set to 15 and 3 psi, respectively. Samples from 0, 2, 5, 7 and 10 h of irradiation were analyzed.

*Analyses of photooxidation products of 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl) ethenyl]benzene (**2**) by liquid chromatography–MS.* A Thermo Fisher Acella UHPLC system (Thermo Fisher Scientific, San Jose, CA) coupled to a photodiode array detector and a high-resolution mass spectrometer (Exactive) was used to analyze the photooxidation products of the reaction of singlet oxygen with 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl) ethenyl]benzene (**2**). Separation was performed using a Kinetex C18 column (1.7 µm, 2.1 mm × 50 mm, 100 Å) from Phenomenex (Torrance, CA) at a flow rate of 0.5 mL min^{-1} at 40°C and eluted species were detected at 254 nm. The mobile phase contained water, acetonitrile and 0.1% formic acid. The gradient elution for all samples was ramped from 5% to 95% acetonitrile over 7 min and then back down to 5% in 0.5 min and then held for 2.5 min. ESI–MS was conducted in the positive ion mode at a spray voltage of 4 kV and the capillary temperature was at 350°C. The sheath and auxiliary gases were set to 55 and 7 psi, respectively.

RESULTS AND DISCUSSION

Singlet oxygen quenching by resveratrol derivatives

In a previous communication, we reported the values for the total singlet oxygen quenching rate constant (k_T value) of resveratrol in deuterated acetonitrile as well as in a 3:2 CD_3OD–D_2O mixture and in D_2O at pH 10 (17) using time-resolved singlet oxygen luminescence spectroscopy. We now report the k_T values for the methylated resveratrol derivative **2** (Fig. 2) as well as for the partially methylated derivatives **3** and **4** and dihydromyricetin (**5**). Singlet oxygen was generated by flash excitation of RB at 532 nm. Compounds **1**–**5** were studied in both protic (1:1 mixture of deuterated acetonitrile and deuterated methanol) and aprotic (deuterated acetonitrile) solvent systems; results are summarized below (Table 1) and additional sample plots of k_{obs} *vs* the concentration of the quencher for compounds **1**–**5** are shown in the Figures S1–S5.

Our results show that in general methylation of the hydroxy groups decreases the singlet oxygen scavenging ability of the resveratrol derivatives. Thus, compound **2**, in which all the hydroxy groups are replaced by methoxy groups, has the lowest k_T value. The effect is, however, rather modest, and even though **3** has one fewer hydroxy group than compound **4**, their k_T values are very similar. Likewise, the results for compounds **3** and **4** in CD_3CN indicate that the location of the methoxy substitutions

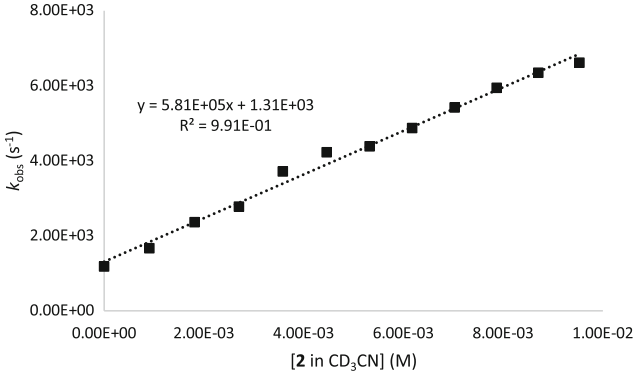

Figure 2. Quenching of singlet oxygen by 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)ethenyl]-benzene (**2**) in CD_3CN.

Table 1. Total rate constant (k_T) of singlet oxygen removal for compounds **1**–**5**.

Compound	Solvent	k_T (M^{-1} s^{-1})
1 (5-[(*E*)-2-(4-hydroxyphenyl) ethenyl]benzene-1,3-diol)	CD_3CN*	$(1.6 \pm 0.1) \times 10^6$
1	CD_3CN-CD_3OD (1:1)†	$(2.1 \pm 0.1) \times 10^6$
1	CD_3OD-D_2O (3:2)*	$(9.2 \pm 0.2) \times 10^6$
1	D_2O (pH = 10)*	$(3.7 \pm 0.2) \times 10^8$
2 (1,3-Dimethoxy-5-[(E)-2-(4-methoxyphenyl)ethenyl]benzene)	CD_3CN†	$(5.8 \pm 0.3) \times 10^5$
2	CD_3CN-CD_3OD (1:1)†	$(3.5 \pm 0.2) \times 10^5$
3 (4-((E)-2-(3,5-dimethoxyphenyl)ethenyl)phenol)	CD_3CN†	$(1.3 \pm 0.1) \times 10^6$
3	CD_3CN-CD_3OD (1:1)†	$(1.4 \pm 0.4) \times 10^6$
4 (5-[(E)-2-(4-methoxyphenyl)ethenyl]benzene-1,3-diol)	CD_3CN†	$(1.2 \pm 0.1) \times 10^6$
4	CD_3CN-CD_3OD (1:1)†	$(2.2 \pm 0.1) \times 10^6$
5 ((2*R*,3*R*)-3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-2,3-dihydrochromen-4-one)	CD_3CN†	$(1.8 \pm 0.1) \times 10^6$
5	CD_3CN-CD_3OD (1:1)†	$(2.6 \pm 0.2) \times 10^6$

*Values from Ref. (17). †This work. Average of three to five plots; error is one standard deviation.

does not have a significant effect on the k_T values. Interestingly, compound **5** which possesses additional hydroxy groups on the aromatic ring bearing the *para*-hydroxy group but lacks the central double bond has a very similar value of k_T as compared to compounds **1**, **3** and **4**.

The addition of protic solvents somewhat increased the k_T values of these derivatives, with the exceptions of the completely methylated derivative **2** and partially methylated derivative **3** (in which the two *meta*-hydroxy groups are methylated). We suggest that this could be due to the higher ionizing ability of the CD_3CN–CD_3OD mixture, as we had previously reported that resveratrol in D_2O at pH 10 has a k_T value more than two orders of magnitude higher than in CD_3CN (17). Consistent with this hypothesis, derivative **2**, which has no hydroxy groups, actually has a smaller k_T value in the CD_3CN–CD_3OD mixture.

Photosensitizer effects on observed quenching behavior

During our initial singlet oxygen quenching (k_T) measurements, we observed that the use of MB (instead of RB) as a photosensitizer led to a slight upward (*i.e.* nonlinear behavior) curvature of plots of k_{obsd} *vs* concentration of the quencher. This effect was observed for compounds **1** and **4**. Similar nonlinear plots were observed in our recent work studying the photooxidation of tryptophan with and without cation-π interaction when MB was used as the photosensitizer. We hypothesize that this results from electron transfer from the quencher to the excited cationic MB photosensitizer, that is, a type I photooxidation process (55). In our previous work, we had been studying a tryptophan model complex. Reducing the electron-donating ability of the quencher (*i.e. via* the presence of a cation-π interaction) resulted in the generation of linear k_T plots. Other groups have made similar observations when using MB as a photosensitizer. The McNeill group demonstrated that phenolic compounds react with MB through a proton-coupled electron transfer mechanism and that more acidic compounds react faster with MB (56), which would be consistent with the methylated compound **2** not showing this effect. Similarly, Jiang *et al.* (57) compared the photooxidation of two fatty acid chains: one that contained two conjugated π bonds (and therefore had a region with more electron density) and one that contained two π bonds that were separated by an sp^3 carbon. The conjugated compound was shown to bleach MB at a faster rate than the nonconjugated compound. These results suggest that the use of MB as a photosensitizer for electron-rich substrates may result in competing Type I photooxidation reactions. The use of RB as a photosensitizer in our k_T experiments resulted in linear plots of k_{obsd} *vs* concentration of the quencher in all cases.

Singlet oxygen production by *trans*-resveratrol and derivatives

As discussed in the introduction, several groups have suggested that resveratrol may be capable of acting as a photosensitizer to generate singlet oxygen while others have suggested that singlet oxygen is only formed from secondary photoproducts formed upon irradiation of *trans*-resveratrol. In all of these examples, singlet oxygen was detected by indirect (*i.e.* trapping) methods. Therefore, we decided to determine whether *trans*-resveratrol and the various derivatives produced singlet oxygen through direct measurement of the singlet oxygen NIR luminescence by laser flash excitation of **1**–**5**. We excited compounds **1**–**5** with the third harmonic of an Nd:YAG laser (355 nm, A_{355} = 0.20 to 0.33 in 1:1 CD_3OD–CD_3CN) and in all cases did not observe any singlet oxygen production, that is, no signal of the characteristic $^1O_2$ NIR luminescence was detected. Liang *et al.* also found that the *primary* photoproduct formed upon direct steady-state excitation of *trans*-resveratrol (in the absence of an external photosensitizer) is the *cis* isomer, without concomitant formation of singlet oxygen products. Taken together with our results from the NIR luminescence experiments, we conclude that the photooxidation products reported in the literature formed upon irradiation of resveratrol must be due to singlet oxygen production from secondary photoproducts, as suggested by Liang *et al.* (18).

Product studies of the reaction of methylated resveratrol with singlet oxygen

Chemical reactions of singlet oxygen quenchers may limit their utility; this is especially the case if the resulting products are toxic and/or highly reactive. In our previous communication (17), we reported that *trans*-resveratrol undergoes two chemical reactions with singlet oxygen leading to an endoperoxide as well as aldehyde products. Detailed characterization of these products by NMR can be found in the supporting information of our previous paper. These reactions account for roughly 25% of the total quenching. The endoperoxide is formed *via* a [4 + 2]

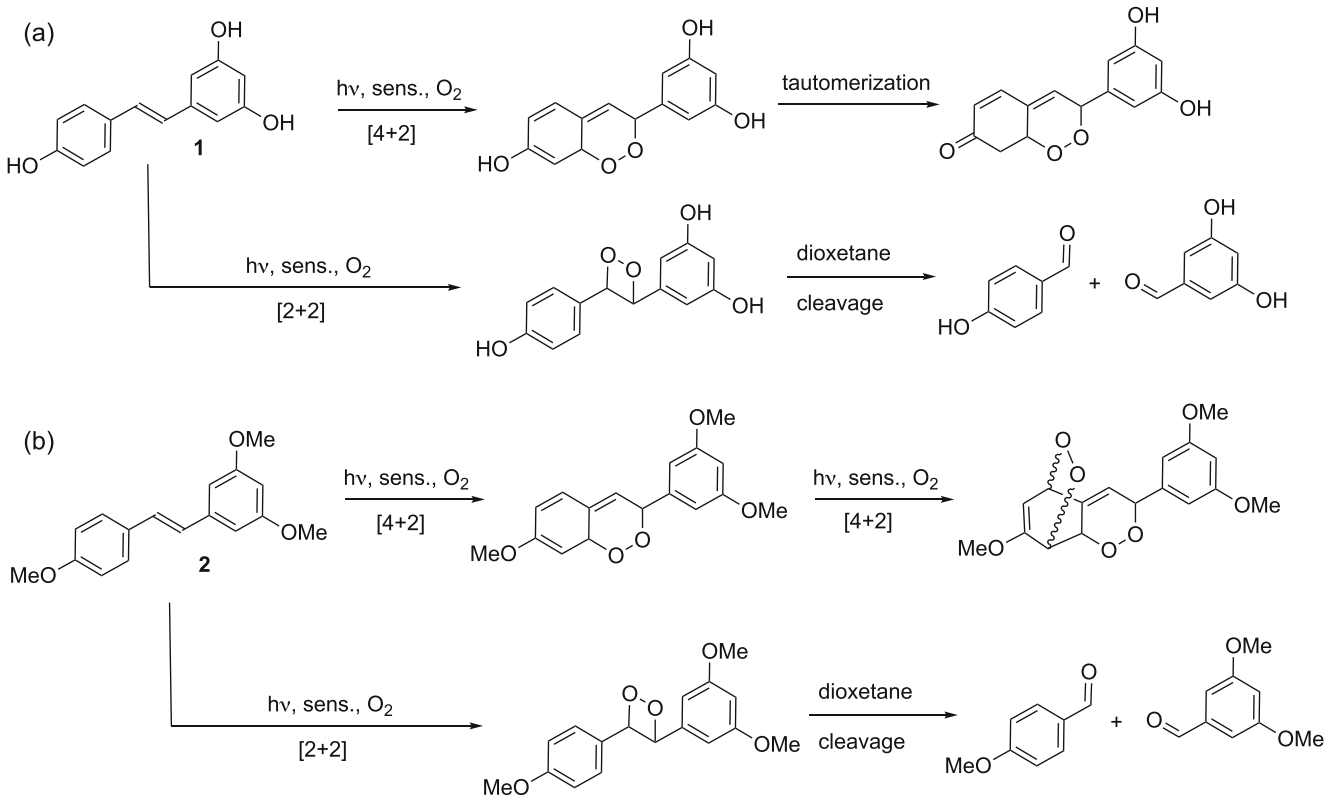

Figure 3. (a) Reactivity pathways of [(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol, (*trans*-.resveratrol, **1**). (b) Reactivity pathways of 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl) ethenyl]benzene (**2**) with singlet oxygen lead to the formation of a diendoperoxide.

cycloaddition pathway, whereas the aldehydes are formed *via* a [2 + 2] addition of singlet oxygen to the trans double bond which produces a transient dioxetane (17). The [4 + 2] cycloaddition involves the *trans*-double bond and the benzene ring bearing the 4′-hydroxy group. This product readily tautomerizes leading to the formation of the corresponding ketone (Fig. 3a). Methylation at the 4′-hydroxy group should prevent this tautomerization, leading instead to a potentially reactive diene as a primary product of the [4 + 2] cycloaddition. Thus, methylation of the 4′ hydroxy group could lead to two sequential [4 + 2] cycloaddition reactions with singlet oxygen with the second one being faster than the initial one. To test this hypothesis, we decided to study the photooxidation products of the methylated resveratrol derivative **2**. Samples of **2** (*ca*. 20 mM) were irradiated in CD_3CN in the presence of MB or RB. Wavelengths below 493 nm were blocked using a cut-off filter to prevent excitation of **2** and possible *trans* to *cis* isomerization. The reaction was initially followed by ^{1}H-NMR. The reaction of **2** with singlet oxygen is exceedingly slow, and even after 10 h of irradiation, ^{1}H NMR analyses indicate that most of the starting material is still present, in addition to a complex mixture of products (Figure S6a). All attempts to separate the various products by column chromatography led to their decomposition. We were therefore unable to quantify which product may predominate. Two new peaks observed at 9.86 and 9.89 ppm (Figure S6b) are consistent with the formation of 4-methoxybenzaldehyde and 3,5-dimethoxybenzaldehyde, respectively, as the aldehyde peaks were not present in the initial solution prior to photooxidation. The addition of authentic samples of 4-methoxybenzaldehyde and 3,5-dimethoxybenzaldehyde (Sigma-Aldrich) to the product mixture led to an increase of both of these peaks confirming their identity. The formation of these aldehydes is consistent with [2 + 2] addition at the *trans* double bond followed by dioxetane cleavage, very similar to what has been observed as one of the two reaction pathways for *trans*-resveratrol. We used ESI–MS to further characterize the products of the reaction of **2** with singlet oxygen. ESI–MS was conducted in the positive ion mode at a spray voltage of 4 kV and capillary temperature of 250°C. The sample prior to irradiation showed the presence of the starting material at m/z = 271.13349 (calculated molecular weight = 270.32). Upon irradiation (from 2 to 10 h; shorter irradiation times than 2 h did not allow us to detect any products), a peak for the addition of two molecules of O_2 was observed (m/z = 335.11394, calculated molecular weight = 334.32). This peak is consistent with two sequential [4 + 2] cycloaddition reactions leading to the formation of diendoperoxides. A similar pathway of two sequential [4 + 2] cycloaddition reactions has been observed for the photooxidation of 4-propenyl anisole by Greer *et al*. (58). To further support the assignment of the peaks obtained from ESI–MS spectrometry, we performed liquid chromatography–MS (LC–MS) experiments. The LC trace again revealed a complex mixture of products (Figure S7). After the separation of the photooxidation products by LC, products were analyzed by a high-resolution mass spectrometer (Exactive, Thermo Fisher). The retention time of the starting material at m/z = 271.12787 was 5.43 min (Figure S8). We were able to observe a small peak at 4.20 min retention time with an m/z value of 303.1227 (Figure S9). This peak could be the initial

[4 + 2] cycloaddition product. The largest peaks of the photooxidation products had retention times of 3.81 and 4.00 min. Both of these peaks had *m/z* values of 335.10753 (Figure S10). This appears to be consistent with our assignment of this peak in the ESI–MS as the double [4 + 2] cycloaddition adduct, since the second [4 + 2] cycloaddition reaction should result in the formation of a mixture of diastereomers. Finally, a peak at 2.87 min was found to have an *m/z* value of 137.059791 which corresponds to 4-methoxybenzaldehyde. We were unable to identify the second aldehyde cleavage product (3,5-dimethoxybenzaldehyde) by LC–MS.

Figure 3b shows a possible reaction pathway for the sequential [4 + 2] additions of **2** with singlet oxygen. It would be of interest to determine the fraction of chemical reaction (k_r) *vs* k_T for compound **2**, but the fact that the primary singlet oxygen product is more reactive with singlet oxygen than the starting compound precludes the use of competition kinetics with a known singlet oxygen acceptor to determine the value of k_r (59).

CONCLUSIONS

While *trans*-resveratrol and its methylated derivatives do not sensitize the production of singlet oxygen, they are moderately strong singlet oxygen quenchers. Methylation of the hydroxy groups of *trans*-resveratrol leads to a decrease in the singlet oxygen scavenging ability of this class of antioxidants. The rate constants of singlet oxygen removal for these compounds are one to two orders of magnitude below that of α-tocopherol (36) but are one to two orders of magnitude higher than the rate constants for the reaction of singlet oxygen with unsaturated fatty acids (35). Thus these compounds may possess some modest photoprotective ability as far as lipid peroxidation by singlet oxygen is concerned. However, the chemical reaction of singlet oxygen with resveratrol and its methylated derivative leads to the formation of aldehydes as well as endoperoxides. In the case of the methylated resveratrol derivative **2**, a diendoperoxide is formed upon two sequential [4 + 2] cycloaddition reactions with singlet oxygen. Such reactions may well limit the utility of these compounds to function as photoprotective agents against singlet oxygen.

Acknowledgements—We thank Narek Darabedian and Prof. Feimeng Zhou (California State University, Los Angeles) for assistance with the LC-MS experiments. B.T.-A. acknowledges support from the NIH-NIGMS MARC program No. T34 GM08228. Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institute of Health under Award Number R25GM061331. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Health. M.S. acknowledges partial support from the NSF-CREST program (No. DRM-1547723) for upgrading and maintaining the singlet oxygen luminescence quenching set up.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article:

Figure S1. Quenching of singlet oxygen by *trans*-resveratrol (**1**, 5-[(*E*)-2-(4-hydroxyphenyl)-ethenyl]benzene-1,3-diol) in 1:1 CD_3CN-CD_3OD, photosensitizer = Rose Bengal.

Figure S2. Quenching of singlet oxygen by 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)-ethenyl]benzene (**2**) in 1:1 CD_3CN-CD_3OD, photosensitizer = Rose Bengal.

Figure S3. Quenching of singlet oxygen by pterosilbene (**3**, 4-((E)-2-(3,5-dimethoxyphenyl)-ethenyl)phenol), in 1:1 CD_3CN-CD_3OD, photosensitizer = Rose Bengal.

Figure S4. Quenching of singlet oxygen by 5-[(E)-2-(4-methoxyphenyl)ethenyl]benzene-1,3-diol (**4**) in 1:1 CD_3CN-CD_3OD, photosensitizer = Rose Bengal.

Figure S5. Quenching of singlet oxygen by (+)-dihydromyricetin (**5**, (2*R*,3*R*)-3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-2,3-dihydrochromen-4-one) in 1:1 CD_3CN-CD_3OD, photosensitizer = Rose Bengal.

Figure S6. (a) Aromatic region of 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)-ethenyl]benzene (**2**), initial reaction mixture and after 10 hours of steady state photooxidation (sensitizer = methylene blue) in CD_3CN. (b) Aldehyde region of 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)-ethenyl]benzene (**2**) after 10 hours of steady state photooxidation (sensitizer = methylene blue) in CD_3CN.

Figure S7. LC of photooxidation product mixture of the reaction of singlet oxygen with 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)-ethenyl]benzene (**2**), irradiation time 10 hours.

Figure S8. LC-MS of 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)-ethenyl]benzene (**2**), retention time 5.43 min.

Figure S9. LC-MS of [4+2] photooxidation product of the reaction of singlet oxygen with 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)-ethenyl]benzene (**2**), retention time 4.20 min.

Figure S10. LC-MS of double [4+2] photooxidation product (diendoperoxide) of the reaction of singlet oxygen with 1,3-dimethoxy-5-[(E)-2-(4-methoxyphenyl)-ethenyl]benzene (**2**), retention time 4.00 min.

REFERENCES

1. Klotz, L., K. Kröncke and H. Sies (2003) Singlet oxygen-induced signaling effects in mammalian cells. *Photochem. Photobiol. Sci.* **2**, 88–94.
2. Kiryu, C., M. Makiuchi, J. Miyazaki, T. Fujinaga and K. Kakinuma (1999) Physiological production of singlet molecular oxygen in the myeloperoxidase-H_2O_2-chloride system. *FEBS Lett.* **443**, 154–158.
3. Steinbeck, M. J., A. U. Khan and M. Karnovsky (1992) Intracellular singlet oxygen generation by phagocytosing neutrophils in response to particles coated with a chemical trap. *J. Biol. Chem.* **267**, 13425–13433.
4. Schweitzer, C. and R. Schmidt (2003) Physical mechanisms of generation and deactivation of singlet oxygen. *Chem. Rev.* **103**, 1685–1758.
5. Di Mascio, P., G. R. Martinez, S. Miyamoto, G. E. Ronsein, M. H. Medeiros and J. Cadet (2019) Singlet molecular oxygen reactions with nucleic acids, lipids, and proteins. *Chem. Rev.* **119**, 2043–2086.
6. Cadet, J., T. Douki and J. Ravanat (2008) Oxidatively generated damage to the guanine moiety of DNA: Mechanistic aspects and formation in cells. *Acc. Chem. Res.* **41**, 1075–1083.
7. Prat, F., C. Hou and C. S. Foote (1997) Determination of the quenching rate constants of singlet oxygen by derivatized nucleosides in nonaqueous solution. *J. Am. Chem. Soc.* **119**, 5051–5052.
8. Frankel, E. N. (1984) Chemistry of free radical and singlet oxidation of lipids. *Prog. Lipid Res.* **23**, 197–221.
9. Matheson, I., R. D. Etheridge, N. R. Kratowich and J. Lee (1975) The quenching of singlet oxygen by amino acids and proteins. *Photochem. Photobiol.* **21**, 165–171.

10. Pospíšil, P., A. Prasad and M. Rác (2019) Mechanism of the formation of electronically excited species by oxidative metabolic processes: Role of reactive oxygen species. *Biomolecules* **9**, 258.
11. Hatz, S., J. D. Lambert and P. R. Ogilby (2007) Measuring the lifetime of singlet oxygen in a single cell: Addressing the issue of cell viability. *Photochem. Photobiol. Sci.* **6**, 1106–1116.
12. Kuimova, M. K., G. Yahioglu and P. R. Ogilby (2009) Singlet oxygen in a cell: Spatially dependent lifetimes and quenching rate constants. *J. Am. Chem. Soc.* **131**, 332–340.
13. Yin, X., J. Yu, Q. Kong and X. Ren (2017) Mechanism of isomers and analogues of resveratrol dimers selectively quenching singlet oxygen by UHPLC-ESI-MS^2. *Food Chem.* **237**, 1101–1111.
14. Li, C., X. Xu, Z. Tao, X. J. Wang and Y. Pan (2015) Resveratrol dimers, nutritional components in grape wine, are selective ROS scavengers and weak Nrf2 activators. *Food Chem.* **173**, 218–223.
15. Kong, Q., X. Ren, J. Qi, J. Yu, J. Lu and S. Wang (2019) Carbon-carbon double bond and resorcinol in resveratrol and its analogues: What is the characteristic structure in quenching singlet oxygen? *Biomolecules* **9**, 268.
16. Lu, W. and J. Liu (2016) Capturing transient endoperoxide in the singlet oxygen oxidation of guanine. *Chem. A Eur. J.* **22**, 3127–3138.
17. Celaje, J. A., D. Zhang, A. M. Guerrero and M. Selke (2011) Chemistry of *trans*-resveratrol with singlet oxygen: [2 + 2] addition, [4 + 2] addition, and formation of the phytoalexin moracin M. *Org. Lett.* **13**, 4846–4849.
18. Zhao, Y., M. Shi, J. Ye, X. Zheng, J. Lu and Y. Liang (2015) Photo-induced chemical reaction of *trans*-resveratrol. *Food Chem.* **171**, 137–143.
19. Fotiou, S., D. Fotiou, A. Alamanou and G. Deliconstantinos (2010) Resveratrol activation of nitric oxide synthase in rabbit brain Synaptosomes: Singlet oxygen ($^1O_2$) formation as a causative factor of neurotoxicity. *In Vivo* **24**, 49–54.
20. Xia, N., A. Daiber, U. Förstermann and H. Li (2017) Antioxidant effects of resveratrol in the cardiovascular system. *Br. J. Pharmacol.* **174**, 1633–1646.
21. Stivala, L. A., M. Savio, F. Carafoli, P. Perucca, L. Bianchi, G. Maga, L. Forti, U. M. Pagnoni, A. Albini, E. Prosperi and V. Vannini (2001) Specific structural determinants are responsible for the antioxidant activity and the cell cycle effects of resveratrol*. *J. Biol. Chem.* **276**, 22586–22594.
22. Hung, L., J. Chen, S. Huang, R. Lee and M. Su (2000) Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc. Res.* **47**, 549–555.
23. Ndiaye, M., C. Philippe, H. Mukhtar and N. Ahmad (2011) The grape antioxidant resveratrol for skin disorders: Promise, prospects, and challenges. *Arch. Biochem. Biophys.* **508**, 164–170.
24. Baxter, R. A. (2008) Anti-aging properties of resveratrol: Review and report of a potent new antioxidant skin care formulation. *J. Cosmet. Dermatol.* **7**, 2–7.
25. Cai, W., L. Zhang, Y. Song, B. Zhang, X. Cui, G. Hu and J. Fang (2011) 3, 4, 4′-trihydroxy-*trans*-stilbene, an analogue of resveratrol, is a potent antioxidant and cytotoxic agent. *Free Radic. Res.* **45**, 1379–1387.
26. Jeong, G. H., E. K. Park and T. H. Kim (2019) Anti-diabetic effects of *trans*-resveratrol byproducts induced by plasma treatment. *Food Res. Int.* **119**, 119–125.
27. Fang, Z., L. Hu, Y. Gao, J. Li and L. Liang (2017) Protection of resveratrol against the photodecomposition of folic acid and photodecomposition-induced structural change of beta-lactoglobulin. *Food Res. Int.* **102**, 435–444.
28. Wang, H., R. Liang, L. Fu, R. Han, J. Zhang and L. H. Skibsted (2014) Nutritional aspects of β-carotene and resveratrol antioxidant synergism in giant unilamellar vesicles. *Food Funct.* **5**, 1573–1578.
29. Kozarski, M., A. Klaus, D. Jakovljevic, N. Todorovic, J. Vunduk, P. Petrović, M. Niksic, M. M. Vrvic and L. Van Griensven (2015) Antioxidants of edible mushrooms. *Molecules* **20**, 19489–19525.
30. Kim, H. J., D. Montenegro, J. Zhao and J. R. Sparrow (2021) Bisretinoids of the retina: Photo-oxidation, iron-catalyzed oxidation, and disease consequences. *Antioxidants* **10**, 1382.
31. Joshi, D., J. Field, J. Murphy, M. Abdelrahim, H. Schönherr, J. R. Sparrow, G. Ellestad, K. Nakanishi and A. Zask (2013) Synthesis of antioxidants for prevention of age-related macular degeneration. *J. Nat. Prod.* **76**, 450–454.
32. Sheu, S., N. Liu and J. Chen (2010) Resveratrol protects human retinal pigment epithelial cells from acrolein-induced damage. *J. Ocul. Pharmacol. Ther.* **26**, 231–236.
33. Sparrow, J. R., H. R. Vollmer-Snarr, J. Zhou, Y. P. Jang, S. Jockusch, Y. Itagaki and K. Nakanishi (2003) A2E-epoxides damage DNA in retinal pigment epithelial cells: Vitamin E and other antioxidants inhibit A2E-epoxide formation. *J. Biol. Chem.* **278**, 18207–18213.
34. Lagunes, I. and Á. Trigos (2015) Photo-oxidation of ergosterol: Indirect detection of antioxidants photosensitizers or quenchers of singlet oxygen. *J. Photochem. Photobiol. B* **145**, 30–34.
35. Vever-Bizet, C., M. Dellinger, D. Brault, M. Rougee and R. V. Bensasson (1989) Singlet molecular oxygen quenching by saturated and unsaturated fatty-acids and by cholesterol. *Photochem. Photobiol.* **50**, 321–325.
36. Foote, C. S., T. Y. Ching and G. G. Geller (1974) Chemistry of singlet oxygen—XVIII. Rates of reaction and quenching of α-tocopherol and singlet oxygen. *Photochem. Photobiol.* **20**, 511–513.
37. Ellis, E. M. (2007) Reactive carbonyls and oxidative stress: Potential for therapeutic intervention. *Pharmacol. Ther.* **115**, 13–24.
38. Li, D., M. Ferrari and E. M. Ellis (2012) Human aldo–keto reductase AKR7A2 protects against the cytotoxicity and mutagenicity of reactive aldehydes and lowers intracellular reactive oxygen species in hamster V79-4 cells. *Chem. Biol. Interact.* **195**, 25–34.
39. Amin, R. P. and G. Witz (2001) DNA-protein crosslink and DNA Strand break formation in HL-60 cells treated with trans,trans-Muconaldehyde, hydroquinone and their mixtures. *Int. J. Toxicol.* **20**, 69–80.
40. Brambilla, G., L. Sciabà, P. Faggin, A. Maura, U. M. Marinari, M. Ferro and H. Esterbauer (1986) Cytotoxicity, DNA fragmentation and sister-chromatid exchange in Chinese hamster ovary cells exposed to the lipid peroxidation product 4-hydroxynonenal and homologous aldehydes. *Mut. Res. Gen. Toxicol.* **171**, 169–176.
41. Song, T., B. Zhou, G. Peng, Q. Zhang, L. Wu, Q. Liu and Y. Wang (2014) Aerobic oxidative coupling of resveratrol and its analogues by visible light using mesoporous graphitic carbon nitride (mpg-C_3N_4) as a bioinspired catalyst. *Chem. A Eur. J.* **20**, 678–682.
42. Rodríguez, R. Á., I. R. Lahoz, O. N. Faza, M. M. Cid and C. S. Lopez (2012) Theoretical and experimental exploration of the photochemistry of resveratrol: Beyond the simple double bond isomerization. *Org. Biomol. Chem.* **10**, 9175–9182.
43. Storniolo, C. E. and J. J. Moreno (2019) Resveratrol analogs with antioxidant activity inhibit intestinal epithelial cancer Caco-2 cell growth by modulating arachidonic acid Cascade. *J. Agric. Food Chem.* **67**, 819–828.
44. Li, J., R. Deng, X. Hua, L. Zhang, F. Lu, T. G. Coursey, S. C. Pflugfelder and D. Li (2016) Blueberry component pterostilbene protects corneal epithelial cells from inflammation via anti-oxidative pathway. *Sci. Rep.* **6**, 19408.
45. Rimando, A. M., M. Cuendet, C. Desmarchelier, R. G. Mehta, J. M. Pezzuto and S. O. Duke (2002) Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. *J. Agric. Food Chem.* **50**, 3453–3457.
46. Remsberg, C. M., J. A. Yáñez, Y. Ohgami, K. Vega-Villa, A. M. Rimando and N. M. Davies (2008) Pharmacometrics of pterostilbene: Preclinical pharmacokinetics and metabolism, anticancer, anti-inflammatory, antioxidant and analgesic activity. *Phytother. Res.* **22**, 169–179.
47. Zheng, L., Q. Wei, Y. Cai, J. Fang, B. Zhou, L. Yang and Z. Liu (2006) DNA damage induced by resveratrol and its synthetic analogues in the presence of cu (II) ions: Mechanism and structure-activity relationship. *Free Rad. Biol. Med.* **41**, 1807–1816.
48. Hsieh, T., C. Wong, D. John Bennett and J. M. Wu (2011) Regulation of p53 and cell proliferation by resveratrol and its derivatives in breast cancer cells: An in silico and biochemical approach targeting integrin αvβ3. *Int. J. Cancer* **129**, 2732–2743.
49. Mikstacka, R., A. M. Rimando, K. Szalaty, K. Stasik and W. Baer-Dubowska (2006) Effect of natural analogues of *trans*-resveratrol on cytochromes P4501A2 and 2E1 catalytic activities. *Xenobiotica* **36**, 269–285.
50. Mikstacka, R., Z. Dutkiewicz, S. Sobiak and W. Baer-Dubowska (2012) The inhibitory effect of natural stilbenes and their analogues on catalytic activity of cytochromes P450 family 1 in comparison with other phenols-structure and activity relationship. In

Phytochemicals-A Global Perspective of their Role in Nutrition and Health (Edited by V. Rao), pp. 519–538. IntechOpen, London, UK.
51. Fu-Yan, L., C. Shu-Mei and Z. Hong-Bing (2021) Investigation of in vitro antioxidant activity of dihydromyricetin and flavonoids rich extract from vine tea (*Ampelopsis grossedentata*). *Trad. Med. Res.* **6**, 7.
52. Teng, J., X. Liu, X. Hu, Y. Zhao, N. Tao and M. Wang (2018) Dihydromyricetin as a functional additive to enhance antioxidant capacity and inhibit the formation of thermally induced food toxicants in a cookie model. *Molecules* **23**, 2184.
53. Xin, M., Y. Ma, W. Lin, K. Xu and M. Chen (2015) Use of dihydromyricetin as antioxidant for polypropylene stabilization. *J. Therm. Anal. Calorim.* **120**, 1741–1747.
54. Guo, Z., X. Chen, Z. Huang, D. Chen, B. Yu, H. Chen, J. Yu, H. Yan, P. Zheng and Y. Luo (2021) Dietary dihydromyricetin supplementation enhances antioxidant capacity and improves lipid metabolism in finishing pigs. *Food Funct.* **12**, 6925–6935.
55. Arevalo, G. E., D. A. Cagan, C. G. Monsour, A. C. Garcia, A. McCurdy and M. Selke (2020) A photoprotective effect by cation-π-interaction? Quenching of singlet oxygen by an indole cation-π model system. *Photochem. Photobiol.* **96**, 1200–1207.
56. Erickson, P. R., N. Walpen, J. J. Guerard, S. N. Eustis, J. S. Arey and K. McNeill (2015) Controlling factors in the rates of oxidation of anilines and phenols by triplet methylene blue in aqueous solution. *J. Phys. Chem. A* **119**, 3233–3243.
57. Jiang, J. and A. Kamal-Eldin (1998) Comparing methylene blue-photosensitized oxidation of methyl-conjugated linoleate and methyl linoleate. *J. Agric. Food Chem.* **46**, 923–927.
58. Greer, A., G. Vassilikogiannakis, K. Lee, T. S. Koffas, K. Nahm and C. S. Foote (2000) Reaction of singlet oxygen with trans-4-propenylanisole. Formation of [2+2] products with added acid. *J. Org. Chem.* **65**, 6876–6878.
59. Higgins, R., C. S. Foote and H. Cheng (1968) Chemistry of singlet oxygen: V. reactivity and kinetic characterization. *Adv. Chem. Ser.* **77**, 102–117.