Heteroatom Chemistry
Volume 18, Number 5, 2007

Ortho-Thioquinones and Mediterranean Diet: The Sulfur Connection

Stefano Menichetti, Chiara Pagliuca, and Caterina Viglianisi

Dipartimento di Chimica Organica "Ugo Schiff" e Laboratorio di Progettazione Sintesi e Studio di Eterocicli Bioattivi (HeteroBioLab), Polo Scientifico e Tecnologico Università di Firenze, Via della Lastruccia 13, I-50019 Sesto Fiorentino, Italy

Received 30 October 2006; revised 13 March 2007

ABSTRACT: *The antioxidant activity of several hydroxy-4-thiaflavanes was determined either by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) bleaching method or by measuring their ability to inhibit the autooxidation of styrene or cumene. Based on this, the role played by the number and position of hydroxy groups and by the oxidation state of sulfur was rationalized and quantified, providing good indications for optimizing the structural features required to make these "double-faced" antioxidants perform at their best. The ability of selected 4-thiaflavanes to protect DNA by oxidative damage in vitro is also discussed.* © 2007 Wiley Periodicals, Inc. Heteroatom Chem 18:489–499, 2007; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20338

INTRODUCTION

Formation of free radicals and other reactive oxygen species (ROS) in the human body is an unavoidable consequence of metabolism [1]. Nowadays it is widely recognized that oxygen-centered free radicals are involved in crucial biochemical transformations [2]. However, it is clear that an anomalous high concentration of ROS is strictly related to tissues, oxidative stress, origin and consequence of large part of the more dangerous diseases, and ageing itself [3]. Evolution provided endogenous and exogenous defenses for keeping the ROS concentration under control. For example, the expression of specific enzymes able to avoid the formation of free radicals (i.e., superoxide dismutase, catalase, and several oxygenases) is one of the endogenous answers to this problem [1]. On the other hand, the intake of small molecules able to break the chain of radical reactions that lead to oxidative damage is the more simple and efficient exogenous solution [1]. A diet rich in antioxidants can play a crucial role in health, most of all in those countries where the factors responsible for dangerously increasing the concentration of ROS (stress, smoke, overalimentation, alcohol and drug abuse, and pollution) are frequent components of the style of living. The most important small molecules with an antioxidant action that are part of the diet are vitamins: ascorbic acid (vitamin C), β-carotenes, and vitamin A; tocopherols (vitamin E); and polyphenolic flavonoids (called vitamin P). These latter compounds, possessing the 2-phenylchromane skeleton (Fig. 1), are almost ubiquitous in vascular plants where they provide color to flowers and leaves and provide protection against insects and microorganisms as well as UV irradiation [4].

Flavonoids are commonly present in our diet (edible plants), with their daily intake ranging from few to hundreds of milligrams. The consumption of these polyphenolic species (such as catechin, Fig. 1) is generally considered healthy [5]. A high intake of

Correspondence to: Stefano Menichetti; e-mail: stefano.menichetti@unifi.it.
Contract grant sponsor: MIUR "Stereoselezione in Sintesi Organica. Metodologie ed Applicazioni.
Contract grant number: 2005035330.

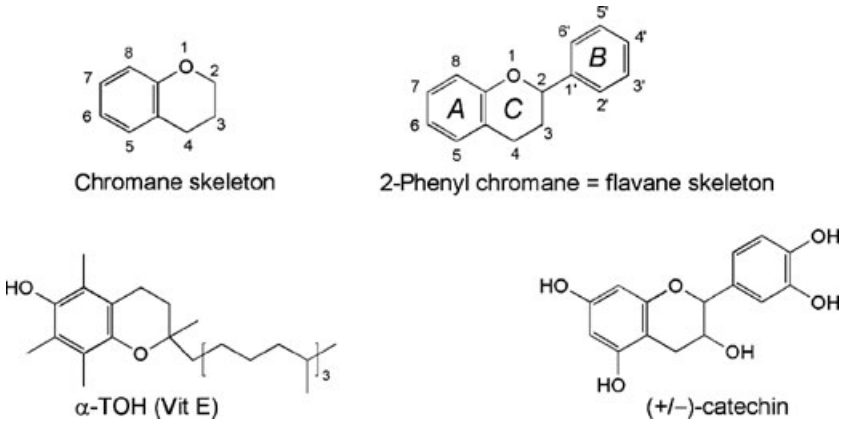


FIGURE 1 Representative antioxidants with a chromane or a flavane skeleton.

flavonoids, usually from red wine, is considered responsible for the unexpected low incidence of cardiovascular diseases, stroke, and several types of cancer in those populations that are considered at high risk for these diseases because of the consumption of animal fats, the so-called "French paradox," [6]. Despite the lack of a definitive evidence for their actual action in vivo, the powerful in vitro antioxidant ability of flavonoids [7] is indicated as the key factor responsible for the beneficial effects in response to a robust intake of these polyphenols [8].

On the other hand, α-tocopherol (α-TOH, Fig. 1), the main component of vitamin E (Vit E hereafter), is known as the best natural chain-breaking lipophilic antioxidant and the foremost factor responsible for providing protection against low-density lipoproteins' (LDL) oxidation in the human body [9].

A tremendous effort is underway for investigating the properties of naturally occurring antioxidants because of their potential clinical relevance.

Much less work has been done to develop synthetic antioxidants designed to optimize the antioxidant activity while satisfying other important criteria such as solubility, bioavailability, and lack of toxicity [10].

RESULTS

Our contribution in this field began while developing a new strategy for the preparation of synthetic benzoxathiin cycloadducts **1**. When properly substituted, it was possible to predict for these compounds [11] the ability to react with radical species miming the behavior of either those flavonoids having a catechol moiety on the B ring, like catechin, and/or the tocopherols, that is, the two most important families of natural antioxidants (Fig. 2). In other words, they possess what we call a "double-faced" antioxidant activity.

Synthesis of these compounds is based on the inverse electron demand hetero Diels–Alder reaction of *ortho*-thioquinones with styrenes. This original methodology foresees the initial reaction of phthalimidesulfenyl chloride (PhtNSCl, Pht = Phthaloyl) with a properly substituted phenol, affording, regiospecifically, an *ortho*-hydroxy-*N*-thiophthalimide that, in the presence of a tertiary amine, usually Et_3N, gives the best results, generates a transient *ortho*-thioquinone trapped in solution as electron-poor diene with a plethora of electron-rich alkenes. Using styrenes as dienophiles, the reaction gives rise to benzoxathiin cycloadducts possessing the 4-thiaflavane skeleton, as reported in Scheme 1 [12].

The reaction is general and allows the synthesis of hydroxyl and methoxy-substituted 4-thiaflavanes **1a–e** tested in this study and reported in Fig. 3. The structural modifications were inspired either by the common substitution patterns typical of the corresponding natural products or by the attempt of understanding the role played by the sulfur atom in the catechin-like and the tocopherol-like antiradical performance. For this reason, 4-thiaflavane-*S*-oxide **3b** and *S*,*S*-dioxides **2a–d** were also prepared by oxidation of the corresponding sulfides with *m*-CPBA [12,13] (Fig. 3). Whether further OH groups were required on either the thioquinone or the styrene moieties, they were protected as *t*-butyldimethylsilyl ethers that revealed themselves to be the most advantageous protecting group in every step of the synthetic sequence. Deprotection with tetra-*n*-butylammonium fluoride (TBAF) hydrate in tetrahydrofuran (THF) was, in any case, the final step of the procedure.

The antiradical and antioxidant activity of compounds **1c–e** was evaluated by using two methods.

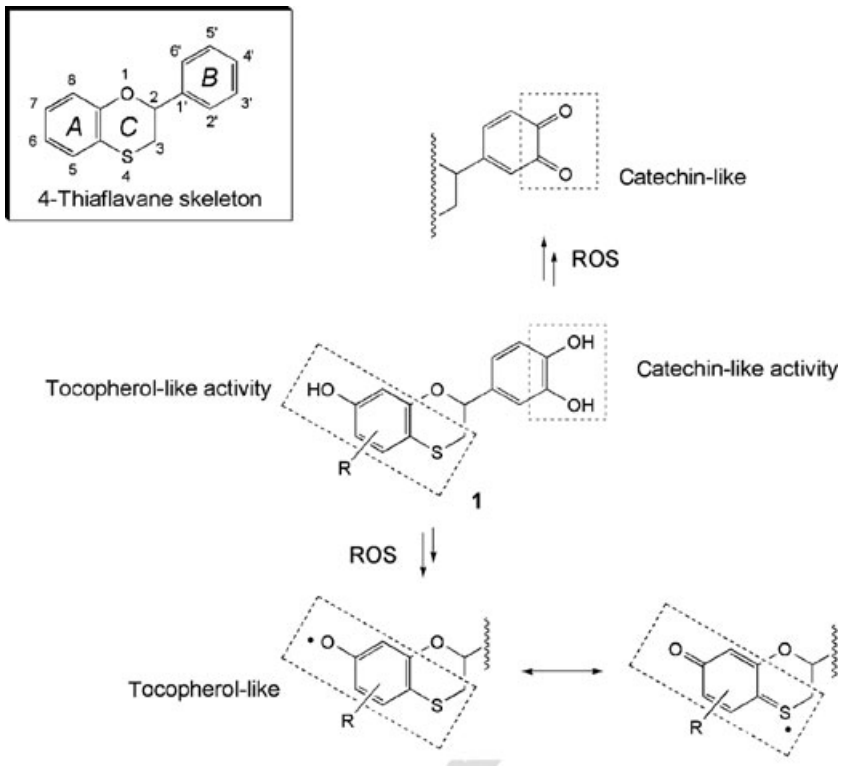
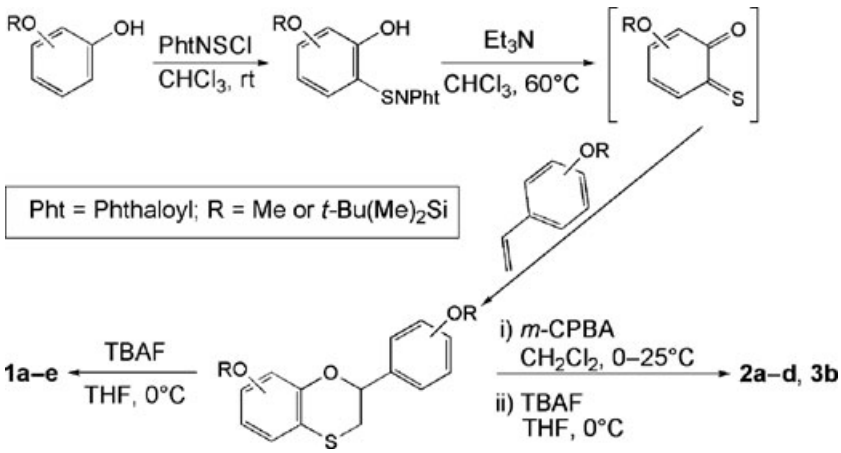


FIGURE 2 4-Thiaflavane skeleton and "double-faced" antioxidant activity of properly hydroxyl-substituted 4-thiaflavanes.

Initially, the ability of thiaflavanes in quenching the purple color of the commercially available 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was expressed as SC_{50}, that is, the micromolar concentration of the sample required to decrease 50% of the absorbance at 515 nm of a 100-μM solution of DPPH in methanol [13]. The double-faced ability of our compounds was verified by comparing their SC_{50} with the corresponding values measured using trolox, a polar Vit E equivalent, and catechin as structural models, certainly operating through the tocopherol-like or the catechin-like mechanism (Fig. 4).

The rate constants of the above-mentioned derivatives for the reaction with peroxyl radicals, k_{inh} (Eq. (5)), were determined by studying the inhibition of the thermally initiated autooxidation of either styrene or cumene [14]. Cumene was employed



SCHEME 1 Synthesis of 4-thiaflavanes tested in this study.

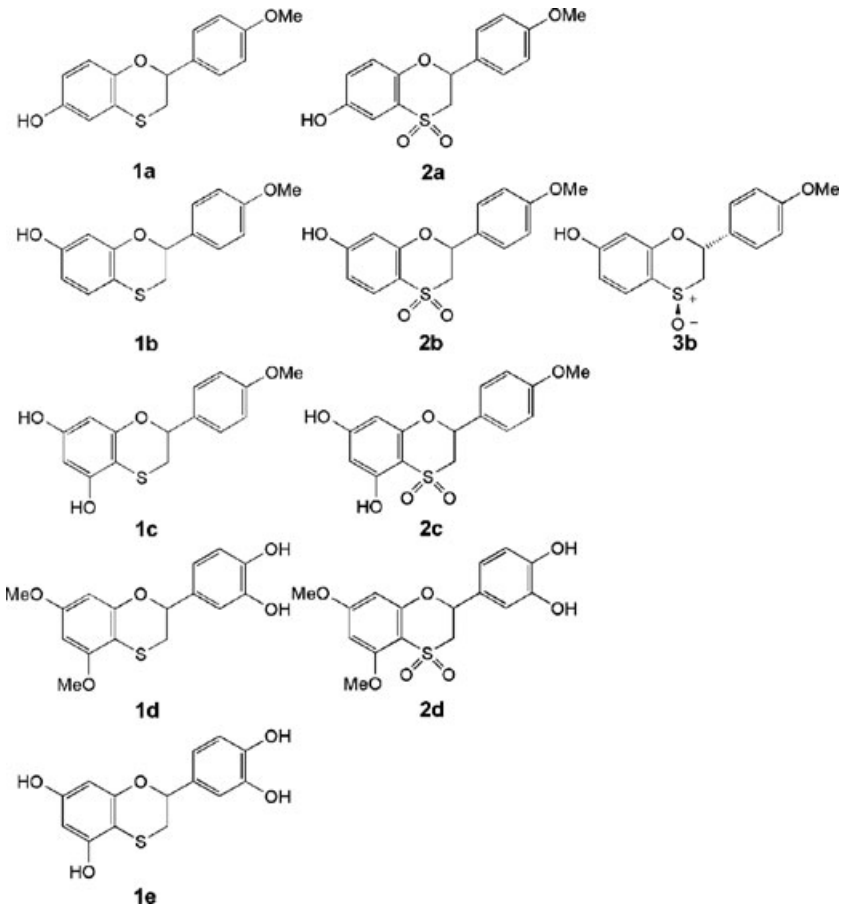


FIGURE 3 Structures of 4-thiaflavanes **1**, **2**, and **3** tested in this study.

because its lower oxidizability magnifies the antioxidant behavior of a given compound, allowing to differentiate more easily the antioxidant activity of moderately effective inhibitors.

$$\text{Initiator} \xrightarrow{R_i} \text{R}^\bullet \quad (1)$$

$$\text{R}\bullet + \text{O}_2 \longrightarrow \text{ROO}^\bullet \quad (2)$$

$$\text{ROO}^\bullet + \text{RH} \xrightarrow{k_p} \text{ROOH} + \text{R}^\bullet \quad (3)$$

$$\text{ROO}^\bullet + \text{ROO}^\bullet \xrightarrow{2k_t} \text{Products} \quad (4)$$

$$\text{ROO}^\bullet + \text{ArOH} \xrightarrow{k_{inh}} \text{ROOH} + \text{ArO}^\bullet \quad (5)$$

$$\text{ROO}^\bullet + \text{ArO}^\bullet \longrightarrow \text{Products} \quad (6)$$

The reaction was followed by monitoring the oxygen consumption during the autooxidation with an automatic recording gas absorption apparatus [15], which uses a commercial differential pressure transducer as detector. The reactions, initiated by the thermal decomposition of AMVN 2,2′-azobis-(2,4-dimethyl)-valeronitrile, were carried out at 30°C under controlled conditions in air-saturated solution

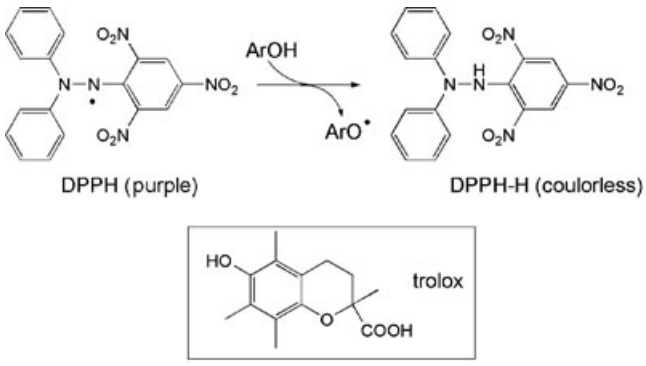


FIGURE 4 Quenching mode of purple color of DPPH and structure of trolox.

of either styrene or cumene in the presence of each antioxidant. α-Tocopherol (α-TOH) was used as a reference chain-breaking inhibitor.

The inhibition rate constants, k_{inh}, were determined by means of a kinetic treatment consisting of the measurement of the initial rates of oxidation of the substrate both in the presence ($-\text{d}[\text{O}_2]/\text{d}t = R_{ox}$)

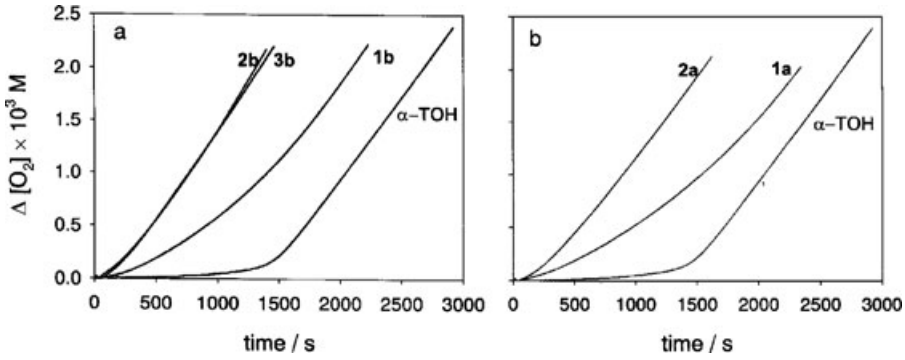


FIGURE 5 Oxygen consumption traces observed at 30°C during the AMVN (5×10^{-3} M) initiated autooxidation of styrene (4.3 M) in chlorobenzene in the presence of α-tocopherol (5.0×10^{-6} M) and thiaflavanes (5.0×10^{-6} M) containing (a) the sulfur atom and (b) the oxygen atom in para position with respect to phenolic OH of A ring.

and in the absence $[(-d[O_2]/dt)_0 = R_{ox,0}]$ of a known amount of antioxidant, ArOH, and calculating k_{inh} from these data by means of Eq. (7) [13].

$$R_{ox,0}/R_{ox} - R_{ox}/R_{ox,0} = nk_{inh}[AH]_0(2k_iR_i)^{1/2} \quad (7)$$

This equation allows the determination of k_{inh} even when the inhibition and termination (Eq. (4)) rates are comparable. The use of Eq. (7) requires knowledge of the initiation rate, R_i, which was determined in preliminary experiments [13], and the termination constant, $2k_t$, for the self-combination of peroxyl radicals, reported in literature as 4.2×10^7 M^{-1} s^{-1} for styrylperoxyl [12] and 4.6×10^4 M^{-1} s^{-1} for cumylperoxyl radicals [16].

The term n represents the stoichiometric coefficient, that is, the number of peroxyl radicals trapped by each antioxidant molecule and can be determined from Eq. (8) by measuring the length of the induction period (τ) during which the rate of the oxygen consumption is strongly reduced. For classical chain-breaking antioxidants, that is, acting by reactions (5) and (6), $n = 2$ is expected.

$$n = R_i\tau/[AH] \quad (8)$$

The experimental traces of oxygen consumption recorded during the oxidation of styrene, reported in Figs. 5 and 6, show that only antioxidants containing the catechol ring (**1d**, **2d**, **1e**) give clearly distinct inhibition periods.

When using the less easily oxidizable cumene (Fig. 7), the inhibition period is more clearly visible even with less reactive phenols, thus allowing the measurement of the n value also for **1a**, **1b**, **2a**, and **1c**. In the case of the thiaflavane **2c**, an n value of 2 was assumed because, even in cumene, no clear induction period was observed.

The experimental values of k_{inh} and the stoichiometric factor, n, measured for derivatives **1a–e**, **2a–d**, and **3b**, as well as the corresponding SC_{50}, are reported in Table 1 together with the data for cathecol, catechin, and trolox, which were used as reference compounds.

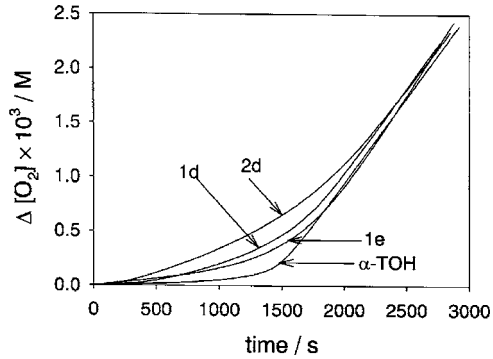


FIGURE 6 Oxygen consumption observed at 30°C during the AMVN (5×10^{-3} M) initiated autooxidation of styrene (4.3 M) in chlorobenzene in the presence of thiaflavanes containing the catechol ring **1d**,**1e**, and **2d** and α-tocopherol (5.0×10^{-6} M).

The more active compounds of this set, namely, derivatives **1c–e**, were also tested to verify their ability to protect against DNA damage induced by both cumene hydroperoxide (CumOOH), a direct generating radical species [18,19], and ferric ions/glutathione (Fe^{3+}/GSH), a system able to initiate the Fenton reaction that causes the formation of highly reactive ROS, including the hydroxyl radical $OH^{\bullet}$.

Data are again reported in association with those obtained using α-TOH and racemic catechin as suitable models to verify whether any activity observed for 4-thiaflavanes on the protection of DNA oxidation could be rationalized considering their double-faced character.

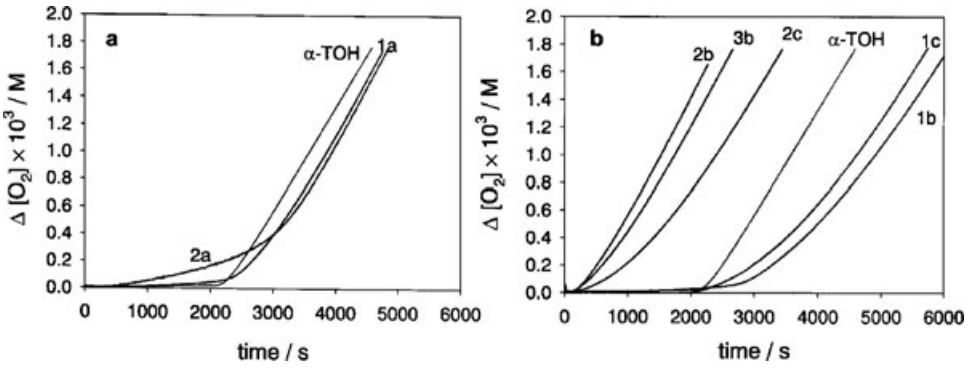


FIGURE 7 Oxygen consumption observed at 30°C during the AMVN (5×10^{-3} M) initiated autooxidation of pure cumene (7.1 M) in the presence of investigated thiaflavanes (5.0×10^{-6} M) containing (a) the oxygen atom and (b) the sulfur atom in para position with respect to phenolic OH of A ring.

Oxidative DNA damage was quantified measuring 8-hydroxy-2′-deoxyguanosine (8-OHdG; Fig. 8) levels by high-performance liquid chromatography and electrochemical detector [20]. The protective effect was measured as the 8-OHdG level in the solution of herring sperm DNA (0.5 mg/mL) incubated for 2 h at 37°C with 20 μM solutions of derivatives **1c–e**, catechin, and Vit E, before adding the oxidative stressor.

In the first set of experiments, we induced oxidative DNA damage with 5 mM CumOOH and found that the 8-OHdG level increased about 2.8-fold compared to that in the control (Fig. 9). In the presence of compounds **1c**, DNA oxidation was not significantly reduced. On the contrary, compounds **1d** and **1e** exerted a similar protective effect, with a reduction of about 30% in CumOOH-induced oxidative DNA damage. Similarly, catechin provided a protection of about 32% while the highest protective effect was obtained in the presence of α-TOH, 61%, (Fig. 9).

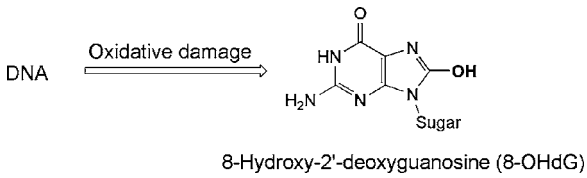


FIGURE 8 Structure of 8-OHdG, the oxidative DNA marker measured to evaluate oxidative damage.

In the second set of experiments, DNA was incubated, under the above-reported conditions, with 3 μM $FeCl_3$ and 15 mM GSH. Significantly, in this case the 8-OHdG level increased to about 20-fold compared to that in the control (Fig. 10).

Hydroxylated 4-thiaflavanes **1c–e** were tested and exerted a certain protective effect on oxidative DNA damage induced by Fe^{3+}/GSH. Although catechin and Vit E reduced the formation of 8-OHdG by 36% and 33%, respectively, the maximum protection was obtained in the presence of compound **1e**, able to reduce of about 82% in the 8-OHdG level (Fig. 10).

TABLE 1 Antioxidant and Antiradical Parameters for Derivatives **1**, **2**, and **3b**

Entry	*Compound*	$k_{inh}(M^{-1}s^{-1})^a$	n^a	SC_{50} $(\mu M)^b$
1	**1a**	$(1.7 \pm 0.3) \times 10^5$	2.2[c]	23
2	**2a**	$(1.3 \pm 0.2) \times 10^4$[c]	2.4[c]	210
3	**1b**	$(1.2 \pm 0.2) \times 10^5$	2.9[c]	18
4	**2b**	$<10^3$	nd	>300
5	**3b**	$<10^3$	nd	>300
6	**1c**	$(3.9 \pm 0.8) \times 10^5$	2.8[c]	12
7	**2c**	$(2.3 \pm 0.5) \times 10^3$[c]	2[e]	>300
8	**1d**	$(5.5 \pm 1.1) \times 10^5$	1.7	16
9	**2d**	$(2.6 \pm 0.5) \times 10^5$	1.8	15
10	**1e**	$(6.8 \pm 1.3) \times 10^5$	2.1	8
11	α-TOH	3.2×10^6[d]	2	–
12	Cathechin	–	–	15
13	Catechol	$(5.3 \pm 0.5) \times 10^5$	1.9	–
14	Trolox	–	–	16

[a]Measured in styrene; mean of three determinations. Error on the determination of *n* is ±0.2.
[b]Antioxidant concentration causing the fading of 50% absorbance of 100 μM DPPH• 20 min after mixing; error ±8% [13].
[c]Obtained in cumene.
[d] After Burton et al. [17].
[e]Assumed (see text).

DISCUSSION

An examination of the kinetic data shows that the presence of a catechol group provides a large contribution to the antioxidant efficacy of these

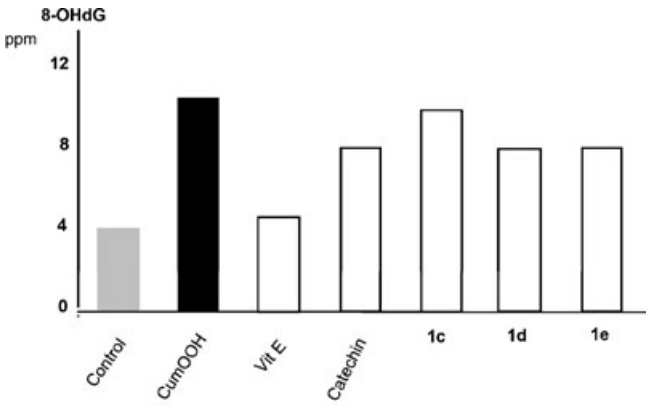


FIGURE 9 Effect of 20-μM solutions of thiaflavanes **1c–e**, α-TOH, and catechin on CumOOH induced formation of 8-OHdG in herring sperm DNA.

compounds independently of the nature of the thiachromane moiety, the presence of hydroxy substituents in this group, and also of the oxidation state of sulfur. Actually, thiaflavanes **1d** (a dimethoxy-substituted A ring derivative), **1e** (a dihydroxy A ring derivative), and **2d** (a dimethoxy-substituted A ring derivative with the sulfone sulfur on C ring), entries 8–10 in Table 1, containing a catechol B ring, have inhibition rate constants ranging from 2.6×10^5 to 6.8×10^5 M^{-1} s^{-1}, that is, the values are very similar to that of catechol itself (5.3×10^5 M^{-1} s^{-1}, entry 13 in Table 1). When taking into account that α-TOH, the best natural chain-breaking antioxidant, has a k_{inh} value of 3.2×10^6 M^{-1} s^{-1} [15] and that 2,6-di-*tert*-butyl-4-methylphenol (BHT) and 2,6-di-*tert*-butyl-4-methoxyphenol (BHA), two of the more common synthetic antioxidants, have k_{inh} values of 1.0×10^4 M^{-1} s^{-1} and 1.1×10^5 M^{-1} s^{-1}, respectively [17], the three compounds **1d**, **1e**, and **2d** can be considered antioxidants characterized by an inhibiting activity ranging from medium to good.

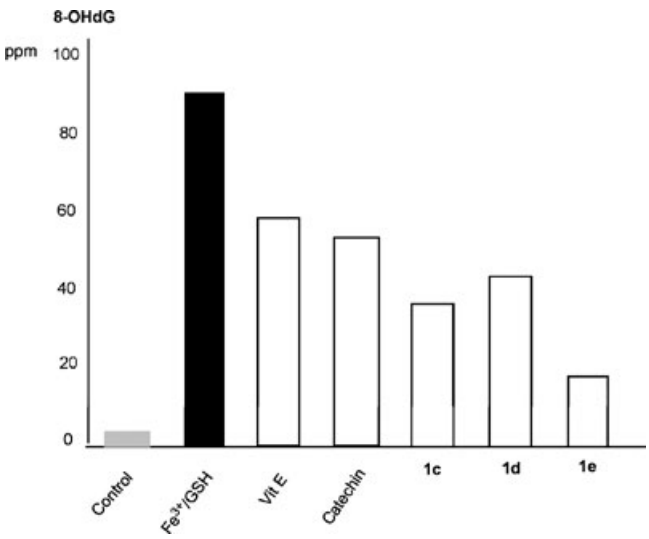


FIGURE 10 Effect of 20-μM solutions of thiaflavanes **1c–e**, α-TOH, and catechin on Fe^{3+}/GSH induced formation of 8-OHdG in herring sperm DNA.

Derivative **1e** is clearly the more active among this set, considering both the SC_{50} and the k_{inh} values; however, while the DPPH quenching suggested an activity double than that of catechin and trolox (entries 10 vs. 12 or 14 in Table 1), the kinetic measurements indicate only a small increase in the overall antioxidant performance with respect to simple catechol (entries 10 vs. 13 in Table 1). This seems to suggest that the DPPH-quenching methodology overestimates the contribution derived from the action of A and C rings, that is, the "tocopherol-like activity.

Hydroxy-thiaflavanes **1a–c**, containing sulfur at its lower oxidation state, represent another set of moderately good antioxidants because their k_{inh} values are in the range from 1.2×10^5 to 3.9×10^5 M^{-1} s^{-1}. Among the mono-hydroxy compounds **1a** and **1b**, the latter one, where the OH group can conjugate with the endocyclic sulfur atom, was slightly less efficient than **1a**, having an oxygen atom in conjugated position. This is in contrast with the DPPH-bleaching method (entries 1 and 3 in Table 1) but in line with previous data on the activity of synthetic thiatocopherol as reported by Ingold and coworkers [21] and with very recent data obtained by us on the antioxidant activity of acyclic thiosubstituted phenols [22]. These data contradict the claim that conjugation with bivalent sulfur should be superior to that with oxygen in stabilizing a phenoxyl radical [23] and the widespread belief that substitution of oxygen with sulfur, or even better, with selenium or tellurium, should be a valuable means for the preparation of more efficient tocopherol-like antioxidants [23,24]. It should, however, be pointed out that the present experiment, that is, thermally initiated oxidations, provides a measure of the chain-breaking activity of antioxidants. Spontaneous oxidations proceeding more slowly might be inhibited efficiently also by sulfur and other chalcogen-containing phenols because of their ability to behave as preventive antioxidants by decomposing hydroperoxides to alcohols. The introduction of an additional OH group in the A ring increases the tocopherol-like antioxidant activity, and derivative **1c** showed a k_{inh} value almost as good as that measured for those compounds possessing a catechol B ring. This increase is likely due to the electron-donating effect introduced by the additional OH group on C5. A dedicated study will be necessary

to understand which of the two OH groups (ortho or para to the endocyclic sulfur atom) is more reactive toward the hydrogen abstraction reaction carried out by the ROO• radicals.

Also, in the case of derivatives **1a–c**, the comparison between k_{inh} and SC_{50} values demonstrates that the DPPH-quenching methodology overvalues the contribution of the tocopherol-like activity, above all when the A ring bears an OH group conjugated with the sulfide sulfur on the C ring. For example, derivative **1b** was found to be more active than **1a**, whereas **1c** was found to possess a SC_{50} that was even lower than that for catechin. An inspection of the significance of parameter n can rationalize these apparently anomalous results (vide infra).

Both methodologies indicated that oxidation of the bivalent sulfur on the C ring to higher oxidation states is critical for the tocopherol-like behavior of thiaflavanes. In fact, sulfones **2a–c** and sulfoxide **3b** did not show significant retarding effect on both styrene and cumene oxidations. This result can be rationalized in consideration of the electron-withdrawing effect [17] exerted by the sulfone or the sulfoxide group that strongly depletes the ability of the phenolic OH to react with peroxyl radicals (Eq. (5)).

Oxidation of sulfides to sulfones, on the other hand, also affects the activity of catechol-containing thiaflavanes although only slightly (see data for **1d** and **2d** entries 8 and 9, respectively, in Table 1). This result indicates that any structural modification, even on positions not directly involved in the interaction with the attacking peroxyl radicals, must be taken into account for fine tuning of the antioxidant activity and that only accurate kinetic measures, such as the k_{inh}, are able to point out such minute differences between the antioxidants.

An analysis of parameter n, that is, the number of peroxyl radicals quenched by each antioxidant molecule, shows that 4-thiaflavanes behave as classical chain-breaking inhibitors. Some exceptions deserve further comment. It appears in Fig. 5b that thiaflavanes with phenolic OH conjugating with the S atom (**1b** and **1c**) show, at the end of strongly inhibited period corresponding to $n = 2$, an oxygen consumption rate slightly lower than expected. This small effect, visible only when using cumene as oxidizable substrate, is likely due to some residual antioxidant activity of the oxidized products. Because the SC_{50} value depends on the actual number of DPPH radicals quenched, the above effect can explain the anomalies reported in the above discussion, such as the overestimation of both the tocopherol-like activity and the contribution of conjugation with the sulfide sulfur.

The examined thiaflavanes can be divided into two groups depending on whether the inhibition rate constants are lower or higher than 10^5 M^{-1} s^{-1}. Correspondingly, the SC_{50} values are much larger than 25 and lower or equal to 25 without any linear dependence on the kinetic rate constants.

To explain this observation, it should be taken into account that the DPPH test involves measuring in alcoholic solution (MeOH) the concentration of antioxidants causing a 50% fading of the absorbance of 100 μM DPPH radical after 20 min. As evidenced by Berset and coworkers [25], with good antioxidants such as ascorbic acid and δ-tocopherol, the reaction quickly reaches a steady state and the SC_{50} value only reflects the stoichiometry of the inhibition, that is, the number of radicals trapped by each molecule of antioxidant. On the contrary, with less efficient antioxidants, the SC_{50} index accounts both for the stoichiometry and the kinetics of inhibition, thus providing an estimate of the reactivity of the tested compound.

In the present case, with highly effective antioxidants able to scavenge two radicals per molecule, the SC_{50} value is expected to be equal to 25 μM, being the initial amount of DPPH 100 μM. Therefore, the SC_{50} values of 23 and 18 μM shown by thiaflavanes **1a** and **1b** seem to correspond to the stoichiometric factors of these antioxidants (2.2 and 2.9, respectively; see Table 1). In the case of the three catechol derivatives, **1d**, **2d** and, above all, **1e**, the anomalous low SC_{50} value (16, 15, and 8 μM, respectively, entries 8–10 in Table 1) is a clear indication that the n factor is higher than 2. This is likely due to the intervention of secondary reactions that regenerate catechol structures from *ortho*-quinones initially formed in the trapping of two DPPH radicals.

The last group of thiaflavanes to consider is that of the sulfones **2a–c** and of the sulfoxide **3b**. Disappointingly, these are too unreactive toward both DPPH and peroxyl radicals to allow any correlation between experimental data. In fact, for three of them, the SC_{50} values lie outside the measurable range, the only exception being **2a**, and for two of them, the inhibition rate constants are too small to be determined. These two techniques only agree about the fact that these thiaflavanes are very poor antioxidants.

Data regarding the protection provided by thiaflavane derivatives **1c–e** against CumOOH-induced DNA damage seem in agreement with their radical-scavenging ability. Since CumOOH-induced DNA oxidation can be mainly ascribed to the formation of peroxyl radicals, the protective effect of a certain compound can be related to its radical-scavenging property. Accordingly, the protection observed with

compounds **1d** and **1e**, having a catechol group on the B ring capable of a catechin-like behavior, is similar to that offered by catechin and is lower than that found with α-TOH, the naturally occurring most efficient chain-breaking antioxidant known. As expected, the weak tocopherol-like action, operative in thiaflavane **1c**, has a minor protective effect on CumOOH-induced damage of DNA, compared to the related activity of Vit E (Fig. 9).

Data obtained by measuring the ability of thiaflavanes **1** to work against DNA oxidation induced by Fe^{3+}/GSH is less obvious yet more interesting. Indeed, the redox reaction of GSH with ferric ions allowed the formation of Fe^{2+} cations, able, in turn, to promote oxidative damage through Fenton chemistry. In these conditions, a mixture of highly reactive and dangerous oxidants, such as perferryl ion species (FeO^{2+} and FeO^{3+}) and, above all, hydroxyl radical ($HO^{\bullet}$), are formed in solution. In such a context, the radical-scavenging ability of an antioxidant will play its role by blocking secondary free radicals generated, for example, from $HO^{\bullet}$, whereas the ability of blocking Fe^{2+} ions by chelation will directly prevent the formation of the dangerous oxidizing cocktail. As a matter of fact, it is known [20] that the addition of EDTA, as an iron chelator, stops the oxidative DNA damage induced by Fe^{3+}/GSH. Thus, we can argue that the observed protective effect offered by α-TOH (Fig. 10), which is unable to chelate any cation, is mostly due to the scavenging of the secondary free radicals. On the other hand, the ability of flavonoids, particularly those bearing a cathecol group, like catechin, to act as metal ions chelators is well known [26,27]. Thus, the fairly similar protective effect observed for catechin and derivative **1d** is possibly derived from their ability to act as radical scavenger and as metal chelator (Fig. 10). Amazingly, compound **1c**, which showed poor efficiency in providing protection against CumOOH-induced DNA damage (see Fig. 9), showed an activity even better than that of **1d** or catechin. To rationalize this result, we assume that compound **1c** might in fact be able to chelate Fe^{2+} ions using the 5-OH group on the A ring and the sulfide sulfur on the C ring. This observation seems soundly corroborated by the remarkable protection (82%) offered by compound **1e** (Fig. 10), which can be explained by considering for this compound an association of the previously verified radical scavenging attitude, with its ability to perform as an efficient dual metal chelating polyphenol by means of both the catecholic B ring and the A/C ring arrangement (Fig. 11).

The presence of the sulfur atom on the C ring is clearly crucial for the chelating ability of derivatives **1c** and **1e**. Indeed, a qualitative yet convincing

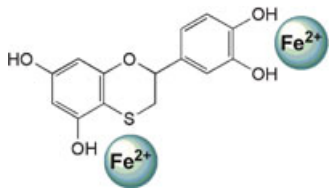


FIGURE 11 Potential dual Fe^{2+} chelating mode for compound **1e**.

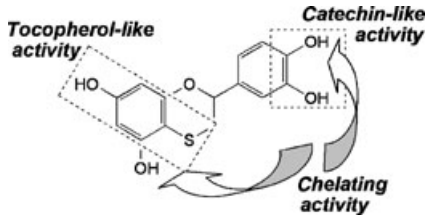


FIGURE 12 Multipotent protective action of 4-thiaflavanes.

validation of these results arose from the UV/visible analyses of compounds **1**. In the presence of Fe^{2+} ions, added at concentrations used for oxidative DNA damage, we observed a hypochromic effect for the cathecol band of compounds **1d** and **1e** and for the resorcinol bands of thiaflavanes **1c** and **1e** as a result of a possible chelation phenomenon. Such a hypochromic effect is indeed removed by adding EDTA to the solution. A dedicated study on the Fe^{2+} chelation ability of 4-thiaflavanes is ongoing.

CONCLUSION

Connecting together the features of different natural antioxidants is a modern trend in drug discovery [28]. In fact, small molecules that protect against ROS face the problem that any new radical formed, even when stabilized, retains a prooxidant effect [29], that is, the possibility of damaging the tissue where it was generated. It is well demonstrated that an effective protection against ROS requires the synergic action of different antioxidants, ensuring a cascade of redox reactions ranging from a highly reactive and dangerous free radical to a safe molecule [30].

This paper demonstrated that 4-thiaflavanes actually show a double-faced antioxidant activity miming the ability of the two most important families of natural antioxidants, that is, flavonoids, vitamin P, for example, from red wine, and tocopherols, Vit E, for example, from olive oil, two of the pillars of the Mediterranean diet.

Moreover, the aptitude of selected hydroxy-4-thiaflavanes in preventing DNA oxidative damage suggests their ability as metal-chelating polyphenols. Thus, these compounds can actually be considered as multipotent protective agents [31] (Fig. 12). Their further optimization is under development in this laboratory.

ACKNOWLEDGMENTS

Authors are indebted to Dr. Antonio Tomaino, University of Messina, Dr. Maura Lodovici, University of Florence, and Prof. Gian Franco Pedulli, University of Bologna, for SC_{50}, DNA oxidative damage, and k_{inh} measurements, respectively.

REFERENCES

[1] Halliwell, B.; Gutteridge, J. M. C. Free Radicals in Biology and Medicine; 3rd ed.; Oxford University Press: London; 1998 and references cited therein.

[2] (a) Stubbe, J.; van der Donk, W. A. Chem Rev 1998, 98, 705–762; (b) Hensley, K.; Robinson, K. A.; Gabbita, S. P.; Salsman, S.; Floyd, R. A Free Radic Biol Med 2000, 28, 1456–1462; (c) Stubbe, J. Chem Commun 2003, 2511–2513.

[3] (a) Vroegop, S. M.; Decker, D. E.; Buxser, S. E. Free Radic Biol Med 1995, 18, 141–151; (b) Breen, A. P.; Murphy, J. A. Free Radic Biol Med 1995, 18, 1033–1077; (c) Pace, G. W.; Leaf, C. D. Free Radic Biol Med 1995, 19, 523–528; (d) Aruoma, O. I. Free Radic Biol Med 1996, 20, 675–705; (e) Schwarz, K. B. Free Radic Biol Med 1996, 21, 641–649; (f) Yu, B. P. Free Radic Biol Med 1996, 21, 651–668.

[4] (a) Harborne, J. B. The Flavonoids Advances in Research Since 1986; Chapman & Hall: London, 1994; (b) Bohm, B. A. Introduction to Flavonoids; Harwood Academic Publishers Amsterdam 1998 and references cited therein; (c) Dixon, R. A.; Ferreira, D. Phytochemistry 2002, 60, 205–211.

[5] Ho, C.-T.; Lee, C. Y.; Huang, M.-T. Phenolic Compounds in Food and Their Effects on Health I and II, ACS Symposium Series 506 and 507; American Chemical Society: Washington, 1992.

[6] (a) Dangles, O.; Fargeix, G.; Dufour, C. J Chem Soc, Perkin Trans 2 2000, 1653–1663; (b) Sun, A. Y.; Simonyi, A.; Sun, G. Y. Free Radic Biol Med 2002, 32, 314–318, and references cited therein.

[7] (a) Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. J Am Chem Soc 1994, 116, 4846–4851; (b) Cotelle, N.; Bernier, J.-L.; Catteau, J.-P.; Pommery, J.; Wallet, J.-C.; Gaydou, E. M. Free Radic Biol Med 1996, 20, 35–43; (c) Dangles, O.; Fargeix, G.; Dufour, C. J Chem Soc, Perkin Trans 2, 1999, 1387–1395; (d) Pietta, P.-G. J Nat Prod 2000, 63, 1035–1042.

[8] (a) Haslam, E. J Nat Prod 1996, 59, 205–215. (b) Saija, A.; Scalese, M.; Lanza, M.; Marzullo, D.; Bonina, F.; Castelli, F. Free Radic Biol Med 1995, 19, 481–486; (c) Sestili, P.; Guidarelli, A.; Dachà, M.; Cantoni, O. Free Radic Biol Med 1998, 25, 196–200; (d) Pouget, C.; Fagnere, C.; Basly, J.-P.; Leveque, H.; Chulia, A.-J. Tetrahedron 2000, 56, 6047–6052; (e) Noguchi, N.; Niki, E. Free Radic Biol Med 2000, 28, 1538–1546; (f) Ishige, K.; Schubert, D.; Sagara, Y. Free Radic Biol Med 2001, 30, 433–446; (g) Moridani, M. Y.; Pourahmad, J.; Bui, H.; Siraki, A.; O'Brien, P. J. Free Radic Biol Med 2003, 34, 243–253.

[9] (a) Burton, G. W.; Ingold, K. U. Acc Chem Res 1986, 19, 194–201; (b) Halliwell, B.; Gutteridge, J. M. C. Antioxidant in Nutrition, Health, and Disease; Oxford University Press: New York, 1996.

[10] Hussain, H. H.; Babic, G.; Durst, T.; Wright, J. S.; Flueraru, M.; Chichirau, A.; Chepelev, L. L. J Org Chem 2003, 68, 7023 and references cited therein

[11] Capozzi, G.; Lo Nostro, P.; Menichetti, S.; Nativi, C.; Sarri, P. Chem Commun 2001, 551–552.

[12] (a) Capozzi, G.; Falciani, C.; Menichetti, S.; Nativi, C. J Org Chem 1997, 62, 2611; (b) Capozzi, G.; Falciani, C.; Menichetti, S.; Nativi, C.; Raffaelli, B. Chem Eur J 1999, 5, 1748; (c) Menichetti, S.; Viglianisi, C. Tetrahedron 2003, 59, 5523.

[13] Menichetti, S.; Aversa, M. C.; Cimino, F.; Contini, A.; Viglianisi, C.; Tomaino, A. Org Biomol Chem 2005, 3, 3066.

[14] Howard, J. A. In Free Radicals; Kochi, J. K. (Ed.); Wiley-Interscience: New York, 1975; Vol. 2, Ch. 12.

[15] Amorati, R.; Pedulli, G. F.; Valgimigli, L.; Attanasi, O. A.; Filippone, P.; Fiorucci, C.; Saladino, R. J Chem Soc, Perkin Trans 2, 2001, 2142.

[16] Enes, R. F.; Tomé, A. C.; Cavaleiro,J. A. S.; Amorati, R.; Fumo, M. G.; Valgimigli, L.; Pedulli, G. F. Chem Eur J 2006, 12, 4646.

[17] Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. J Am Chem Soc 1985, 107, 7053.

[18] Hix, S.; Kadiiska, M. B.; Mason, R. B.; Augusto, O. Chem Res Toxicol 2000, 13, 1056.

[19] Parrado, J.; Absi, E. H.; Machado, A.; Ayala, A. Biochim Biophys Acta 2003, 1624, 139.

[20] Lodovici, M.; Guglielmi, F.; Meoni, M.; Dolara, P. Food Chem Toxicol 2001, 39, 1205.

[21] Zahalka, H. A.; Robillard, B.; Hughes, L.; Lusztyk, J.; Burton, G. W.; Janzen, E. G.; Kotake, Y.; Ingold, K. U. J Org Chem 1988, 53, 3739.

[22] Amorati, R.; Fumo, M. G.; Mugnaini, V.; Pedulli, G. F.; Menichetti, S. J Org Chem 2006, 71, 6325.

[23] Malmström, J.; Jonsson, M.; Cotgreave, I. A.; Hammarström, L.; Sjödin, M.; Engman, L. J Am Chem Soc 2001, 123, 3434.

[24] Shanks, D.; Amorati, R.; Fumo, M. G.; Pedulli, G. F.; Valgimigli, L; Engman L. J Org Chem 2006, 71, 1033.

[25] Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Food Sci Technol 1995, 28, 25.

[26] (a) Mira, L.; Fernandez, M. T.; Santos, M.; Rocha, R.; Florencio, M. H.; Jennings, K. R. Free Radic Res 2001, 36, 1199; (b) Khokhar, S.; Apenten, R. K. O. Food Chem 2003, 81, 133.

[27] Hider, R. C.; Liu, Z. D.; Khodr, H. H. Z. Methods Enzymol 2001, 335, 190.

[28] (a) Bebbington, D.; Monck, N. J. T.; Gaur, S.; Palmer, A. M.; Benwell, K.; Harvey, V.; Malcolm C. S.; Porter, R. H. P. J Med Chem 2000, 43, 2779–2782; (b) Koufaki, M.; Calogeropoulou, T.; Detsi, A.; Roditis, A.; Kourounakis, A. P.; Papazafiri, P.; Tsiakitzis, K.; Gaitanaki, C.; Beis, I.; Kourounakis, P. N. J Med Chem 2001, 44, 4300–4303; (c) Palozza, P.; Piccioni, E.; Avanzi, L.; Vertuani, S.; Calviello, G.; Manfredini, S. Free Radic Biol Med 2002, 33, 1724–1735.

[29] (a) Cao, G.; Sofic, E.; Prior, R. L. Free Radic Biol Med 1997, 22, 749–760; (b) Bowry, V. W.; Ingold, K. U. Acc Chem Res 1999, 32, 27–34; (c) Skibola, C. F.; Smith, M. T. Free Radic Biol Med 2000, 29, 375–383; (d) Dangles, O.; Dufour, C.; Fargeix, G. J Chem Soc, Perkin Trans 2, 2000, 1215–1222.

[30] (a) Chen, H.; Tappel, A. L. Free Radic Biol Med 1995, 18, 949–953; (b) Böhm, F.; Edge, R.; Land, E. J.; McGarvey, D. J.; Truscott, T. G. J Am Chem Soc 1997, 119, 621–622; (c) Jia, Z.-S. B. Zhou, Z.-S.; Yang, L.; Wu, L.-M.; Liu, Z.-L. J Chem Soc, Perkin Trans2, 1998, 911–915; (d) Zhou, B.; Jia, Z.-S.; Chen, Z.-H.; Yang, L.; Wu, L.-M.; Liu, Z.-L. J Chem Soc, Perkin Trans 2, 2000, 785–791; (e) Amorati, R.; Ferroni, F.; Lucarini, M.; Pedulli, G. F.; Valgimigli, L. J Org Chem 2002, 67, 9295–9303; (f) Shang, F.; Lu, M.; Dudek, E.; Reddan, J.; Taylor, A. Free Radic Biol Med 2003, 34, 521–530.

[31] Antonello, A.; Hrelia, P.; Leonardi, A.; Marucci, G.; Rosini, M.; Tarozzi, A.; Tumiatti, V.; Melchiorre, C. J Med Chem 2005, 48, 28.