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Synthesis and antioxidant activity of [60]fullerene–flavonoid conjugates

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ABSTRACT

Chalcone, flavone, and arylideneflavanone derivatives bearing one or two 3,5-di-*tert*-butyl-4-hydroxyphenyl groups were synthesized from 2′,4′-dihydroxyacetophenone and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde. These flavonoids were converted into the corresponding malonates and then reacted with C_{60} to yield the title compounds. The O–H bond dissociation enthalpies (BDE) and the chain-breaking antioxidant activity of the flavonoid derivatives and the corresponding C_{60} conjugates were evaluated. These results are consistent with the phenolic moiety being the main responsible for the reaction with peroxyl radicals, while the C_{60} part of the molecule acts synergically by trapping alkyl radicals under reduced O_2 partial pressure. These novel C_{60}–flavonoid conjugates are therefore promising leads for broad-spectrum radical scavengers.

1. Introduction

Oxidative stress plays a significant pathological role in many human diseases, namely in all inflammatory and ischemic diseases and also in the neurologic ones such as Alzheimer's and Parkinson diseases, multiple sclerosis, and amyotrophic lateral sclerosis.[1] Cancer diseases are also associated with oxidative stress; malignant cells in general are more active than normal cells in the production of superoxide.[2] These diseases result from the overproduction of reactive oxygen species (ROS), generated endogenously during the cellular metabolic activities, which lead to cellular damage including lipid peroxidation, DNA adduct formation, protein oxidation, and enzyme inactivation, which can all ultimately lead to cell death.[2] The detrimental effect of ROS can be prevented by antioxidants. It is unanimously accepted that diets rich in food containing antioxidants (legumes, vegetables, and fresh fruit) have beneficial effects on human health.[3–5] The beneficial effects of natural polyphenolic antioxidants have been reviewed recently.[6–8]

Among the natural compounds with high antioxidant activity, flavonoids, a widely distributed class of phytochemicals, have a central role.[9–11] Natural flavonoids also show anticancer, antiatherogenic, antimicrobial, and anti-inflammatory properties.[12–18] The biological activity and the healthy effects of flavonoids have been reviewed recently.[19–21]

In recent years it has been shown that fullerene derivatives display a range of biological activities and, specifically, may be used as protective drugs against diseases related with oxidative stress.[22] In fact, fullerenes and their organic derivatives are regarded as 'radical sponges' as they are able to trap multiple radicals per molecule.[23,24] For instance, it has been shown that C_{60} is a more effective antioxidant than α-tocopherol (vitamin E) on the prevention of lipid peroxidation induced by superoxide and hydroxyl radicals.[25] In addition, aqueous C_{60} suspensions not only have no acute or subacute toxicity in rodents but they also protect their livers in a dose dependent manner against free radical damage.[26] Poly-hydroxylated fullerenes [$C_{60}(OH)_n$] are also excellent antioxidants able to reduce the free radical damage of neuronal tissues.[27,28] Carboxyfullerene derivatives have also been successfully used in vivo as protective drugs against neurodegenerative diseases related with oxidative stress.[29–32] The water-soluble fullerene derivative $C_{60}(ONO_2)_{7\pm2}$ attenuates ischemia-reperfusion-induced lung injury in young Wistar rats due to its antioxidant properties.[33] Water-soluble fullerene derivatives are able to protect human skin keratinocytes from UV irradiation by scavenging ROS.[34,35] Recent studies indicate that dendritic water-soluble C_{60} derivatives, especially the anionic ones, exhibit high antioxidant activity against superoxide anions,[36] and fullerene-derivatized amino acids also show potent antioxidant activities.[37,38]

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Recently, we reported the synthesis of new fullerene derivatives having flavonoid moieties of synthetic and natural origin.[39–41] We also reported the synthesis and antioxidant activity of fullerene derivatives bearing 3,5-di-*tert*-butyl-4-hydroxyphenyl groups,[42] i.e., compounds structurally related with BHT,[43] a phenolic antioxidant largely used in the food industry.[44,45] It has been demonstrated that C_{60} alone is not a powerful chain-breaking antioxidant as believed, as it does not efficiently trap peroxyl radicals that are involved in the propagation step of the peroxidative chain reaction.[42] Instead, flavonoids bearing a 3,5-di-*tert*-butyl-4-hydroxyphenyl moiety are powerful scavengers of peroxyl radicals. They exhibit high antioxidant protective activity against LDL peroxidation induced by metal ions or peroxyl radical and do not exhibit prooxidant activity.[46] It is thus expected that the addition of a C_{60} moiety to flavonoids incorporating groups structurally related to BHT could, eventually, show increased antioxidant and radical scavenging activities. In this paper, we report the synthesis of C_{60}–flavonoid conjugates incorporating one or two 3,5-di-*tert*-butyl-4-hydroxyphenyl groups and the evaluation of their antioxidant activities by measuring the O–H bond dissociation enthalpies and the rate constants for their reaction with peroxyl radicals.

2. Results and discussion

2.1. Synthesis of the flavonoids

A conventional route to flavonoids (chalcones, flavanones, and flavones) involves the aldol condensation of a 2′-dihydroxyacetophenone with a benzaldehyde. These reactions lead to 2′-dihydroxychalcones which can be converted into flavanones, by cycloisomerization, or into flavones, by oxidative cyclization.[47] To prepare flavonoids adequately functionalized to be linked to C_{60} we used 2,′4′-dihydroxyacetophenone (**1**) and 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (**3**), both commercially available, as starting materials. Our idea was to functionalize acetophenone **1** with a hydroxyalkyl group in order to use it, in a later stage, to prepare malonate derivatives which could be linked to C_{60} by cyclopropanation (see Schemes 4–6). Selective alkylation of the 4′-hydroxyl group of acetophenone **1** with 3-iodopropan-1-ol leads to **2** in 82% yield (Scheme 1). Acid-catalyzed condensation of this compound with aldehyde **3** affords a 3:1 mixture of the chalcone **4** and the arylideneflavanone **5**, resulting from the cyclization of **4** to the corresponding flavanone and subsequent reaction with another molecule of aldehyde **3**.[48]

The low yield of the acid-catalyzed reaction prompted us to carry out a base-catalyzed aldol condensation. However, since under alkaline conditions aldehyde **3** does not behave as an electrophile (it predominates as a quinone methide anion),[48] it was necessary to protect the hydroxyl group. Reaction of aldehyde **3** with an excess of methoxyethylmethyl chloride (MEM-Cl)[49] in dried THF, using NaH as base, afforded aldehyde **6** in 87% yield (Scheme 2). Condensation of aldehyde **6** with acetophenone **2** in the presence of a 60% NaOH aqueous solution afforded the MEM-protected chalcone **7** in 35% yield. The cleavage of the MEM protecting group afforded a mixture of chalcone **4** and the corresponding isomeric flavanone. Because of that, chalcone **7** was used in the following steps and the cleavage of the MEM protecting group was left for the final synthetic step. In that way the expected products were obtained in good yields.

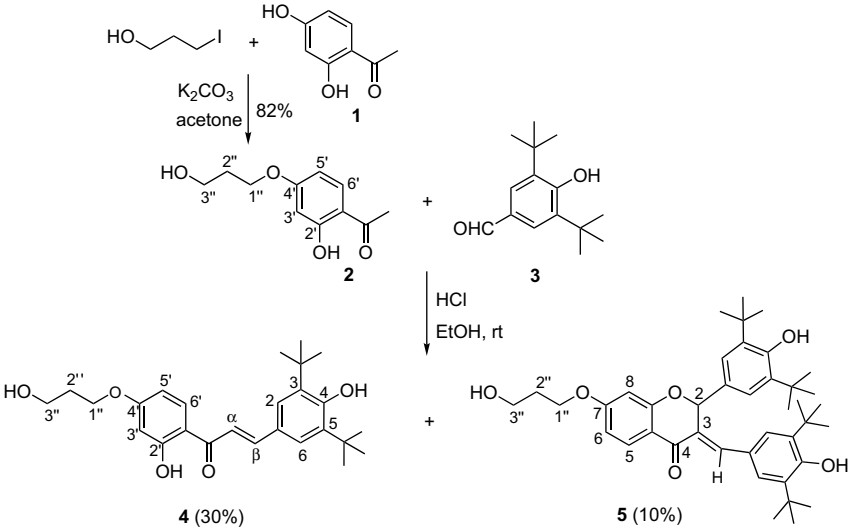
The oxidative cyclization of chalcone **7** with dimethylsulfoxide in the presence of a catalytic amount of iodine[47] afforded directly the unprotected flavone **8** (Scheme 3). The cleavage of the MEM group is probably due to the formation of HI during the cyclization step.

2.2. Synthesis of the C_{60}–flavonoid conjugates

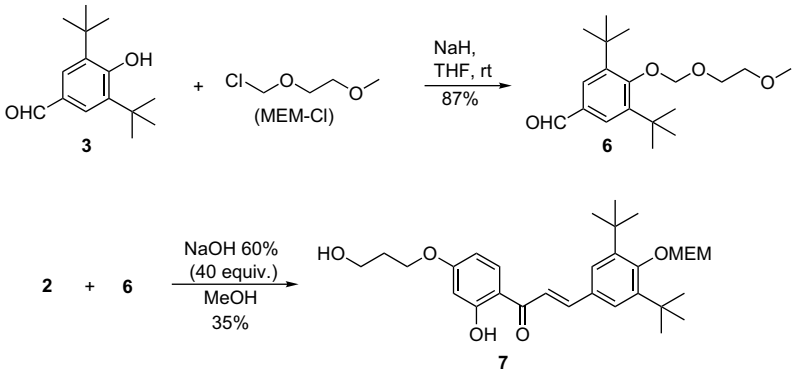
The C_{60}–flavonoid conjugates were synthesized by the cyclopropanation of C_{60} with the malonates obtained in the reaction of 3-chloro-3-oxopropanoate with the 3-hydroxypropyl derivatives **5**, **7**, and **8**. The malonates, obtained in excellent yields (80–98%), were coupled to C_{60} following a variation[50] of the Bingel methodology.[51] The reaction of C_{60} with the malonates, in the presence of CBr_4 and DBU, afforded the expected C_{60}–flavonoid conjugates in moderate yields (21–48%) (Schemes 4–6). Finally, compound **11** was obtained in an almost quantitative yield (97%) by acid catalyzed cleavage of the protecting MEM group (Scheme 4).

2.3. Structural characterization of the synthesized compounds

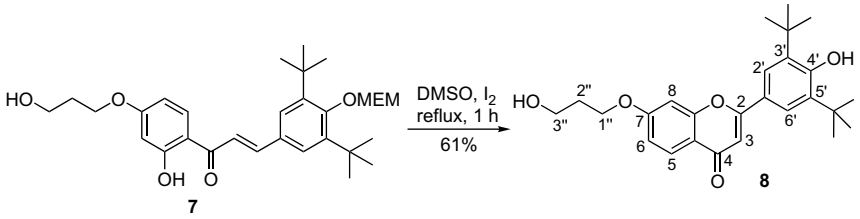
All compounds were characterized by ^1H and ^{13}C NMR spectroscopy and mass spectrometry. The ^1H NMR spectra of all synthesized flavonoids show a singlet at δ 1.48–1.51 ppm corresponding to the *tert*-butyl groups. In the case of arylideneflavanone derivatives, two singlets at δ 1.28–1.29 ppm and 1.36 ppm are observed. The spectra of the chalcone derivatives are characterized by the presence of two doublets at δ 7.37–7.44 ppm and at δ



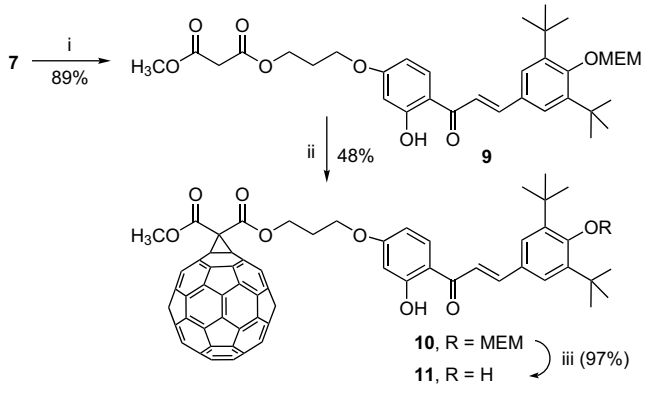
Scheme 1.



Scheme 2.



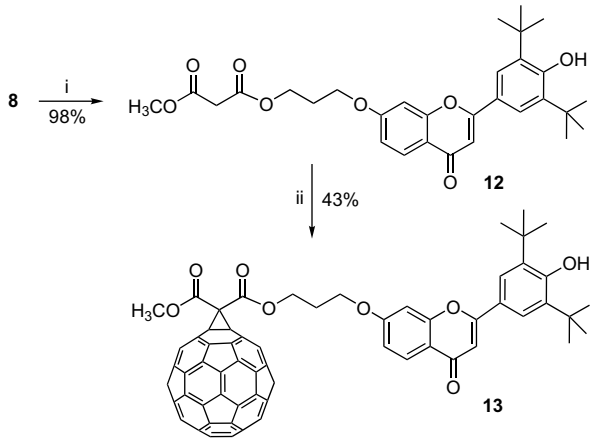
Scheme 3.



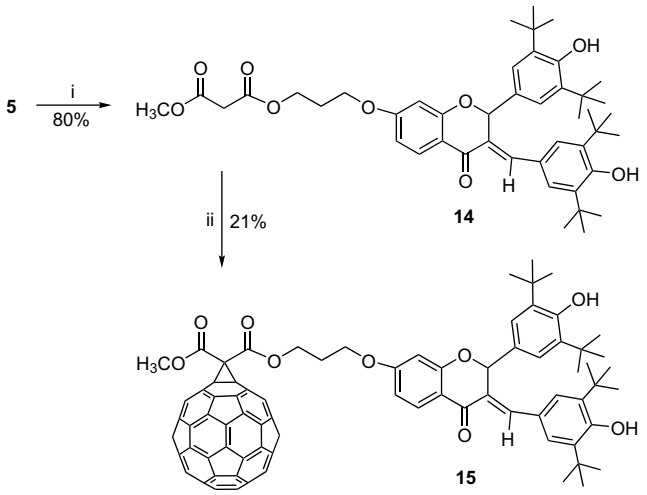
Scheme 4. (i) $ClCOCH_2COOMe$, NEt_3, CH_2Cl_2, 0 °C to rt; (ii) C_{60}, CBr_4, DBU, toluene, rt; (iii) HCl 10% in methanol, 70 °C.

7.82–7.91 ppm corresponding to the resonances of protons H-α and H-β, respectively. The coupling constant of 15.4 Hz confirms a trans configuration for the C–C double bond. The singlet at ca. 13.5 ppm, due to the resonance of the 2′-OH proton, is also distinctive of all chalcone derivatives. The singlet corresponding to the 4-OH proton appears typically at δ 5.0–5.5 ppm; this signal is absent in the ^{1}H NMR spectra of the compounds bearing the MEM group. The ^{1}H NMR spectra of the flavone derivatives show a characteristic singlet at δ 6.67–6.70 ppm corresponding to the resonance of proton H-3. The ^{1}H NMR spectra of the arylideneflavanone derivatives **5**, **14**, and **15** show the expected singlets corresponding to the resonances of protons H-2 and C=C*H*Ar at δ 6.50–6.51 and 8.01–8.05 ppm, respectively. The chemical shifts of the C=C*H*Ar protons ($\delta \sim 8$ ppm) indicate that these compounds have an *E*-configuration.[52,53]

The resonance of the ester methyl group of malonate derivatives appears as a singlet at δ 3.73–3.74 ppm while in the C_{60}–flavonoid



Scheme 5. (i) $ClCOCH_2COOMe$, NEt_3, CH_2Cl_2, 0 °C to rt; (ii) C_{60}, CBr_4, DBU, toluene, rt.



Scheme 6. (i) $ClCOCH_2COOMe$, NEt_3, CH_2Cl_2, 0 °C to rt; (ii) C_{60}, CBr_4, DBU, toluene, rt.

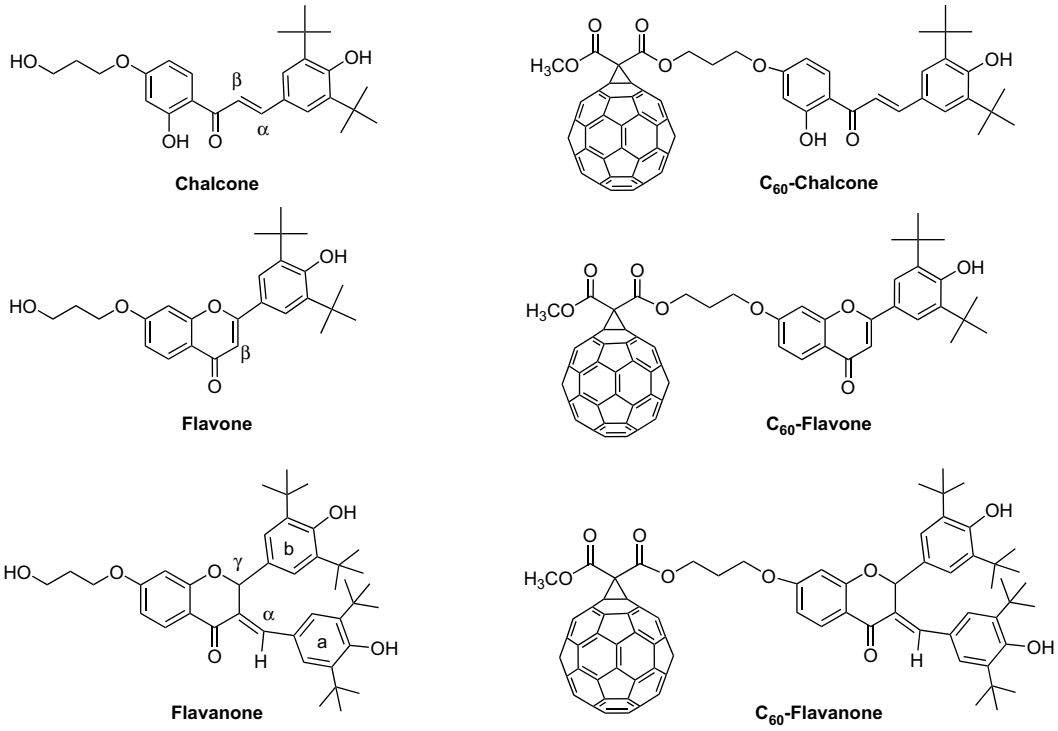


Figure 1. Flavonoids and C_{60}–flavonoid conjugates used in the antioxidant activity studies.

derivatives it appears at δ 3.95–4.08 ppm. The ^{13}C NMR spectra of the C_{60}–flavonoid conjugates show the expected signals corresponding to the methano bridge at $\delta \sim 54$ ppm and to the sp^3 carbons of C_{60} at δ 69.1–72.5 ppm. The mass spectra of the C_{60}–flavonoid conjugates show the $[M+H]^+$ ion, confirming the proposed structures.

2.4. Antioxidant activity of the C_{60}–flavonoid conjugates

The antioxidant activity of the flavonoid derivatives and of the corresponding C_{60} conjugates, shown in Figure 1, was evaluated for each compound by determining two physico-chemical parameters that provide a good estimate of this property that is the O–H bond dissociation enthalpies (BDE) and the rate constant for their reaction with peroxyl radicals. The BDE values were measured by using the electron paramagnetic resonance (EPR) radical equilibration technique,[54] as described below.

2.4.1. EPR spectra

Phenoxyl radicals were produced at room temperature inside the cavity of an EPR spectrometer by reacting the compounds shown in Figure 1 with alkoxyl radicals generated photolytically from di-*tert*-butyl peroxide in deoxygenated benzene solution. The measured *g* factors were typical of phenoxyl radicals (see Table 1 for spectral parameters). In the case of *flavanone* derivatives, only the radical from the phenolic moiety **a** (see Fig. 1) was detectable.

When photolyzing solutions of *C_{60}–chalcone*, only a singlet (g=2.0033; peak-to-peak line width ≈ 0.7 G), whose intensity

Table 1
EPR spectral parameters for the phenoxyl radicals obtained by abstraction of the OH hydrogen atom from the title compounds

Molecule	$a(2H_m)$/G	a(other)/G	g
Chalcone	1.82	3.01 ($1H_\alpha$); 6.02 ($1H_\beta$)	2.0043
C_{60}–chalcone	—[a]	—	—
Flavone	1.95	3.63 ($1H_\beta$)	2.0045
C_{60}–flavone	1.95	3.63 ($1H_\beta$)	2.0045
Flavanone[b]	1.81	2.70 ($1H_\alpha$); 5.43 ($1H_\gamma$)	2.0044
C_{60}–flavanone[b]	1.83	2.70 ($1H_\alpha$); 5.52 ($1H_\gamma$)	2.0044

[a] See text.
[b] Referred to ring **a**.

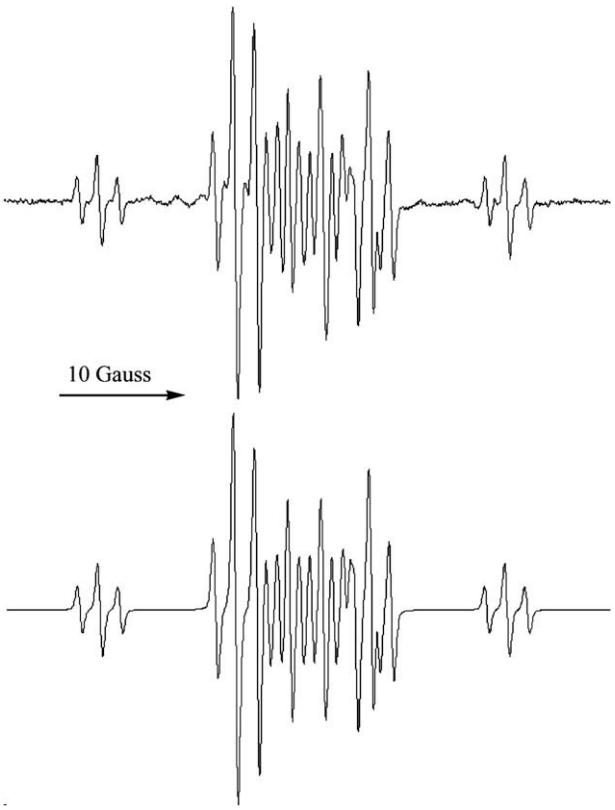


Figure 2. Experimental (upper) and computer simulated EPR spectra observed at room temperature when photolyzing a deoxygenated benzene solution of *flavanone* and BHT in a concentration ratio of 5.2:1.

Table 2
O–H BDE values of the title compounds measured at room temperature in benzene containing 10% di-*tert*-butyl peroxide, rate constants k_{inh} for their reaction with peroxyl radicals in cumene at 30 °C, and number of radicals n trapped by each antioxidant molecule

Molecule	BDE(O–H)[a]/kcal mol^{-1}	k_{inh}[b]/10^3 M^{-1} s^{-1}	n[b]
Chalcone	81.0	13	2.0
C_{60}–chalcone	—	13	2.0
Flavone	80.6	8.1	1.8
C_{60}–flavone	—	8.9	1.8
Flavanone	80.9[c]	9.3	3.7
C_{60}–flavanone	80.8[c]	10	3.8
BHT	79.9[d]	11[e]	2.0[e]

[a] Error±0.2 kcal mol^{-1}.
[b] Error: ±10%.
[c] Referred to the ring **a**.
[d] Ref. 56.
[e] Ref. 42.

increased during irradiation, was observed. This line was also visible when photolyzing *C_{60}–flavone* solutions, together with the spectrum of the corresponding phenoxyl radical. Since the *g* factor and the line width do not correspond to those typical of the addition of alkoxyl radicals to C_{60},[42,55] we tentatively attribute this singlet to a persistent adduct to the fullerene moiety of trichloromethyl radicals, which might be formed by H-atom abstraction from chloroform, employed in little amount (10%) to enhance the solubility of *C_{60}–chalcone* and *C_{60}–flavone*.

2.4.2. BDE(O–H)

The determination of the O–H bond dissociation enthalpies was done by measuring, by means of EPR spectroscopy, the equilibrium constant K_1 for the hydrogen atom transfer reaction between two phenols and the corresponding phenoxyl radicals (Eq. 1) generated under continuous photolysis (as an example, see Fig. 2). The BDEs for the species ArOH were calculated, with the assumption that the entropic term can be neglected,[56] by means of Eq. 2 from K_1 and the known BDE value of a reference species Ar′OH, which in the present case was 2,6-di-*tert*-butyl-4-methylphenol (BHT) whose recently revised BDE(O–H) value is 79.9 kcal mol^{-1}.[56,57]

$$\mathrm{ArOH} + \mathrm{Ar'O^{\bullet}} \overset{K_1}{\rightleftharpoons} \mathrm{ArO^{\bullet}} + \mathrm{Ar'OH} \quad (1)$$

$$\mathrm{BDE(ArO{-}H)} = \mathrm{BDE(Ar'O{-}H)} - \mathrm{RTln}(K_1) \quad (2)$$

This approach could not be applied in the cases of *C_{60}–chalcone* and *C_{60}–flavone* due to the presence of the singlet line at g=2.0033, whose intensity increased under irradiation at the expenses of the phenoxyl radicals.

The results, reported in Table 2, show that the flavonoid compounds are characterized by a BDE(O–H) value of about 1 kcal mol^{-1} larger than that of BHT, and that these values are not significantly influenced by the covalent link to C_{60}.

2.4.3. Autoxidation studies

The chain-breaking antioxidant activity of the title compounds was evaluated by studying the inhibition of the thermally initiated autoxidation of cumene or styrene (RH) in chlorobenzene, (Eqs. 3–8). This reaction, initiated by the thermal decomposition of azobis(isobutyronitrile) (AIBN) at 30 °C, was followed by monitoring the oxygen consumption (see Fig. 3) with an automatically recording gas-absorption apparatus, built in our laboratory and described previously, which uses as detector a commercial differential pressure transducer.[58] The solutions of cumene or styrene were either air-saturated, or bubbled with a gas mixture containing N_2 and 1% oxygen; BHT was used as reference chain-breaking inhibitor.

$$\mathrm{Initiator} \xrightarrow{R_i} \mathrm{R^{\bullet}} \quad (3)$$

$$\mathrm{R^{\bullet} + O_2 \rightarrow ROO^{\bullet}} \quad (4)$$

$$\mathrm{ROO^{\bullet} + RH} \xrightarrow{k_p} \mathrm{ROOH + RO^{\bullet}} \quad (5)$$

$$\mathrm{ROO^{\bullet} + ROO^{\bullet}} \xrightarrow{2k_t} \text{non-radical products} \quad (6)$$

$$\mathrm{ROO^{\bullet} + ArOH} \xrightarrow{k_{inh}} \mathrm{ROOH + ArO^{\bullet}} \quad (7)$$

$$\mathrm{ROO^{\bullet} + ArO^{\bullet} \rightarrow} \text{non-radical products} \quad (8)$$

In the presence of the investigated compounds, the oxidation of cumene was strongly retarded (induction period), the oxygen uptake being given by Eq. 9, where k_p is the rate constant for the propagation step (0.32 M^{-1} s^{-1}),[42] k_{inh} the inhibition rate constant of the antioxidant and τ the length of the induction period.[59] The value of k_{inh} was obtained from the slope of the linear plot of $-\Delta[O_2]$ versus $\ln(1-t/\tau)$. The stoichiometric factor (that is the number of peroxyl radicals trapped by each antioxidant molecule),

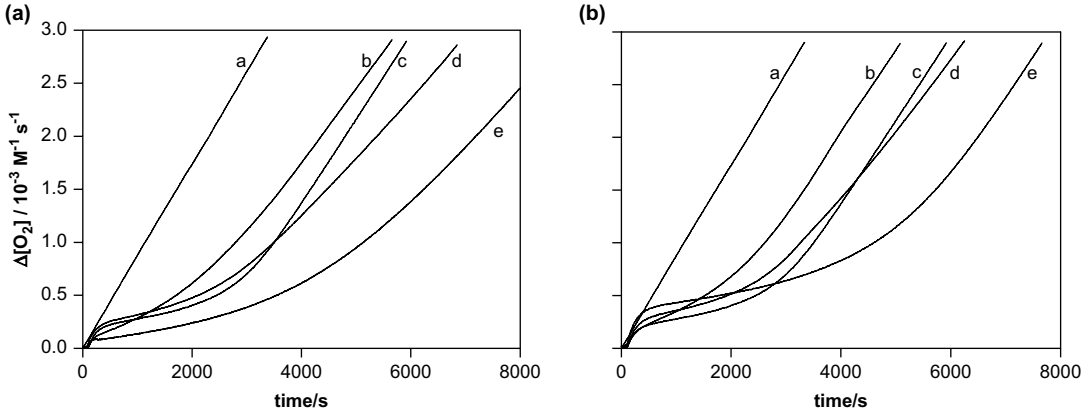


Figure 3. Oxygen consumption traces observed during AIBN (0.05 M) initiated cumene autoxidation (6.2 M) in chlorobenzene solution at 30 °C, in the absence (a) and in the presence of 6.3×10^{-6} M of the investigated compounds. Panel **a**: (b) *C_{60}–flavone*; (c) BHT; (d) *C_{60}–chalcone*; (e) *C_{60}–flavanone*. Panel **b**: (b) *flavone*; (c) BHT; (d) *chalcone*; (e) *flavanone*.

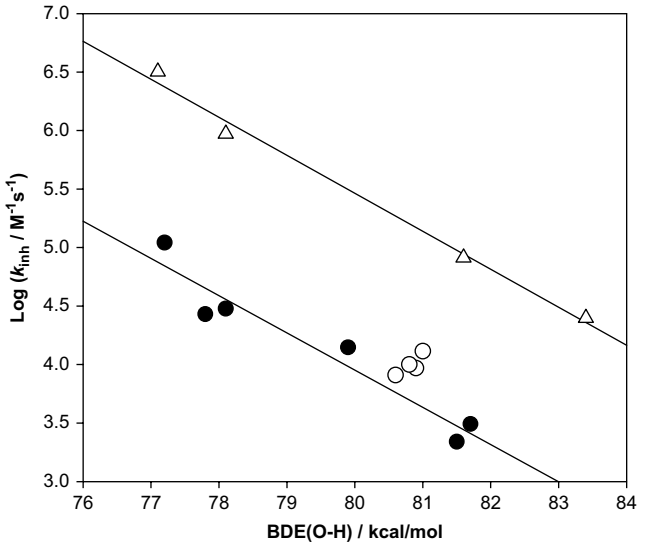


Figure 4. Plot of Log(k_{inh}) versus the BDE(O–H) values of the investigated compounds (empty circles). Similar plots are also reported for 2,6-di-*tert*-butyl (●) and 2,6-dimethyl (△) 4-substituted phenols.[56,60]

n, was determined from the length of τ with respect to BHT, for which n=2.0.[59]

$$-\Delta[O_2]_t = \frac{k_p[RH]}{k_{inh}}\ln(1 - t/\tau) \quad (9)$$

The results obtained in the case of cumene, reported in Table 2 and Figure 3, show that all the investigated compounds possess a k_{inh} value comparable to that of BHT and a stoichiometric coefficient of about 2, with the exception of *flavanone* and *C_{60}–flavanone*, which trap ca. 4 peroxyl radicals due to the presence of two phenolic moieties. In the case of C_{60}–flavonoids, at the end of the induction period the oxygen consumption is still slightly retarded with respect to the uninhibited reaction (see Fig. 3a), suggesting that the fullerene moiety possesses some antioxidant activity.[42] Figure 4 shows that the BDE(O–H) and k_{inh} values obtained in the present work are in reasonable agreement with those reported in the literature for 2,6-di-*tert*-butylphenols.[60]

Instead, when using styrene as oxidizable substrate, only a slight reduction of the oxygen consumption rate was observed, this being in line with the k_p value of styrene (41 M^{-1} s^{-1})[60] much higher than that of cumene. Figure 5a, reporting the relative reduction of the reaction rates as a function of inhibitor concentration, shows that *C_{60}–flavone* possesses an antioxidant activity smaller than BHT, but larger than C_{60}, in agreement with the results obtained in cumene.

The autoxidation of styrene was also performed at low oxygen concentration, by bubbling in the reaction vessel, before starting the reaction, a N_2/O_2 mixture containing 1% O_2 (Fig. 5b). In these conditions, C_{60} behaves as a better inhibitor than under air, presumably because at low O_2 concentration the C_{60} moiety is able to compete with O_2 for R$^\bullet$, thus interrupting some radical chains.[61] Interestingly, at low O_2 partial pressure, *C_{60}–flavone* is more effective in reducing the oxidation rate not only than C_{60}, but also than BHT, differently to what observed under air. This result can be rationalized by admitting that the fullerene moiety behaves as a good trap toward alkyl radicals, and that this action is synergic with the ROO$^\bullet$ scavenging by the flavonoid part of the molecule. This is consistent with the reported reactivity of alkyl radicals toward C_{60} (1.4×10^7 M^{-1} s^{-1} for benzyl radical),[62] larger than that with BHT (5×10^4 M^{-1} s^{-1} for neophyl radical).[63]

3. Conclusions

Flavonoid derivatives bearing one or two 3,5-di-*tert*-butyl-4-hydroxyphenyl groups were synthesized and linked covalently to fullerene C_{60} via cyclopropanation reactions. The chain-breaking antioxidant activity of these compounds was evaluated by measuring the O–H bond dissociation enthalpies (BDE) and the rate constant for the reaction with peroxyl radicals (k_{inh}). From the comparison of the BDE and k_{inh} values of flavonoids with those of the corresponding C_{60} conjugates, it is evident that the ROO$^\bullet$ trapping ability of the studied compounds in air-saturated solutions is due to the phenolic moiety, similar to that of the synthetic inhibitor BHT. On the other hand, under reduced O_2 partial pressure, the investigated compounds showed better antioxidant activity than C_{60} and BHT alone, because in these conditions the ability of the fullerene moiety to trap alkyl radical acts synergically with the peroxyl radical scavenging of the phenolic part of the molecule. This property may become important in the case of the autoxidation of unsaturated lipids, since at the relatively low oxygen partial pressure present in most living cells, alkyl radicals are not quantitatively converted to peroxyl radicals (Eq. 4).[64] Furthermore, the use of C_{60}–flavonoid conjugates may have other biological benefits relatively to the flavonoids alone: a better lipophilicity of the conjugates, the spherical shape of C_{60}, which could adapt to active sites of enzymes,[22d] and the fact that C_{60} may act as a 'passport' for the intracellular delivery of drugs[65] and as a slow-release system.[66] These biological properties induced by the fullerene moiety make the use of C_{60}–antioxidant conjugates particularly interesting in medical research.

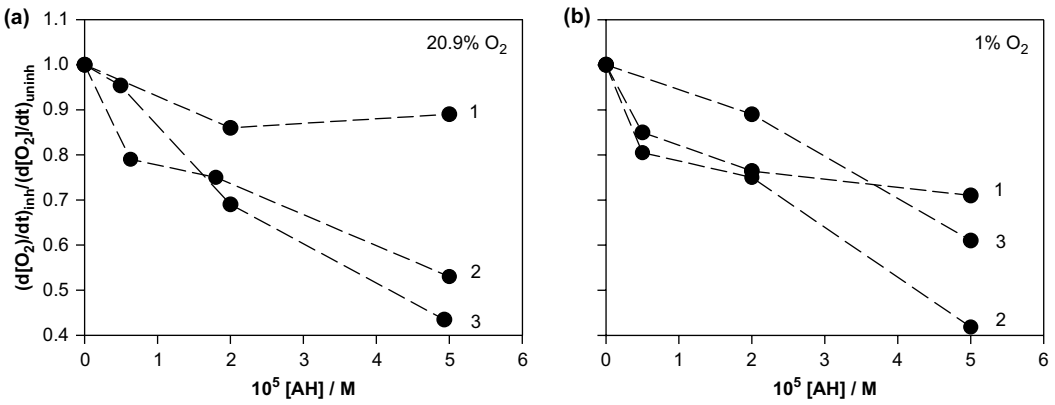


Figure 5. Relative decrease of the rate of oxygen consumption during the autoxidation of styrene initiated by AIBN at 30 °C in chlorobenzene in the presence of increasing amounts of: (1) C_{60}, (2) *C_{60}–flavone*, (3) BHT; in air (**a**) and 1% O_2 (**b**).

4. Experimental section

4.1. General

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300 and 500 spectrometers at 300 or 500 MHz and 75 or 125 MHz, respectively. $CDCl_3$ was used as solvent and TMS as internal reference. Chemical shifts (δ) are quoted in parts per million relative to TMS and the coupling constants (J) are expressed in hertz (Hz). Mass spectra were recorded on a 4800 MALDI TOF/TOF Analyser (Applied Biosystems). Flash chromatography was carried out with silica gel 0.032–0.063 mm. Melting points were measured in a Büchi Melting Point B-540 apparatus.

4.2. Synthesis of flavonoid derivatives

*4.2.1. 2′-Hydroxy-4′-(3-hydroxypropoxy)acetophenone, **2***

2′,4′-Dihydroxyacetophenone (**1**) (600 mg) and K_2CO_3 (1.6 g, 3 equiv) were stirred in acetone (15 mL) for 15 min, at room temperature. To this mixture was added 3-iodopropan-1-ol (0.45 mL, 1.2 equiv). The reaction mixture was heated at reflux for 14 h. The reaction mixture was filtered and the solid residue was washed with acetone. Most part of the solvent was evaporated and the residue was added to water and acidified to pH 4–5 with a 10% HCl aqueous solution. The resulting mixture was extracted with ethyl acetate (3×30 mL). The organic phase was concentrated and the acetophenone **2** was purified by column chromatography using a mixture of chloroform/acetone (8.5:1.5) as the eluent. Acetophenone **2** was crystallized from ethyl ether/petroleum ether, in the freezer, yielding 673 mg (82%) of white solid with mp 46–47 °C. ^{1}H NMR (500.13 MHz, $CDCl_3$): δ=2.06 (quintet, J=6.0 Hz, 2H, H-2″), 2.55 (s, 3H, CH_3), 3.86 (t, J=6.0 Hz, 2H, H-3″), 4.15 (t, J=6.0 Hz, 2H, H-1″), 6.42 (d, J=2.5 Hz, 1H, H-3′), 6.44 (dd, J=8.7, 2.5 Hz, 1H, H-5′), 7.62 (d, J=8.7 Hz, 1H, H-6′). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=26.2, 31.7, 59.7, 65.5, 101.4, 107.8, 113.9, 132.3, 165.1, 165.3, 202.6 (C=O). MS (EI$^+$): m/z (%): 210 ($M^{\bullet+}$, 41), 195 (33), 137 (100). HRMS-ESI m/z calcd for $C_{11}H_{15}O_4$ $(M+H)^+$ 211.0965, found 211.0965.

4.3. 3,5-Di-*tert*-butyl-2′,4-dihydroxy-4′-(3-hydroxypropoxy)-chalcone (4) and arylideneflavanone 5

These compounds were prepared as described in the literature[48] using acetophenone **2** (273 mg, 1.3 mmol) and aldehyde **3** (316 mg, 1.3 mmol). Compounds **4** and **5** were separated by crystallization from a chloroform/methanol mixture.

*4.3.1. Chalcone **4***

Yield 135 mg (30%), mp 140–141 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.49 (s, 18H, *t*-Bu), 2.08 (quintet, J=6.0 Hz, 2H, H-2″), 3.88 (t, J=6.0 Hz, 2H, H-3″), 4.18 (t, J=6.0 Hz, 2H, H-1″), 5.02 (s, 1H, 4-OH), 6.48–6.51 (m, 2H, H-3′ and H-5′), 7.41 (d, J=15.4 Hz, 1H, H-α), 7.50 (s, 2H, H-2 and H-6), 7.82–7.91 (m, 2H, H-6′ and H-β), 13.63 (s, 1H, 2′-OH). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=30.1 (C-2″), 31.7 [C(CH_3)$_3$], 34.3 [C(CH_3)$_3$], 59.9, 65.6, 101.6, 107.7, 114.2, 116.8, 126.1, 131.1, 136.5, 146.0, 156.7, 165.1, 166.5, 191.9 (C=O). MS (MALDI-TOF): m/z: 427 $(M+H)^+$. HRMS-ESI m/z calcd for $C_{26}H_{35}O_5$ $(M+H)^+$ 427.2479, found 427.2488.

*4.3.2. Arylideneflavanone **5***

Yield 45 mg (10%), mp 180–181 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.28 (s, 18H, *t*-Bu), 1.36 (s, 18H, *t*-Bu), 2.02 (quintet, J=6.0 Hz, 2H, H-2″), 3.81–3.86 (m, 2H, H-3″), 4.10 (t, J=6.0 Hz, 2H, H-1″), 5.21 (s, 1H, OH), 5.47 (s, 1H, OH), 6.35 (d, J=2.4 Hz, 1H, H-8), 6.51 (s, 1H, H-2), 6.52 (dd, J=8.9, 2.4 Hz, 1H, H-6), 7.07 (s, 2H, H-2′ and H-6′), 7.27 (s, 2H, H-2′ and H-6′), 7.90 (d, J=8.9 Hz, 1H, H-5), 8.05 (s, 1H, C=C*H*–Ar). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=30.0 (C-2″), 30.2 and 31.7 [C(CH_3)$_3$], 34.3 and 34.4 [C(CH_3)$_3$], 59.9, 65.6, 79.2, 102.2, 109.8, 116.2, 124.9, 125.9, 128.0, 128.8, 129.3, 129.5, 135.8, 136.1, 139.6, 153.9, 155.4, 161.0, 165.1, 181.3 (C=O). MS (MALDI-TOF): m/z: 643 $(M+H)^+$. HRMS-ESI m/z calcd for $C_{41}H_{55}O_6$ $(M+H)^+$ 643.3993, found 643.4001.

4.4. 3,5-Di-*tert*-butyl-4-[(2-methoxyethoxy)methoxy]benzaldehyde, 6

NaH (197 mg, 4 equiv) was added to a solution of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (**3**) (500 mg, 2.05 mmol) in dry THF (20 mL) and the mixture was stirred for 15 min at room temperature under a nitrogen atmosphere. MEM-Cl (0.70 mL, 3 equiv) was added to the reaction mixture and it was stirred for 3 h. The reaction mixture was poured into ice and neutralized to pH 6–7 with a 10% HCl aqueous solution. The resulting mixture was extracted with chloroform (3×30 mL) and the organic phase was concentrated. The aldehyde **6** was purified by preparative TLC using a mixture of chloroform/petroleum ether (6:4) as the eluent. Compound **6** (575 mg, 87% yield) was obtained as an oil. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.48 (s, 18H, *t*-Bu), 3.43 (s, 3H, OCH_3), 3.64–3.67 (m, 2H, O$CH_2$$CH_2$O), 3.98–4.01 (m, 2H, OCH_2CH_2O), 5.03 (s, 2H, OCH_2O), 7.80 (s, 2H, Ar–H), 9.92 (s, 1H, CHO). ^{13}C NMR (125.77 MHz, $CDCl_3$): δ=31.8 [C(CH_3)$_3$], 35.9 [C(CH_3)$_3$], 59.1, 69.3, 99.8, 128.5, 131.5, 145.7, 160.2, 192.1. MS (EI$^+$): m/z (%): 322 ($M^{\bullet+}$, 10), 247 (100). HRMS-ESI m/z calcd for $C_{19}H_{31}O_4$ $(M+H)^+$ 323.2217, found 323.2208.

4.5. 3,5-Di-*tert*-butyl-2′-hydroxy-4′-(3-hydroxypropoxy)-4-[(2-methoxyethoxy)methoxy] chalcone, 7

NaOH aqueous solution (60%, 40 equiv) was added to an ice cooled solution of acetophenone **2** (420 mg, 2.0 mmol) and aldehyde **6** (587 mg, 1.2 equiv) in methanol (10 mL). The reaction mixture was stirred for 15 min at room temperature and then it was heated at reflux for 8 h using an oil bath. The reaction mixture was poured into ice, acidified to pH 3–4 with a 10% HCl aqueous solution, and extracted with chloroform (3×30 mL). Compound **7** was purified by preparative TLC using a mixture of petroleum ether/ethyl acetate (1:1) as the eluent. Chalcone **7** was crystallized from ethyl ether/petroleum ether yielding yellow crystals (305 mg, 35%) with mp 99–100 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.48 (s, 18H, *t*-Bu), 2.08 (quintet, J=6.0 Hz, 2H, H-2″), 3.43 (s, 3H, OCH_3), 3.65–3.68 (m, 2H, O–CH_2 CH_2–O), 3.85–3.90 (m, 2H, H-3″), 3.99–4.02 (m, 2H, O–CH_2CH_2–O), 4.18 (t, J=6.0 Hz, 2H, H-1″), 5.02 (s, 2H, O–CH_2–O), 6.49–6.52 (m, 2H, H-3′ and H-5′), 7.44 (d, J=15.4 Hz, 1H, H-α), 7.55 (s, 2H, H-2 and H-6), 7.84 (d, J=9.6 Hz, 1H, H-6′), 7.86 (d, J=15.4 Hz, 1H, H-β), 13.52 (s, 1H, 2′-OH). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=31.7 (C-2″), 31.9 [C(CH_3)$_3$], 35.8 [C(CH_3)$_3$], 59.1, 59.8, 65.6, 69.1, 71.6, 99.7, 101.6, 107.8, 114.1, 118.7, 127.2, 129.5, 131.2, 145.2, 157.1, 165.1, 166.4, 166.5, 166.9, 191.9 (C=O). MS (EI$^+$): m/z (%): 514 ($M^{\bullet+}$, 9), 438 (24), 137 (23), 89 (100). Elemental analysis (%) calcd for $C_{30}H_{42}O_7$: C 70.01, H 8.23; found: C 70.15, H 8.09.

4.6. 3′,5′-Di-*tert*-butyl-4′-hydroxy-7-(3-hydroxypropoxy)-flavone, 8

A solution of chalcone **7** (60 mg, 0.12 mmol) and iodine (1.2 mg, 0.04 equiv) in DMSO (2 mL) was heated at reflux for 1 h, under a nitrogen atmosphere. The reaction mixture was poured into water, and the iodine was reduced with an aqueous solution of 5% $Na_2S_2O_3$ (20 mL). The resulting solution was extracted with ethyl acetate (3×25 mL), the organic phase was concentrated and purified by preparative TLC (silica gel) using a mixture of chloroform/ethyl acetate (8:2) as the eluent. Compound **8** was crystallized from $CHCl_3$/MeOH giving white crystals (30 mg, 61% yield) with mp 176–177 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.51 (s, 18H, *t*-Bu), 2.13 (quintet, J=6.0 Hz, 2H, H-2″), 3.89–3.94 (m, 2H, H-3″), 4.26 (t, J=6.0 Hz, 2H,

H-1″), 5.66 (s, 1H, OH), 6.70 (s, 1H, H-3), 6.96–7.00 (m, 2H, H-6 and H-8), 7.73 (s, 2H, H-2′ and H-6′), 8.12 (d, J=9.5, 1H, H-5). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=30.1 [C(CH_3)$_3$], 31.8 (C-2″), 34.5 [C(CH_3)$_3$], 59.7, 65.9, 101.1, 106.0, 114.3, 117.8, 122.8, 123.5, 127.0, 136.5, 157.0, 157.9, 163.2, 164.4, 178.0 (C=O). MS (MALDI-TOF): m/z: 425 (M+H)$^+$. MS (EI$^+$): m/z (%): 424 (M$^{\bullet+}$, 100), 409 (61), 137 (17). Elemental analysis (%) calcd for $C_{26}H_{32}O_5$: C 73.56, H 7.60; found: C 73.77, H 8.00.

4.7. Synthesis of malonate derivatives

General procedure. A solution of methyl 3-chloro-3-oxopropanoate (2 equiv) in anhydrous CH_2Cl_2 (2 mL) was added dropwise to an ice cooled solution of chalcone **7** (100 mg, 0.19 mmol), flavone **8** (58 mg; 0.12 mmol), or arylideneflavanone **5** (40 mg, 0.085 mmol) and anhydrous NEt_3 (4 equiv) in anhydrous CH_2Cl_2 (10 mL). The ice bath was removed and the reaction mixture was stirred at room temperature for 3 h. It was then washed with an aqueous solution of 0.5% HCl (2×15 mL) and with a saturated aqueous solution of $NaHCO_3$ (2×15 mL). The organic phase was dried (Na_2SO_4) and the solvent was evaporated under vacuum. Malonates **9**, **12**, and **14** were purified by preparative TLC (silica gel) using a 95:5 mixture of $CHCl_3$/AcOEt as the eluent.

4.7.1. Malonate *9*

Obtained as an yellow oil (106 mg, 89% yield). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.48 (s, 18H, *t*-Bu), 2.18 (quintet, J=6.1 Hz, 2H, H-2″), 3.41 (s, 2H, OC–CH_2–CO), 3.43 (s, 3H, OCH_3), 3.63–3.68 (m, 2H, O$CH_2$$CH_2$O), 3.74 (s, 3H, CO_2CH_3), 3.99–4.02 (m, 2H, OCH_2CH_2O), 4.11 (t, J=6.1 Hz, 2H, H-3″), 4.37 (t, J=6.1 Hz, 2H, H-1″), 5.03 (s, 2H, OCH_2O), 6.46 (d, J=2.5 Hz, 1H, H-3′), 6.50 (dd, J=8.9, 2.5 Hz, 1H, H-5′), 7.44 (d, J=15.4 Hz, 1H, H-α), 7.84 (d, J=8.9 Hz, 1H, H-6′), 7.86 (d, J=15.4 Hz, 1H, H-β), 13.53 (s, 1H, 2′-OH). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=28.1 (C-2″), 31.8 [C(CH_3)$_3$], 35.8 [C(CH_3)$_3$], 41.2, 52.5, 59.1, 62.0, 64.4, 69.1, 71.6, 99.6, 101.4, 107.9, 114.1, 118.6, 127.2, 129.5, 131.2, 145.2, 157.1, 165.1, 166.5 (2malonate C=O), 191.8 (chalcone C=O). MS (MALDI-TOF): m/z: 615 (M+H)$^+$.

4.7.2. Malonate *12*

Obtained as a colorless oil (55 mg, 98% yield). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.50 (s, 18H, *t*-Bu), 2.23 (quintet, J=6.1 Hz, 2H, H-2″), 3.42 (s, 2H, OC–CH_2–CO), 3.73 (s, 3H, CO_2CH_3), 4.19 (t, J=6.1 Hz, 2H, H-3″), 4.41 (t, J=6.1 Hz, 2H, H-1″), 5.66 (s, 1H, OH), 6.70 (s, 1H, H-3), 6.96–7.00 (m, 2H, H-6 and H-8), 7.74 (s, 2H, H-2′ and H-6′), 8.12 (d, J=8.8 Hz, 1H, H-5). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=28.3 (C-2″), 30.1 [C(CH_3)$_3$], 31.8 [C(CH_3)$_3$], 34.5, 41.2, 59.1, 62.0, 64.7, 101.2, 106.0, 114.1, 117.9, 122.8, 123.5, 127.0, 136.5, 157.0, 157.9, 163.0, 164.4 (2malonate C=O), 177.9 (flavone C=O). HRMS-ESI m/z calcd for $C_{30}H_{37}O_8$ (M+H)$^+$ 525.2483, found 525.2496.

4.7.3. Malonate *14*

Colorless oil (53 mg, 80% yield). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.28 (s, 18H, *t*-Bu), 1.36 (s, 18H, *t*-Bu), 2.12 (quintet, J=6.1 Hz, 2H, H-2″), 3.38 (s, 2H, OC–CH_2–CO), 3.68 (s, 3H, CO_2CH_3), 4.03 (t, J=6.1 Hz, 2H, H-3″), 4.33 (t, J=6.1 Hz, 2H, H-1″), 5.22 (s, 1H, OH), 5.48 (s, 1H, OH), 6.33 (d, J=2.4 Hz, 1H, H-8), 6.51 (dd, J=8.8, 2.4 Hz, 1H, H-6), 6.52 (s, 1H, H-2), 7.06 (s, 2H, H-2′ and H-6′), 7.27 (s, 2H, H-2′ and H-6′), 7.89 (d, J=8.8 Hz, 1H, H-5), 8.05 (s, 1H, C=CH–Ar). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=28.2 (C-2″), 30.0 and 30.2 [C(CH_3)$_3$], 34.3 and 34.4 [C(CH_3)$_3$], 41.3, 52.5, 62.1, 64.3, 79.2, 102.3, 109.7, 116.3, 124.9, 125.9, 128.0, 128.9, 129.3, 129.5, 135.9, 136.1, 139.6, 153.9, 155.4, 160.9, 165.0, 166.4, 166.9, 181.3 (C=O).

4.8. Synthesis of C_{60} derivatives

General procedure. To a solution of malonate **9** (31 mg, 0.061 mmol), malonate **12** (22 mg, 0.046 mmol), or malonate **14** (30 mg, 0.040 mmol) and C_{60} (1.5 equiv) in anhydrous toluene (50 mL) were added CBr_4 (1 equiv) and DBU (2 equiv). The reaction mixture was stirred for 18 h at room temperature, under a nitrogen atmosphere, and then it was concentrated and purified by flash chromatography (silica gel) using a (9:1) or (98:2) mixture of toluene/ethyl acetate as the eluent. Compounds **10**, **13**, and **15** were crystallized from $CHCl_3$/MeOH.

4.8.1. C_{60}–chalcone conjugate *10*

Black solid (39 mg, 48% yield), mp >300 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.48 (s, 18H, *t*-Bu), 2.37 (quintet, J=5.9 Hz, 2H, H-2″), 3.41 (s, 3H, OCH_3), 3.65–3.68 (m, 2H, O$CH_2$$CH_2$O), 3.99–4.02 (m, 2H, OCH_2CH_2O), 4.07 (s, 3H, CO_2CH_3), 4.19 (t, J=5.9 Hz, 2H, H-3″), 4.73 (t, J=5.9 Hz, 2H, H-1″), 5.03 (s, 1H, OCH_2O), 6.43 (d, J=2.5 Hz, 1H, H-3′), 6.52 (dd, J=9.0, 2.5 Hz, 1H, H-5′), 7.39 (d, J=15.4 Hz, 1H, H-α), 7.80 (d, J=9.0 Hz, 1H, H-6′), 7.84 (d, J=15.4 Hz, 1H, H-β), 13.47 (s, 1H, 2′-OH). ^{13}C NMR (125.77 MHz, $CDCl_3$): δ=28.1 (C-2″), 31.9 [C(CH_3)$_3$], 35.8 [C(CH_3)$_3$], 54.1 (methano bridge), 59.1, 63.6, 64.0, 69.1 (C_{60}-sp^3), 71.4, 71.6 (C_{60}-sp^3), 99.7, 101.6, 107.9, 114.4, 118.7, 127.2, 129.5, 131.4, 138.4, 139.4, 140.9, 141.7, 141.8, 142.2, 142.9, 143.0, 143.7, 143.9, 144.4, 144.5, 144.60, 144.62, 144.65, 144.77, 144.81, 144.86, 145.09, 145.14, 145.18, 145.18, 145.23, 145.27, 145.4, 157.1, 163.6, 164.1, 165.0, 166.6 (2malonate C=O), 191.9 (chalcone C=O). HRMS-ESI m/z calcd for $C_{94}H_{45}O_{10}$ (M+H)$^+$ 1333.3007, found 1333.2978.

4.8.2. C_{60}–chalcone conjugate *11*

A solution of 10% HCl in methanol (2 mL) was added to a solution of compound **10** (19 mg, 0.014 mmol) in chloroform (3 mL). The mixture was stirred at 70 °C for 3 h, it was neutralized with an aqueous solution of Na_2CO_3 and then compound **11** was extracted with chloroform (2×25 mL). The organic layer was dried (Na_2SO_4) and concentrated. Compound **11** was crystallized from $CHCl_3$/MeOH affording a black solid (17 mg, 97% yield) with mp >300 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.49 (s, 18H, *t*-Bu), 2.33–2.41 (m, 2H, H-2″), 4.07 (s, 3H, CO_2CH_3), 4.19 (t, J=5.8 Hz, 2H, H-3″), 4.73 (t, J=5.8 Hz, 2H, H-1″), 5.61 (s, 1H, 4-OH), 6.42 (d, J=2.5 Hz, 1H, H-3′), 6.51 (dd, J=9.0, 2.5 Hz, 1H, H-5′), 7.37 (d, J=15.4 Hz, 1H, H-α), 7.81 (d, J=9.0 Hz, 1H, H-6′), 7.86 (d, J=15.4 Hz, 1H, H-β), 13.59 (s, 1H, 2′-OH). ^{13}C NMR (125.77 MHz, $CDCl_3$): δ=28.1 (C-2″), 30.2 [C(CH_3)$_3$], 34.4 [C(CH_3)$_3$], 54.1 (methano bridge), 63.6, 64.0, 71.4 (C_{60}-sp^3), 101.6, 107.8, 114.4, 116.8, 126.1, 126.3, 136.5, 138.5, 141.0, 141.7, 141.8, 142.16, 142.18, 142.90, 142.97, 143.04, 143.7, 143.9, 144.45, 144.52, 144.63, 144.67, 144.80, 144.83, 144.87, 145.10, 145.15, 145.20, 145.23, 146.1, 156.7, 163.6, 164.1, 164.9, 166.5 (2malonate C=O), 192.0 (chalcone C=O). MS (MALDI-TOF): m/z: 1245 (M+H)$^+$, 720 ($C_{60}^{\bullet+}$). HRMS-ESI m/z calcd for $C_{90}H_{37}O_8$ (M+H)$^+$ 1245.2483, found 1245.2447.

4.8.3. C_{60}–flavone conjugate *13*

Black solid (25 mg, 43% yield), mp >300 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.49 (s, 18H, *t*-Bu), 2.42 (quintet, J=5.9 Hz, 2H, H-2″), 4.08 (s, 3H, CO_2CH_3), 4.27 (t, J=5.9 Hz, 2H, H-3″), 4.77 (t, J=5.9 Hz, 2H, H-1″), 5.63 (s, 1H, OH), 6.67 (s, 1H, H-3), 6.89 (d, J=2.4 Hz, 1H, H-8), 7.00 (dd, J=8.8, 2.4 Hz, 1H, H-6), 7.62 (s, 1H, H-2′ and H-6′), 8.11 (d, J=8.8 Hz, 1H, H-5). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=28.1 (C-2″), 30.2 [C(CH_3)$_3$], 34.5 [C(CH_3)$_3$], 52.0 (methano bridge), 54.1, 63.4, 64.2, 71.3 (C_{60}-sp^3), 101.1, 106.2, 114.3, 118.1, 122.8, 123.6, 127.1, 136.5, 138.3, 139.4, 140.85, 140.87, 141.6, 141.8, 142.1, 142.82, 142.88, 142.90, 142.95, 143.03, 143.6, 143.8, 144.2, 144.4, 144.55, 144.59, 144.60, 144.63, 144.71, 144.84, 145.0, 145.07, 145.12, 145.20, 157.0, 157.9, 163.0, 163.5, 164.0, 164.5 (2malonate C=O), 177.8 (flavone C=O). MS (MALDI-TOF): m/z: 1243 (M+H)$^+$. HRMS-ESI m/z calcd for $C_{90}H_{35}O_8$ (M+H)$^+$ 1243.2326, found 1243.2321.

4.8.4. C_{60}–flavanone conjugate *15*

Black solid (12 mg, 21% yield), mp >300 °C. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ=1.29 (s, 18H, *t*-Bu), 1.36 (s, 18H, *t*-Bu), 2.18–

2.36 (m, 2H, H-2″), 3.95 (s, 3H, CO_2CH_3), 4.10–4.14 (m, 2H, H-3″), 4.65–4.72 (m, 2H, H-1″), 5.21 (s, 1H, OH), 5.63 (s, 1H, OH), 6.32 (d, J=2.3 Hz, 1H, H-8), 6.50 (s, 1H, H-2), 6.54 (dd, J=8.8, 2.3 Hz, 1H, H-6), 7.04 (s, 2H, H-2′ and H-6′), 7.26 (s, 2H, H-2′ and H-6′), 7.89 (d, J=8.8 Hz, 1H, H-5), 8.01 (s, 1H, C=CH–Ar). ^{13}C NMR (75.47 MHz, $CDCl_3$): δ=28.1 (C-2″), 30.0 and 30.2 [$C(CH_3)_3$], 34.3 and 34.4 [$C(CH_3)_3$], 54.0 (methano bridge), 63.4, 63.6, 63.9, 72.5 (C_{60}-sp^3), 79.2, 102.1, 109.8, 116.4, 124.9, 125.9, 128.0, 128.9, 129.4, 129.5, 135.9, 136.1, 138.6, 139.3, 139.8, 140.9, 141.7, 141.8, 142.2, 143.0, 143.8, 143.9, 144.5, 144.6, 144.7, 144.8, 144.9, 145.10, 145.14, 145.19, 145.23, 153.9, 155.4, 161.0, 163.5, 165.0 (malonate C=O), 178.5 (flavanone C=O). MS (MALDI-TOF): m/z: 1461 $(M+H)^+$. HRMS-ESI m/z calcd for $C_{105}H_{57}O_9$ $(M+H)^+$ 1461.3997, found 1461.3966.

4.9. EPR and thermochemical measurements

Deoxygenated benzene solutions containing the title compounds (0.05 M) and di-*tert*-butyl peroxide (5% v/v) were sealed under nitrogen in a suprasil quartz EPR tube. The sample was inserted in the cavity of an EPR spectrometer, and photolyzed with the unfiltered light from a 500 W high pressure mercury lamp at room temperature. The EPR spectra were recorded on a spectrometer equipped with a microwave frequency counter for the determination of the g factors, which were corrected with respect to that of the perylene radical cation in concentrated H_2SO_4 (g=2.00258). The BDE values were determined by photolyzing concentrated solutions of the reference phenols and the title compounds in the presence of di-*tert*-butyl peroxide (5% v/v). The molar ratio of the two equilibrating radicals, obtained from the EPR spectra, was used to calculate the equilibrium constant, K_1. Different concentration ratios of starting phenols were used in order to check if the equilibrium was reached. Spectra were recorded few seconds after starting to irradiate in order to avoid significant consumption of the phenols during the course of the experiment. Relative radical concentrations were determined by comparison of the digitized experimental spectra with computer simulated ones as previously described.[56]

4.10. Autoxidation experiments

The rate constants for the reaction of the title compounds with peroxyl radicals were measured by following the autoxidation of cumene (7.1 M) or styrene (4.3 M) in chlorobenzene at 30 °C using as initiator AIBN (0.05 M). The reactions were performed in an oxygen uptake apparatus built in our laboratory and based on a differential pressure transducer. The entire apparatus was immersed in a thermostatted bath, which ensured a constant temperature within ±0.1 °C. In a typical experiment, an air-saturated solution of styrene or cumene in chlorobenzene containing AIBN was equilibrated with a reference solution of the same composition also containing an excess of the very strong antioxidant 2,2,5,7,8-pentamethyl-6-chromanol (1×10^{-4} M). In the cases of autoxidation conducted under reduced O_2 partial pressure, a gas mixture containing 1% O_2 and N_2 was bubbled into the reaction vessels for about 15 min. When constant oxygen consumption was reached, a small amount of the antioxidant in chlorobenzene was added to the sample and the oxygen consumption was measured from the differential pressure between the two channels recorded as function of time. This instrumental setting allowed us to have the N_2 production and the oxygen consumption derived from the azo-initiator decomposition already subtracted from the measured reaction rates.

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