Antioxidant properties and free radical-scavenging reactivity of a family of hydroxynaphthalenones and dihydroxyanthracenones

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Abstract—This study was undertaken to investigate the free radical-scavenging and antioxidant activities of various structurally related hydroquinones including hydroxynaphthalenones and dihydroxyanthracenones. Electron spin resonance spectroscopy and spin trapping techniques were used to evaluate the ability of hydroquinones to scavenge hydroxyl, diphenylpicrylhydrazyl, and galvinoxyl radicals. In addition, the oxygen radical absorbing capacity assay using fluorescein (ORAC-FL) was used to obtain the relative antioxidant capacity of these radicals. The rate constants of the first H atom abstraction by 2,2-diphenyl-2-picrylhydrazyl (k_2), were obtained under pseudo-first-order conditions. The free radical-scavenging activities and k_2 values discriminate well between hydroxynaphthalenones and dihydroxyanthracenones, showing that the latter have better antioxidant properties. The aforementioned experimental data agree with quantum-chemical results demonstrating the relevance of intramolecular H bonding to radical-scavenging activities.

1. Introduction

Reactions of free radicals and reactive oxygen species (ROS) with biological molecules in vivo play an important physiological role in many diseases such as cancer,[1,2] gastric ulcers,[3,4] Alzheimer's disease, arthritis, and ischemia–reperfusion tissue damage.[5] ROS are entities containing one or more reactive oxygen atoms including hydroxyl radical (·OH), superoxide anion radical ($O_2^{\cdot -}$), and hydrogen peroxide (H_2O_2). Their formation is an unavoidable consequence of respiration in aerobic organisms. These species are very unstable and react rapidly with other substances in the body, leading to cell or tissue injury.

Antioxidants are defined as substances that, when present at low concentrations compared with those of an oxidizable substrate, significantly delay or prevent oxidation of that substrate.[6] Small-molecule antioxidants can be present extra- and intracellularly. Antioxidants work by preventing the formation of new free radical species, by converting existing free radicals into less harmful molecules, and by preventing chain reactions.

Organic molecules such as phenolic compounds may stimulate or inhibit oxidative damage to biomolecules and are believed to behave as either antioxidants or pro-oxidants.[7–10] Although, after absorption into the bloodstream, phenolic compounds may undergo chemical modifications such as glycosylation, methylation, and glucuronidation, their availability and ability to exert biological activity remain.[9,10]

The cytotoxicity of phenols has been associated with their pro-oxidative activity, which can accelerate oxidative damage either to DNA or to proteins and carbohydrates, depending on the structure, dose, target molecule, and environment. This type of compound has been reported to have antiproliferative and cyto-

Abbreviations: ROS, reactive oxygen species; QH_2, p-hydroquinone(s); Q, quinone(s); $LO_2^{\cdot}$, lipoperoxy radical(s); $SQ^{\cdot}$, ubisemiquinone; ESR, electron spin resonance; AUC, area under the curve; ORAC-FL, oxygen radical absorbing capacity assay using fluorescein; DPPH, 2,2-diphenyl-2-picrylhydrazyl; BDE, bond dissociation enthalpy; HAT, H atom transfer.

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toxic properties in several tumor cell lines.[11–16] For example, some polyphenolic antioxidants exhibit dose-dependent toxicity against human promyelocytic leukemia cells (HL-60), and it has been suggested that their toxicity is related to their pro-oxidant character.[13] Moreover, inhibition of L1210 cancer cell growth has been proposed as a striking example of toxicity occurring via phenoxyl radicals.[17]

On the other hand, substituted *p*-hydroquinones (QH_2) are among the most potent chain-breaking antioxidants and are of special interest with respect to biomedical and food chemistry. *p*-hydroquinones are generally less thermodynamically stable than their oxidized form, quinones (Q). However, quinones can be effectively converted into QH_2 by several one- and two-electron mechanisms,[18,19] and hence, *p*-hydroquinones coexist with quinones in biological systems. The pronounced antioxidant activity of *p*-hydroquinones is determined by their ability to terminate radical chain reactions due to their reactivity with lipoperoxy radicals $LO_2^{\cdot}$.[20]

$$LO_2^{\cdot} + QH_2 \rightarrow LOOH + QH^{\cdot} \quad k_1 \qquad (1)$$

For some *p*-hydroquinones, antioxidant capacity has been reported previously in the oxidation of a model styrene[21] and in the oxidation of biologically relevant lipids in aqueous microheterogeneous systems.[22,23] The antioxidant capacity of ubiquinol (a *p*-hydroquinones) in peroxidizing lipid membranes demonstrated the existence of ubisemiquinone (SQ**.**) as the first reaction product of ubiquinol.[24]

It seems noteworthy that a phenolic aryl ketone group is a feature common to many of these biologically active compounds. In addition, we have demonstrated that compounds incorporating a carbonyl group at an *ortho* position with respect to a phenol group inhibit tumor cell respiration in the TA3 and multidrug-resistant TA3-MTX-R cell lines.[25]We have reported that 5,8-dihydroxy-4,4-dimethyl(4*H*)naphthalene-1-one (QH 3) and a series of derivatives inhibit mitochondrial respiration at low micromolar to sub-micromolar concentrations in the TA3 and TA3-MTX-R cell lines, suggesting that the phenoxyl radicals derived from these compounds remain inside the tumor cells at levels sufficient to inhibit oxygen uptake.[25]

Considering that alkylation of the hydroquinone moiety should stabilize the semiquinone free radical presumably involved in the inhibition of cellular respiration, we also tested 9,10-dihydroxy-4,4-dimethyl-5,8-dihydro-1(4*H*) anthracenone (QH 4), an analogous compound that incorporates a third ring into its molecular structure, blocking the free positions of the aromatic ring. The activities reported for QH 3 and QH 4 indicate that dialkylation of QH 3 raises its activity by a factor of 15 in the TA3 cell line and by 26-fold in the TA3-MTX-R subline. IC_{50} (μM) values for growth inhibition of the human U937 cell line show a similar trend: QH 3 (40.39) >QH 4 (7.96). The results obtained with these two compounds support the hypothesis that an increase in the stability of the free radical derived from the hydroquinone substrate, due to incorporation of a third ring **(C)**, leads to increased activity.

Some analogs of QH 4 were also tested against TA3 and TA3-MTX-R. Members of this series have exhibited antifungal activity against *Botrytis cinerea*.[25]

During the past few years, interest in the effect of intramolecular interactions on the reactivity of phenolic groups has increased considerably. Experimental studies have indicated that phenolic hydrogens involved in intramolecular H bonding are less reactive toward peroxyl radicals than free hydroxyl groups. It has also been demonstrated that their reactivity is less affected by H bond-accepting solvents, and that the stabilization of phenol is lost in the phenoxyl radical, so that the energy needed to abstract the hydrogen atom is greater than in non-H-bonded phenols.[26] Conjugated carbonyl groups would also be expected to increase oxidation potential,[27] thus hindering free radical formation by electron transfer.

In this article, we report the antioxidant capacity of two families of compounds, hydroxynaphthalenones and dihydroxyanthracenones. Two hypotheses were evaluated: First, phenolic hydrogens involved in intramolecular H bonding are less reactive toward free radical species than toward free hydroxyl groups. Second, an increase in the stability of the free radical derived from the hydroquinone substrate, due to incorporation of a third ring **(C)**, leads to increased antioxidant capacity. Electron spin resonance (ESR) spectroscopy, bleaching of the diphenylpicrylhydrazyl radical, and the oxygen radical absorbing capacity assay using fluorescein (ORAC-FL) were used to measure the hydrogen-donating ability of these families of compounds. The experimental results were correlated with the results of quantum-mechanical calculations.

2. Results and discussion

The free radical-scavenging capacity of a series of hydroquinones that inhibit oxygen uptake by the TA3 mouse carcinoma cell line and its multidrug-resistant variant TA3-MTX-R[25,28] has been evaluated through their direct scavenging activity against a variety of reactive oxygen and nitrogen species such as hydroxyl, peroxyl, galvinoxyl, and 2,2-diphenyl-2-picrylhydrazyl (DPPH) radicals. Hence, in the present study, we used the ORAC-FL and ESR spectroscopy, spin trapping techniques, to directly assess the mechanisms by which hydroquinones might display antioxidant capacity. In addition, kinetic information was obtained from the second-order rate constants under pseudo-first-order conditions.

DPPH and galvinoxyl are stable free radicals. They accept an electron or hydrogen radical to become stable diamagnetic molecules. In addition, DPPH and galvinoxyl are often used as substrates to evaluate the antioxidant capacity of an antioxidant (the unpaired electron is delocalized over N atoms and over O atoms, respec-

tively). In Figure 1 are the ESR spectra of DPPH treated with various concentrations of QH 6. Similar results were obtained for all compounds. QH 6 scavenged the DPPH radical by more than 93% at 2.0 mM. When galvinoxyl was used as a substrate to evaluate antioxidant capacity at various concentrations (data not shown), the behavior was the same as for the DPPH radical, indicating that the scavenging pattern in both cases was concentration dependent.

ESR results for the DPPH radical are illustrated in Figure 2, where it can be seen that 1.0 mM hydroquinones scavenged between 4.0 and 69.7% of the DPPH radical. Similarly, the compounds under study scavenged between 0.0 and 48.3% of the galvinoxyl radical. Hydroxyl radicals were generated by the Fenton reaction ($Fe^{2+} + H_2O_2$), and trapped by DMPO, forming spin adducts that could be detected with an ESR spectrometer, and the typical 1:2:2:1 ESR signal of the hydroxyl adduct (DMPO-OH) (see Fig. 3) was observed. After addition of hydroquinones, the decrease in the amount of DMPO–OH adduct was reflected in the ESR spectra (Fig. 3). ESR results demonstrated that at 0.6 mM, the hydroquinones scavenged between 47.5 and 72.0% of the hydroxyl radical.

All of the compounds in this study, hydroxynaphthalenones and dihydroxyanthracenones, proved to react with hydroxyl radicals and exhibited the same scavenging pattern observed for DPPH and galvinoxyl radicals. In addition, the differences observed in their scavenging activity only showed that the hydroxynaphthalenones QH 1–QH 3 are less reactive than the dihydroxyanthracenones QH 4–QH 6. In this sense, the ESR results, under our study conditions, provided us with qualitative information on the antioxidant capacity of these hydroquinones. Therefore, there seem to be few apparent differences in the ESR studies of these two groups of hydroquinones. We used other methods, such as ORAC-FL, kinetic studies, and O–H bond dissociation energy (BDE) calculations, to quantify and extract more information on the slight differences between these compounds.

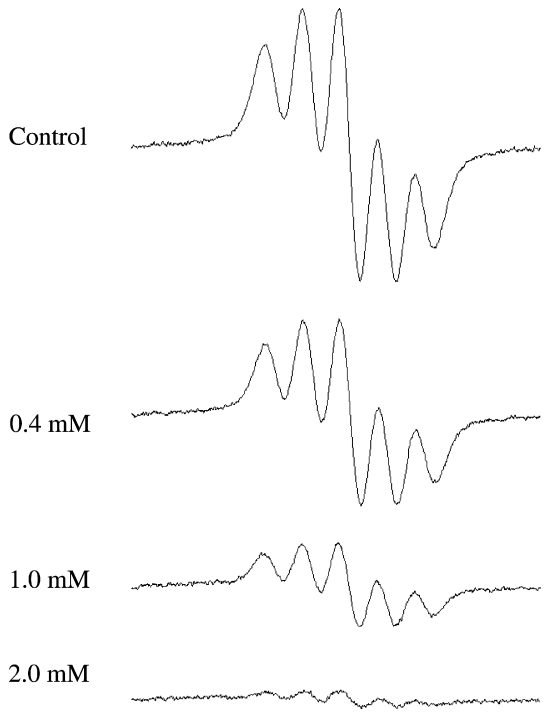


Figure 1. ESR spectra of DPPH in the absence and presence of QH 6.

On the other hand, in this study it was assumed that under our work conditions, the formation of semiquinone radical by oxidation of phenol groups with DPPH, galvinoxyl or hydroxyl radicals does not contribute to the ESR signal, because of decay by disproportionation, which has previously been described for the phenoxyl radical.[29]

Previous reports suggested that the electron-withdrawing character of the carbonyl group, whether *ortho* or *para*, could weaken the O–H bond and stabilize a hypothetical free radical generated. This effect should be maximal with the carbonyl group in a strictly coplanar relationship with the hydroxylated aromatic ring. As an additional factor, the intramolecular hydrogen bond to carbonyl also weakens the O–H bond, as do hydrogen bond-accepting solvents.[25,30,31] In contrast, when we compared ORAC-FL and k_2 values for QH 1 and QH 2, we observed that the –OH group, which forms an intramolecular hydrogen bond with the carbonyl group, is less reactive than the free hydroxyl group toward the free radicals studied. Furthermore, this is in agreement with our calculations, which indicate that the O–H BDE is higher for QH 1, 49.13 kcal/mol, than for QH 2, 34.54 kcal/mol. In this sense, the result for QH 1 strongly suggests that an intermolecular hydrogen bond between the hydroxyl group and the oxygen atom of the carbonyl group, in hydroxynaphthalenones and dihydroxyanthracenones, might not contribute significantly to their free radical-scavenging activity or antioxidant properties. On the other hand, the presence of an intramolecular hydrogen bond, determined by ^{1}H NMR spectroscopy,[32] could be an important factor in their antioxidant capacity (in Trolox equivalent) and k_2 values. Calculations of the optimized structures indicate that, first, the hydrogen bonds (forming six-membered rings) are shorter in the hydroxynaphthalenones and dihydroxyanthracenones, around 1.80 and 1.70 Å, respectively, and second, the O–H–O angle is about 136.0° and 138.0°, respectively.

The slight difference in antioxidant capacity and k_2 values (see Table 1) observed between QH 5 and QH 6 could be explained by the presence of another intramolecular hydrogen bond in QH 6 (forming a seven-membered ring, see Scheme 1). In accordance with previous NMR results,[32] the ^{1}H NMR spectra of QH 5 and QH 6 exhibited signals due to OH groups at 4.50 (s, 1 H, OH), 13.50 (s, 1 H, OH), 7.97 (s, 1 H, OH), and 13.16 (s, 1 H, OH), respectively, where the signal at 7.97 of QH 6 can be assigned to a weakly chelated proton. To determine if this conformation is stable, we performed an energy scan along a dihedral angle in the molecule. The dihedral angle (HOCC) was chosen

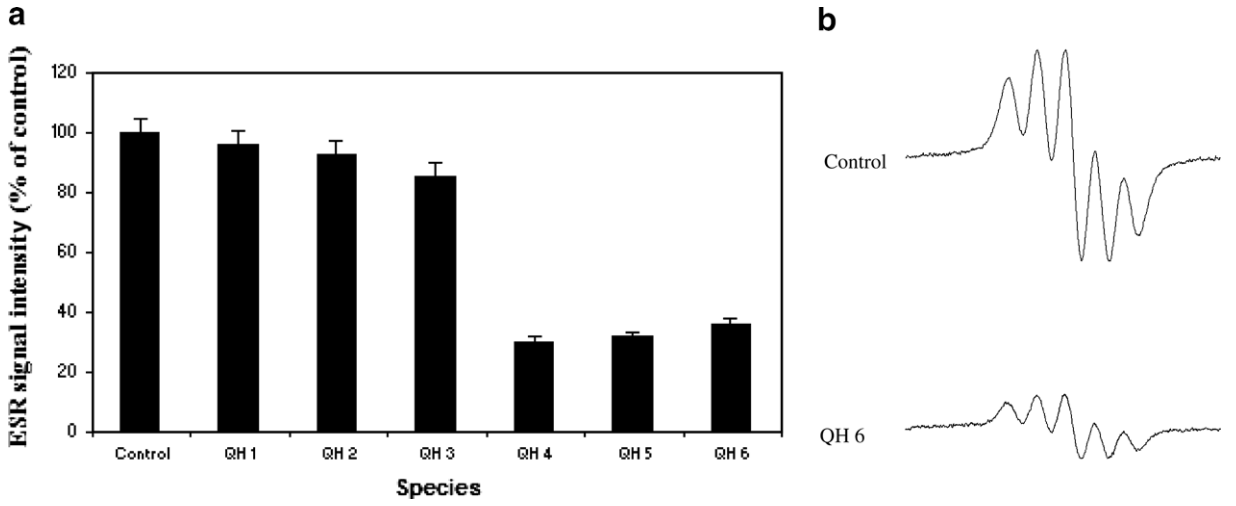


Figure 2. Scavenging effect of hydroxynaphthalenones or dihydroxyanthracenones on DPPH radicals. (a) The reaction mixture contained 1.0 mM DPPH in the presence or absence of 1.0 mM concentrations of the compounds under study. (b) Changes in radical signal intensity under the experimental conditions used in (a).

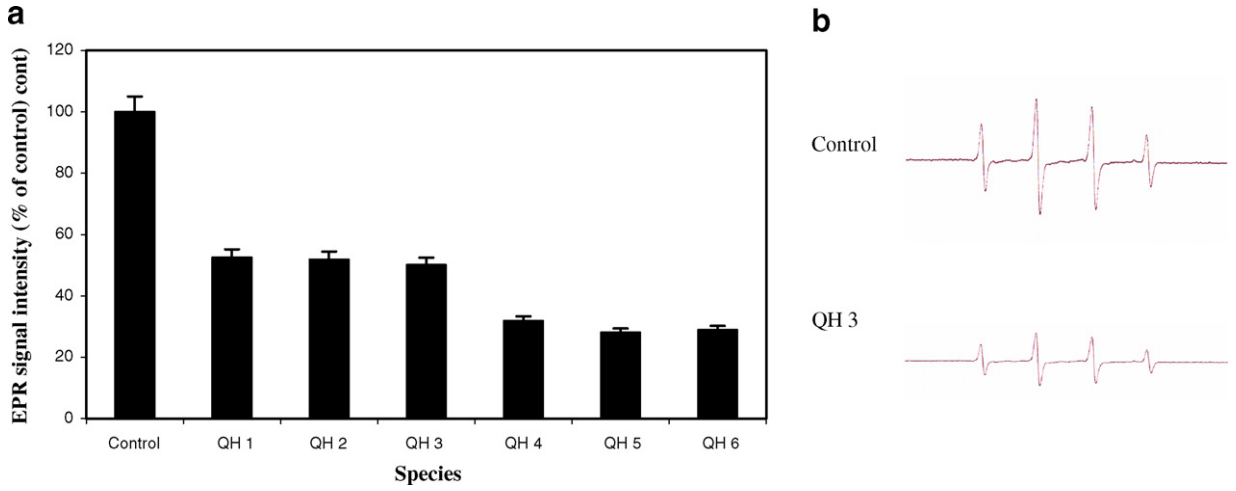


Figure 3. Scavenging effect of hydroxynaphthalenones or dihydroxyanthracenones on hydroxyl radicals. (a) The reaction mixture contained 1 mM Fe^{2+}, 1.0% H_2O_2, and 200 mM DMPO in the presence or absence of a 0.6 mM concentration of the compound under study. (b) Changes in radical signal intensity under the experimental conditions used in (a).

because of the possibility of its changing the conformation of the molecule from one with a second intramolecular hydrogen bond to one without it (Fig. 4). We found that the most stable conformation has a minimum stabilized by a new intramolecular hydrogen bond (seven-membered ring) that hypothetically should be weaker than the one forming a six-membered ring in the same molecule. This hypothesis agrees with the ^{1}H NMR data, which show this proton to be less chelated. Furthermore, in our conformational scan results, it can be seen that this conformation is the most stable and must overcome an energy barrier of approximately 12 kcal/mol, which corresponds to breakage of the intramolecular hydrogen bond forming a seven-membered ring.

Table 1. Hydrogen atom-donating ability, k_2, and antioxidant capacity (%) of scavenging of hydroxyl radical (ESR) and Trolox equivalent (ORAC-FL), for dihydroxynaphthalenones and dihydroxyanthracenones

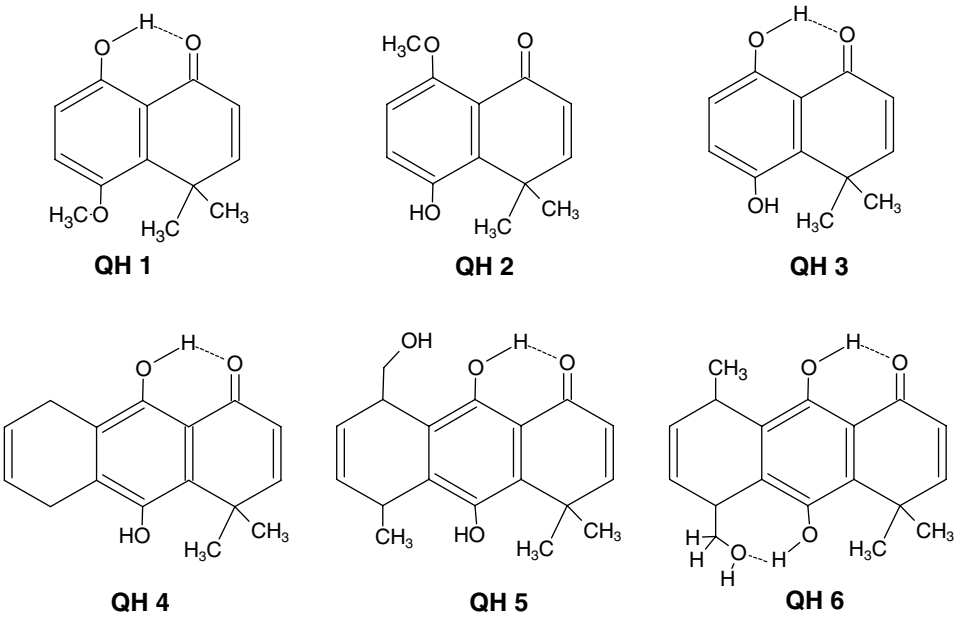
	k_2[a] (M^{-1} s^{-1})	Trolox equiv.[b]	·OH scavenging (%)[c]
QH 1	8.81 (±0.22)× 10^{-9}	1.56 (±0.15)	47.5
QH 2	2.70 (±0.09)× 10^{-6}	3.42 (±0.10)	48.2
QH 3	5.21 (±0.12)× 10^{-6}	3.76 (±0.12)	50.0
QH 4	1.81 (±0.09)× 10^{-5}	5.86 (±0.17)	68.2
QH 5	2.80 (±0.18)× 10^{-5}	6.41 (±0.12)	72.0
QH 6	1.52 (±0.13)× 10^{-5}	5.10 (±0.20)	71.3

[a] The H-atom-donating ability to the DPPH· radical in acetonitrile at 25 °C, with their respective SD.

[b] Expressed as μmol of Trolox equivalent/μmol of pure compound, with their respective SD.

[c] Scavenging conditions are the same as for Figure 1.

All the results demonstrated significant differences between the two families studied, hydroxynaphthalenones and dihydroxyanthracenones; for the latter, it was suggested that the presence of a third ring (**C**) increases their reactivity toward free radicals. O–H BDE values calculated using ab initio methodology



Scheme 1. Structures of hydroxynaphthalenones QH 1–3 and dihydroxyanthracenones QH 4–6.

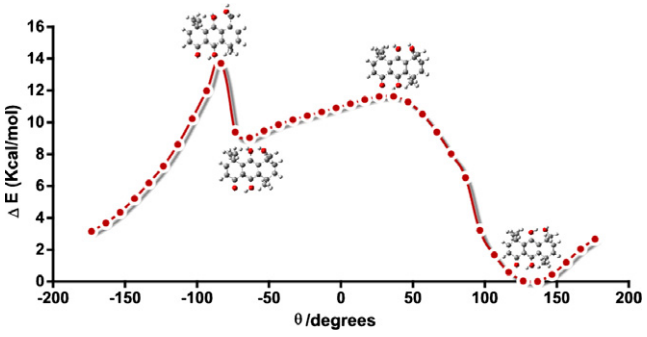


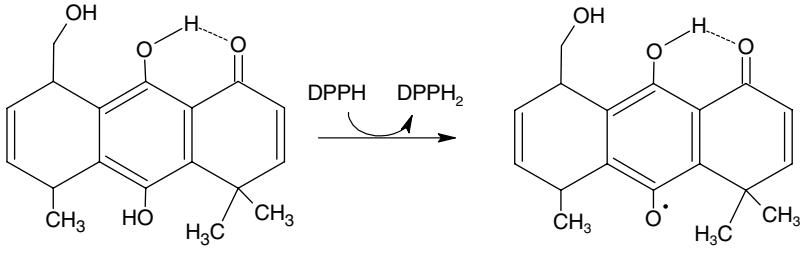
Figure 4. Conformational scan of compound QH 6.

demonstrated that the more effective H atom donor is the free phenolic group in ring B (Scheme 2), without an intramolecular hydrogen bond. To rationalize the H atom-donating ability of QH 4, QH 5, and QH 6, we performed quantum-chemical calculations. The theoretical results indicated that the energy needed to abstract a hydrogen atom from a H-bonded phenolic group is greater than that needed to abstract a hydrogen atom from a non-H-bonded phenolic group. Comparison of QH 5 and QH 6 demonstrated that the O–H BDE value in QH 6 is lower than that in QH 5 (Table 2), in agreement with the hypothetical intramolecular hydrogen bond (forming a seven-membered ring), which should stabilize the nonradical species, and explaining a slight difference in the antioxidant capacity (Table 1). When experimental k_2 values are listed from least active to most active, we obtain 1 <2 <3 <6 ≈4 <5, which, in general, is in agreement with the order of the calculated O–H BDEs. These results support a hydrogen atom transfer (HAT) mechanism as the rate-determining step for all reactions.

Table 2. Calculated homolytic bond dissociation enthalpies (BDEs) of O–H bonds and dihedral angle of the phenolic group of dihydroxyanthracenones, $T = 298.15$ K

	H_{ArOH}[a]	$H_{ArO\cdot}$[b]	O–H BDE (kcal/mol)	Dihedral angle HOCC[c]
QH 4	−839.128275	−838.570379	37.64	−0.250°
QH 5	−991.907255	−991.349352	37.64	35.065°
QH 6	−991.916368	−991.347722	44.39	−50.956°

[a] Sum of electronic and thermal enthalpies for parent molecules.
[b] Sum of electronic and thermal enthalpies for radicals formed after H atom abstraction.
[c] Dihedral angle HOCC of dihydroxyanthracenones.



Scheme 2. Radical scavenging process of QH 5.

3. Conclusion

The H bond-donating ability of these hydroquinones is a biologically important property, along with the capacity of these molecules to convert potentially damaging ROS (oxyl and peroxyl radicals) into nontoxic species. The formation of the semiquinone free radical is presumably involved in the inhibition of cellular respiration in tumor cells. As a first approach, the H atom-donating capacity of these hydroquinones can be conveniently assessed through free radical-scavenging activity (ESR), antioxidant capacity (ORAC-FL), and kinetics of DPPH quenching. Although the overall mechanism is complex, simple kinetic analysis readily gives access to the rate constant of the first H atom abstraction (k_2).

We have studied the effect of intramolecular interactions on the reactivity of phenolic groups, demonstrating experimentally that the phenolic hydrogens involved in intramolecular H bonds are less reactive toward hydroxyl, peroxyl, diphenylpicrylhydrazyl, and galvinoxyl radicals than toward free hydroxyl groups and have lower k_2 values, so that the energy needed to abstract a hydrogen atom is greater than in non-H-bonded phenols. Our results also indicate that the phenolic hydrogens involved in intramolecular H bonds may not contribute significantly to the free radical-scavenging activity or antioxidant properties of the compounds containing them.

The hydroxynaphthalenones proved to be less reactive toward hydroxyl, peroxyl, diphenylpicrylhydrazyl, and galvinoxyl radicals and have smaller k_2 values than the dihydroxyanthracenones, which is in agreement with the hypothesis that an increase in the stability of the free radical derived from the hydroquinone substrate leads to increased reactivity toward free radical species, where the presence of a third ring (**C**) would be an important factor. Our work supports a HAT mechanism as the rate-determining step for all reactions studied here.

4. Experimental

4.1. Chemicals

Hydroxynaphthalenones and dihydroxyanthracenones (hydroquinones) were synthesized according to described procedures.[25,32] 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), 2,2-diphenyl-2-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-α-(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-*p*-tolyoxyl (galvinoxyl), fluorescein (FL), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) were purchased from a commercial supplier (Sigma). Other chemicals and solvents used were of the highest analytical grade.

4.2. ESR experiments

Hydroxyl, DPPH, and galvinoxyl radicals were detected by ESR spectroscopy. ESR spectra were recorded in the X band (9.7 GHz) using a Bruker ECS 106 spectrometer with a rectangular cavity and 50-kHz field modulation. The reaction mixtures described below were introduced into a quartz capillary and ESR spectra were recorded over time. All experiments were performed at room temperature, and ESR signal intensity was calculated using the double integral of the ESR spectra. All ESR spectra were recorded 1 min after mixing the samples with each radical species.

4.2.1. Assay for DPPH radical. The DPPH radical-scavenging capacity of individual selected hydroxynaphthalenones and dihydroxyanthracenones was determined with an ESR spectrometry method.[33]Each hydroquinone solution was mixed with DPPH stock solution to initiate the antioxidant–radical reaction. All reaction mixtures contained 1.0 mM DPPH and 1.0 mM hydroquinones, and the control solution contained no antioxidant. Both DPPH and hydroquinone solutions were prepared in acetonitrile. ESR signals were recorded 1 min following the start of the reaction. Spectrometer conditions were: microwave frequency, 9.72 GHz; microwave power, 20 mW; modulation amplitude, 0.98 G; receiver gain, 59 db; and sweep time, 20.972 s.

The scavenging activity of each hydroquinone was estimated by comparing the DPPH signals in the antioxidant–radical reaction mixture and the control reaction at the same reaction time, and expressed as percentage DPPH remaining. The DPPH radical-scavenging rate of test compounds was calculated using the formula scavenging rate = $[(A_0 - A_x)/A_0] \times 100\%$, where A_x and A_0 are the double-integral ESR for the first line of samples in the presence and absence of test compounds, respectively.

4.2.2. Assay for galvinoxyl radical. The galvinoxyl radical-scavenging capacity of individual selected bicyclic hydroquinones was determined with an ESR spectrometry method. The hydroquinones (0.1 mM) were mixed with 0.5 mM galvinoxyl in all reaction mixtures, and the control solution contained no antioxidant. Both galvinoxyl and hydroquinone solutions were prepared in acetonitrile. ESR signals were recorded 1 min following the start of the reaction. Spectrometer conditions were the same as described above.

4.2.3. Assay for hydroxyl radical in the fenton system. Hydroxyl radical ($HO^{\bullet}$)-scavenging capacity of the hydroquinones was determined by ESR. The ESR assay was based on the competition between the trapping agent and the antioxidative hydroquinone. $HO^{\bullet}$ was generated by a Fenton reaction, and DMPO was used as the trapping agent. Acetonitrile was used as the solvent to dissolve individual hydroxynaphthalenones and dihydroxyanthracenones. The reaction mixture contained 50 μl of 1 mM freshly prepared $FeSO_4$, 50 μl of 200 mM DMPO, 20 μl of 0.5 mM H_2O_2, and 50 μl of hydroxynaphthalenone or dihydroxyanthracenone solution or solvent for the blank. The final concentration was 1 mM for all compounds under study. The ESR measurements were conducted 1 min after preparing each reaction mixture, at room temperature. Spectrom-

eter conditions were the same as described above, except for receiver gain, which was set at 30 dB.

4.3. UV–Vis assay for DPPH

The H-transfer reactions from an antioxidant to DPPH were monitored using a UV2 UNICAM UV–Vis spectrometer (optical path length, 1 cm). The temperature in the cell was kept at 25 °C by means of a thermostated bath. In a typical procedure, to 2 mL of a freshly prepared 2×10^{-4} M solution of DPPH in acetonitrile, placed in the spectrometer cell, was added 25–125 μL of a freshly prepared 1×10^{-3} M solution of the antioxidant in the same solvent. Spectra were recorded every 0.5 s over 1–2 min for the determination of rate constants.

4.3.1. Kinetic UV–Vis assay for DPPH. Second-order anti-radical kinetic determinations were obtained using DPPH and hydroquinones:[34]

$$-\frac{\mathrm{d[DPPH]}}{\mathrm{d}t} = K_2[\mathrm{DPPH}][\mathrm{QH}] \tag{2}$$

The second-order rate constant (k_2) was determined with the anti-radical compound [QH] in large excess as compared with the radical compound [DPPH], thus forcing the reaction to behave as first order in DPPH:

$$-\frac{D[\mathrm{DPPH}]}{\mathrm{d}t} = K_1[\mathrm{DPPH}] \tag{3}$$

where

$$K = K_2[\mathrm{QH}] \tag{4}$$

[QH] is assumed to remain constant throughout the reaction and can be modified to obtain different k_1 values. Therefore, DPPH was depleted from the medium under pseudo-first-order conditions following the equation

$$[\mathrm{DPPH}] = [\mathrm{DPPH}]_0 \mathrm{e}^{K_1 t} \tag{5}$$

where [DPPH] is the radical concentration at any time (t), $[\mathrm{DPPH}]_0$ is the radical concentration at time zero, and k_1 is the pseudo-first-order rate constant. This constant (k_1) is linearly dependent on the concentration of the antioxidant, and from the slope of these plots, k_2 is determined.[34]

Kinetic studies were conducted by measuring the disappearance of DPPH in acetonitrile at 515 nm under pseudo-first-order conditions at 25 °C. Determinations of k_1 were conducted in triplicate using different hydroquinone concentrations for each sample.

4.4. ORAC-FL

The assay was based on the procedure described by Dávalos et al.[35] Antioxidant curves (fluorescence vs time) were first normalized to the curve of the blank corresponding to the same assay by multiplying original data by the factor

$$\mathrm{fluorescence}_{\mathrm{blank},t=0}/\mathrm{fluorescence}_{\mathrm{sample},t=0}$$

From the normalized curves, the area under the fluorescence decay curve (AUC) was calculated as

$$AUC = 1 + \sum_{i=1}^{i=80} f_i/f_0$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The net AUC corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. Regression equations between net AUC and antioxidant concentration were calculated for all samples. ORAC-FL values were expressed as Trolox equivalent by using the standard curve calculated for each assay. Final results were in micromoles of Trolox equivalent/micromoles of pure compound for hydroquinones. In all cases, the ORAC-FL was conducted in triplicate. SD values are given in Table 1.

4.5. Calculation methods

The Gaussian 98 program[36] was used for calculations of homolytic BDEs and to scan the dihedral angle (HOCC) at the HF/6-31g level for the parent and radical species. For each optimized structure, a frequency analysis at the same level of theory was performed to verify that it corresponded to a stationary point on the potential energy surface. Employing the sum of electronic and thermal enthalpies (H) in the gas phase at 298.15 K, the BDE is equal to $H_r + H_h - H_p$ where H_r is the enthalpy of the radical generated after H atom abstraction, H_h is the enthalpy of the H atom (0.497912 Hartree), and H_p is the enthalpy of the parent molecule.

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