



Antioxidant Potential of Natural and Synthesised Polypropenylated Hydroquinones

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Abstract—The metabolites 2-octaprenyl-1,4-hydroquinone (**1**) and 2-(24-hydroxy)-octaprenyl-1,4-hydroquinone (**2**), isolated from the sponge *Ircinia spinosula*, along with a series of synthetic derivatives, were evaluated for their antioxidant capacity, in order to establish a potential relationship between structural characteristics and antioxidant activity. The antioxidant potential of both natural and synthesised compounds was evaluated in vitro by their ability: (1) to interact with the stable free 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and (2) to inhibit the peroxidation, induced by the Fe⁺⁺/ascorbate system, of heat inactivated hepatic microsomal membrane lipids. Metabolite **1** presented a strong interaction with DPPH and had a moderate effect on lipid peroxidation, while metabolite **2** interacted extensively with DPPH and exhibited a significant effect against lipid peroxidation. All derivatives retaining the free 1,4-hydroquinone system maintained fully or partly the free radical scavenging capacity. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

Oxidative damage is considered to contribute to atherosclerosis, cardiovascular diseases, and cancer, pathological conditions that are the major causes of mortality in industrialised countries. Certain metabolites derived from natural sources could play a preventive role due to their antioxidant properties. Within the context of our investigations towards the isolation of bioactive natural products with prospects for therapeutic use, we recently studied the black massive sponge *Ircinia spinosula* found in shallow Mediterranean marine ecosystems.

The extracts of several *Ircinia* species have been found to exert analgesic, muscle relaxant, anti-inflammatory, and anti-microbial effects.^{1–4} In particular, a number of biological activities of *I. spinosula* have been attributed to polypropenyl-benzoquinones and polypropenyl-hydroquinones,^{5–8} which constitute the majority of its chemical content. Among this class of metabolites, triterpenyl- and tetraterpenyl-hydroquinones are con-

sidered to be the most abundant representatives. Biological studies conducted on several polypropenyl-hydroquinones showed them: (1) to be effective inhibitors of phospholipase A₂;³ (2) to exert an interesting anti-inflammatory action through inhibition of 5-lipoxygenase activity;⁹ (3) to have a moderate anti-microbial action^{2,4} and (4) to act as general inhibitors of retroviral reverse transcriptases as well as of cellular DNA polymerases.¹⁰

In addition, terpenoid 1,4-benzoquinones, such as tocopherols, ubiquinones and plastoquinones, along with their reduced forms, often major metabolites in the plants, participate in electron transport, photosynthesis, and play an important role as endogenous cellular antioxidants.^{11,12}

In continuation of our studies on the biological activities of hydroquinones, intrigued by the aforementioned distinctive activities, we decided to evaluate the antioxidant potency of two natural hydroquinones and a series of synthesised derivatives, in an attempt to relate the structural features of the metabolites with the exerted activity (Fig. 1).

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Results

The metabolites 2-octaprenyl-1,4-hydroquinone (**1**) and 2-(24-hydroxy)-octaprenyl-1,4-hydroquinone (**2**) along with a series of oxidised, hydrogenated, and acetylated derivatives, afforded *via* chemical modifications, were evaluated for their antioxidant activity in order to establish the relationship between structural characteristics and their antioxidant potential.

In the assays employed, natural product **1** presented a strong interaction with DPPH (Fig. 2A) and a moderate effect on lipid peroxidation (Fig. 3A, Table 1). It was found that acetylation of the phenolic hydroxyl groups (**3**) as well as the combination of acetylation and hydrogenation of the octaprenyl side chain (**6**) resulted in significant reduction of its antioxidant activity (Fig. 2A, Table 1). On the other hand, derivatives **5** and **9**, that maintain the free aromatic hydroxyl groups and the semi-hydroquinone system, respectively, retain fully or partly their free radical scavenging activity (Fig. 2A).

Natural product **2** interacted extensively with DPPH (Fig. 2B) and exhibited a significant effect against lipid peroxidation with an IC_{50} of 220 μ M (Fig. 3B, Table 1). Its antioxidant action was greater than that of compound **1**. Similar to metabolite **1**, acetylation of the aromatic hydroxyl groups of compound **2** (**4** and **8**) resulted in a dramatic reduction of the antioxidant

activity (Fig. 2B, Table 1). The derivative that retained most of the lipid peroxidation activity (IC_{50} of 350 μ M) possessed a hydrogenated side chain, a free chain hydroxyl group and an acetylated aromatic ring (**7**), (Fig. 3C, Table 1). However, the same derivative (**7**) does not retain the initial DPPH activity exhibited by compound **2** (Fig. 2B). The oxidised derivative (**10**) exhibits a moderate DPPH activity (Fig. 2B) and is completely inactive on lipid peroxidation (Table 1).

Discussion

The results obtained from the DPPH as well as the lipid peroxidation assay employed to test the antioxidant activity of the hydroquinones, demonstrated that natural product **2** is the best antioxidant among the compounds tested (Figs 2 and 3, Table 1). Our results are analogous to earlier data that showed enhanced anti-inflammatory, anti-microbial and cytotoxic activity for the hydroquinones bearing an additional hydroxyl group on the side chain.^{3,4} In all above mentioned cases, the presence of the hydroxyl group on the side chain enhances remarkably the activity although no satisfactory explanation has yet been found. In an attempt to explain this, we have considered and estimated (by theoretical calculations) the lipophilicity of the molecules and the influence of the absence or presence of the hydroxyl group on the side chain. Although in general

Table 1. IC_{50} values of the different compounds tested for their lipid peroxidation activity

Compounds	IC_{50} (mM) ^a
1	0.99
2	0.22
7	0.35
9	0.99
3, 4, 5, 6, 8, 10	> > 1

^aValues are means of three independent experiments, which did not differ more than 2–8%, and are estimated after incubation of the reaction mixture at 37 °C for 45 min.

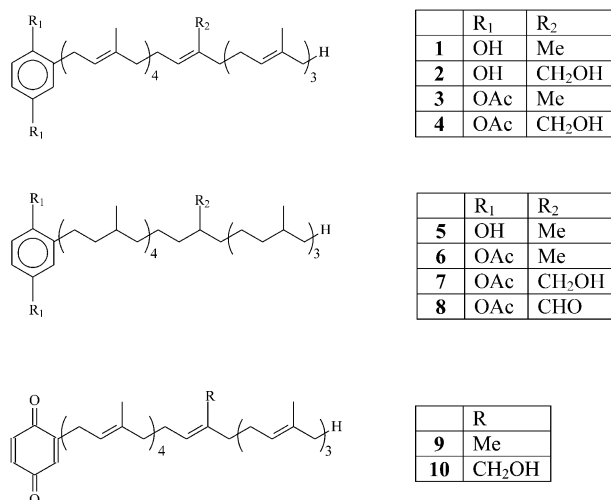


Figure 1. Chemical structures of the natural and synthesised hydroquinones.

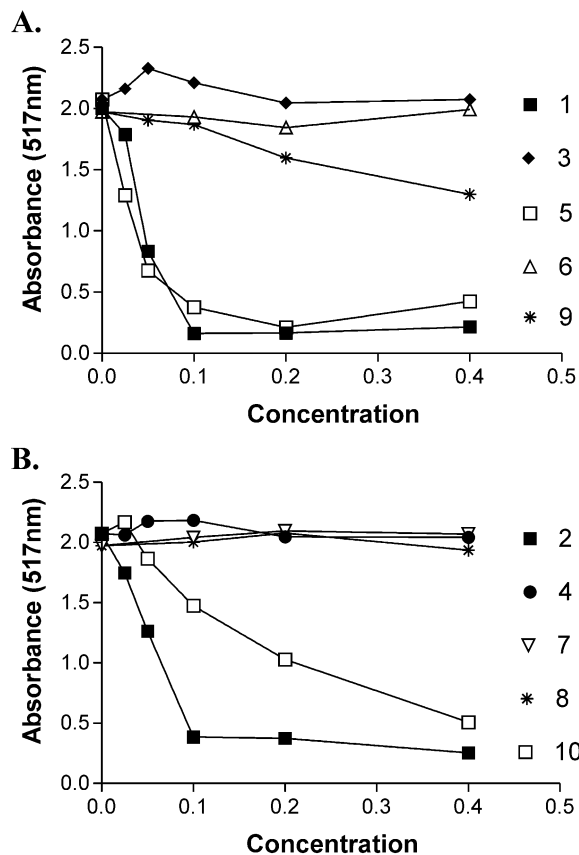


Figure 2. Interaction at 30 min of compounds **1** (A) and **2** (B) along with their derivatives, with 0.2 mM of the stable free radical DPPH (each value is the mean of three independent measurements, which did not differ more than 5–12%).

we cannot find any significant correlation of activity with the calculated lipophilicity of the molecules, perhaps this aspect is worth further exploring in a longer series of derivatives.

Acetylation of the phenolic hydroxyl groups resulted in reduction of the antioxidant activity of both natural compounds (Fig. 2, Table 1), exhibiting the same lack of action as in earlier studies regarding the anti-microbial action of polyprenylated hydroquinones and their synthetic derivatives.⁴

A series of anti-microbial and cytotoxic activity studies concerning the biological action of polyprenyl hydroquinones revealed that hydrogenation of the side chain double bonds enhanced the pharmacological potency of the molecules,^{2,4} while, we found that hydrogenation *per se* does not affect the antioxidant activity of the compounds tested, since derivative **5** exerts the same action as compound **1** (Fig. 2A).

Despite previous studies showing that quinone derivatives exert a better anti-microbial effect,^{2,4} the oxidised derivative (**10**) exhibits a moderate DPPH activity (Fig. 2B) and is completely inactive on lipid peroxidation (Table 1). Our results suggest that the necessary and sufficient condition for the interaction of the compounds with the stable free radical DPPH is the presence of the semi-hydroquinone system. These results also point to the involvement of the double bonds conjugation with the ring oxo-groups, to facilitate electron

delocalisation, exerting in that way radical scavenging capacity as has been suggested for flavonoids.¹³

Our findings also indicate that the combined presence of phenolic hydroxyls and the side-chain hydroxyl group enhances the protective activity against lipid peroxidation (**1**, **2**) (Figs 3A and 2B, Table 1). It is well known that p-hydroquinone systems participate in oxidoreductive cycles in the presence of $\text{Fe}^{++(+)}$. In particular, there are indications¹⁴ pointing to either the hydroquinone or semiquinone as the active intermediates in scavenging mechanisms involved in lipid peroxidation.

Hydroquinones are probably able to reduce highly oxidising free radicals by hydrogen atom donation. Some activity is also observed for the quinone system (**9**), which may be attributed to the above mentioned properties. Finally, although the activity of **7** is unexpected, it could be explained by partial hydrolysis of the acetoxy groups during the incubation period of the assay.

Conclusion

In addition to the significant antioxidant capacity of the investigated natural products, this study revealed some interesting structural requirements for this activity. From the results obtained, we can attribute the properties of the active compounds to the free 1,4-hydroquinone system in combination with the presence of a

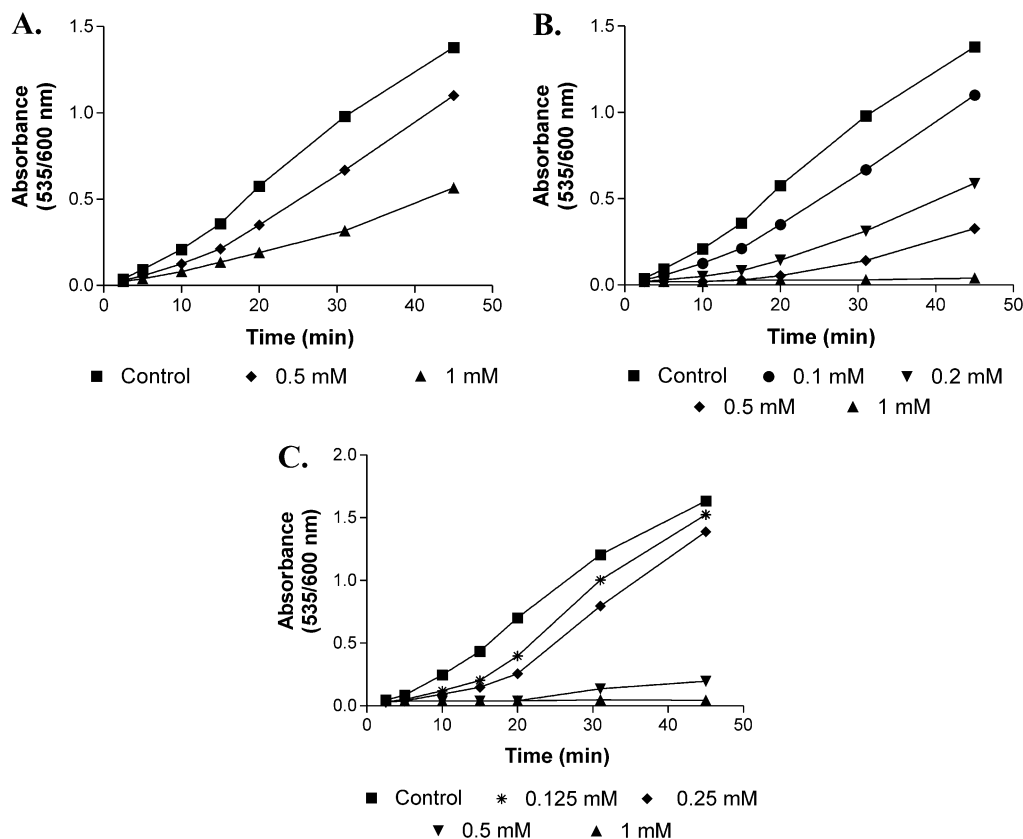


Figure 3. Representative graphs of the time course of lipid peroxidation as affected by various concentrations of compounds **1** (A), **2** (B) and **7** (C) (each value is the mean of three independent measurements, which did not differ more than 2–8%).

hydroxy group on the octaprenyl side chain as an enhancing moiety. It was found that changes in the prenyl chain result in important alterations of their antioxidant activity. These structural requirements promote the antioxidant potency of these compounds that may be potentially interesting antioxidant supplement candidates.

Experimental

Chemistry

General details. UV spectra were determined in spectroscopic grade C_6H_{14} on a Shimadzu UV model 160A. IR spectra were obtained using a Paragon 500 Perkin-Elmer spectrophotometer. 1H and ^{13}C NMR spectra were recorded using a Bruker AC 200 and DRX 400 spectrometers. Chemical shifts are given on a δ (ppm) scale using TMS as internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). High resolution FAB Mass Spectra data were recorded on a JEOL AX505HA Mass Selective Detector and were provided by the University of Notre Dame, Department of Chemistry and Biochemistry, Notre Dame, Indiana. Column chromatography was performed with Kieselgel 60 (Merk); TLCs were performed with Kieselgel 60 F₂₅₄ (Merk aluminum support plates).

Sponge material. *I. spinosula* was collected by Scuba (2–15 m) from Saronikos Gulf, Greece and kept frozen until analysed. A voucher specimen is deposited at the Herbarium of the Laboratory of Pharmacognosy (ATPH/MO/35).

Extraction and isolation. The organism was initially freeze dried (452.0 g) and then exhaustively extracted at room temperature with mixtures of CH_2Cl_2 /MeOH (3:1, v/v). The organic extract (21.3 g) after evaporation of the solvents was subjected to vacuum column chromatography using silica gel and a step gradient system ranging from 100% CH_2Cl_2 to 100% EtOAc. Metabolites **1** (4.28 g) and **2** (3.36 g) were isolated as oils from the non polar fractions, and their structural confirmation was based on previously reported spectral data.^{4,5}

Chemical modifications and spectral data. Hydroquinone derivatives were prepared by simple chemical manipulations, such as oxidation, acetylation of the hydroxyl groups, and hydrogenation of the chain double bonds (Fig. 1). Polyprenylated hydroquinones **1** and **2** were treated with Ac_2O in dry pyridine to afford the corresponding acetylated products, 2-octaprenyl-1,4-diacetoxy-benzene (**3**) and 2-[24-hydroxy]-octaprenyl-1,4-diacetoxy-benzene (**4**). Reduction of the metabolite **1**, as well as of the previously referred products **3** and **4**, with H_2 using 10% Pd/C as a catalyst produced the saturated analogues 2-octaisopentyl-1,4-hydroquinone (**5**), 2-octaisopentyl-1,4-diacetoxy-benzene (**6**) and 2-[24-hydroxy]-octaisopentyl-1,4-diacetoxy-benzene (**7**), respectively. Oxidation of **1**, **2** and **7** with CrO_3 in 70% acetic acid led to the formation of quinones 2-octaprenyl-1,4-quinone (**9**), 2-[24-hydroxy]-octaprenyl-1,4-qui-

none (**10**) and the aldehyde 2-[24-oxy]-octaisopentyl-1,4-diacetoxy-benzene (**8**), respectively. The structures of the derivatives were confirmed by their spectral characteristics.

Derivatives **3–5** and **10** were prepared as earlier described, and their spectral features were in accordance with previously reported data.⁴ Derivatives **4**, **7** and **8** are reported for first time and their spectroscopic data are shown below.

2-[24-hydroxy]-octaprenyl-1,4-diacetoxy-benzene (4). Ac_2O (200 μ L) was added to a solution of **2** (669.4 mg, 1.0 mmol) in pyridine (5 mL) in a molar ratio of 2.1:1, and the mixture was stirred overnight at room temperature. In the resulting mixture EtOAc and H_2O were added, and the organic layer was separated, washed with water, dried over anhydrous Na_2SO_4 , and concentrated in vacuo. The residue (706.2 mg) was chromatographed on silica gel (cyclohexane/EtOAc) to afford 538.7 mg of **4** (71% yield), as a colorless oil; IR (in $CHCl_3$): 3611, 2927, 1759, 1490, 1447, 1371, 1224, 1170, 1013 cm^{-1} ; UV (C_6H_{14}): λ_{max} (ϵ) = 267 (803) nm; 1H NMR ($CDCl_3$): δ 6.89–7.02 (3H, m), 5.28 (1H, t, J = 7.3 Hz), 5.20 (1H, t, J = 7.3 Hz), 5.09 (6H, m), 4.08 (2H, s), 3.20 (2H, d, J = 6.9 Hz), 2.27 (3H, s), 2.26 (3H, s), 1.98–2.11 (28H, m), 1.65 (6H, s), 1.57 (18H, br); ^{13}C NMR ($CDCl_3$): δ 169.4 (s), 169.3 (s), 148.2 (s), 146.3 (s), 138.3 (s), 137.6 (s), 135.4 (s), 135.2 (s, $\times 2$), 134.9 (s, $\times 2$), 134.4 (s), 131.3 (s), 128.6 (d), 124.9 (d), 124.3 (d), 124.2 (d), 124.0 (d), 123.9 (d, $\times 2$), 122.9 (d), 122.6 (d), 120.6 (d), 119.9 (d), 60.3 (t), 39.9 (t), 39.7 (t, $\times 4$), 35.2 (t, $\times 2$), 28.5 (t), 26.9 (t), 26.7 (t, $\times 2$), 26.6 (t, $\times 2$), 26.5 (t), 26.2 (t), 25.7 (q), 21.1 (q), 20.8 (q), 17.7 (q), 16.2 (q), 16.0 (q).

2-[24-hydroxy]-octaisopentyl-1,4-diacetoxy-benzene (7). A solution of **4** (480.6 mg, 0.64 mmol) in EtOH (10 mL) was hydrogenated using 10% Pd/C under H_2 atmospheric pressure. The mixture was stirred overnight at 50 °C. Then the catalyst was removed by filtration, and the solvent was evaporated to give 489.8 mg of pure **7** (99% yield), as a colorless oil; IR (in $CHCl_3$): 3660, 2928, 1759, 1463, 1371, 1220, 1209, 1171 cm^{-1} ; UV (C_6H_{14}): λ_{max} (ϵ) = 267 (1046) nm; FABHRMS m/z 771.6874 [$M + H$]⁺ (calcd. for $C_{50}H_{90}O_5$ 770.6792); m/z (% rel. int.) 123 (100), 165 (78), 207 (9), 263 (6), 333 (4), 403 (2), 473 (1) 557 (1), 668 (20), 686 (6), 711 (3), 728 (14), 753 (1); 1H NMR ($CDCl_3$): δ 6.89–7.02 (3H, m), 3.52 (2H, d, J = 4.75), 2.44 (2H, m), 2.29 (3H, s), 2.26 (3H, s), 1.05–1.43 (52H, m), 0.86 (3H, d, J = 6.5 Hz), 0.84 (18H, br), 0.81 (3H, d, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$): δ 169.4 (s), 169.3 (s), 148.2 (s), 146.2 (s), 136.2 (s), 123.0 (d, $\times 2$), 122.8 (d, $\times 4$), 119.7 (d, $\times 2$), 65.7 (t), 39.3 (t), 37.4 (t, $\times 4$), 37.3 (t), 37.2 (t), 37.1 (t), 32.8 (t, $\times 4$), 32.7 (t, $\times 3$), 28.0 (t), 27.8 (t), 24.8 (t, $\times 2$), 24.5 (t, $\times 2$), 24.4 (t), 24.3 (t), 22.7 (q), 22.6 (q), 21.1 (q), 20.9 (q), 19.7 (q, $\times 4$), 19.6 (q), 19.5 (q).

2-[24-oxy]-octaisopentyl-1,4-diacetoxy-benzene (8). A quantity of **7** (360.2 mg, 0.47 mmol), dissolved in acetone (3 mL) was added to a solution of CrO_3 (100 mg, 1.0 mmol) in 70% HOAc (10 mL). The resulting mix-

ture was stirred at 50 °C overnight, and after completion of the reaction CH_2Cl_2 and H_2O were added, the organic layer was separated, washed with water, dried over anhydrous Na_2SO_4 , and concentrated in vacuo. The residue (358.9 mg) was chromatographed on silica gel (cyclohexane/EtOAc) to afford 67.8 mg of **8** (21% yield) as a colorless oil; (**8**): IR (in CHCl_3): 3660, 2928, 1759, 1491, 1463, 1371, 1232, 1199, 1171 cm^{-1} ; UV (C_6H_{14}): λ_{max} (ϵ) = 271 (535) nm; FABHRMS: m/z 769.6673 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{50}\text{H}_{88}\text{O}_5$ 768.66354); m/z (% rel. int.) 123 (100), 165 (86), 207 (11), 263 (5), 333 (4), 403 (2), 473 (1), 557 (1), 755 (5); ^1H NMR (CDCl_3): δ 9.53 (1H, d, $J = 3.4$), 6.87–7.02 (3H, m), 2.46 (2H, m), 2.29 (3H, s), 2.26 (3H, s), 1.05–1.46 (52H, m), 0.86 (3H, d, $J = 6.5$ Hz), 0.83, (18H, br), 0.81 (3H, d, $J = 6.5$ Hz); ^{13}C NMR (CDCl_3): δ 189.2 (d), 169.4 (s), 169.3 (s), 148.2 (s), 146.2 (s), 136.2 (s), 123.0 (d, $\times 2$), 122.8 (d, $\times 3$), 119.7 (d, $\times 2$), 39.4 (t), 37.5 (t, $\times 2$), 37.4 (t, $\times 2$), 37.3 (t), 37.2 (t), 37.1 (t), 32.8 (t, $\times 5$), 32.6 (t, $\times 2$), 28.0 (t), 27.8 (t), 24.8 (t), 24.7 (t), 24.6 (t), 24.5 (t, $\times 3$), 22.7 (q), 22.6 (q), 21.1 (q), 20.9 (q), 19.7 (q, $\times 3$), 19.6 (q, $\times 2$), 19.5 (q).

Activity

Interaction of the test compounds with the stable radical DPPH. The test compounds were dissolved in absolute ethanol [analytical grade, iron content was less than $10^{-5}\%$ (w/v)], added to an equal volume of an ethanolic solution of DPPH (final concentration 0.2 mmol/L) at various concentrations (0.1, 0.2 and 0.4 mmol/L) and kept at room temperature ($22 \pm 2^\circ\text{C}$). Absorbance (517 nm) was recorded at 45 min.¹⁵ DPPH was purchased from Aldrich-Chemie (Steinheim, Germany).

In vitro lipid peroxidation. Hepatic microsomal fraction from untreated female Fischer-344 rats (180–220 g) was prepared as described earlier.¹⁶ The incubation mixture contained heat inactivated (90 °C for 90 s) hepatic microsomal fraction, corresponding to 2.5 mg protein/mL (final concentration) or 4 mmol/L fatty acid residues,¹⁷ ascorbic acid (0.2 mmol/L) in Tris–HCl/KCl buffer (50 mmol/L/150 mmol/L, pH 7.4) and various concentrations (1–0.05 mmol/L) of the test compounds dissolved in dimethylsulphoxide (DMSO) or DMSO alone (Control). The reaction was started by the addition of a freshly prepared FeSO_4 solution (10 $\mu\text{mol/L}$), and the mixture was incubated at 37 °C for 45 min.

Aliquots (0.3 mL) from the incubation mixture were taken at various time intervals. Lipid peroxidation was assessed spectrophotometrically (535 against 600 nm) by the determination of the 2-thiobarbituric acid (TBA) reactive material.¹⁸ Under the above experimental conditions, all compounds, as well as DMSO, were tested and found not to interfere with the assay. TBA was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

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