Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 229–235

BIOORGANIC & MEDICINAL CHEMISTRY

New Bis-Catechols 5-Lipoxygenase Inhibitors

Romain Dupont,[a] Jean-François Goossens,[b] Nicole Cotelle,[a] Laurence Vrielynck,[c] Hervé Vezin,[a] Jean-Pierre Hénichart[b] and Philippe Cotelle[a,*]

[a]*Laboratoire de Chimie Organique et Macromoléculaire, UPRESA CNRS 8009, USTL, 59655 Villeneuve d'Ascq, France*
[b]*Institut de Chimie Pharmaceutique Albert Lespagnol, Université de Lille 2, EA 2692, 3 rue J. Laguesse, 59006 Lille, France*
[c]*Laboratoire de Spectrochimie Infra-Rouge et Raman, UMR CNRS 8516, USTL, 59655 Villeneuve d'Ascq, France*

Received 29 May 2000; accepted 24 August 2000

Abstract—Three polyhydroxy-2-phenylnaphthalenes (**1**–**3**) and the oxy analogue of tetrahydroxypavinan (**4**) were prepared and evaluated for their antioxidant properties (inhibition of diphenylpycrylhydrazyl radical (DPPH), reduction of iron (III) ion) and inhibition of 5-lipoxygenase (5-LO) activity. Their three-dimensional structures were established on the basis of spectroscopic data and semi-empirical calculations. Compounds **1** and **2** were found as potent 5-LO inhibitors as nordihydroguaiaretic acid (NDGA), whereas **4** is 2.5 times less potent than NDGA. The reliability of the 3-D structures with the 5-LO inhibition properties is discussed. Their antioxidant properties show that tested compounds are expected to act as redox inhibitors.

Introduction

Leukotrienes are potent mediators of inflammation derived from arachidonic acid through the action of 5-lipoxygenase enzyme (5-LO).[1] The peptidoleukotrienes, LTC_4,LTD_4 and LTE_4, are powerful spasmogens, which have been implicated in the inflammation and allergy processes.[2] Thus, the control of leukotriene biosynthesis through the inhibition of 5-LO represents a potential new method for the treatment of diseases such as asthma or rheumatoid arthritis.[3] Among the known inhibitors of the 5-LO are a variety of polyhydroxylated natural products such as caffeic acid,[4] NDGA[5] and flavonoids[6] (Scheme 1). The rational design of new drugs requires the maximum of knowledge about the target itself (structure, role, mechanism of the 5-LO) and the action mechanism of the known inhibitors.[7] In the case of 5-LO, answers to most of the fundamental structural and mechanistic questions have remained elusive due to the lack of an atomic resolution model. Most of our knowledge about lipoxygenases is based upon studies on soybean lipoxygenase-1 closely related to mammalian lipoxygenases.[8–10]

In our effort to synthesise polyhydroxylated natural products, we have prepared a series of naphthalenic derivatives[11–13] and a polycyclic compound, which is the oxy analogue of tetrahydroxypavinan[14] (Scheme 1). These molecules possess rigid or semi-rigid three-dimensional structures, which will be demonstrated in this paper. They have been tested for their ability to inhibit the 5-lipoxygenase in order to draw new insights into the flexibility of the natural substrate, arachidonic acid, for its access to the iron (III) ion in the active site of the enzyme. We also present the reactivities of our molecules with iron (III) ion, DPPH radical and their ability to give stable radicals under aqueous basic conditions. Our compounds were compared to NDGA, a well-known lipoxygenase inhibitor.

Results

Chemistry

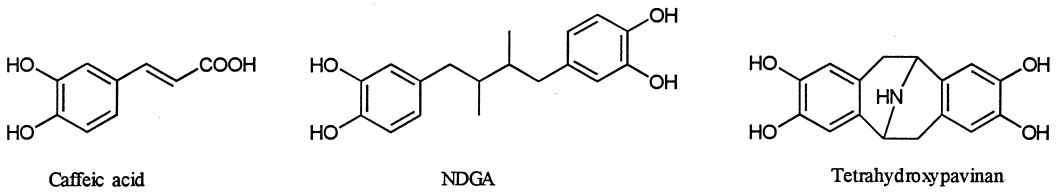
Compounds **1**–**4** (Scheme 2) were obtained by treatment of arylethanals and arylacetones with BBr_3 or HCl–dioxane. Compound **1**[12] was obtained in a two-step procedure from 3,4-dimethoxyphenylethanal, i.e. reaction with HCl in dioxane to promote the formation of the naphthyl nucleus followed by a demethylation with BBr_3. Compounds **2** and **3** were obtained directly by treatment of 3,4-dimethoxy- or 4-methoxyphenylacetone with BBr_3 in 77 and 83% yield respectively.[11–13] Compound **4** was obtained in 78% yield from 3,4-dimethoxyphenylethanal and BBr_3.[12]

5-LO inhibition

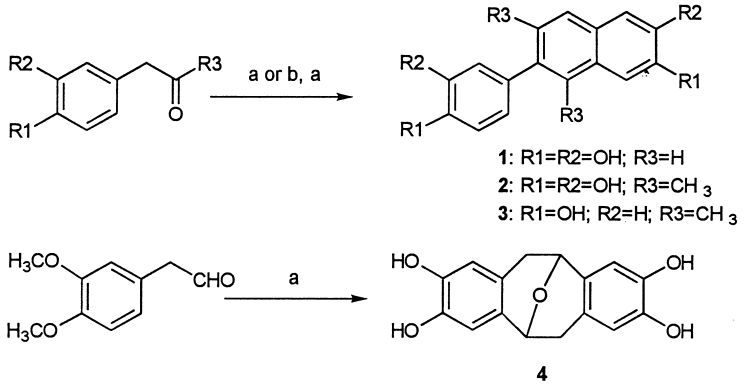
The 5-LO activity was determined by using a rat basophilic leukaemia cell line (RBL-1 cells). The 5-LO product

*Corresponding author. Tel.: +33-(0)320337231; fax: +33-(0)320336309; e-mail: philippe.cotelle@univ-lillel.fr

0968-0896/01/$ - see front matter
PII: S0968-0896(00)00258-3



Scheme 1.



a. BBr_3, CH_2Cl_2, RT, 1h; b. HCl, dioxane

Scheme 2.

5-hydroxyeicosatetraenoic acid (5-HETE) was quantified by HPLC analysis on Chromspher C18 column. Detection of 5-HETE was carried out by UV absorbance at 230 nm.[15] The results are reported in Table 1.

Inhibition of DPPH

The antioxidant activities of the tested compounds were measured using the classical DPPH method.[16] Results are given in Table 1.

EPR spectroscopy

Since the radical scavenging properties are associated with the capability of an antioxidant to give a stable radical, the autoxidation of the compounds under basic conditions has been undertaken. As expected, the autoxidation of **1**, **2** and **4** in aqueous basic conditions gives the stable primary radicals (Fig. 1). The hyperfine constants are given in Scheme 3. The spectra were simulated according to Janzen.[17]

Iron-reducing activity

The capacity of the compounds to reduce Fe (III) was assessed by a modified ferrozine (3-(2-pyridyl)-5,6-bis-(4-phenylsulfonic acid)-1,2,4-triazine) method.[18] Results are given in % of reduction of Fe (III) at two pH values and are reported in Table 1.

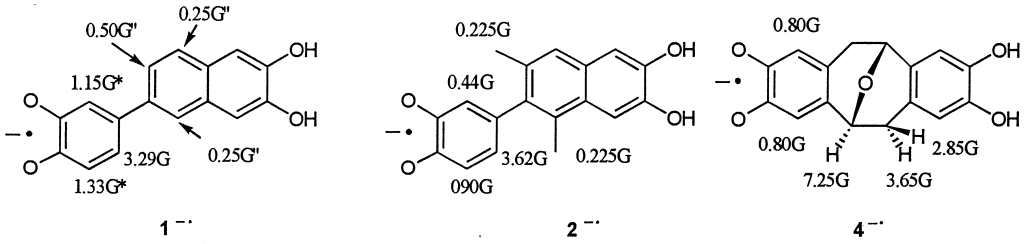
Three-dimensional structures of 1, 2, 4 and NDGA

Theoretical quantum chemical calculations have been investigated and correlated with several structural and spectroscopic properties of the tested compounds. Three-dimensional structures of **1**, **2**, **4** and NDGA have been calculated using the semi-empirical method AM1 at the Hartree–Fock level with RHF formalism. The geometry was fully optimised starting without any geometry constraints. Some of the calculated angles can be obtained by Karplus calculations[19,20] of the dihedral angles from the ^3J and ^2J coupling constants (Table 2). Similarly, the hyperfine constants of the methylene protons

Table 1. 5-LO and DPPH inhibitions and Fe (III) reduction of compounds **1**–**4** and NDGA

Tested compound	5-LO inhibition	DPPH inhibition		Iron (III) reduction (in%)	
	IC_{50} (μM)[a]	EC_{50} (μM)	Log Z[b]	pH = 2.0	pH = 4.2
1	0.95±0.05	17.5	3.32	51	72
2	0.80±0.01	7.0	3.62	19	40
3	40.0±0.1	430	1.64	25	38
4	2.50±0.03	13.7	3.22	62	57
NDGA	1.00±0.01	7.7	3.74	72	60

[a]IC_{50} values are based on tests at seven concentrations; each in triplicate twice.
[b]The rate constants k (obtained from plots of 1/[C] versus time) were plotted against the ratio [antioxidant]/[DPPH]. Linear regression ($r^2 > 0.9$) gave the parameter Z (slope of the line in L mol^{-1} s^{-1}).



Scheme 3. * and ″ denote that the attributions can be inverted.

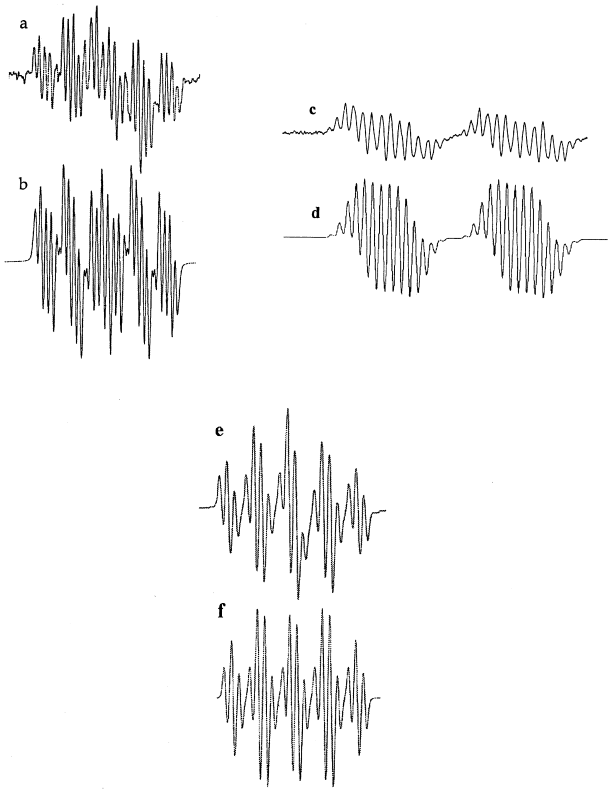


Figure 1. a, c, e: EPR spectra obtained from 250 µl of an aerated solution of **1**, **2** and **4** respectively (1.6×10^{-3} M in DMSO/H_2O 50/50) and 50 µl of 0.1 M NaOH solution. b, d, f: Simulated spectra using a line width of 0.4 G.

may give information about the dihedral angle between the orbital of the unpaired electron (orthogonal to the aromatic ring) and the C−H bond of the methylene group using the usual $B\cos^2\theta$ relationship with B = 9.64 G according to Felix and Sealy.[21] Calculated dihedral angles are given in Table 2. Homonuclear Overhauser experiments (Table 3) confirm that **4** also adopts a folded structure in solution.

Table 2. Some calculated dihedral angles of **4**

Coupling constants	Calculated dihedral angles from the experimental coupling constants	Calculated dihedral angles from semi-empirical method AM1
$^3J_{(H2H3)} = 5.6$ Hz	≈40°[a]	36°
$^3J_{(H2H4)} = 0$ Hz	≈80°[a]	81°
$^2J_{(H3H4)} = 15.8$ Hz	≈30°[b]	22°
2 85 G	≈65°[c]	48°
3.65 G	≈75°[c]	70°
7.25 G	≈40°[c]	43.5°

[a]Calculated dihedral angles using the Karplus equation.[18]
[b]Calculated dihedral angle using the Karplus equation.[19]
[c]Calculated dihedral angles using the Felix and Sealy equation.[21]

Discussion

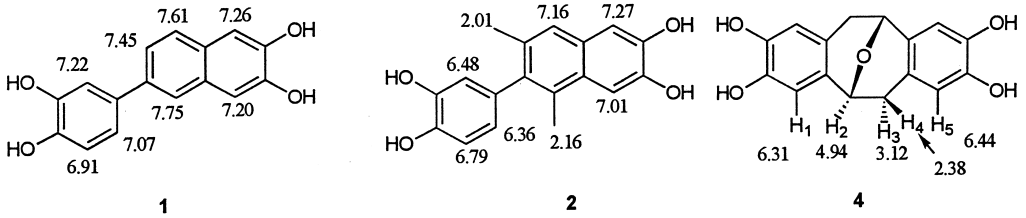
The AM1 semi-empirical calculations show that compounds **1** and **2** present a preferred 3-D structure where the naphthyl and the phenyl rings form an angle of about 40° and 80° respectively. These results are confirmed by the observed strong shielding (0.7 ppm) for the ^{1}H NMR chemical shift of the H2′ and H6′ from **1** to **2** (in DMSO-d_6) (Scheme 4). This shielding suggests that H2′ and H6′ are near the ring current of the naphthyl ring due to a larger twist between the naphthyl and the phenyl rings in the case of **2**. The comparison of the hyperfine constants obtained from the EPR spectra of **1** and **2** also shows a notable difference between **1** and **2** which can be attributed to the presence of the two methyl groups. Calculated 3-D structure of **4** shows an angle of about 90° between the two aryl rings. This result is in accordance with the X-ray crystallographic data of the unsubstituted tetracycle.[22] Furthermore, the 3-D structure of **4** in solution was confirmed by the calculations of some angles from the NMR and EPR coupling constant data and by homonuclear Overhauser experiments. As expected, the semi-empirical calculations predict the staggered conformation as the structure of lowest potential energy for NDGA where the aryl rings are parallel, the methyl groups are antiperiplanar as well as the (3,4-dihydroxyphenyl)methyl groups. However, the energy barriers are low enough to consider that NDGA is a flexible molecule.

The stable free radical DPPH is a useful reagent to investigate the radical scavenging activity of phenols or catechols. The first step of such a reaction mechanism involved the abstraction of a hydrogen atom from a phenol donor to give the hydrazine and a phenoxyl radical. The phenoxyl radical may undergo further reactions such as coupling, fragmentation and addition, which may affect the reliability of the EC_{50} values. Indeed, the traditional EC_{50} parameter describes only the global reactivity of a compound towards DPPH. Consequently, we calculated the kinetic parameter log Z, which should be influenced only by the hydrogen abstraction mechanism. As expected, the bis-catechols **1**, **2**, **4** and NDGA give high log Z values (> 3) whereas compound **3** gives a low log Z value (< 2), which indi-

Table 3. Homonuclear Overhauser effects on **4**

Irradiated signal	Corresponding proton	Homonuclear Overhauser effects (in % of enhancement of the signal)									
		H1	dH1Hx[a]	H2	dH2Hx	H3	dH3Hx	H4	dH4Hx	H5	dH5Hx
4.94 ppm	H2	12%	2.57 Å	—	—	28%	2.37 Å	8%	2.67 Å	0%	4.87 Å
3.12 ppm	H3	1.5%	3.87 Å	7%	2.37 Å	—	—	42%	1.82 Å	4%	2.86 Å
2.38 ppm	H4	7%	2.71 Å	7%	2.67 Å	29%	1.82 Å	—	—	10%	2.59 Å

[a]Distance between the atom which presents an enhancement of its signal and the irradiated proton.



Scheme 4.

cated that the phenoxyl radical of **3** is less stable than the semiquinonic radicals of **1**, **2**, **4** or NDGA. The difference between the EC_{50} values of **1**, **2**, **4** and NDGA show that the two catechol units are totally independent in **2** (twisted angle $>45°$) and NDGA (separated by Csp3), but strongly dependent in **1** and **4**. This dependence is expected to be due mainly to conjugation in the case of **1** (twisted angle $<45°$) and spatial proximity in the case of **4**. These results are in accordance with the calculated angles between the phenyl and the naphthyl nucleus in **1** and **2**.

The EPR spectra of the radicals formed by autoxidation of **1**, **2** and **4** in mild basic aerated solution revealed a great stability of the primary semiquinonic radical. In the case of **1** and **2**, the radical is formed on the phenyl ring. This result is in accordance with previous work[23] on the autoxidation of 2,3-dihydroxynaphthalene.

The reduction of Fe (III) by the tested compounds was monitored using ferrozine, a reagent that avidly binds Fe (II), forming a complex with a very high extinction coefficient at 562 nm. Using this technique, **1**, **2**, **3**, **4** and NDGA were found to reduce Fe (III) rapidly at pH = 2 and 4.2 but very slowly at pH = 7.8. This reactivity towards iron (III) ions at low pH is interesting since it is known[7] that the behaviour of ferric soybean lipoxygenase-1 towards catechols is similar to that of Fe (III) in acidic aqueous solution (pH = 1–2).

From numerous articles[24] on lipoxygenases (and notably 3-D structure of soybean lipoxygenase)[20] we know that: (i) lipoxygenases contain one atom of iron per molecule; (ii) the iron atom shows an octahedral symmetry; (iii) the known ligands of iron are three His (Nε1) and one Ile (O1); (iv) two cavities are connected to the iron atom (one for the approach of molecular oxygen, the other for the approach of arachidonic acid). Inhibitors of 5-lipoxygenase can be classified under three headings:[25] redox inhibitors, iron–ligand inhibitors and non-redox inhibitors. Our results show that **1**, **2**, **4** and NDGA are potent Fe (III) reductors, inhibit efficiently free radicals such as DPPH and give very stable semiquinonic radicals. Consequently it was not surprising that **1**, **2**, **4** and NDGA are potent redox inhibitors of 5-lipoxygenase. Nevertheless, it was unexpected that there was no significant difference between **1**, **2** and NDGA on one hand and **4** on the other hand. The decrease of the number of free rotation degrees (five between the two catechol units in NDGA, one between the naphthyl and the phenyl rings in **1** and **2**) does not alter the 5-lipoxygenase inhibition, whereas the totally rigid and bulkiest molecule **4** is only 2.5 times less active than NDGA. This result suggests that the cavity devoted to the approach of arachidonic acid is sufficiently flexible to accept bulky inhibitors such as **4**.

We are currently attempting to synthesise analogues of **2** where two hydroxyl groups are absent, i.e. 5,7-dimethyl-6-phenylnaphthalene-2,3-diol and 2-(3,4-dihydroxyphenyl)-1,3-dimethylnaphthalene in order to determine if the two catechol moieties are necessary in the 5-LO inhibition and which catechol moiety gives the best result.

Experimental

Chemistry

Compound **1**[26] was obtained as previously described by us.[12] Compounds **2** and **3** were obtained directly by treatment of 3,4-dimethoxy- or 4-methoxyphenylacetone with BBr_3 in 77 and 83% yield respectively.[11–13] Compound **4** was obtained in 78% yield from 3,4-dimethoxyphenylethanal and BBr_3.[12]

4: mp = 270 °C (acetone); ^{1}H NMR (DMSO-d_6): 2.38 (1H, d, $^2J = 15.8$ Hz, $^3J = 0$ Hz), 3.12 (1H, dd, $^2J = 15.8$ Hz, $^3J = 5.6$ Hz), 4.94 (1H, d, $^3J = 5.6$ Hz, $^3J = 0$ Hz),

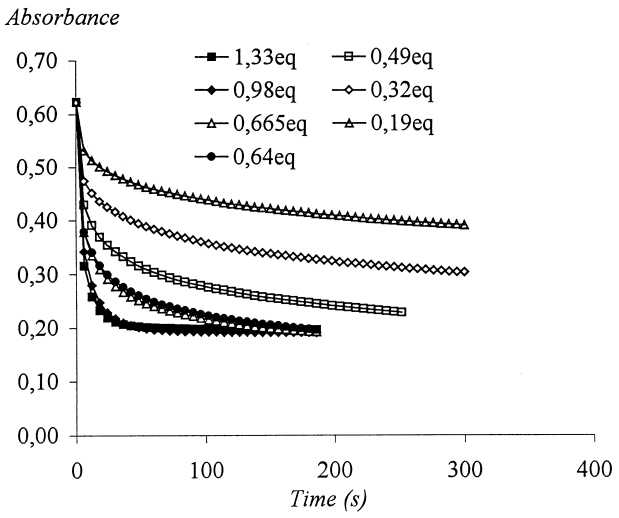


Figure 2. Reaction of **1** with DPPH, monitored at 515 nm.

6.31 (2H, s), 6.44 (1H, s), 9.40 (1H, bs), 9.60 (1H, bs); ^{13}C NMR (DMSO-d_6): 35.2 (t), 68.2 (d), 112.0 (d), 115.1 (d), 121.9 (s), 128.4 (s), 143.4 (s), 144.1 (s), MS: 286 (3), 268 (13), 124 (21), 110 (77), 84 (77), 49 (100).

5-LO inhibition

5-LO activity was measured in 1000 g supernatants of RBL-1 cells. Washed cells at a concentration of 5×10^6 cells/mL were suspended in a sodium phosphate/$CaCl_2$ buffer, pH 7.0. The cells were equilibrated at 37 °C for 5 min, 10 μM arachidonic acid and 5 μM calcium ionophore A 23187 were added and the cells were incubated for an additional 2 min at 37 °C. The primary 5-LO product, 5-HETE, was isolated by ethyl acetate extraction (solution at 4 °C). Lipoxygenase products synthesised in RBL-1 cells were also analysed by reversed phase HPLC as previously described.[14] Varying concentrations of test

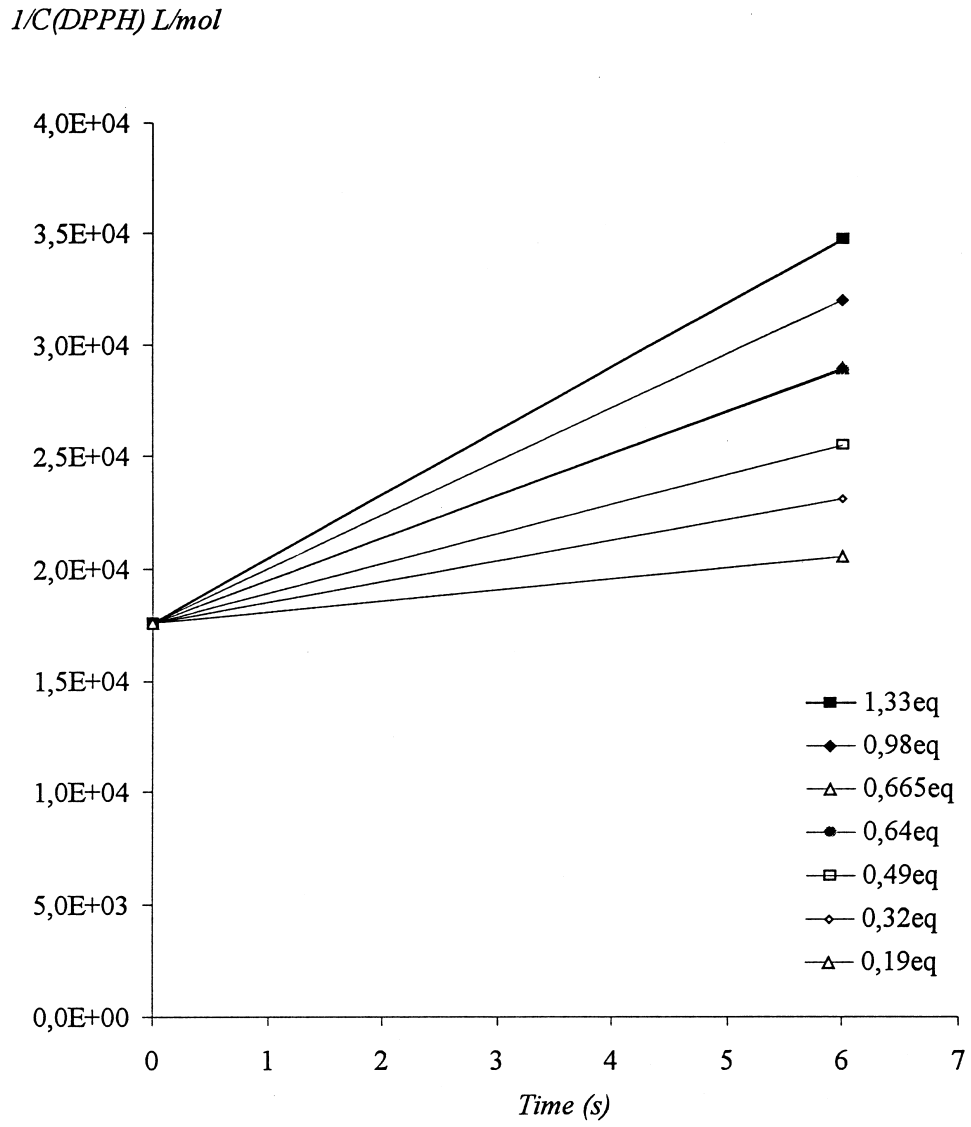


Figure 3. Kinetics of the reaction of **1** with DPPH.

compounds and RBL-1 cells were pre-incubated for 5 min at 37 °C before AA stimulation.

EPR spectroscopy

EPR spectra were recorded using a Varian E-109 spectrometer operating at 9.5 GHz with a 100 kHz high frequency modulation amplitude ranging from 0.1 to 0.4 G. The sample solutions were examined in a flat quartz cell inserted in an E-238 cavity operating in the TM_{110} mode for the EPR spectra recorded at room temperature.

Iron-reducing activity

The reaction mixtures comprised 50 mM sodium acetate (pH = 7.8), 50 mM γ-morpholinopropanesulfonic acid (Mops) (pH = 4.2) or 50 mM Mops + HC1 (pH = 2.0), 1 mM ferrozine, 25 μM tested compound and 100 μM $FeCl_3$ in a final volume of 1.5 mL. The tested compounds were dissolved in ethanol. The reaction was started by addition of $FeCl_3$ and the decrease of absorbance at 562 nm after 3 min was recorded using control lacking the tested compound.

DPPH inhibition

Spectrophotometric measurements were performed with an Uvikon 932 spectrophotometer. Calibration curves for DPPH were performed in triplicate and the initial DPPH concentration was then calculated from eq (1).

$$A_{517\ nm} = 0.109 \times C_{DPPH} \times 10^5 + 0.0013 \quad (1)$$

Standard solutions of the antioxidants were prepared in ethanol and rapidly mixed (volumes from 0.02 mL to 0.4 mL) with an ethanol solution of DPPH taken between 50 and 63 μM. The decrease in absorbance was recorded every 6 s for 5 min. Seven measurements per potential antioxidant were recorded with [antioxidant]/[DPPH] ratios varying from 0.19 to 1.7. In parallel a blank solution of DPPH was screened to estimate DPPH decomposition during the time of measurement. The decrease in absorbance was then plotted against time, after correction for DPPH decomposition (Fig. 2). The effective concentration ratio (EC_{50}) is the concentration of antioxidant producing a 50% decrease in DPPH at steady state. The initial reaction followed second-order kinetics; the rate constants k (obtained from plots of 1/[C] versus time) (Fig. 3) were plotted against the ratio [antioxidant]/[DPPH] (Fig. 4). Linear regression ($r^2 > 0.9$) gave the parameter Z (slope of the line in L mol^{-1} s^{-1}) which was examined as a quantification of radical scavenging activity.

Computational method

The molecular modelling semi-empirical calculations have been performed at the RHF level with the Austin Model 1 method (AM1) using the HYPERCHEM[27] program (version 5). The energy global minimum search was started from standard geometrical parameters using the Polak–Ribiere conjugate gradient algorithm. No constraint was set on the free molecular systems. The termination conditions of calculation for the energy minimisation were imposed by the set of a root-mean-square gradient to 0.008 kJ/(Å·mol).

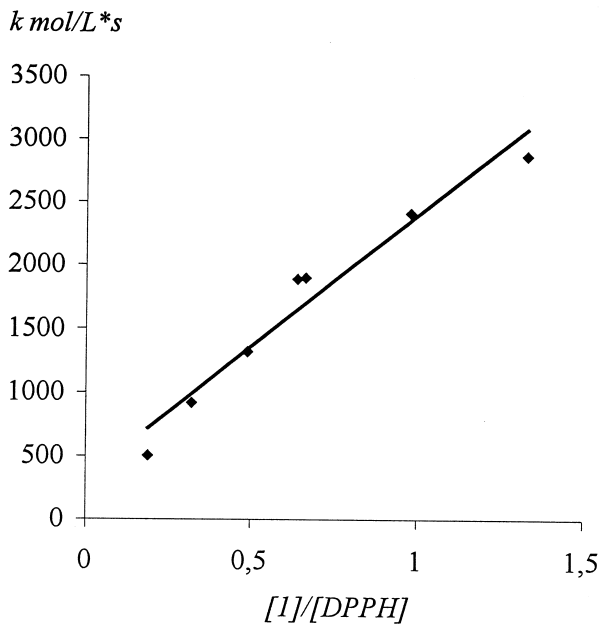


Figure 4. Calculation of Z for **1**.

Acknowledgements

This work was supported by grants from the Centre National de la Recherche Scientifique (CNRS), the Fond Européen pour le Développement de la Recherche (FEDER) and the Région Nord-Pas-de-Calais.

References

1. (a) Musser, J. H.; Kreft, A. F. *J. Med. Chem.* **1992,** *35,* 2501. (b) Brooks, C. D. W.; Summers, J. B. *J. Med. Chem.* 1996, *39,* 2629.
2. (a) Samuelsson, B. *Science* **1983,** *220,* 568. (b) Lewis, R. A.; Austen, K. F. *Nature* **1981,** *293,* 103.
3. (a) Shaw, A.; Krell, R. D. *J. Med. Chem.* **1991,** *34,* 1235. (b) McMillan, R. M.; Walker, E. R. H. *Trends Pharm. Sci.* **1992,** *13,* 323.
4. Koshihara, Y.; Neichi, T.; Murota, S. I.; Lao, A. N.; Fujimoto, Y.; Tatsuno, T. *Biochim. Biophys. Acta* **1984**, *792*, 92.
5. Yoshimoto, T.; Furukawa, M.; Yamamoto, S.; Horie, T.; Watanabe-Kohno, S. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 612.
6. Baumann, J.; Von Bruchhausen, F.; Wurm, G. *Prostaglandins* **1980**, *20*, 627.
7. Young, R. N. *Eur. J. Med. Chem.* **1999**, *34*, 671.
8. Kemal, C.; Louis-Flamberg, P. L.; Krupinski-Olsen, R.; Shorter, A. L. *Biochemistry* **1987**, *26*, 7064.
9. Nelson, M. J. *Biochemistry* **1988**, *27*, 4273.
10. Boyington, J. C.; Gaffney, B. J.; Amzel, L. M. *Science* **1993**, *260*, 1482.

11. Cotelle, P.; Catteau, J. P. *Tetrahedron Lett*. **1997**, *38*, 2969.
12. Dupont, R.; Cotelle, P. *Tetrahedron Lett*. **1998**, *39*, 8457.
13. Dupont, R.; Cotelle, P. *Synthesis* **1999**, 1651.
14. Brossi, A.; Rice, K. C.; Mak, C. P.; Reden, J.; Jacobson, A. E.; Nimitkitpaisan, Y.; Skolnick, P.; Daly, J. *J. Med. Chem*. **1980**, *23*, 648.
15. (a) McColl, S. R.; Betts, W. H.; Murphy, G. A.; Cleland, L. G. *J. Chromatography* **1986,** *378,* 444. (b) Bennett, C.; Chiang, M.; Monia, B.; Crooke, S. *Biochem. J*. **1993,** *289,* 33.
16. Cotelle, N.; Bernier, J. L.; Catteau, J. P.; Pommery, J.; Wallet, J. C.; Gaydou, E. *Free Radic. Biol. Med*. **1996**, *20*, 35.
17. Oehler, U. M.; Janzen, E. G. *Can. J. Chem*. **1982**, *60*, 1542.
18. Moran, J. F.; Klucas, R. V.; Grayer, R. J.; Abian, J.; Becana, M. *Free Radic. Biol. Med*. **1997**, *22*, 861.
19. Colucci, W. J.; Jungk, S. J.; Gandour, R. D. *Magn. Reson. Chem*. **1985**, *23*, 335.
20. Abraham, R. J.; Bakke, J. M. *Org. Magn. Reson*. **1973**, *11*, 373.
21. Felix, C. C.; Sealy, R. C. *J. Am. Chem. Soc*. **1981**, *103*, 2831.
22. Zabel, V.; Watson, W. H.; Kagan, J.; Agdeppa, D. A.; Chen, S. A. *Cryst. Struct. Commun*. **1978**, *7*, 727.
23. Ashworth, P.; Dixon, W. T. *J. Chem. Soc., Perkin Trans. 2* **1974**, 739.
24. Lehmann, W. D. *Free Radic. Biol. Med*. **1994**, *16*, 241.
25. McMillan, R. M.; Walker, E. R. H. *Trends Pharm. Sci*. **1992**, *13*, 323.
26. Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron* **1994**, *50*, 13583.
27. Pazun, J. L. *J. Chem. Inf. Compt. Sci*. **1993**, *33*, 931.