Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 1647–1658

BIOORGANIC & MEDICINAL CHEMISTRY

Novel 4,5-Diaryl-3-hydroxy-2(5*H*)-furanones as Anti-Oxidants and Anti-Inflammatory Agents

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Received 22 May 2001; revised 20 June 2001; accepted 28 January 2002

Abstract—In order to study the effect of phenol moieties on biological activities of ascorbic acid derivatives, we synthesized 13 novel 4,5-diaryl-3-hydroxy-2(5*H*)-furanones **5a**–**m** with various substitution patterns. Compound **5g** bearing a 2,3-dihydroxy phenyl ring on the 5-position of the heterocycle appeared to be the most powerful anti-oxidant furanone with reducing activity against DPPH ($IC_{50} = 10.3$ μM), superoxide anion quenching capacity ($IC_{50} = 0.187$ mM) and lipid peroxidation inhibitory effect ($IC_{50} = 0.129$ mM). To ascertain determinant molecular features for anti-oxidant activities, structure–activity relationships were studied. Lipophilicity and molecular parameters related to electron distribution and structure (difference in heats of formation between the compound and its radical or its cation radical, energy of the highest occupied molecular orbital, HOMO) were found to correlate with the anti-oxidant action of compounds **5** in the different tests used. Oxygen-derived free radicals are known to contribute to inflammatory disorders; therefore we have investigated effects of compounds **5** in two models of inflammation: phorbol ester-induced ear edema in mice (TPA-test) and carrageenan-induced paw edema in rat. At 100 mg/kg ip in the TPA-test, the anti-inflammatory activity of compounds **5** was potent compared with that of indomethacin and ketorolac and all the results suggested a cyclooxygenase inhibition in the emergence of such properties. The combined pharmacological actions of compounds **5** associated with a favorable therapeutic index prompt with interesting perspectives for their use in heart and brain disorders as well as in inflammatory diseases.

Introduction

Multiple medical conditions[1,2] such as myocardial infarction, carcinogenesis and neurologic trauma, to name a few, could be heightened by the presence of oxygen free radicals. These radicals appear to be toxic mainly because they initiate the chain reaction of lipid peroxidation. These small molecular units could split DNA, RNA, proteins, cellular membranes and cellular organization. Under normal conditions, aerobic organisms have evolved anti-oxidant defences to protect them against free radicals: enzymes such as superoxide dismutase, glutathione peroxidase, anti-oxidants and radical scavengers such as α-tocopherol (vitamin E), β-carotene, glutathione and ascorbic acid (vitamin C).[3] Ascorbate is one of the most potent naturally occuring anti-oxidants because it works at the same time directly by reaction with aqueous peroxyl radicals and indirectly by restoring the anti-oxidant properties of fat-soluble vitamin E.[4] Furthermore, it plays an important part in the prevention of a large number of chronic diseases.[5,6] However, the low solubility of ascorbic acid in lipophilic environments as well as its susceptibility to thermal and oxidative degeneration has led to interest in the synthesis of derivatives with better lipophilicity and increased stability.[7–17]

Due to the biological interest of this class of compounds, we previously reported the syntheses and the anti-oxidant properties of lipophilic ascorbic acid analogues **1** and **2**

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0968-0896/02/$ - see front matter
PII: S0968-0896(02)00053-6

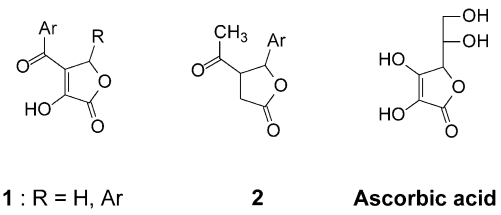


Chart 1.

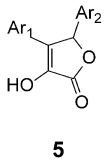


Chart 2.

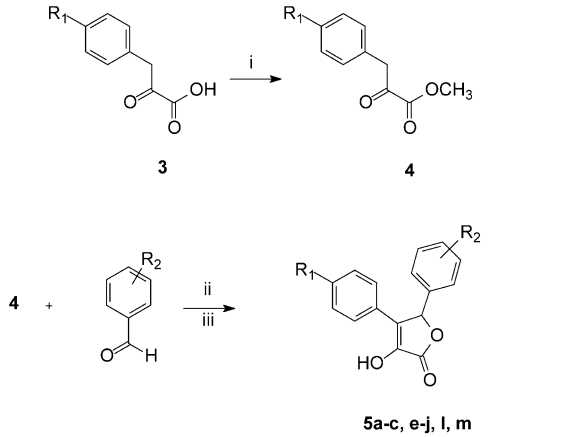
(Chart 1).[18,19] Although these furanone derivatives were more effective than ascorbic acid both as superoxide anion scavengers and as lipid peroxidation inhibitors, the substitution of the furan nucleus by either a benzoyl or an acetyl group in the 4-position appeared to enhance toxicity ($LD_{50} \leq 200$ mg/kg ip) with regard to vitamin C ($LD_{50} > 800$ mg/kg ip). Consequently, we investigated here the anti-oxidant properties of a series of novel 3-hydroxy furanones **5** (Chart 2) having aromatic substituents directly linked to the heterocycle in the 4- and 5-positions, in order to identify suitable features for optimal activity and minimal toxic effects.

Thus, anti-oxidant effects of these new derivatives have been evaluated in vitro by means of three tests: measurement of the reducing activity on the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), superoxide anion scavenging assay and lipid peroxidation assay.

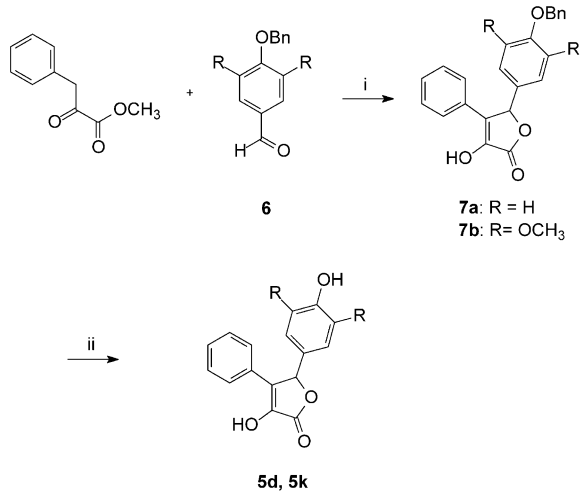
Furthermore, free radicals are well known to play an important part in the inflammatory process.[20] Superoxide anion radicals, hydrogen peroxide and hydroxyl radicals, produced by activation of phagocytes, are considered to be implicated in inflammation and tissue destruction. Free radicals are also involved in the biosynthesis of prostaglandins, which are important mediators in inflammatory process.[21] Therefore, the evaluation of anti-inflammatory properties of furanones **5** seemed to be of great interest.

Chemistry

The synthesis of 4,5-diaryl-3-hydroxyfuranones **5a–c**, **e–j**, **l–m** was accomplished by reaction of methyl arylpyruvates **4** with appropriate benzaldehydes in the presence of 1,5-diazabicyclo[5.4.0]undecene (DBU) in dimethylformamide,[22–24] as shown in Scheme 1. Methyl arylpyruvates **4** were prepared by methylating arylpyruvic acids **3** according to the procedure described by Namiki et al.[22] Preparation of **5d** and **5k** from arylpyruvates **4** failed so these compounds were then obtained by hydrogenolysis of **7a** and **7b** (Scheme 2). Structural



Scheme 1. Reagents and conditions: (i) DBU, CH_3I, DMF, O°C; (ii) DBU, DMF, 0 °C; (iii) HCl.



Scheme 2. Reagents and conditions: (i) DBU, DMF, 0 °C; (ii) Pd/C, H_2, EtOH/THF.

determination of furanones **5** was achieved by IR, ^1H and ^{13}C NMR spectral studies and purity of products was confirmed by elemental analysis. All compounds **5** were obtained as racemates.

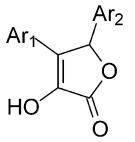
Results and Discussion

In vitro anti-oxidant activities

Anti-oxidants can act at several different levels in the oxidative sequence and their mechanisms of action can be multiple.[25] So, we chose to investigate anti-oxidant activities of furanones **5** by means of three different tests (Table 1).

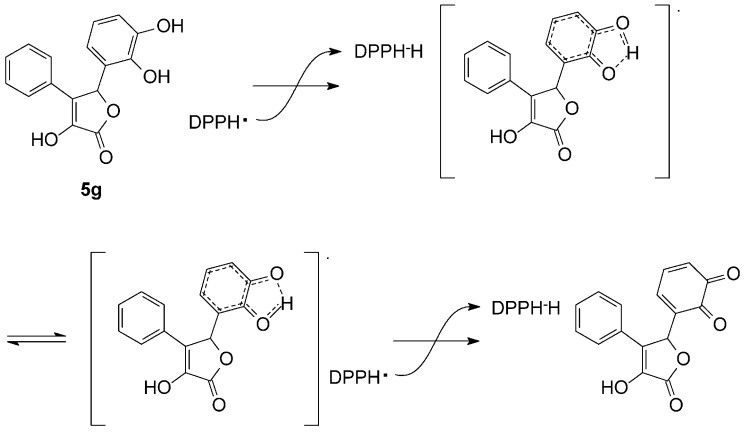
First, the free radical scavenging properties of tested compounds were evaluated by decrease in the absorption of the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) at 517 nm. This bleaching of DPPH absorption occurs when the odd electron of the radical is paired. Thus, it is representative of the capacity of furanones **5** to scavenge free radicals, independently from any enzymatic

Table 1. Anti-oxidant activities and lipophilicity of derivatives **5**



Compd	Ar_1	Ar_2	Reducing activity on DPPH IC_{50} (μM)	Superoxide anion scavenging activity IC_{50} (mM)	Lipid peroxidation IC_{50} (mM)	log k'
5a	C_6H_5	C_6H_5	970.0	NT	NT	1.75
5b	C_6H_5	2-OH C_6H_4	34.0	0.499	1.219	1.59
5c	C_6H_5	3-OH C_6H_4	698.0	0.277	0.436	1.46
5d	C_6H_5	4-OH C_6H_4	586.0	0.705	28.0 ± 2.1[a]	1.45
5e	C_6H_5	2-OCH_3 C_6H_4	165.0	NT	NT	1.80
5f	C_6H_5	4-OCH_3 C_6H_4	762.0	NT	NT	1.90
5g	C_6H_5	2,3-diOH C_6H_3	10.3	0.187	0.129	1.43
5h	C_6H_5	2,5-diOH C_6H_3	12.2	0.559	0.210	1.07
5i	C_6H_5	2-OH, 3-OCH_3 C_6H_3	20.4	>0.8	0.413	1.58
5j	C_6H_5	2-OH, 5-OCH_3 C_6H_3	23.2	0.277	0.152	1.54
5k	C_6H_5	3,5-diOCH_3, 4-OH C_6H_2	39.5	0.886	0.170	1.15
5l	4-OH C_6H_4	C_6H_5	20.5	NT	NT	1.44
5m	4-OH C_6H_4	4-OCH_3 C_6H_4	22.0	NT	NT	1.53
Ascorbic acid			46.6	24.0 ± 1.5[b]	Pro-oxidant	—
Vitamin E			19.6	NT	NT	—

NT, not tested.
[a]% inhibition at 0.25 mg/mL.
[b]% inhibition at 1 mg/mL.



Scheme 3.

activity. The presence of substituents on either aromatic ring bound to the furan skeleton led to more potent derivatives **5b**–**m** ($10.3 \leq IC_{50} \leq 762$ μM) than the unsubstituted parent compound **5a** ($IC_{50} = 970$ μM). Obviously, the increased anti-oxidant activity of derivatives **5b**, **5g**–**m** is essentially due to the presence of a phenol moiety on the olide ring. The presence of a hydroxyl group at C-2′ (**5b**) in ring Ar_2 is associated with a high reducing activity against DPPH ($IC_{50} = 34$ μM), which is confirmed by the weak one of the corresponding compound **5e** ($IC_{50} = 165$ μM) having a methoxy group at this position. Moreover, the IC_{50} values of **5c** (698 μM) and **5d** (586 μM) were far higher than that of **5b**, suggesting that a *meta*- or *para*-substituent on the phenyl nucleus is unfavourable for activity. In addition, the IC_{50} values of diphenols **5g**, **5h** (10.3 and 12.2 μM, respectively) were found to be 2 to 3 times weaker than that of monophenol **5b** or polysubstituted compounds **5i**–**k** ($20 < IC_{50}$ values < 40 μM). The potent activity of compound **5g** could be due to the weakness of OH linkages related to the mesomeric electron-donating effect of the OH substituent in the *ortho*-position with regard to the phenoxyl radical. Thus, **5g** could form a semiquinonic intermediate stabilized by electronic delocalization and, consequently, easily oxidized to produce a stable orthoquinonic compound as previously observed with catechin (Scheme 3) by Sawaï and Sakata.[26] In addition, this semiquinonic intermediate is stabilized by hydrogen bonding which prevents such a radical to generate secondary radical reactions. The formation of the semiquinonic radical of **5g** was corroborated by electron spin resonance (ESR) measurement.

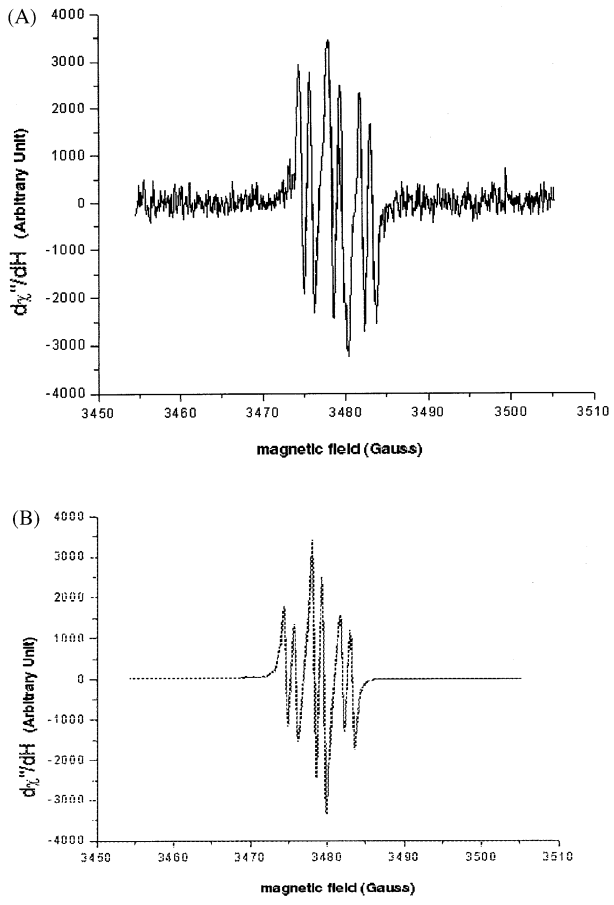
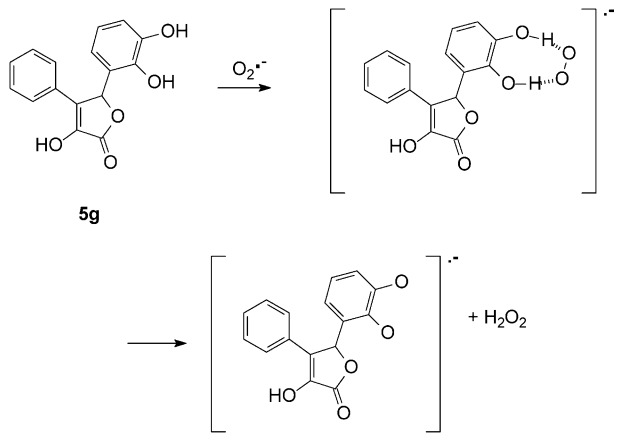


Figure 1. ESR spectrum of stable semiquinone radical generated from compound **5g**. (A) Experimental spectrum recorded after NaOH addition. Spectrometer setting: microwave power, 10 mW, modulation amplitude, 0.5 gauss, scan range, 100 gauss, frequency, 9.42 GHz. (B) Simulated ESR spectrum.

Thus, addition of NaOH to aerated solution of **5g** generated a stable radical, which is presented in Figure 1 under our experimental conditions, **5g** with a catechol function was sensitive to autoxidation and gave rise to a stable free radical. The ESR spectrum consists of a six-line spectrum: a small hyperfine coupling constant of 1.38 gauss and two coupling constant of 3.75 gauss. The result clearly indicated the formation of the semiquinonic radical of **5g**.[27] Similarly, 2,5-diOH substitution on the phenyl ring in derivative **5h** could lead to a stable parasemiquinonic derivative which find expression in potent reducing activity. The presence of a hydroxyl group in the 4-position on either Ar_1 or Ar_2 seems to differently affect anti-oxidant properties in this series of derivatives. Thus, the superior activity of **5l**, **5m** versus **5d** could be explained by the fact that these two compounds are vinylogous analogues of the enolic form of ascorbic acid. Finally, compared with conventional anti-oxidants, diphenols **5g** and **5h** were slightly superior to vitamin C and vitamin E ($IC_{50} = 46.6$ and 19.6 μM, respectively).

The first product of the univalent reduction of oxygen is the superoxide anion, $O_2^{\bullet -}$, which is generated in many biological processes.[28,29] So, compounds able to scavenge



Scheme 4.

$O_2^{\bullet -}$ could be protecting agents against cellular injury during reperfusion of ischemic tissues. Therefore, superoxide anion scavenging activity of furanones **5** was explored using a non-enzymic biological generator of superoxide anion radical, for instance phenazine methosulfate NADH system.[30] This test was limited by the solubility of products in aqueous solution. So, it was realized on compounds **5b**, **c**, **d**, **g**, **h**, **j**, **k**, whose solubility is remarkably increased by the introduction of a hydroxyl group on the phenyl nucleus in the 5-position. Unfortunately, it could not have been done on derivatives **5a**, **e**, **f**, **l** and **5m** due to their poor solubility and **5i** was investigated only at doses up to 0.25 mg/mL. Except compound **5i**, all tested furanones exhibited noteworthy scavenging activity with IC_{50} values ranging from 0.187 to 0.886 mM (Table 1). Although clear structure–activity relationships are not evident, the presence of a hydroxyl group in the 3-position on Ar_2 appeared to increase the efficiency of molecules (**5c** vs **5b**, **5d** and **5g** vs **5h**, **5i**). Furanone **5g** was again found to be the most active compound in this test, what could be explained by the capacity of superoxide anions to deprotonate diphenolic compounds,[31] as shown in Scheme 4 for furanone **5g**. In addition, all furanones exhibited a better superoxide anion scavenging activity than ascorbic acid considered as a reference, with only 24% inhibition at a concentration of 1 mg/mL. Vitamin E was not tested because of its insolubility in aqueous medium.

Excessive lipid peroxidation in biomembranes is thought to be related to several abnormal states producing peroxyl radicals $ROO^{\bullet}$ resulting from chain reactions carried by free radicals.[32–35] Because phenolic compounds and ascorbic acid have $ROO^{\bullet}$ trapping and $z^{\bullet}OH$ scavenging abilities,[34,35] the effects of furanones **5** (Table 1) were investigated on the Fe^{2+}/ADP-ascorbate induced lipid peroxidation of rat liver microsomes. We observed potent antioxidative activities for most compounds (**5g**–**k**). Therefore, di- or trisubstitution on Ar_2 seems to be required for efficient free radical quenching by furanones as it is proved by comparing **5g**–**k** (IC_{50} values ≤ 0.413 mM) with **5b**–**d** (IC_{50} values ≥ 0.436 mM). Methylating one hydroxyl group of **5g**, providing **5i**, decreased the efficiency of scavenging activity,

nevertheless, **5i** remained much more effective than **5b**. An electron-donating methoxy group in the *ortho*-position could increase the stabilization of the aryloxyl radical, formed by the abstraction of hydroxyl hydrogen, through electron delocalization. This hypothesis appears again consistent when comparing **5k** ($IC_{50} = 0.170$ mM) and **5d** (28% inhibition at 0.25 mg/mL). However, the nature of substituent (hydroxy or methoxy group) in the 5-position on Ar_2 seems to moderately influence anti-oxidant effect in the test (**5j** versus **5h**). As previously reported,[35–37] vitamin C underwent autoxidative destruction in presence of iron initiating lipid peroxidation. However, this 'pro-oxidant' activity in vitro has obviously no significance in vivo and ascorbic acid works as anti-oxidant under most circumstances.[36,37]

The hydrophilic or lipophilic character of anti-oxidants greatly influenced them to act as radical scavengers in aqueous phase or as chain-breaking anti-oxidants in biological membranes.[38,39] Therefore, we chose to determine capacity factors (log k′) from reversed-phase high-performance liquid chromatography, which estimate the hydrophobicity of compounds. A mobile phase composition containing 50% MeOH appeared to be most reliable to predict the conventionally hydrophobic parameter log P value for molecules possessing hydrogen-bonding substituents.[40] Although lipophilicity has a marked impact on activity, no clear correlation was found between lipophilicity and anti-oxidant properties whatever the test used (Table 1).

Compound **5g** appears to possess ideal structural features for anti-oxidant activities, considering its reactivity as hydrogen or electron-donating agent, its superoxide anion scavenging property and its possible interaction with hydroxyl and lipid peroxyl radicals. In addition, its propensity for metal chelations, particularly iron and copper, supports the role of any polyphenolic derivative[41] and, thus, the role of **5g** as preventive anti-oxidant in terms of inhibiting transition metal catalysed free radical formation.

Structure–activity relationships

In order to find a possible explanation for the observations described above and to provide a better understanding of the influence of structure on the anti-oxidant activity in a molecular way, different physicochemical parameters were calculated using Sybyl 6.6 software[42] and examined, in particular the enthalpy of homolytic H–O bond cleavage ($\Delta\Delta H_f$), the energy of electron abstraction ($\Delta\Delta H_{ox}$) and the energy of the HOMO (Table 2). The enthalpy of homolytic H–O bond cleavage ($\Delta\Delta H_f$) was determined as the difference in heats of formation between the radical formed after abstraction of hydroxyl enolic or phenolic hydrogen by drugs and the parent compound. The more stable the radical, the weaker will be the H–O bond in the enol and/or phenol groups. The weaker this bond, the more readily it will be cleaved by an attacking active oxygen species and stronger will be the activity. Thus, in the case of diphenols **5g** and **5h**, only the lowest $\Delta\Delta H_f$ phenol values were considered for the study. The energy of electron abstraction ($\Delta\Delta H_{ox}$) was calculated as the difference in heats of formation between the cation radical and the parent compound. This parameter was used because anti-oxidant action could involve single-electron transfer followed by proton transfer as an alternative or in addition to H-abstraction. The energy of the HOMO (highest occupied molecular orbital) was computed and determines the ionization potential and, therefore, will correlate with the ability of enol and/or phenol moieties to donate electrons.

Table 2. Computed molecular properties of derivatives **5**

Compd	ΔH_f molecule (kcal mol^{-1})	$\Delta\Delta H_f$ enol (kcal mol^{-1})	$\Delta\Delta H_f$ phenol (kcal mol^{-1})	$\Delta\Delta H_{ox}$ (kcal mol^{-1})	E_{HOMO} molecule (eV)
5a	−39.74	25.22	—	192.85	−9.05
5b	−81.37	24.90	38.66	187.90	−9.04
5c	−83.66	25.13	39.31	193.31	−9.05
5d	−84.08	25.23	39.52	192.99	−9.05
5e	−74.73	24.63	—	188.16	−8.88
5f	−77.78	25.24	—	192.94	−9.02
5g	−127.94	25.11	32.25 (OH en 2) 33.49 (OH en 3)	190.58	−8.96
5h	−127.25	25.22	33.58 (OH en 2) 36.28 (OH en 5)	187.39	−9.01
5i	−121.67	25.05	36.10	190.33	−8.93
5j	−121.03	25.11	33.43	194.94	−8.92
5k	−156.98	25.03	34.58	197.20	−8.98
5l	−84.26	25.63	37.44	187.22	−8.77
5m	−122.46	24.56	37.47	186.54	−8.76

First, a number of statistical investigations were conducted with the aim of finding correlations between DPPH reactivity on the one hand and calculated molecular and reactivity properties as well as lipophilicity on the other. Eq 1 was obtained from the 13 tested furanones:

$$\begin{aligned}\log(1/IC50) = &-0.027\ (\pm0.020)\ \Delta\Delta H_f\ (\text{phenol } Ar_1)\\ &+0.015\ (\pm0.012)\ \Delta\Delta H_f\ (\text{phenol } Ar_2)\\ &-0.074\ (\pm0.048)\ \Delta\Delta H_{ox}\\ &+7.269\ (\pm2.512)\ E_{HOMO}\\ &-1.279\ (\pm0.828)\log k' + 85.101\ (\pm23.482)\end{aligned} \quad (1)$$

$$n = 13,\ r^2 = 0.774,\ s = 0.470,\ F = 4.798,\ p = 0.0318$$

In this equation, the number in parentheses is the 95% confidence interval associated with the coefficients, n is the number of compounds used, r^2 is the squared correlation coefficient corresponding to the fraction of observed variance accounted for, s is the standard deviation and F (as also p) the statistical significance of fit. Calculated electron abstraction energy and ionization potential are important electronic parameters to predict the reactivity towards DPPH, although lipophilicity also plays a determinant part. Surprisingly, $\Delta\Delta H_f$ (enol) did not interfere in eq 1, contrary to $\Delta\Delta H_f$ (phenol), what

was explained by the fact that this former parameter was practically constant in the studied molecules (range 24.25–25.63). This last result also corroborates the importance of phenol moieties and supports our hypothesis about a part of the action mechanism evoked for **5g** and **5h**.

However, the modest quality of eq 1 suggests that other complex interactions, not encoded in the parameters used (e.g., intermolecular and intramolecular H-bonds),[43,44] could influence the reaction.

Concerning superoxide anion scavenging activity, regression analysis resulted in non-significance of equations with the same set of variables as for DPPH study. From these data, the activity of **5c** appeared to be fundamentally different from the calculated value and is certainly notably influenced by other factors than those considered in establishing correlations. Consequently, excluding **5c**, a predictive model described by eq 2 was found using stepwise multilinear regression:

$$\log(1/IC_{50}) = -0.131\ (\pm 0.027)\ \Delta\Delta H_f\ (\text{phenol } Ar_2) - 3.291\ (\pm 1.531)\ E_{HOMO} + 1.375\ (\pm 0.221)\ \log k' - 23.494\ (\pm 13.097) \quad (2)$$

$$n = 6,\ r^2 = 0.971,\ s = 0.071,\ F = 22.722,\ p = 0.0424$$

This analysis reveals three parameters to be the best predictors of superoxide anion scavengers for the studied compounds. Eq 2 shows that $\Delta\Delta H_f$ (phenol Ar_2) is the less influencial property whereas the ability of the molecule to donate electrons predominates.

Finally, the best correlation for lipid peroxidation is given by eq 3:

$$\log(1/IC_{50}) = 1.160\ (\pm 0.284)\ \Delta\Delta H_f\ (\text{enol}) - 0.086\ (\pm 0.010)\ \Delta\Delta H_f\ (\text{phenol } Ar_2) + 0.037\ (\pm 0.007)\ \Delta\Delta H_{ox} - 29.574\ (\pm 7.511) \quad (3)$$

$$n = 7,\ r^2 = 0.985,\ s = 0.061,\ F = 64.939,\ p = 0.0032$$

Examination of eq 3 leads to the conclusion that activity is governed by the enthalpy of homolytic H–O bond cleavage. Contrary to all expectations, no or less significant correlations were found with lipophilicity.

Despite of the useful indications furnished by eqs 1–3, the traditional QSAR approach has revealed its limitations on the key structural elements that can modulate the anti-oxidant activity of furanones. For example, as far as $\Delta\Delta H_f$ values are concerned in the emergence of scavenging activity, the difference in sign between the slopes of the equations gives evidence for a complex multifactorial mechanism of action, whatever the test considered. However, similarly to a study published by Ancerewiz et al.,[45] it is interesting to note that the potency of **5** to inhibit the oxidation of lipids can be usefully predicted, using a simple in vitro reactivity test, as exemplified by eq 4:

$$\log(1/IC_{50})_{lipo} = -0.423\ (\pm 0.043)\log(1/IC_{50})_{DPPH} - 0.187(\pm 0.010)\ \Delta\Delta H_f\ (\text{phenol } Ar_2) + 12.055(\pm 0.513) \quad (4)$$

$$n = 7,\ r^2 = 0.991,\ s = 0.041,\ F = 211.572,\ p < 0.0001$$

Table 3. Anti-inflammatory activity of derivatives **5**

Compound	TPA-induced ear edema test % inhibition of edema at		Carrageenin-induced paw edema test % inhibition of edema at	
	100 mg/kg ip	200 mg/kg po	100 mg/kg ip	200 mg/kg po
5a	48.4±5.6[b]	NT	35.5±0.4[a]	NT
5b	23.8±4.5[a]	NT	NT	NT
5c	24.7±5.5[a]	NT	NT	NT
5d	52.4±5.4[b]	NT	39.4±0.4[b]	NT
5e	58.3±7.1[b]	5.8±5.5 (NS)	48.4±6.5[b]	29.8±1.8 (NS)
5f	29.0±10.3[a]	NT	NT	NT
5g	48.3±1.4[b]	8.7±5.5 (NS)	52.6 ±7.5[b]	Inactive
5h	62.8±9.9[b]	5.3±5.7 (NS)	73.8±6.5[b]	Inactive
5i	6.2±7.2 (NS)	NT	NT	NT
5j	27.7±12.5 (NS)	NT	NT	NT
5k	44.2±8.4[b]	NT	NT	NT
5l	21.7±4.5[a]	NT	NT	NT
5m	77.5±7.7[b]	5.6±5.5 (NS)	59.7±4.9[b]	Inactive
Indomethacin	13.8±10.1 (NS)	18.8±6.4 (NS)	54.8±0.4[b,c]	52.6±8.8[a]
Ketorolac	10.6±8.2 (NS)	9.3±7.3 (NS)	53.3±9.1[b,c]	45.6±5.3[a]
Dexamethasone	93.3±2.0[b,d]	83.9±4.1[b,d]	76.5±5.4[b,d]	49.1±10.5[a]

NT, not tested; NS, not significant.
[a]$0.001 < p < 0.05$.
[b]$p < 0.001$.
[c]Tested at 15 mg/kg ip.
[d]Tested at 1 mg/kg ip.

In vivo anti-inflammatory activities

Reactive oxygen species are well known to be implicated in the induction and prolongation of inflammatory process.[20,21,46,47] In addition, a number of commercially available non steroidal anti-inflammatory drugs (NSAIDs) and analgesics such as salicylates, acetaminophen, indomethacin and nimesulide, have been shown to possess radical scavenging properties.[48–51] All these observations prompted us to investigate derivatives **5** as anti-inflammatory drugs.

At first, behavioral effects and intraperitoneal acute toxicity of compounds **5** were examined in mice. Neither of tested drugs produced significant behavioral effects, even at doses up to 800 mg/kg ip and all animals were still alive after an observation period of 1 week. Consequently, these results enhance the interest of ascorbic acid derivatives **5** as anti-oxidant drugs compared to previously described products **1** and **2**, the therapeutic margin of which was fairly weak.

With regard to evaluation of furanones **5** as anti-inflammatory compounds, they were first administered ip at the dose of 100 mg/kg in the phorbol 12-myristate 13-acetate (TPA)-induced ear edema test (Table 3). Only six furanones, **5a**, **5d**, **5e**, **5g**, **5h**, **5m**, exhibited a noteworthy activity and produced more than 45% inhibitory effects. If compounds **5e**, **5g**, **5h**, **5m**, which demonstrated anti-oxidant properties in either of the tests used, were active to diminish TPA inflammation, surprisingly, the unsubstituted parent compound **5a**, the least efficient to reduce DPPH, exhibited significant anti-inflammatory activity. Moreover, lipophilicity did not seem to play a major role since one of the highest lipophilic compound **5e** ($\log k' = 1.80$) and the lowest one **5h** ($\log k' = 1.07$) were equipotent with about 60% inhibition of edema. In contrast, classical NSAIDs indomethacin and ketorolac, failed to produce significant effect after ip administration, since maximum reduction of ear thickness was only 13.8% at 100 mg/kg. These results, consistent with literature data,[52] demonstrate the particular interest of furanones **5** as potent anti-inflammatory compounds, even though their effectiveness remained clearly less important than that of steroid dexamethasone (93.3% inhibition at 1 mg/kg ip). Considering that the TPA-induced ear edema test is primarily mediated by PGE_2,[53,54] derivatives **5** could be assumed to possess cyclo-oxygenase inhibitory activity. However, to confirm this hypothesis, the in vivo anti-inflammatory efficacy of furanones **5a**, **5d**, **5e**, **5g**, **5h** and **5m** was assessed by using the functional model of carrageenan-induced paw edema in the rat.[55] This test is particularly efficient in detecting compounds whose activity is the result of inhibition of prostaglandin amplification. At 100 mg/kg ip compounds **5g**, **5h** and **5m** demonstrated a significant inhibition of the edema similar to that of indomethacin administered at the low dose of 15 mg/kg ip (Table 3). These results corroborate those obtained in the TPA-induced ear edema test in the mouse. Potency of furanones **5** in the TPA-induced ear edema test compared to that of reference compounds is probably due to a multiple mechanism of action resulting from both anti-oxidant and anti-inflammatory activities. However, compounds **5** did not exhibit significant anti-inflammatory effects when administered by oral route, suggesting a possible low bioavailability due to glucuronidation, as was classically observed for phenol derivatives.[56]

Conclusions

Our study provides evidence that furanones **5** exhibit interesting anti-oxidant properties expressed either by their reducing activity against the stable radical DPPH or their capacity to scavenge superoxide anion and to inhibit lipid peroxidation. Appropriate modifications of previous structures **1** and **2** lead to products with a favorable therapeutic index. In this series, free radical scavenging activity is favored by the presence at the same time of enol and phenol moieties as shown by compound **5g**. Moreover, the introduction of two aromatic rings on the furanone skeleton enhances the lipophilicity of compounds **5** compared to vitamin C and, thus, is expected to contribute to their effective access, retention and interaction with biological membranes. So, derivatives **5** appeared to be related to tocopherol, especially with respect to lipid peroxidation. This fact suggests interesting perspectives for the use of compound **5g** in the treatment of heart and brain disorders involving free radicals, such as myocardial infraction or stroke. Preliminary studies indicate that some furanones **5** possess, by ip route, anti-inflammatory activity comparable with that of classical NSAIDs and even higher in the TPA-induced ear edema test. The combined anti-oxidant, anti-inflammatory and lipophilic effects of compound **5g** could also have therapeutic value in inflammatory diseases, such as rheumatoid arthritis, in which synovial lipid hydroperoxides provide a reservoir of potential oxygen-derived free radicals.

Experimental

General chemical methods

All starting materials used for synthesis were reagent-grade from Janssen Chimica (Noisy-le-Grand, France). Reagents for biochemical assays were purchased from Sigma (Montluçon, France). Melting points were determined on a Reichert apparatus (Isi, Paris, France) and were uncorrected. Column chromatography was carried out on SDS silica gel 60 (70–200 mesh). The infrared (IR) spectra were recorded with a FTIR-Nicolet Impact 410 spectrophotometer (Thermo Optek, Montigny le Bretonneux, France). The proton and carbon nuclear magnetic resonance (^1H NMR and ^{13}C NMR) spectra were recorded on a Brüker AC-400 (400 MHz) spectrometer (Brüker, Wissembourg, France). Chemical shifts are reported in parts per million (δ ppm) relative to tetramethylsilane used as an internal standard. Abbreviations used for signal patterns are: br s, broad singlet; s, singlet; d, doublet; m, multiplet. The ultra violet (UV) measurements were carried out with a DU-70 spectrometer (Beckman Instruments, Fullerton, USA).

Elemental analysis were done by the Service Central d'Analyses, CNRS, Vernaison, France.

4,5-Diaryl-3-hydroxy-2(5*H*)-furanones (5). General procedure. To a cold (0 °C) solution of a mixture of methyl 2-oxo-3-phenylpropionate **4** (5.27 mmol) and appropriate benzaldehyde (1.3 equiv, 6.85 mmol) in dry DMF (24mL) was added DBU (0.80 mL, 5.35 mmol) under stirring. The mixture was stirred for 3–5 h at 0 °C and then poured into a mixture of ethyl acetate (10 mL) and HCl 1M (50 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt. The organic layers were combinated, dried over Na_2SO_4 and evaporated in vacuo. The oily residue was then purified either by column chromatography (AcOEt/cyclohexane) or by precipitation in water, filtration and crystallization from ethyl ether.

4,5-Diphenyl-3-hydroxy-2(5*H*)-furanone (5a). This was prepared from methyl 2-oxo-3-phenylpropionate and benzaldehyde. Precipitation in water and crystallization from ethyl ether led to compound **5a** in 72% yield; mp 202 °C; IR (KBr) ν 3285, 1731, 1676, 1454 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.09 (br s, 1H), 7.63 (d, 2H), 7.42–7.23 (m, 8H), 6.58 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 169.1 (C-2), 138.8 (C-3), 136.6, 128.0 (C_{ipso}), 130.5 (C-4), 129.3, 129.0, 128.5, 128.4, 127.9, 127.4 (CH aromatic), 79.8 (C-5). Anal. calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.32; H, 4.78.

3-Hydroxy-5-(2-hydroxyphenyl)-4-phenyl-2(5*H*)-furanone (5b). This was prepared from methyl 2-oxo-3-phenylpropionate and 2-hydroxybenzaldehyde. Precipitation in water and crystallization from ethyl ether afforded compound **5b** in 56% yield; mp 166 °C; IR (KBr) ν 3268, 1750, 1674, 1605, 1497, 1459 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.94 (br s, 1H), 10.10 (br.s, 1H), 7.62 (d, 2H), 7.34 (t, 2H), 7.25 (t, 1H), 7.15 (t, 1H), 6.97 (d, 1H), 6.89 (d, 1H), 6.73 (d, 1H), 6.71 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 169.6 (C-2), 155.9 (*C*-OH), 138.8 (C-3), 130.9 (C-4), 127.9, 122.1 (C_{ipso}), 130.3, 128.5, 128.2, 128.0, 127.0, 119.4, 116.0 (CH aromatic), 74.6 (C-5). Anal. calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.39; H, 4.50.

3-Hydroxy-5-(3-hydroxyphenyl)-4-phenyl-2(5*H*)-furanone (5c). This was prepared from methyl 2-oxo-3-phenylpropionate and 3-hydroxybenzaldehyde. Purification by column chromatography, eluting with EtOAc/cyclohexane (3/7), gave compound **5c** in 65% yield; mp 215 °C; IR (KBr) ν 3392, 1726, 1682, 1604, 1458 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.05 (br s, 1H), 9.54 (s, 1H), 7.62 (d, 2H), 7.34 (t, 2H), 7.27 (t, 1H), 7.17 (t, 1H), 6.86 (d, 1H), 6.73 (m, 2H), 6.48 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 169.2 (C-2), 157.7 (*C*-OH), 138.7 (C-3), 137.9, 128.0 (C_{ipso}), 130.6 (C-4), 130.6, 128.5, 128.4, 127.4, 118.8, 116.4, 114.0 (CH aromatic), 79.7 (C-5). Anal. calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.39; H, 4.49.

5-(4-Benzyloxyphenyl)-3-hydroxy-4-phenyl-2(5*H*)-furanone (7a). This was prepared from methyl 2-oxo-3-phenylpropionate and 4-benzyloxybenzaldehyde **6a**.[57,58] Purification by column chromatography, eluting with EtOAc/cyclohexane (2/8), gave compound **7a** in 34% yield; mp 223 °C; IR (KBr) ν 3306, 1747, 1681, 1608, 1512 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.02 (br s, 1H), 7.61 (d, 2H), 7.42–7.20 (m, 10H), 6.99 (d, 2H), 6.52 (s, 1H), 5.06 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 169.2 (C-2), 159.0 (*C*-OBn), 138.8 (C-3), 130.7 (C-4) 136.8, 128.7, 127.8 (C_{ipso}), 129.4, 128.6, 128.5, 128.3, 127.9, 127.8, 127.5 (CH aromatic), 79.5 (C-5), 69.3 (CH_2).

3-Hydroxy-5-(4-hydroxyphenyl)-4-phenyl-2(5*H*)-furanone (5d). 0.10 g of 10% Pd/C was added to 5-(4-benzyloxyphenyl)-3-hydroxy-4-phenyl-2(5*H*)-furanone **7a** (0.40 g, 1.1 mmol) dissolved in THF/EtOH and submitted to a hydrogen atmosphere for 4 h. After filtration of the catalyst and evaporation of the solvent, crystallization from ethyl ether afforded 0.26 g (88%) of **5d**; mp 232 °C; IR (KBr) ν 3254, 1732, 1679, 1614, 1519, 1449 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.05 (br s, 1H), 9.73 (br s, 1H), 7.63 (d, 2H), 7.36 (t, 2H), 7.28 (t, 1H), 7.21 (d, 2H), 6.77 (d, 2H), 6.48 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 169.2 (C-2), 158.2 (*C*-OH), 138.8 (C-3), 130.7 (C-4), 129.3, 128.4, 128.2, 127.4, 115.6 (CH aromatic), 127.7, 126.6 (C_{ipso}), 79.8 (C-5). Anal. calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.43; H, 4.50.

3-Hydroxy-5-(2-methoxyphenyl)-4-phenyl-2(5*H*)-furanone (5e). This was prepared from methyl 2-oxo-3-phenylpropionate and o-anisaldehyde. Precipitation in water and crystallization from ethyl ether led to compound **5e** in 22% yield; mp 217 °C; IR (KBr) ν 3274, 1736, 1676, 1600, 1584, 1490, 1461 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.99 (br s, 1H), 7.57 (d, 2H), 7.34–7.24 (m, 4H), 7.10–7.03 (m, 2H), 6.88–6.86 (m, 1H), 6.74 (s, 1H), 3.89 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 169.4 (C-2), 157.7 (*C*–OCH_3), 139.0 (C-3), 130.8, 128.5, 128.3, 128.1, 127.0, 120.8, 111.9 (CH aromatic), 127.6 (C-4), 127.9, 123.9 (C_{ipso}), 74.3 (C-5), 55.9 (OCH_3). Anal. calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.61; H, 4.99.

3-Hydroxy-5-(4-methoxyphenyl)-4-phenyl-2(5*H*)-furanone (5f). This was prepared from methyl 2-oxo-3-phenylpropionate and p-anisaldehyde. Precipitation in water and crystallization from ethyl ether gave compound **5f** in 32% yield; mp 188 °C; IR (KBr) ν 3293, 1742, 1674, 1615, 1517 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.07 (br s, 1H), 7.63 (d, 2H), 7.34–7.27 (m, 5H), 6.93 (d, 2H), 6.54 (s, 1H), 3.73 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 169.5 (C-2), 159.9 (*C*–OCH_3), 138.8 (C-3), 130.6 (C-4), 129.3, 128.5, 128.3, 127.4, 114.3 (CH aromatic), 128.4, 128.0 (C_{ipso}), 79.5 (C-5), 55.1 (OCH_3). Anal. calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.53; H, 4.98.

5-(2,3-Dihydroxyphenyl)-3-hydroxy-4-phenyl-2(5*H*)-furanone (5g). This was prepared from methyl 2-oxo-3-phenylpropionate and 2,3-dihydroxybenzaldehyde. Purification by column chromatography, eluting with a gradient of EtOAc/cyclohexane, afforded compound **5g** in 44% yield; mp 189 °C; IR (KBr) ν 3357, 1720, 1672, 1604 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.89 (br s, 1H), 9.64 (br s, 1H), 9.02 (br s, 1H), 7.64 (d, 2H), 7.34 (t, 2H), 7.25 (t, 1H), 6.77 (d, 1H), 6.73 (s, 1H), 6.56 (t, 1H), 6.43 (d, 1H); ^{13}C NMR (DMSO-d_6) δ 169.6 (C-2), 145.6, 144.6 (*C*–OH), 138.8 (C-3), 131.0 (C-4), 128.5,

128.2, 127.1, 119.3, 117.8, 115.8 (CH aromatic), 128.1, 122.9 (C_{ipso}), 74.9 (C-5). Anal. calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25. Found: C, 67.45; H, 4.26.

5-(2,5-Dihydroxyphenyl)-3-hydroxy-4-phenyl-2(5*H*)-furanone (5h). This was prepared from methyl 2-oxo-3-phenylpropionate and 2,5-dihydroxybenzaldehyde. Purification by column chromatography, eluting with a gradient of EtOAc/cyclohexane, led to compound **5h** in 49% yield; mp 176 °C; IR (KBr) ν 3365, 1696, 1664, 1521, 1455 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.99 (br s, 1H), 9.47 (br s, 1H), 8.78 (br s, 1H), 7.64 (d, 2H), 7.36 (t, 2H), 7.27 (t, 1H), 6.76 (d, 1H), 6.67 (s, 1H), 6.59 (d, 1H), 6.36 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 169.7 (C-2), 150.1, 148.4 (*C*–OH), 138.8 (C-3), 130.9 (C-4), 128.6, 128.4, 127.1, 117.4, 117.0, 113.2 (CH aromatic), 128.2, 122.6 (C_{ipso}), 74.25 (C-5). Anal. calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.25. Found: C, 67.82; H, 4.25.

3-Hydroxy-5-(2-hydroxy-3-methoxyphenyl)-4-phenyl-2(5*H*)-furanone (5i). This was prepared from methyl 2-oxo-3-phenylpropionate and 2-hydroxy-3-methoxybenzaldehyde. Purification by column chromatography, eluting with a gradient of EtOAc/cyclohexane, gave compound **5i** in 39% yield; mp 202 °C; IR (KBr) ν 3280, 1735, 1677, 1450, 1484 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.91 (br s, 1H), 9.32 (br s, 1H), 7.61 (d, 2H), 7.31 (m, 2H), 7.24 (t, 1H), 6.92 (d, 1H), 6.74 (s, 1H), 6.69 (t, 1H), 6.55 (d, 1H), 3.80 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 169.5 (C-2), 147.8, 145.2 (*C*-OH, *C*–OCH_3), 138.7 (C-3), 130.9 (C-4), 128.5, 128.2, 127.0, 119.2, 112.1 (CH aromatic), 128.0, 122.5 (C_{ipso}), 74.4 (C-5), 55.8 (OCH_3). Anal. calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.57; H, 4.72.

3-Hydroxy-5-(2-hydroxy-5-methoxyphenyl)-4-phenyl-2(5*H*)-furanone (5j). This was prepared from methyl 2-oxo-3-phenylpropionate and 2-hydroxy-5-methoxybenzaldehyde. Precipitation in water and crystallization from ethyl ether afforded compound **5j** in 72% yield; mp 126 °C; IR (KBr) ν 3282, 1738, 1650, 1505 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.94 (br s, 1H), 9.65 (br s, 1H), 7.63 (d, 2H), 7.33 (t, 2H), 7.26 (t, 1H), 6.82 (m, 2H), 6.68 (s, 1H), 6.50 (s, 1H), 3.73 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 169.5 (C-2), 152.1, 149.8 (*C*–OH, *C*–OCH_3), 138.8 (C-3), 130.8 (C-4), 128.5, 128.3, 127.0, 116.8, 115.5, 113.1 (CH aromatic), 127.9, 122.8 (C_{ipso}), 74.5 (C-5), 55.3 (OCH_3). Anal. calcd for $C_{17}H_{14}O_5$, H_2O: C, 64.55; H, 5.10. Found: C, 64.68; H, 5.11.

5-(4-Benzyloxy-3,5-dimethoxyphenyl)-3-hydroxy-4-phenyl-2(5*H*)-furanone (7b). This was prepared from methyl 2-oxo-3-phenylpropionate and 4-benzyloxysyringaldehyde **6b**.[59,60] Precipitation in water and crystallization from ethyl ether led to compound **7b** in 52% yield; mp 166 °C; IR (KBr) ν 3302, 1750, 1674, 1592, 1504 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.05 (br s, 1H), 7.65 (d, 2H), 7.46–7.26 (m, 8H), 6.73 (s, 2H), 6.48 (s, 1H), 4.87 (s, 2H), 3.71 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 169.1 (C-2), 138.9 (C-3), 153.3, 153.4 (*C*–OBn, *C*–OCH_3), 137.7, 136.9, 132.2 (C_{ipso}), 130.7 (C-4), 128.5, 128.3, 128.0, 128.1, 127.9, 127.7, 127.3, 105.2 (CH aromatic), 80.0 (C-5), 73.9 (CH_2), 56.1 (OCH_3).

5-(3,5-Dimethoxy-4-hydroxyphenyl)-3-hydroxy-4-phenyl-2(5*H*)-furanone (5k). This was synthesized like **5d** in 97% yield from 5-(4-benzyloxy-3,5-dimethoxyphenyl)-3-hydroxy-4-phenyl-2(5*H*)-furanone **7b** (0.5 g); mp 197 °C; IR (KBr) ν 3342, 1747, 1672, 1620, 1573, 1449 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.97 (br s, 1H), 8.65 (br s, 1H), 7.65 (d, 2H), 7.38 (t, 2H), 7.29 (t, 1H), 6.67 (s, 2H), 6.45 (s, 1H), 3.73 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 169.1 (C-2), 148.1, 136.5 (*C*–OH, *C*–OCH_3), 138.7 (C-3), 130.8 (C-4), 128.4, 128.2, 127.4, 105.5 (CH aromatic), 127.7, 126.2 (C_{ipso}), 80.4 (C-5), 56.1 (OCH_3). Anal. calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 66.02; H, 4.90.

3-Hydroxy-4-(4-hydroxyphenyl)-5-phenyl-2(5*H*)-furanone (5l). This was prepared from methyl 2-oxo-3-(4-hydroxyphenyl)propionate and benzaldehyde. Precipitation in water and crystallization from ethyl ether gave compound **5l** in 21% yield; mp 258 °C; IR (KBr) ν 3338, 1740, 1709, 1664, 1641, 1607, 1581 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.61 (br s, 1H), 9.79 (br s, 1H), 7.48 (d, 2H), 7.37–7.35 (m, 5H), 6.72 (d, 2H), 6.46 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 169.4 (C-2), 157.8 (*C*–OH), 137.0 (C-3), 136.5, 121.5 (C_{ipso}), 129.2, 129.1, 128.9, 127.8, 115.3 (CH aromatic), 79.6 (C-5). Anal. calcd for $C_{16}H_{12}O_4$, $1/2H_2O$: C, 69.31; H, 4.72. Found: C, 69.45; H, 4.71.

3-Hydroxy-4-(4-hydroxyphenyl)-5-(4-methoxyphenyl)-2(5*H*)-furanone (5m). This was prepared from methyl 2-oxo-3-(4-hydroxyphenyl)propionate and 4-methoxybenzaldehyde. Precipitation in water and crystallization from ethyl ether gave compound **5m** in 44% yield; mp 256 °C; IR (KBr) ν 3333, 1705, 1667, 1608, 1582 cm^{-1}; ^1H NMR (DMSO-d_6) δ 10.58 (br s, 1H), 9.79 (br s, 1H), 7.46 (d, 2H), 7.28 (d, 2H), 6.91 (d, 2H), 6.72 (d, 2H), 6.41 (s, 1H), 3.72 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 169.4 (C-2), 159.8, 157.7 (*C*–OH, *C*–OCH_3), 136.5 (C-3), 129.0 (C-4), 129.2, 115.4, 114.3 (CH aromatic), 128.8, 121.6 (C_{ipso}), 79.4 (C-5), 55.1 (OCH_3). Anal. calcd for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73. Found: C, 68.67; H, 4.74.

Anti-oxidant assays

Measurement of the reducing activity against the stable radical DPPH. A solution of 4 mL of DPPH (10^{-4} M, 2 mg) in EtOH (50 mL) was added to 1 mL of a solution of the test compound in DMF, to prepare a 5×10^{-3} M sample solution. The absorbance at 517 nm was measured each 5 mn during 3 h. The absorbance of the control sample without test compound was measured simultaneously. The difference in absorbance between the control and the test compound was taken as the reducing activity.

Superoxide anion scavenging assay. The technique of Slater and Eakins[30] utilizing the interactions of NADH, phenazine methosulfate (PMS), molecular oxygen and nitro blue tetrazolium (NBT) was used for evaluating superoxide anion scavenging. The NADH/PMS/O_2/NBT system involves the intermediate formation of the

superoxide anion radical ($O_2^{\bullet -}$) from the interaction of reduced PMS with O_2; the superoxide anion radical then reduced NBT to the highly colored formazan. The reaction can be followed by measuring the absorbance of formazan at 578 nm. The incubation mixture contained disodium hydrogen phosphate buffer (200 μL, 19 mM, pH = 7.4), PMS (200 μL, 10.8 μM), NBT (200 μL, 172 μM) and NADH + H^+ (200 μL, 360 μM). In order to estimate their activity, the compounds were dissolved in the buffer at different concentrations and 200 μL of buffer in the reaction mixture was replaced by 200 μL of these solutions of compounds. In parallel, blanks were realized by remplacing 200 μL of NADH + H^+ by 200 μL of buffer. Each assay was performed after 5 min incubation.

Lipid peroxidation assay. Iron-dependent peroxidation of male rat liver microsomes was assayed as previously described.[61] Liver microsomes were prepared according to the technique of May and McCay.[62] Lipid peroxidation was initiated by the ADP-Fe^{2+}/ascorbate system[63] which produced hydroxyl radical ($^{\bullet}OH$). Incubations of 200 μL microsomal fractions (1 mg protein/mL) were carried out at 37 °C with 200 μL potassium phosphate buffer (0.1 M, pH = 7.4), 200 μL of ADP solution in buffer (2 mM), 200 μL of aqueous $FeSO_4$ solution (16 μM). Lipid peroxidation was started by adding 200 μL aqueous ascorbate solution (0.5 mM) to the above incubation mixture. The extent of polyunsaturated fatty acid peroxidation was measured spectrophotometrically by the rate of malondialdehyde formed after 90 min microsomial incubation. The reaction was quenched by the addition of 0.5 mL of 20% trichloroacetic acid; after centrifugation, the supernatant fraction was collected and then boiled with 0.5 mL of 0.67% thiobarbituric acid solution for 20 min. After cooling, samples were diluted with 1 mL of distilled water and extinction was read at 535 nm. In order to estimate their activity, the compounds were dissolved in disodium hydrogen phosphate buffer (19 mM, pH = 7.4) at different concentrations and 200 μL of buffer in the reaction mixture was replaced by 200 μL of these solution of compounds. In parallel, blanks were realized by replacing 200 μL of ascorbate by 200 μL of buffer.

Electron spin resonance measurement

ESR spectrum was recorded with a Varian (USA) E-109 spectrometer operating at 9.42 GHz with a 100 kHz high-frequency modulation amplitude of 0.5 gauss. The sample solution was examined in a flat quartz cell inserted in an E-238 cavity operating in the TM_{110} mode. The semiquinonic radical was obtained spontaneously by addition of NaOH (120 μL, 2 M) to 1 mL aerated solution of tested compound **5g** in methanol (1 mM). The spectrum was simulated with the public WinSim EPR software.[64] Coupling constants and signal intensity were adjusted using simplex method.

Lipophilicity measurements

Lipophilicity was determined by reversed-phase high-performance liquid chromatography using literature procedures.[65,66] A Varian 5000 liquid chromatograph equipped with a detector operating at 254 nm was employed. A Varian CDS 111L integrator was used for peak registration and calculation of retention times. An ODP column (15 cm × 6 mm ID), prepacked with octadecyl copolymer gel, particle size 5 μm, was used as a non-polar stationary phase. Mobile phases were prepared volumetrically from 65:35 to 95:5 combinations of methanol/acetic acid (0.085 N, pH = 3). The flow rate was 1 mL/min. Isocratic capacity factors k_i were defined as $k_i = (t_r - t_0)/t_0$ where t_r is solute retention time and t_0 is column dead time, determined by using methanol as the non-retained compound. Log k', used as the lipophilic index, was obtained by linear extrapolation of log k_i to 50% water.

Molecular modeling

All computations were performed using SYBYL 6.6 software package on a Silicon Graphics SGI Power Indigo R 8000 workstation. Structures were built within SYBYL and minimized with the Tripos force field, Maximin 2, in vacuo conditions, to provide reasonable standard geometries. Molecules were deemed to be minimized until there was a minimum energy change of less than 5×10^{-3} kcal mol^{-1} for one iteration. The BFGS method was used for minimization.

The conformational spaces of **5a**–**m** were explored using the SYBYL search facility. Torsion angles were defined around the single bonds C_4–$C_{ipso}Ar_1$ and C_5–$C_{ipso}Ar_2$ of **5**, and a gridsearch was performed allowing these bonds to rotate with a 180° and a 360° revolution by 15° increments for unsubstituted and substituted aromatic rings, respectively. The lowest energy conformers thus obtained were submitted to AM1 calculations (MOPAC version 6.0)[67] to optimize their geometry and calculate physicochemical parameters: enthalpy of formation ΔH_f and energy of the highest occupied molecular orbital (HOMO). The radical corresponding to each lowest energy conformer was obtained by removing the enolic or phenolic hydrogen. The calculations of open shell systems were performed using the Unrestricted Hartree-Fock method (UHF keyword). To avoid spin contamination and because the UHF energy cannot be compared with the restricted Hartree Fock (RHF) energy of the mother structure, a SCF (1SCF keyword) Restricted Open Shell calculation was performed on the UHF-optimized radical structure. The calculated enthalpies of formation were used to calculate the enthalpies of reaction of H-atom abstraction ($\Delta\Delta H_f$). Similarly, the enthalpies of reaction of single electron transfer ($\Delta\Delta H_{ox}$) were calculated after geometry optimizations of radical cations using AM1 method (Charge +1, UHF keyword and then 1SCF calculation on the UHF-optimized geometry).

Pharmacological assays

All tests were performed in accordance with the recommendations and policies of the International Association for the Study of Pain (IASP). [68]

Acute toxicity in mice. The compounds were administered ip as suspensions in saline (0.9% NaCl) at doses of 200, 400, 600 and 800 mg/kg. Swiss male mice purchased from Elevage Depre (Saint-Doulchard, France) weighting 18–22 g were used. Mice were kept in groups of 10 in a temperature controlled room with a 12 h light/dark cycle. Food and water were available ad libitum during the time of the experiment. The animals were observed for 8 days in order to detect any sign of toxicity.

TPA-induced ear edema. Male Swiss mice (20–22 g) were used (Elevage Depre, Saint-Doulchard, France). The anti-inflammatory activity of the drugs studied was evaluated according to the procedure of Carlson et al.[52] Mice were divided into groups of 10. Phorbol 12-myristate 13-acetate (TPA) (Sigma-Aldrich, Saint-Quentin Fallavier, France) was dissolved in 80% aqueous ethanol at concentration of 250 μg/mL. Drugs or saline were given intraperitoneally (0.25 mL/20 g) or orally (0.50 mL/20 g). Thirty minutes after intraperitoneal drug administration or 1 h after oral drug administration, TPA was applied topically by an automatic pipette to the right ear (10 μL) and left ear only received the vehicle (10 μL of ethanol/water 80:20). Inflammation was allowed to develop for 3 h 30 min after TPA. Edema measurements were taken with an Oditest Calipers (Oditest S 5010-Kreoplin, Gmbh, Germany). The thickness of the right and left ears was measured in units of 0.01 mm. Ear edema was calculated by substracting the thickness of the left ear (vehicle control) from the right one (treated ear).

Carrageenan-induced paw edema in rat. Male Sprague–Dawley rats (120–140 g) were used (Elevage Depre, Saint-Doulchard, France). The anti-inflammatory activity of drugs studied was evaluated according to the procedure of Winter et al.[55] Rats were divided into groups of six and treated intraperitoneally (0.25 mL/100 g) or orally (0.50 mL/100 g) with compounds or saline. Thirty min after intraperitoneal drug administration or 1 h after oral drug administration, 0.05 mL of a w/v 1% suspension of carrageenan (Sigma-Aldrich, Saint-Quentin Fallavier, France) in deionized water was given by intraplantar injection into the right paw. Animals were returned to the cage and 3 h later, edema was measured with a plethysmometer (V.G.O. Basile-Apelex 05.7150). The edema means of the compound-treated groups were compared to the vehicle to give the percentage of inhibition of paw swelling.

Data analysis. Statistical analysis of the results was performed using the method of Schwartz.[69] Data were analyzed by using the Student's *t*-test. All values were expressed as mean ± SD.

Acknowledgements

The authors would like to thank Prof. Philip Hoggan from Université Blaise Pascal, Clermont-Ferrand, for fruitful discussions, and Anne Marie Privat from Laboratoire de Pharmacologie, Université d'Auvergne for technical assistance.

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