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Effect of different C3-aryl substituents on the antioxidant activity of 4-hydroxycoumarin derivatives

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ABSTRACT

The antioxidant activity of 4-hydroxycoumarin synthetic derivatives and 4-methylumbelliferone were determined taking 4-hydroxycoumarin as the reference compound. Six 3-aryl-4-hydroxycoumarin derivatives were synthesized from 4-hydroxycoumarin as precursor in order to evaluate changes in their antioxidant properties due to C3-aryl substituent nature. Free radical scavenging capacities of these compounds against two different species DPPH· and $ABTS^{\cdot+}$ and the protecting ability towards the β-carotene-linoleic acid co-oxidation enzymatically induced by lipoxygenase were measured. In addition, the relationship between the activities of these molecules against DPPH radical and the bond dissociation energy of O–H (BDE) calculated using methods of computational chemistry was evaluated.

1. Introduction

Coumarins include a vast array of biologically active compounds ubiquitous in green plants. Many of them have been used in traditional medicine for thousand years. Coumarin basic structure consists of a fused benzene with a α-pyrone ring known as 1,2-benzopyrone. Among them, hydroxy-coumarin derivatives constitute an important group of low-molecular weight phenolics[1] which have widely been used for the prevention and treatment of venous thromboembolism, myocardial infarction and strokes.[2] The medicinal properties of coumarins include inhibition of platelet aggregation, cytochrome P450, and steroid 5a-reductase, spasmolytic action, anticoagulant, antibacterial, anticancer, and anti HIV activities.[3–5] Some natural coumarins extracted from the dried bark of *Fraxinus* spp. (Cortex Fraxini) were found to prevent the formation or to scavenge oxygen and nitrogen reactive species; therefore exhibiting tissue protective antioxidant properties.[6]

Antioxidants are compounds that can delay, inhibit, or prevent the oxidation of sensitive compounds by different mechanisms as scavenging free radicals, inhibiting prooxidant enzymes or chelating active metal ions according to the nature of the oxidizing agent.[7] Free radicals have a key role in the initiation and advance of serious diseases like heart condition, and cancer. Besides, they are also able to promote DNA damage. Oxidation in biological systems may lead to food quality loss, cellular membrane dysfunction, and aging.[8–11]

Antioxidant properties of hydroxycoumarins have attracted great interest of several research groups due to their bioactivity and promising pharmacological properties. However, their extraction from natural sources, as plants, is sometimes inconvenient since it is time consuming and requires high-technology equipment. Therefore, synthetic methods to obtain coumarin derivatives have strongly been encouraged in recent years. Moreover, the possibility of varying the chemical structures by introducing specific substituents allows obtaining more active molecules.

For instance, in the case of phenolic compounds, their antioxidant activity is strongly related to their molecular structure, more precisely to the presence, location and number of hydroxyl groups, and to conjugation and resonance effects.[12] The phenolic substitution pattern is the key factor which defines the ability of the phenolic compound to scavenge free radicals by transferring its hydrogen atom in a single or a sequential process to the electron unpaired species.

Different families of hydroxycoumarins have already been synthesized and their antioxidant properties investigated to conclude that the number and position of the hydroxyl groups are essential for their activity.[13–15] However, only basic structures have been studied and the relation between substituent presence and their reactivity towards different oxidizing agents has not been completely elucidated yet. Most of these studies are devoted to coumarin derivatives bearing the hydroxyl groups in positions 5, 6, 7, or/and 8, which means that they are phenolic groups and, in some cases, catechol ones. In the case of 4-hydroxycoumarins, the

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hydroxyl group is bound to a vynilic carbon, in consequence, a different chemical behavior can be expected. Despite of the fact that their medicinal properties have been recognized, 4-hydroxycoumarins have been little studied as antioxidants. Recently, Stanchev et al. reported the antioxidant activity in vitro in hypochlorous system of a series of 4-hydroxycoumarin derivatives based on the luminol-dependent chemiluminescence.[16] Differently substituted 4-hydroxycoumarins were tested for free radical scavenging activity in the system of 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radical.[17]

The aim of this study was to determine the free radical scavenging activity, antioxidant action and prooxidant-enzyme inhibition of six C3-aryl-4-hydroxycoumarin synthetic derivatives with those of the basic 4-hydroxycoumarin, in order to evaluate the effect of different aryl substituents in C3, and with those of 4-methylumbelliferone, as a typical 7-hydroxycoumarin.

2. Materials and methods

2.1. Chemicals

The reference compound, 4-hydroxycoumarin, was obtained from Sigma Aldrich and 4-methylumbelliferone was synthesized according to the slightly modified Pechmann method.[18] Others derivatives of 4-hydroxycoumarin were synthesized as previously described.[19]

The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·), Tween-20 (polyoxyethylene-sorbitan monolaurate) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Aldrich (Buenos Aires, Argentina). Soybean lipoxygenase type I-B, and β-carotene, were provided by Sigma (Buenos Aires, Argentina), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was from Fluka (Buenos Aires, Argentina), linoleic acid (99%) was from Riedel de Haën (Buenos Aires, Argentina). All other reagents (potassium persulphate, borate sodium, methanol, acetic acid, hydrochloric acid and chloroform) were supplied by Ciccarelli (Buenos Aires, Argentina).

2.2. DPPH·scavenging capacity assay

Radical consumption fraction by coumarin action was determined according to Brand-Williams et al.[20] Typical procedure consisted of adding an aliquot of the sample to a cuvette containing 3 ml of ca. 85 μM DPPH· solution. Reaction progress was followed by UV–Vis spectrophotometry and measuring the absorbance at 515 nm in cycles for 10 min. Radical consumption was expressed as percentage of antiradical activity (ARA) as proposed by Burda and Oleszek[21] and calculated according to the following (Eq. (1)):

$$\%\mathrm{ARA} = 100 \times [1 - A_{SS}/A_0] \quad (1)$$

where A_0 is the absorbance of the DPPH· solution before adding the antioxidant and A_{SS} is the absorbance at the steady state estimated by mathematical fitting of the kinetic curves. Percentages of radical consumption for different antioxidant concentrations were measured. EC_{50} value corresponds to the concentration that scavenges the 50% of the radicals, expressed as the antioxidant/DPPH· mole ratio.

2.3. ABTS·+ scavenging capacity assay

An ABTS stock solution (7 mM) was mixed with 2.45 mM potassium persulphate solution and incubated at room temperature in the dark for 16 h.[12] Afterwards, the solution was diluted with water to an absorbance value of 0.7 ± 0.1 AU at 734 nm. An aliquot of coumarin sample was added to a cuvette containing 3 ml of ABTS·+ solution. Radical consumption was monitored by spectrophotometry at 734 nm. Results were expressed as Trolox equivalent antioxidant capacity (TEAC)[22] by using Trolox as a reference compound for calibration purposes.

2.4. Antioxidant activity in the β-carotene-linoleic acid co-oxidation enzymatically induced by soybean lipoxygenase

The experiment was carried out according to Chaillou and Nazareno[23] with minor modifications. Linoleic acid solution was prepared by mixing this compound with Tween-20 and diluting with 0.01 M borate buffer pH 9 up to a 330 μg/ml concentration. An aliquot of 500 μL a saturated solution of β-carotene in chloroform was mixed with the same amount of Tween-20. Chloroform was removed using a nitrogen stream. β-Carotene solutions were prepared by adding pH 9 buffer to a final carotene absorbance equal to 1.00. β-Carotene and linoleic acid solutions were mixed in a 3 ml cuvette; then, an aliquot of the coumarin sample was added. Finally, 200 μL of 1000 μg/ml LOX solution were added to initiate the reaction, which was measured by monitoring the absorbance at 464 nm during 10 min. The same procedure excluding sample addition was done for control. All assays were carried out in triplicate at room temperature (25 ± 1 °C). Antioxidant activity (AOA) was calculated[21] as the percentage of inhibition of β-carotene bleaching of the samples compared to that of the control using the (Eq. 2):

$$\%\mathrm{AOA} = 100 \times \left[1 - \left(A_s^0 - A_s^t\right)/\left(A_c^0 - A_c^t\right)\right] \quad (2)$$

Where A_s^0 is the absorbance of the sample at 0 min, A_c^0 is the absorbance of the control at 0 min. A_c^t and A_s^t are the absorbances at t = 10 min of the control and the sample, respectively. All determinations were performed by triplicate.

2.5. Theoretical calculations

The details of our methodology and those needed to obtain the BDE, are given here. All calculations reported in the present study were carried out employing the density functional theory, as implemented in the GAUSSIAN 03 package.[24] B3LYP[25] level of density functional theory was used. The geometry optimization of radicals and neutral species was performed with UB3LYP and the restricted B3LYP, respectively, by using the 6-31G(d) and 6-31++G(d,p) basis set. In the computations, no constrains were imposed on the geometry. All possible conformers for the ArOH and ArO· were investigated. The conformer with the lowest electronic energy was used in this work. All structures were true minima on the calculated potential surface, verified by frequency calculations. Vibrational frequencies were computed at the same level of theory for all the optimized structures.

The enthalpy was obtained by thermal correction to the electronic energy by adding zero-point energy (ZPE), translational, rotational, and vibrational contribution.

3. Results and discussion

Six 4-hydroxy-3-aryl-coumarins were selected to analyze their antioxidant activities taking as reference the unsubstituted 4-hydroxycoumarin. Besides, 7-hydroxy-4-methylcoumarin (4-methylumbelliferone) was also measured to compare the reactivities between 4-hydroxy and 7-hydroxycoumarins. The Figure 1 shows the chemical structures of the studied compounds.

3.1. Free radical scavenging capacity of 4-hydroxycoumarins derivatives

In order to determine the relative ability of these substances to scavenge free radicals, two spectrophotometric methods were

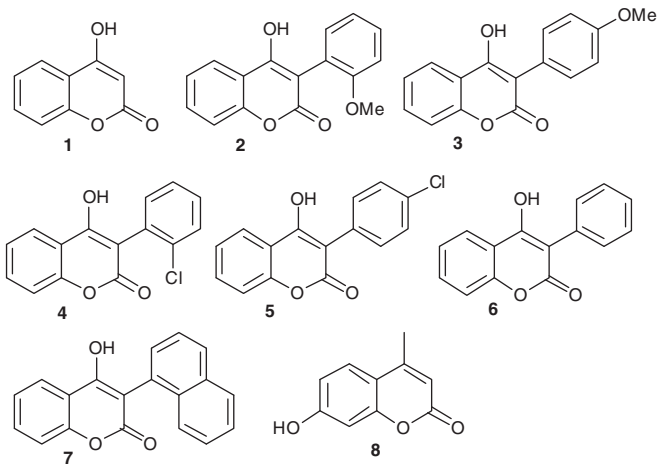


Figure 1. Chemical structures of the studied coumarins.

used. The antiradical capacities against two different reactive species, DPPH· and $ABTS^{\cdot+}$, were measured by monitoring radical consumptions by the coumarin action. DPPH· is a synthetic free radical which can be readily obtained dissolving the solid before use, while $ABTS^{\cdot+}$ must be previously generated by a chemical reaction of ABTS.

3.1.1. Antiradical activity towards DPPH·

The kinetic profiles for DPPH· disappearance by addition of the synthetic hydroxycoumarin (**3**) is shown in Figure 2.

The inset shows a linear variation of the ARA with the concentration of **3** in the system. Similar behavior was observed for every coumarin of the family indicating that the compounds studied behaved as dose-dependent antioxidants.

Table 1 shows the effective concentration to reduce the 50% of the radical (EC_{50}) of the studied compounds. The increasing activity order observed in this system (inverse to the EC_{50} values) was **8** < **7** < **6** ≈ **5** ≈ **4** < **1** < **2** < **3**. The compounds bearing *o*- and *p*-OMe groups as substituent of the aryl ring in C3 had the highest activity whilst the lowest one corresponded to 4-methylumbelliferone and **3** is seven times more active than **1.**

These results indicated that 4-hydroxycoumarins, although they are not phenolic compounds, are more active than a typical

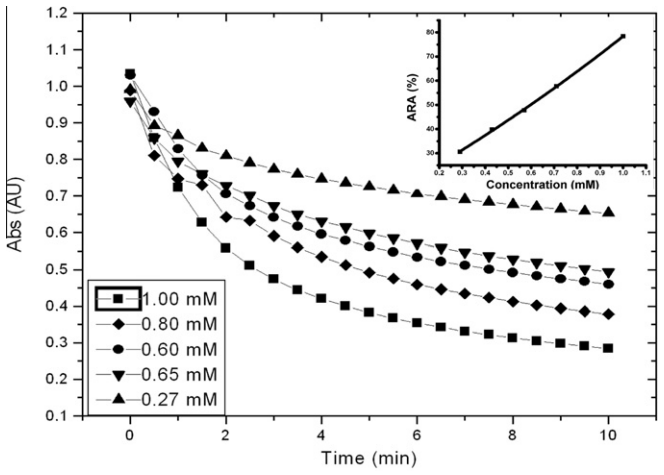


Figure 2. Kinetic profiles at 515 nm corresponding to DPPH· consumption by additions of the coumarin derivative **3**. Inset: antiradical activity of the coumarin derivative **3**.

7-hydroxycoumarin. Previously reported data indicate that the substitution in the pyrone ring of the coumarin moiety has little influence on the antioxidant activity of coumarins with a phenolic group.[26] However, the substitution at C3 of **1** with electron-donating groups (OPh, benzyl) increased twice the scavenger activity but did not change with phenyl group.[17]

We studied the influence of substituents on the 3-phenyl group, not directly over the pyrone ring. When the substituents in C3 position of the 4-hydroxycoumarin structure were phenyl (**6**), 1-naphthyl (**7**), 1-(4-chlorophenyl) (**5**) and 1-(2-chlorophenyl) (**4**), the activity was lower than 4-hydroxycoumarin (**1**). All of them have as a common feature that they behave as electron-withdrawing groups. Compounds **4**, **5** and **6** presented all similar activities. The chlorine substituent of the aryl group bound to C3 did not change notoriously the behavior refer to **6**. Compound **7** has a more voluminous group than the other 3-aryl substituted coumarins, in consequence, its lower activity can be ascribed to a greater steric hindrance than **5** to react with DPPH· radical. The behavior of this family depends markedly on the nature of the C3 substituent group. Two main factors have to be taken into account: first, the electronic characteristics (the electron releasing or electron withdrawing character of the groups) and second, the steric hindrance of the molecules because the steric accessibility to the DPPH· is determinant of the reaction, since it has been found that small molecules that have better access to the radical site have relatively higher antioxidant capacity.[27]

It has been proposed that DPPH· radical reacts with phenols essentially via two different mechanisms: (i) a direct abstraction of phenolic H-atom by DPPH· (HAT reaction) and (ii) an electron transfer process from the phenoxide anion to DPPH· (SPLET reactions), as shown in Scheme 1.[28]

SPLET mechanism would be dramatically influenced by the pH in the reaction medium. At basic pH, SPLET mechanism would be promoted since the phenol ionizes and prevails as an anionic species; in contrast to the situation at acidic pH where the neutral species would be the predominant one and, therefore, the main mechanism would be HAT (Scheme 1).

The first experimental analyses to establish the differences between these mechanisms were those reported by Litwinienko and Ingold,[28] and Foti et al.[29] They found that the reaction rates of different phenols towards DPPH· were modified by adding different concentrations of acetic acid to the system, the reaction being generally slower.

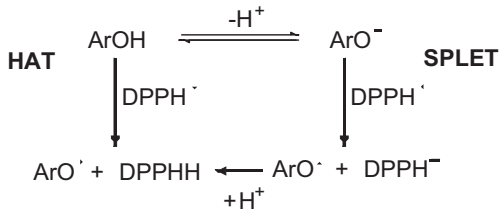
In order to evaluate the influence of SPLET mechanism in the global reactivity of the family of hydroxycoumarins against DPPH·, the EC50 values for 4-hydrocycoumarin were determined with the addition of acetic acid to final concentration of 10 and 100 mM.[30] The EC_{50} value of compound **1** was slightly modified in both cases (10 mM EC_{50} = 96% and 100 mM EC_{50} = 94%) respect to the EC_{50} without any addition (in the standard conditions of system). This experiment allows to determine the contribution of SPLET mechanism to the global reaction, being this participation of about 5%, and to propose the HAT pathway as the primary mechanism of the reaction of hydroxycoumarins with DPPH· in the present experimental conditions.

3.1.2. Antiradical activity towards $ABTS^{\cdot+}$

This method was used to measure the ability of antioxidant substances to scavenge free radicals ($ABTS^{\cdot+}$) in aqueous solution in order to compare with the activity obtained towards DPPH· in a methanolic medium. Figure 3 shows the kinetic profiles for the radical disappearance for different additions of sample **3**. According to these results and using the calibration curve done for Trolox (R = 0.99919 for a range of 5–20 μM), the antiradical activities determined for the coumarins derivatives studied, expressed as μmol Trolox/g sample (TEAC), are shown in Table 1.

Table 1
Antioxidant activity

Compound	DPPH· EC_{50} (mM)	$ABTS^{\cdot+}$ TEAC (μmol Trolox/g)	β-Carotene linoleic acid co-oxidation (%AOA $mmol^{-1}$)
1	3.80 ± 0.05	186 ± 3	2.07 ± 0.02
2	1.92 ± 0.04	567 ± 5	25.54 ± 0.03
3	0.54 ± 0.01	699 ± 7	23.07 ± 0.04
4	4.14 ± 0.01	50 ± 1	nd
5	4.15 ± 0.03	50 ± 4	5.94 ± 0.01
6	4.19 ± 0.02	46.6 ± 0.3	5.79 ± 0.04
7	6.41 ± 0.03	56.8 ± 0.6	4.26 ± 0.02
8	8.90 ± 0.02	54.8 ± 0.4	<0.50



Scheme 1.

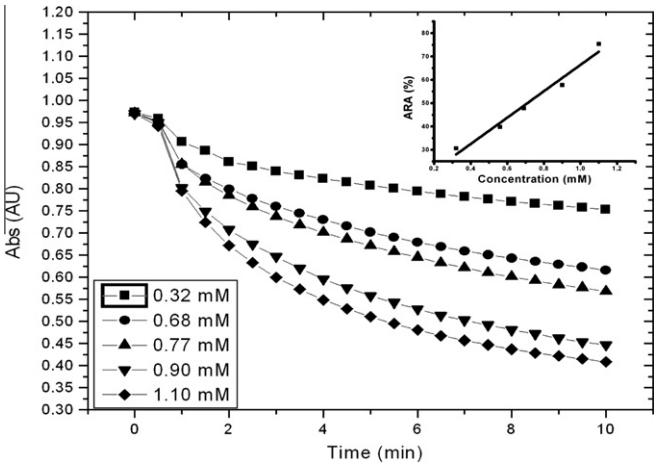


Figure 3. Kinetic profiles of $ABTS^{\cdot+}$ consumption by sample additions. Inset: antiradical activity of the coumarin derivative **3**.

The order of increasing activities for the series was **6** < **7** < **4** ≈ **5** < **7** ≈ **8** < **1** < **2** < **3**. This trend was the same as that observed towards DPPH·, and the compounds with *o*-OMe or *p*-OMe groups as substituents in the C3-aryl ring having the highest activity. Although in the case of the other compounds, a little variation in the sequence was found. In this system the compounds with electron withdrawing groups (**4**–**7**) and **8** presented similar activities. The lowest one corresponded to compound **6**. This result indicated that the electronic properties (inductive effect) of the substituent group have a key role to determine the behavior of the family.

3.1.3. Antioxidant activity in β-carotene-linoleic acid co-oxidation reaction induced by LOX

The antioxidant activity of the coumarins derivatives was assayed towards the enzymatically induced co-oxidation of linoleic acid and β-carotene in a micelle system. This methodology that mimics biological systems considers the ability of a compound to reduce carotene consumption as result of breaking of the chain propagation reaction by scavenging peroxyl radicals or/and preventing initiation step of lipoperoxidation by enzymatic inhibition.[22] Oxidation reaction progress was monitored by spectrophotometry as the carotene bleaching at 460 nm. Figure 4 shows the kinetic behavior of β-carotene disappearance with and without additions of compounds **2** and **3**.

Similar behavior was observed with the other compounds studied. In Table 1, the values of antioxidant activity per mmol (%AOA/mmol of hydroxycoumarin) are summarized. The order of increasing activity of the coumarin derivatives in this system was **8** < **1** < **7** < **5** ≈ **6** ≪ **3** < **2**.

The highest activities in the LOX system were found for compounds **2** and **3** having *o*-OMe or *p*-OMe groups respectively as electron donor substituents in the C3-aryl ring. In contrast to the order of reactivity observed against DPPH· and $ABTS^{\cdot+}$ radicals, **2** was more active than **3**. The other 3-aryl-4-hydroxycoumarins studied presented lower activities than **2** and **3** whilst 4-methylumbelliferone was the less active.

Lipoxygenase (LOX) is an iron-containing enzyme that catalyses the dioxygenation of polyunsaturated fatty acids in lipids. LOX has recently become of interest, as it is considered the key enzyme in the biosynthesis of leukotrienes that have been postulated to play an important role in the pathophysiology of several inflammatory and allergic diseases.[31]

Roussaki et al.[32] compared the behaviour of other family of 3-arylcoumarins in two lipoperoxidation systems: linoleic acid oxidation induced by an azocompound and the reaction induced by LOX. They showed that the presence of OH group is required for activity against DPPH· but not for enzyme inhibition. In general some good radical scavengers of DPPH· were not active as LOX inhibitors. This shows that the mechanism of enzyme inhibition is different from that of breaking the radical propagation chain but has a marked influence on the overall antioxidant activity of compounds.

3.1.4. Comparison of the three tested methods

In order to compare three different experimental systems, the relationships between the activities of all molecules assayed and compound **1** were plotted, as Figure 5 shows. The highest activities were found for the compounds **2** and **3**.

The compound **3** was more effective as scavenger than **2** against both DPPH· and $ABTS^{\cdot+}$. However, **3** presented a remarkably higher activity than that of **2** in the DPPH· system. This may be explained

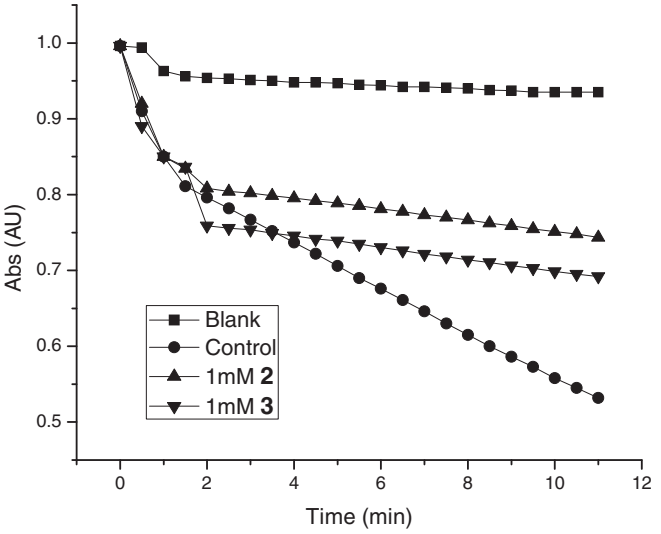


Figure 4. Protective effects of coumarins against β-carotene consumption induced by lipoxygenase in a co-oxidation system with linoleic acid. Absorbance was monitored at 460 nm.

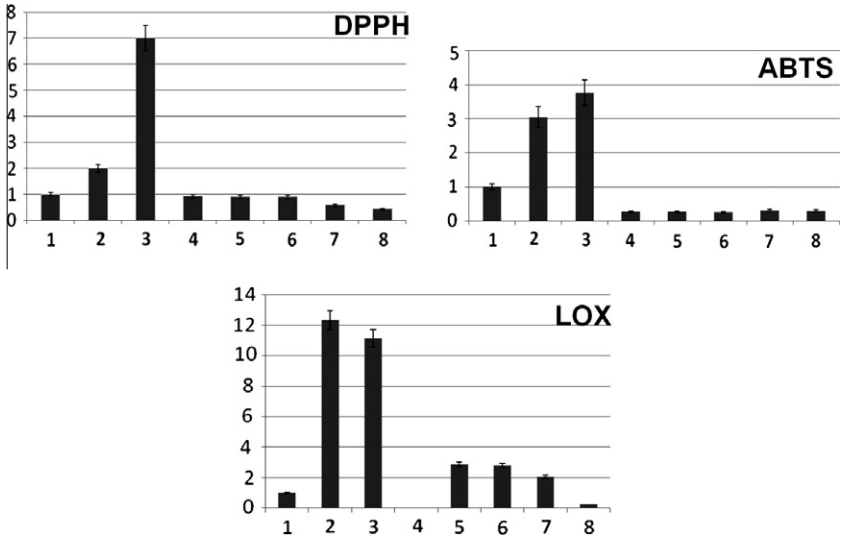


Figure 5. Comparison of the relative antioxidant activities of the studied compounds (**2**–**8**) referred to **1**.

due to the largest steric hindrance of **2** to react with DPPH· and not with ABTS·+ because DPPH· presents bulky groups linked to the radical center (NO_2 groups, for example), while ABTS·+ has only an ethyl group as a substituent of the radical center and hence, a more accessible structure than DPPH·. However, in the enzymatic system the activity supremacy was reversed, being **2** more active that **3**. Moreover, the relative activity of these compounds respect to **1** increased considerably in the enzymatic system. This behavior clearly indicated that the inhibition of LOX prevails over the radical scavenging process.

Compounds **4**, **5**, **6** and **7** presented lower activities in the ABTS·+ system than in the DPPH· and LOX systems. This behavior might be explained as their lower solubility in water, the solvent used in these experiments. Here again, the enzymatic inhibition was the prevalent mechanism of these compounds to prevent co-oxidation reaction in the system induced by LOX.

The lowest activity in all systems was presented by **8** among the compounds assayed which exhibited an extremely low capacity to inhibit LOX or react towards the free radicals.

3.1.5. Theoretical calculation

According to current knowledge of the processes of ArOH radical scavenging and considering our experimental results, HAT is the mechanism that mainly occurs in the reactions of DPPH radical with hydroxycoumarins. Antiradical properties of ArOH are related to their ability to transfer their phenolic H-atom to a free radical. Numerous authors have suggested that BDE is an excellent primary descriptor of the antioxidant activity. The HAT mechanism corresponds to the homolytic dissociation of an O–H bond. This mechanism depends of two bond dissociation enthalpies (BDEs), the O–H BDE of ArOH and the H–R BDE of the radical. The O–H BDE can be calculated by the following equation[33]

$$BDE_{ArO-H} = \Delta_f H(ArO^{\cdot}) + \Delta_f H(H^{\cdot}) - \Delta_f H(ArOH) \quad (3)$$

where $\Delta_f H$ (ArO·) is the enthalpy of formation of the radical of hydroxycoumarin generated after H-abstraction, $\Delta_f H$ (H·) is the enthalpy of formation of the hydrogen atom, and $\Delta_f H$ (ArOH) is the enthalpy of formation of the antioxidant molecule. A lower BDE value is usually attributed to a greater ability to donate a hydrogen atom from the hydroxyl group and resulting in an easier free radical scavenging reaction. The generated radical is a relatively stable free radical.

On the other hand, the density functional theory (DFT)-based approaches are able to compute reasonably accurate BDEs.[34,35]

In order to obtain the $\Delta_f H$ for different species, we calculated the most stable conformer of the neutral and radical form of **2** y **3**. The structures of these are shown in Figure 6.

In this work, BDEs of studied compounds were determined using two approaches. In the first, BDEs were approximated from the calculated total electronic energies, E_0. The main reason of this approach application was the effort to omit any corrections. In the following text and tables, BDEs approximated from total electronic energies will be denoted "BDE_{E0}". Moreover, the plausibility of this hypothesis has been confirmed for a large number of phenols, tocopherols and chromans.[35] In the second approach, BDEs were calculated on the basis of (Eq. 3) to obtain gas phase values at 298 K.

We have calculated BDE_{E0} and BDE of coumarins (as Table 2 shows). The gas phase BDE are lower than BDEE0 values by 6–7 kcal/mol but the relative energy (ΔBDE) between the substituted coumarins **2**–**8** respect to **1** are the same order in both cases.

In general, all 3-aryl substituted compounds would be better antioxidants because they have lower dissociation energies of the 4-hydroxycoumarin.

However, the substituent location in the phenyl moiety has an evident influence on the activity; the same group in *ortho*-position decreasing the activity with respect to *para* substitution as indicates the comparison between **2** and **3**, or between **4** and **5**. This behavior is ascribed to the steric hindrance that reduces the possibility of DPPH· to abstract the hydrogen of the OH group.

The Figure 7 shows the activity dependence versus BDE. Three different trends were found: (i) without phenyl substituents, (ii) substituents in *para*-position of C3-aryl group of the 4-hydroxy-

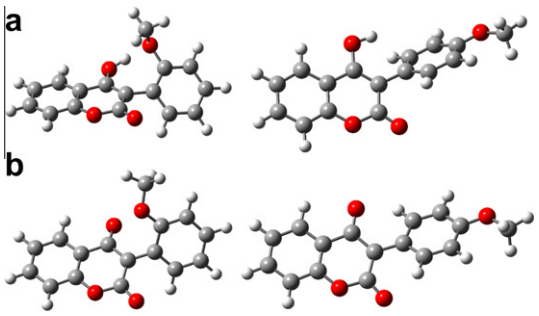


Figure 6. The structures of ArOH (a) and ArO· (b) of **2** and **3** activities of all molecules.

Table 2
The BDEs of coumarin derivatives

Compound	BDE_{E0} (kcal/mol)	ΔBDE_{E0} (kcal/mol)	BDE (kcal/mol)	ΔBDE (kcal/mol)
1	88.50	0	81.44	0
2	86.03	−2.47	79.36	−2.08
3	79.37	−9.13	72.90	−8.54
4	87.15	−1.35	80.34	−1.11
5	82.03	−6.47	75.38	−6.07
6	82.66	−5.84	75.99	−5.47
7	85.28	−3.22	78.51	−2.93
8	87.41	−1.09	80.41	−1.04

BDE = H(ArO·) + (−0.49791 eV) − H(ArOH).
$BDE_{E0} = E_0(ArO^{\cdot}) + (-0.50027\ eV) - E_0(ArOH)$.

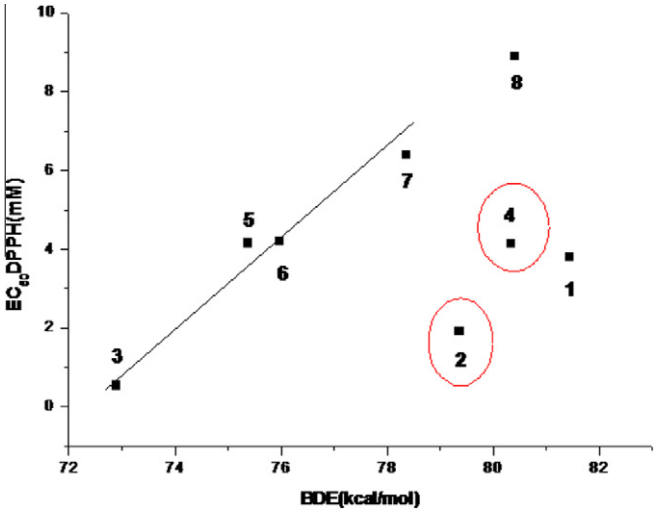


Figure 7. BDE of coumarins versus EC_{50} DPPH·.

coumarin and (iii) substituents in *ortho*-position of C3-aryl group of the 4-hydroxycoumarin.

The results obtained show that the activity to DPPH· is in agreement with the BDE of the 3-aryl-4-hydroxycoumarin. Various studies are now in progress in order to extend these theoretical studies including the solvent model.

4. Conclusion

The antioxidant activity of the new 3-aryl substituted 4-hydroxycoumarins was evaluated using two antioxidant assays: the radical scavenging ability of the compounds was tested against the DPPH· radical and the ABTS radical cation, and their ability to inhibit the enzymatically induced co-oxidation of linoleic acid and β-carotene.

It is evident, for this series of 4-hydroxycoumarin compounds that the presence of the 3-aryl substituents is crucial for the activity increase: the corresponding methoxylated analogues **2** and **3** being the most active radical scavengers. The activity was remarkably enhanced in the LOX-induced oxidation.

Not only the presence of 3-aryl substituents, but also their electron-donor character and steric-hindrance properties, had strong influence enhancing the antioxidant activity of the coumarins. Although, they are not directly bound on the coumarin basic structure but in the aryl system in position 3, are able to stabilize the electrophilic radical formed; unless, the steric hindrance due to bulky group in *ortho*-position produce a twisting of plane of the aromatic ring preventing delocalization of the odd electron.

An excellent correlation between computed bond dissociation energies and experimental EC_{50} (DPPH·) values of C3-aryl 4-hydroxycoumarins has been established. This information could be valuable to predict the EC_{50} values of differently substituted coumarins in order to select the most active compounds before chemical synthesis.

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