

Remarkable antioxidant properties of a series of hydroxy-3-arylcoumarins



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ABSTRACT

In the present work we synthesized a series of hydroxy-3-arylcoumarins (compounds **1–9**), some of them previously described as MAO-B selective inhibitors, with the aim of evaluating their antioxidant properties. Theoretical evaluation of ADME properties of all the derivatives was also carried out. From the ORAC-FL, ESR and CV data it was concluded that these derivatives are very good antioxidants, with a very interesting hydroxyl, DPPH and superoxide radicals scavenging profiles. In particular compound **9** is the most active and effective antioxidant of the series (ORAC-FL = 13.5, capacity of scavenging hydroxyl radicals = 100%, capacity of scavenging DPPH radicals = 65.9% and capacity of scavenging superoxide radicals = 71.5%). Kinetics profile for protection fluorescein probe against peroxy radicals by addition of antioxidant molecule **9** was also performed. Therefore, it can operate as a potential candidate for preventing or minimizing the free radicals overproduction in oxidative-stress related diseases.

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1. Introduction

Phenolic compounds are bioactive substances widely distributed in the vegetable kingdom. Generally, this group of compounds has one or more aromatic rings in their structure and one or more hydroxyl groups. They have been described to act as natural antioxidants and their presence contributes to prevent or minimize several types of oxidative processes.¹ Due to their antioxidant activity their ingestion is correlated with interesting benefits to health. Therefore, the research and characterization of new bioactive phenolic substances from diet has been intensified in the last years, either for the development of nutraceuticals or medicines.¹ Due to their antioxidant properties they can protect cells from the oxidative damage of the reactive oxygen species (ROS). In fact, the overproduction of free radicals have been related to cellular membrane and DNA damage, and indirectly with aging and oxidative-stress related diseases like cancer, cardiovascular and

neurodegenerative pathologies.² Therefore, antioxidants are very important for protecting the organisms from oxidative disorders, in which ROS are also involved.^{3,4} Antioxidants are capable of decrease or prevent oxidation processes through different mechanisms, such as scavenging free radicals, inhibition of pro-oxidant enzymes or chelation of transition metal ions.⁵

An increasing number of reports suggested the involvement of oxidative stress in neurodegenerative diseases (ND), where the increased formation of ROS can contribute to neuronal damage and cell death.^{3,4}

Suggestion has been made that the etiology of Parkinson's (PD) and Alzheimer's (AD) diseases may be closely linked to biochemical changes resultant from this oxidative stress.^{6–8} Dopamine (DA) auto-oxidation naturally produces oxidative species and may contribute to ND such as PD and ischemia/reperfusion-induced damage. Monoamine oxidase (MAO) enzyme (particularly MAO-B) is responsible for metabolizing DA and plays an important role in oxidative stress through altering the redox state of neuronal and glial cells, leading to neuronal death.⁹ Consequences are an over-production of MAO and non-MAO initiated hydrogen peroxide (H₂O₂) by proliferated reactive microglia and inability of neurons to dispose of H₂O₂ and other reactive species like peroxy radicals.¹⁰ H₂O₂ produces highly toxic ROS, namely hydroxyl

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radical, by Fenton reaction that is catalyzed by iron and neuro-melanin.¹¹ Concerning the mechanism of the clinical efficacy of MAO-B inhibitors in PD, the inhibition of DA degradation (a symptomatic effect) and also the prevention of the formation of neurotoxic DA degradation products, that is, ROS and DA derived aldehydes have been speculated.¹² The neuroprotective effect of rasagiline, a well-known MAO-B inhibitor, might be explained through multiple mechanisms, possibly due to reduction of DA catabolism with a subsequent increased activity on dopaminergic D₂ receptors and suppressing the action of ROS as well.¹³ So, the possible mechanism of neuroprotection of MAO-B inhibitors may be related not only to MAO-B inhibition but also to induction and activation of multiple factors related with oxidative stress and apoptosis.¹⁴

Coumarins are a family of compounds widely distributed in the nature.¹⁵ Due to their structural features, and biological properties, namely anticancer, anti-inflammatory, antioxidant, antithrombotic, vasorelaxant, antiviral and enzymatic inhibition agents, they have been ascribed as important building blocks in Organic Chemistry and Medicinal Chemistry.^{16–23}

Recently, it was shown by our group that 3-substituted aryl coumarins are potent and selective MAO-B inhibitors.^{24–30} In addition, it has been found that hydroxycoumarins are antioxidants scavenging ROS and/or chelating transition metals, exhibiting tissue-protective properties.^{6,31–33} The complementarity of these activities for 3-arylcoumarins was not previously studied and described. The versatility of the used reactions allowed obtaining a family of compounds with hydroxyl and/or methyl substituents in different positions of the molecule. The election of these derivatives has considered the previously MAO-B inhibitory pharmacological evaluation and the low cost of the commercial reagents to begin with. Also, the influence of the substituents in the desired activity was taken into account.

2. Materials and methods

2.1. Chemistry

Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ¹H and ¹³C NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as internal standard (chemical shifts in δ values, *J* in Hz). Mass spectra were obtained using a Hewlett–Packard 5988A spectrometer. Elemental analyses were performed using a Perkin–Elmer 240B microanalyser and were within $\pm 0.4\%$ of calculated values in all cases. Silica gel (Merck 60, 230–00 mesh) was used for flash chromatography (FC). Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm).

2.1.1. General procedure for the preparation of methoxy-3-arylcoumarins

To a solution of the conveniently substituted *ortho*-hydroxybenzaldehyde (7.34 mmol) and the corresponding phenylacetic acid (9.18 mmol) in dimethyl sulfoxide (15 mL), *N,N*-dicyclohexylcarbodiimide (11.46 mmol) was added. The mixture was heated at 110 °C for 24 h. Then, ice (100 mL) and acetic acid (10 mL) were added to the reaction mixture. After keeping it at room temperature for 2 h, the mixture was extracted with ether (3 \times 25 mL). The organic layers were combined and washed with sodium bicarbonate solution (50 mL, 5%) and water (20 mL). Subsequently, the solvent was evaporated under vacuum and the dry residue was purified by flash chromatography (hexane/ethyl acetate 9:1), to give the desired methoxy-3-arylcoumarins.^{23,28}

2.1.2. General procedure for the preparation of hydroxy-3-arylcoumarins

To a solution of a methoxy-3-arylcoumarin (0.50 mmol) in acetic acid (5 mL) and acetic anhydride (5 mL), at 0 °C, hydriodic acid 57% (10 mL) was added dropwise. The mixture was stirred under reflux, for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by crystallization (CH₃CN).^{23,28,34}

2.1.2.1. 3-(3',4'-Dihydroxyphenyl)-6-methylcoumarin (4).

Yield: 92%; mp 199–200 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.43 (s, 3H, –CH₃), 6.43 (s, 1H, H-2'), 7.01 (d, *J* = 7.1 Hz, 1H, H-5'), 7.14 (d, *J* = 7.0 Hz, 1H, H-6'), 7.28–7.32 (m, 2H, H-7, H-8), 7.57 (d, *J* = 2.2 Hz, 1H, H-5), 7.83 (s, 1H, H-4), 10.40 (s, 2H, –OH); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 20.7, 100.1, 110.7, 111.6, 115.7, 119.4, 120.1, 127.3, 127.4, 132.1, 133.7, 138.8, 146.5, 146.8, 151.3, 161.6; EI MS *m/z*: 269 (13), 268 (M⁺, 100), 241 (31), 240 (70), 239 (22), 165 (30), 125 (12), 111 (10); Anal. Calcd for C₁₆H₁₂O₄: C, 71.64; H, 4.51. Found: C, 71.60; H, 4.49.

2.1.2.2. 3-(3',4'-Dihydroxyphenyl)-8-methylcoumarin (5).

Yield: 85%; mp 205–206 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.50 (s, 3H, –CH₃), 6.80 (d, *J* = 8.3 Hz, 1H, H-5'), 7.05 (dd, *J* = 8.2, *J* = 2.2 Hz, 1H, H-6'), 7.22 (d, *J* = 2.2 Hz, 1H, H-2'), 7.25 (d, *J* = 7.6 Hz, 1H, H-6), 7.44 (dd, *J* = 7.4, *J* = 1.0 Hz, 1H, H-7), 7.57 (dd, *J* = 7.6, *J* = 1.1 Hz, 1H, H-5), 8.09 (s, 1H, H-4), 9.09 (s, 1H, –OH), 9.24 (s, 1H, –OH); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 14.9, 115.4, 116.0, 119.5, 119.9, 124.1, 124.6, 125.7, 126.1, 126.5, 132.2, 138.7, 144.8, 146.2, 150.9, 159.9; EI MS *m/z*: 270 (12), 269 (76), 268 (M⁺, 82), 241 (47), 240 (100), 239 (57), 211 (23), 166 (19), 165 (58), 152 (18), 139 (14), 125 (30), 111 (28), 82 (19); Anal. Calcd for C₁₆H₁₂O₄: C, 71.64; H, 4.51. Found: C, 71.63; H, 4.49.

2.1.2.3. 3-(3',5'-Dihydroxyphenyl)-8-methylcoumarin (6).

Yield: 90%; mp 180–181 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.49 (s, 3H, –CH₃), 6.70 (s, 3H, H-2', H-4', H-6'), 7.26 (t, *J* = 7.6 Hz, 1H, H-6), 7.48 (d, *J* = 7.8 Hz, 1H, H-7), 7.62 (d, *J* = 7.7 Hz, 1H, H-5), 8.11 (s, 1H, H-4), 10.27 (s, 2H, OH); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 15.3, 103.3, 107.2, 119.6, 124.5, 125.1, 126.8, 127.2, 133.1, 136.6, 140.9, 151.6, 158.5, 160.0; EI MS *m/z*: 269 (21), 268 (M⁺, 100), 241 (12), 240 (63), 239 (26); Anal. Calcd for C₁₆H₁₂O₄: C, 71.64; H, 4.51. Found: C, 71.60; H, 4.50.

2.1.2.4. 3-(3',4',5'-Trihydroxyphenyl)-8-methylcoumarin (7).

Yield: 82%; mp 189–190 °C. ¹H NMR (300 MHz, DMSO-*d*₆): δ 2.49 (s, 3H, –CH₃), 6.95 (s, 2H, H-2', H-6'), 7.20 (t, *J* = 7.4 Hz, 2H, H-6, H-7), 7.38 (d, *J* = 7.4 Hz, 1H, H-5), 7.79 (s, 1H, H-4), 10.55 (s, 2H, –OH), 10.60 (s, 1H, –OH); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 15.6, 106.1, 119.4, 124.2, 125.4, 125.7, 127.7, 130.4, 132.7, 135.6, 139.8, 146.7, 146.9, 160.6; EI MS *m/z*: 285 (16), 284 (M⁺, 100), 283 (84), 256 (32), 181 (10), 141 (10); Anal. Calcd for C₁₆H₁₂O₅: C, 67.60; H, 4.25. Found: C, 67.61; H, 4.28.

2.2. Antioxidant assays

2.2.1. Oxygen radical antioxidant capacity-fluorescein (ORAC-FL)

The ORAC analyses were carried out on a Synergy HT multi detection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, USA), using white polystyrene 96-well plates, purchased from Nunc (Denmark). Fluorescence was read from the top, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by Gen 5 software. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and 200 μ L final volume. FL (70 nM, final concentration) and hydroxy-3-arylcoumarin solutions in methanol with

a range of concentration between 0.3 and 2 μM were placed in each well of 96-well plate. The mixture was pre-incubated for 15 min at 37 $^{\circ}\text{C}$, before rapidly adding the AAPH solution (18 mM, final concentration). The microplate was immediately placed in the reader and automatically shaken prior to each reading. The fluorescence was recorded every 1 min for 120 min. A blank with FL and 2,2'-azobis(2-methylpropionamide)dihydrochloride (AAPH) using methanol instead of the antioxidant solution were used in each assay. Five calibration solutions using Trolox (0.5–2.5 μM) as antioxidant were also done. The inhibition capacity was expressed as ORAC values and is quantified by integration of the area under the curve (AUC_{NET}). All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. The area under the fluorescence decay curve (AUC) was calculated integrating the decay of the fluorescence where F_0 is the initial fluorescence read at 0 min and F is the fluorescence read at time. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank. Data processing was performed using Origin Pro 8 SR2 (Origin Lab Corporation, USA).

2.2.2. Hydroxyl radical scavenging assay using electron spin resonance (ESR)

Reactivity of all the hydroxy-3-aryl coumarin derivatives against the hydroxyl radical was investigated using the non-catalytic Fenton type method. ESR spectra were recorded in the X band (9.7 GHz) using a Bruker ECS 106 spectrometer with a rectangular cavity and 50 kHz field modulation, equipped with a high-sensitivity resonator at room temperature. Spectrometer conditions were: microwave frequency 9.81 GHz, microwave power 20 mW, modulation amplitude 0.91 G, receiver gain 59 db, time constant 81.92 ms and conversion time 40.96 ms. The scavenging activity of each derivative was estimated by comparing the DMPO-OH adduct signals in the antioxidant–radical reaction mixture and the control reaction at the same reaction time, and is expressed as scavenging percent of hydroxyl radical.

To prepare the samples, 150 μL of *N,N*-dimethylformamide (DMF) and 50 μL of NaOH (3 mM) were mixed, followed by the addition of 50 μL of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) spin trap (30 mM final concentration) and finally 50 μL of hydrogen peroxide 30%. The mixture was put in an ESR cell and the spectrum was recorded after five minutes of reaction. All the compounds were studied to 4 mM final concentration (300 μL final volume).

2.2.3. DPPH radical scavenging assay using ESR

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging capacity^{35–38} of the hydroxy-3-aryl coumarin derivatives was determined by an ESR spectrometry method.^{39–43} Each hydroxy-3-aryl coumarin solution was mixed with DPPH stock solution to initiate the antioxidant–radical reaction. All the reaction mixtures contained 1.0 mM of DPPH and 1.0 mM of the studied compound. The control solution was prepared in absence of the studied compounds. Both DPPH and compounds solutions were prepared in acetonitrile. ESR signals were recorded after five minutes of reaction. Spectrometer conditions were: microwave frequency 9.81 GHz, microwave power 20 mW, modulation amplitude 0.95 G, receiver gain 59 db, time constant 81.92 ms and conversion time 40.96 ms. The scavenging activity of each compound was estimated by comparing the DPPH signals in the antioxidant–radical reaction mixture and the control reaction at the same reaction time, and was expressed as scavenging percent of DPPH.

2.2.4. Superoxide antioxidant assay using cyclic voltammetry (CV)

Cyclic voltammetry (CV) measurements were performed in a Metrohm 693VA instrument with a 694VA stand convertor and a

693VA processor, at room temperature, using a three-electrode cell. A glassy carbon (GC) electrode presenting an area of 0.03 cm^2 was used as the working electrode. The electrode surface was polished to a mirror finish with alumina powder (0.3 and 0.05 μM) before use and after each measurement. Platinum wire was used as auxiliary electrode and silver–silver chloride (Ag/AgCl, 3 M KCl) of Metrohm Company with a plastic tip was used as a reference electrode. The CV experiments were carried out in dimethyl sulfoxide (DMSO) of analytical grade (Sigma–Aldrich) with 0.1 M of tetrabutylammonium perchlorate (TBAP) as supporting electrolyte. Superoxide anion radical was generated in DMSO containing TBAP 0.1 M. The scan rate was kept 30 mV/s and potential window was -1.0 to -0.0 V. The atmospheric solubility of oxygen in DMSO was 2.1 mM.⁴⁴ The final concentration of each derivative (30 μM) was achieved by additions of the corresponding aliquot of stock solution (10 mL final volume). Finally, the antioxidant activity was assessed from the change in the cathodic current of the voltammograms in absence and present of the derivatives, using pertinent mathematical formulations.

2.2.5. Data statistical analysis

Statistical analyses were conducted using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA). The data are expressed as means \pm SD. The experimental data were analyzed by one-way analysis of variance (ANOVA), and differences between groups were assessed using Tukey's post-test. The level of significance was set at $p < 0.05$, and all experiments were replicated 3 times.

3. Results

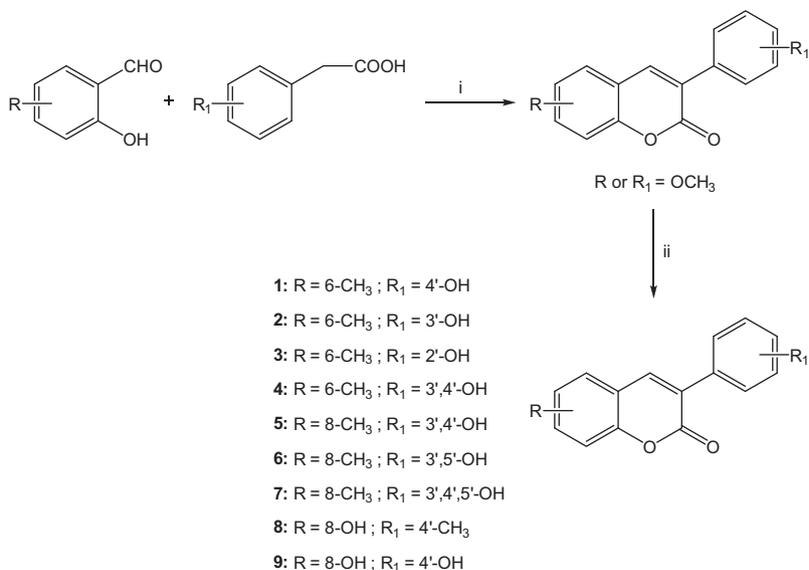
3.1. Chemical synthesis

Coumarin derivatives **1–9** were efficiently synthesized according to the protocol outlined in Scheme 1. The general reaction conditions and the characterization data of the new compounds were described in the experimental section. Perkin condensation of different *ortho*-hydroxybenzaldehydes with the adequate arylacetic acid, using *N,N*-dicyclohexylcarbodiimide (DCC) as dehydrating agent,²⁸ afforded the methoxy-3-aryl coumarins. Hydroxyl derivatives were obtained from the above-mentioned methoxy substituted precursors by acidic hydrolysis, using hydriodic acid 57% in the presence of acetic acid and acetic anhydride.²⁸

3.2. Antioxidant capacity assays

The evaluation of the antioxidant activity of the studied compounds was performed towards different types of reactive oxygen or nitrogen species—peroxyl, hydroxyl, superoxide and DPPH radicals. ORAC-FL, ESR and CV assays were the techniques used to obtain the desired results.

The peroxyl radical scavenging activity of the synthesized hydroxy-3-aryl coumarins was evaluated by the oxygen radical absorbance capacity (ORAC) method⁴⁵ This assay use a fluorescence-based technology (ORAC-FL) and allow obtaining a relative antioxidant index by using as reference trolox, a hydrosoluble vitamin E derivative. The exposition of the fluorophore, in this case fluorescein (FL) to the peroxyl radical lead to an oxidation process reflected as a decay of fluorescence emission through time. In ORAC assays, the loss of fluorescence of FL generally corresponds to an induction time and is reliant on antioxidant capacity of a compound. In fact, it refers to the time in which the FL is protected against the oxidative damage of peroxyl radicals and this behavior is associated to a competitive reaction between the radical and the antioxidant.^{46,47} ORAC data take into account the induction time,



Scheme 1. Reagents and conditions: (i) DCC, DMSO, 110 °C, 24 h; (ii) HI 57%, AcOH, Ac₂O, reflux, 3 h.

Table 1

ORAC-FL index and hydroxyl, DPPH and superoxide radical scavenging data obtained for compounds **1–9**

Compd	ORAC-FL index	% Scavenging hydroxyl radicals ^a	% Scavenging DPPH radicals ^a	% Scavenging superoxide radicals ^b
1	6.1	100	27.7	17.4
2	6.7	5.2	3.4	17.5
3	8.4	100	10.6	25
4	5.3	6.05	28.3	28.9
5	5.7	100	66.7	77.3
6	5.5	75	11.2	22.6
7	5.3	100	100	76.5
8	6.3	16	28.6	17.4
9	13.5	100	65.9	71.5
Trolox	1.0	—	—	—
Quercetin	7.28 ^c	—	20.0 ^d	—
Catechin	6.76 ^c	—	44.5 ^d	—

^a The scavenging activity of hydroxyl and DPPH radicals effect was calculated as follows: $[(A_0 - A_x)/A_0] \times 100$, where A_x and A_0 are the double-integral ESR for the first line of samples in the presence and absence of test compounds, respectively.

^b The scavenging activity of superoxide radical effect was calculated as follows: $[(I_{pc} \text{ blank} - I_{pc} \text{ aox})/I_{pc} \text{ blank}] \times 100$, where $I_{pc} \text{ blank}$ is cathodic current in the absence of the studied compounds and $I_{pc} \text{ aox}$ is the cathodic current in the presence of the studied compounds.

^c Data collected from Ref. 45.

^d Data collected from Ref. 49.

initial rate and the range of total antioxidant inhibition in one value.⁴⁶

Results expressed as ORAC-FL values are presented in Table 1. All the obtained ESR results for the % scavenging of the hydroxyl and DPPH radicals are also illustrated in Table 1. In addition, % of superoxide radical scavenging, performed by CV, are also represented in Table 1.

The ORAC-FL profile, intensity of fluorescence at 528 nm versus the incubation time was obtained for all derivatives. Compounds **3** and **9** (8.4 and 13.5, respectively) display the highest ORAC-FL indexes, comparing with the flavonoids quercetin and catechin that are very well known natural antioxidant compounds. Figure 1 shows the kinetic profile for protection of FL probe against peroxy radicals obtained in presence of increasing concentrations of compound **9**. It was obtained a profile of fluorescence measure at 528 nm versus the incubation time at different concentration, for all derivatives.

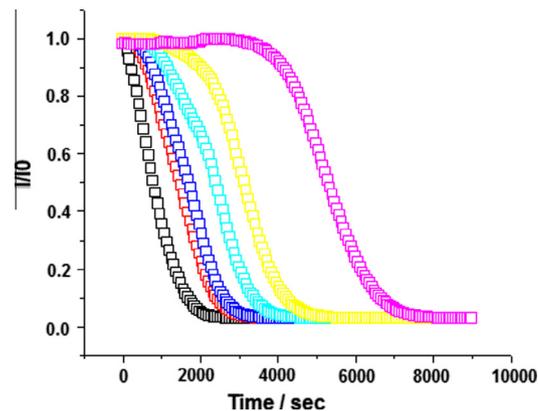


Figure 1. ORAC-FL profile (kinetic profile for protection of FL probe against peroxy radicals) for compound **9**.

In order to study the antioxidant reactivity of all the synthesized hydroxy-3-aryl coumarin derivatives towards hydroxyl radicals, a non-catalytic and competitive type Fenton system in which the DMPO spin trap was performed.⁴⁸ The ESR spin-trapping spectrum obtained in the control assay (DMPO + *N,N*-dimethylformamide + NaOH + H₂O₂) presents four hyperfine lines, due to the DMPO-OH adduct formation, as it is shown in Figure 2 (red line). For each putative antioxidant coumarin compounds ESR spectra were also acquired to check their capacity of scavenging hydroxyl radicals. The data obtained with compound **2** is depicted in Figure 2 (black line).

The intensity of the spectra decreases when the hydroxy-3-aryl coumarin derivatives were added into the system. For compound **9**, 100% of scavenging of hydroxyl radicals was obtained (Fig. 3—black line). This type of response was observed for all derivatives, reflecting different percentage of the hydroxyl radical scavenging activity (Table 1).

The stable free radical DPPH assay has been used for detecting the antioxidant activity in several chemical analyses.^{35–38} Currently, DPPH assay is considered an easy and accurate method, appropriate for measuring the antioxidant capacity of fruits, vegetables, juices or extracts.³⁹ This is due to the electronic properties shared by DPPH and peroxy radicals (the unpaired electron is delocalized through the pair of nitrogen or oxygen atoms, respec-

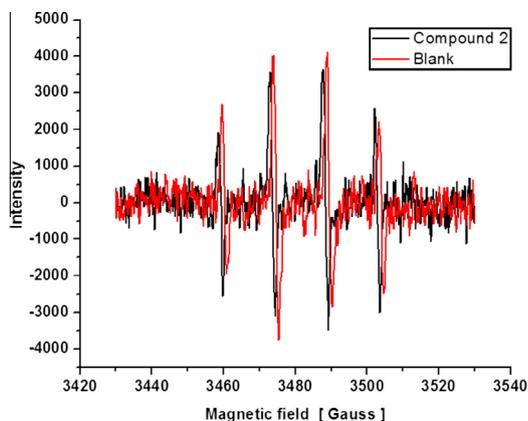


Figure 2. ESR spectra obtained for the control (adduct DMPO-OH without antioxidant molecule—red line) and for adduct DMPO-OH in the presence of compound **2** (black line).

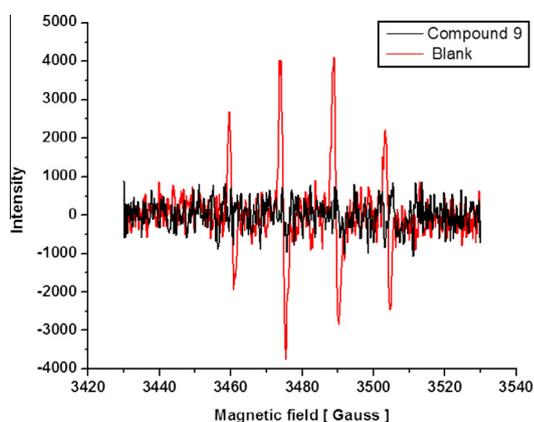


Figure 3. ESR spectra obtained for the control (adduct DMPO-OH without antioxidant molecule—red line) and for adduct DMPO-OH in the presence of compound **9** (black line).

tively), in such way that the reaction rate between DPPH and several antioxidants provides a good approximation for scavenging activities with lipid peroxy radicals.^{40,41} ESR spectra of DPPH in absence and presence of compounds **4**, **7** and **9** are showed in Figure 4.

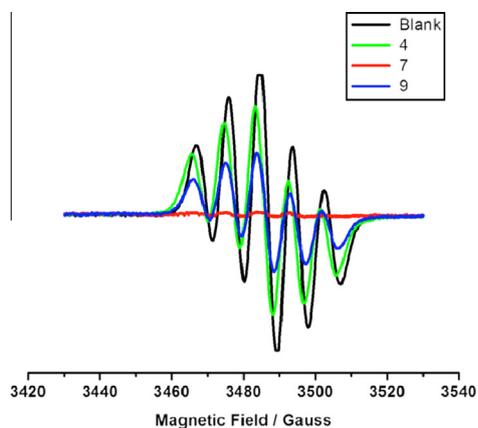


Figure 4. ESR signal from DPPH radical in absence (blank) and presence of compounds **4**, **7** and **9**.

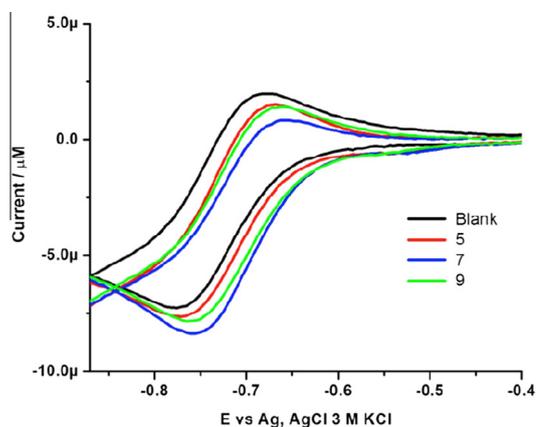


Figure 5. Cyclic voltammograms of superoxide radical in absence (blank) and presence of compounds **5**, **7** and **9** in DMSO + TBAP 0.1 M, on GC (working electrode) versus Ag/AgCl, at room temperature, with scan rate of 30 mV/s.

Superoxide anion radical was generated by one electron reduction of the atmospheric molecular oxygen dissolved in DMSO at room temperature (25 °C). Then, the voltammetric performance of the nine compounds was studied, one by one, in DMSO and TBAP 0.1 M. The resultant CV responses for derivative **5**, **7** and **9**, and in absence of any derivative (blank) are represented in Figure 5.

3.3. Theoretical evaluation of ADME properties

In order to better understand the overall properties of the described compounds, the lipophilicity (expressed as the octanol/water partition coefficient and herein called $\log P$), was calculated using the Molinspiration property calculation program.⁵⁰ The theoretical prediction of ADME properties (molecular weight, $\log P$, number of hydrogen donors and acceptors) of all the compounds was carried out and is presented in Table 2.^{51,52}

4. Discussion

In previous works, 3-arylcoumarin derivatives were described as potent and selective MAO-B inhibitors.²⁸ This family of compounds and their remarkable data on the selective inhibition of MAO-B isoenzyme and putative application for ND therapy were the inspiration for this work. In particular, compounds **1–3** were previously described as potent and selective MAO-B inhibitors, with IC_{50} values between 120 and 650 nM.²⁸ On the other hand the recent medicinal chemistry paradigms in the drug design, namely the rational discovery of multi-target drugs, as a promising strategy to combat this type of multifactorial diseases prompted us to look for other properties for this type of coumarins. Based on this data, a new family of derivatives sharing the same scaffold and type of substituents was designed and synthesized.

The evaluation of the antioxidant activity of the hydroxy-3-arylcoumarin compounds was performed towards different types of reactive oxygen or nitrogen species—peroxy, hydroxyl, superoxide and DPPH radicals. ORAC-FL, ESR reactivity and CV assays were the techniques used to achieve the goals. From the obtained data, it was concluded that the antioxidant scavenging activity is related with the type of substituents presented in the 3-arylcoumarin skeleton.

Compound **9** was found to be the most interesting coumarin of the series. This compound has two hydroxyl groups in its structure, one at position 8 and another at position 4' of the 3-arylcoumarin scaffold. The other compounds have structural combinations of two types of substituents (methyl and one, two or three hydroxyl groups). Compounds **1**, **3**, **5** and **7** have ORAC-FL values between

Table 2
Theoretical structural properties of the hydroxy-3-aryl coumarins derivatives **1–9**^a

Compd	log <i>P</i>	Molecular weight	TPSA	<i>n</i> -OH acceptors	<i>n</i> -OHNH donors	Volume
1	3.68	252.27	50.44	3	1	224.57
2	3.66	252.27	50.44	3	1	224.57
3	3.89	252.27	50.44	3	1	224.57
4	3.19	268.27	70.67	4	2	232.59
5	3.17	268.27	70.67	4	2	232.59
6	3.11	268.27	70.67	4	2	232.59
7	2.88	284.27	90.90	5	3	240.61
8	3.92	252.27	50.44	3	1	224.57
9	2.99	254.24	70.67	4	2	216.03

^a log*P*—octanol/water partition coefficient; TPSA—topological polar surface area; *n*-OH—number of hydrogen acceptors; *n*-OHNH—number of hydrogen bond donors. The data was determined with Molinspiration calculation software.⁵⁰

5.3 and 8.4 and the higher scavenging activity towards hydroxyl radicals (100% – total scavenging). From these derivatives, compound **7** presented the best DPPH and superoxide radicals scavenging (100 and 76.5%, respectively). Compounds **1** and **3** have on their structure a methyl group at position 6 of the coumarin moiety and a hydroxyl group in the 3-aryl ring. The ORAC-FL indexes are 6.1 and 8.4, respectively, but the position of the hydroxyl group in the exocyclic aromatic ring seems to have no influence on the hydroxyl radical scavenging capacity (100%, in both cases). The DPPH radical scavenging values of these compounds are, respectively, 27.7% and 10.6% and superoxide radical scavenging values are, respectively, 17.4% and 25%. Their profiles are, therefore, very similar. Compounds **5** and **7** have a methyl group at position 8 of the coumarin moiety, and two or three hydroxyl groups, respectively, in the 3-aryl ring. Both ORAC-FL values (5.7 and 5.3, respectively), hydroxyl radical scavenging capacity (100%) and superoxide radical scavenging capacity (77.3% and 76.5%, respectively) are similar, proving that the presence of the extra hydroxyl group does not seem to significantly affect the peroxy, hydroxyl and superoxide radicals scavenging activities. On the other hand, DPPH radical scavenging capacity is 66.7% for compound **5** and 100% for compound **7**. Compounds **2**, **4** and **8**, with ORAC-FL values between 5.3 and 6.7, presented the lowest hydroxyl scavenging capacity (between 5.2% and 16%). Compound **2** presented also the lowest DPPH radical scavenging capacity (3.4%) and compounds **1** and **8** the lowest superoxide radical scavenging capacities (17.4%). Comparing the data obtained for compound **4** (3',4'-dihydroxy substituted) and for compound **1** (4'-hydroxy substituted), it can be concluded that the presence of two hydroxyl groups led to the decline in the ORAC-FL index (6.1–5.3) and the hydroxyl radical scavenging capacity (100%–6.05%). Comparing compound **4** with **2** (3'-hydroxy substituted), it is also noted a decrease in the ORAC-FL index (from 6.7 to 5.3), caused by the presence of two hydroxyl groups in the molecule. However, the hydroxyl radical scavenging capacity is almost the same (6.05% and 5.2%, respectively). Superoxide radical scavenging capacity is also similar (28.9 and 17.5, respectively). Compounds **4** and **5** have the same substitution pattern, changing only the position of the methyl group from 6 to 8. This modification strongly affects the hydroxyl radical scavenging capacity (from 6.05% to 100%), the DPPH radical scavenging capacity (from 28.3% to 66.7%) and superoxide radical scavenging capacity (from 28.9% to 77.3%). Compound **6**, with a methyl group at position 8 of the coumarin moiety and two hydroxyl groups at positions 3' and 5' of the 3-aryl ring, in spite of presenting an ORAC-FL value of 5.5 and low DPPH and superoxide radicals scavenging (11.2 and 22.6, respectively), evidence a significant tendency to scavenge hydroxyl radicals (75%).

As said before, the highest ORAC-FL values were found for compounds **3** and **9** (8.4 and 13.5, respectively). The results are very interesting comparing with the ORAC-FL values of catechin (6.76) and quercetin (7.28), very well known natural antioxidants. Com-

paring with trolox (ORAC-FL = 1.0), the interesting ORAC-FL values of compounds **3** and **9** make them promising antioxidant molecules. It is important to notice that compound **5**, **7** and **9** presented good trends against all the studied radicals.

From the obtained data, it is also remarkable that all the coumarin derivatives possess log*P* values compatible with those required to cross membranes. TPSA, described to be a predictive indicator of membrane penetration, is also found to be positive. In addition, it can be observed that no violations of Lipinski's rule (molecular weight, log*P*, number of hydrogen donors and acceptors) were found. This is important information about the promising potential of these derivatives.

All the synthesized compounds, in spite of presenting different chemical substituents, disclose interesting ORAC-FL values, in most cases accompanied by a remarkable ability to scavenge hydroxyl, DPPH and superoxide radicals. Therefore, the data acquired so far are relevant allowing proposing hydroxy-3-aryl coumarins as a valid scaffold for the design of novel antioxidants.

5. Concluding remarks

In conclusion, in the current work coumarins presenting very promising antioxidant profiles were described. Compound **9** proved to be the most interesting molecule of the whole series, with an ORAC-FL of 13.5, 100% of scavenging of hydroxyl radicals, 65.9% of scavenging of DPPH radicals and 71.5% of scavenging of superoxide radicals. This derivative has presented good antioxidant capacity towards different types of reactive oxygen or nitrogen species—peroxy, hydroxyl, superoxide and DPPH radicals. Compound **3**, previously describe as very good selective MAO-B inhibitor, presented also a very interesting antioxidative profile. It is important to notice that compound **5** and **7** also presented good trends against all the studied radicals. In addition, it can be observed that no theoretical violations of Lipinski's rule were observed for all the studied derivatives. Therefore, the described compounds seem to present desirable ADME properties. Based on these results, it can be concluded that especially compounds **3** and **9** are potential candidates for a further optimization process and could be successfully employed in the prevention or minimization of the oxidative damage caused by overproduction of oxygen free radicals.

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