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

Maria João Matos a,b,*, Fernanda Pérez-Cruz c, Saleta Vazquez-Rodriguez a, Eugenio Uriarte a, Lourdes Santana a, Fernanda Borges b, Claudio Olea-Azar c,a

a Department of Organic Chemistry, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain
b CIQUP/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal
c Department of Inorganic and Analytical Chemistry, Laboratory of Free Radicals and Antioxidants, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago de Chile, Chile

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A B S T R A C T

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 peroxyl 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 nutraceutics 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 peroxyl radicals.10 H2O2 produces highly toxic ROS, namely hydroxyl
radical, by Fenton reaction that is catalyzed by iron and neuro-
melanin.\textsuperscript{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.\textsuperscript{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
D\textsubscript{2} receptors and suppressing the action of ROS as well.\textsuperscript{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.\textsuperscript{14}

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

Recently, it was shown by our group that 3-substituted aryl
coumarins are potent and selective MAO-B inhibitors.\textsuperscript{24–30} In addi-
tion, it has been found that hydroxycoumarins are antioxidants
scavenging ROS and/or chelating transition metals, exhibiting
tissue-protective properties.\textsuperscript{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 deriva-
tives has considered the previously MAO-B inhibitory pharmacol-
ogical 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 Koffler ther-
man and or in capillary tubes on a Büchi 510 apparatus and are
uncorrected. \textsuperscript{1}H and \textsuperscript{13}C NMR spectra were recorded on a Bruker
AMX spectrometer at 300 and 75.47 MHz, respectively, using
TMS as internal standard (chemical shifts in \textit{\textit{\textit{\textit{\textit{\ensuremath{\delta}}}}}} values, \textit{\textit{\textit{\textit{\textit{\textit{\textit{J}}}}}} Hz). Mass
spectra were obtained using a Hewlett–Packard 5988A spectrome-
ter. Elemental analyses were performed using a Perkin–Elmer 240B
spectrometer at 300 and 75.47 MHz, respectively, using
\textsuperscript{1}H NMR (75 MHz, DMSO-
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 °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-methylpropionamidine)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). 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 F0 is the initial fluorescence read at 0 min and F is the fluorescence read at time. The net AUC corresponding to 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).

Reactivity of all the hydroxy-3-arylcoumarin 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.

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-arylcoumarins was evaluated by the oxygen radical absorbance capacity (ORAC) method. 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. ORAC data take into account the induction time,
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 peroxyl radicals. It was obtained a profile of fluorescence measure at 528 nm versus the incubation time at different concentration, for all derivatives.

In order to study the antioxidant reactivity of all the synthesized hydroxy-3-arylcoumarin derivatives towards hydroxyl radicals, a non-catalytic and competitive type Fenton system in which the DMPO spin trap was performed. The ESR spin-trapping spectrum obtained in the control assay (DMPO+\textsubscript{N,N}-dimethylformamide + NaOH + H\textsubscript{2}O\textsubscript{2}) 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. Currently, DPPH assay is considered an easy and accurate method, appropriate for measuring the antioxidant capacity of fruits, vegetables, juices or extracts. This is due to the electronic properties shared by DPPH and peroxyl radicals (the unpaired electron is delocalized through the pair of nitrogen or oxygen atoms, respec-

### Table 1

<table>
<thead>
<tr>
<th>Compd</th>
<th>ORAC-FL index</th>
<th>% Scavenging hydroxyl radicals(^a)</th>
<th>% Scavenging DPPH radicals(^b)</th>
<th>% Scavenging superoxide radicals(^\text{ii})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.1</td>
<td>100</td>
<td>27.7</td>
<td>17.4</td>
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<tr>
<td>2</td>
<td>6.7</td>
<td>5.2</td>
<td>3.4</td>
<td>17.5</td>
</tr>
<tr>
<td>3</td>
<td>8.4</td>
<td>100</td>
<td>10.6</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>5.3</td>
<td>6.05</td>
<td>28.3</td>
<td>28.9</td>
</tr>
<tr>
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<td>5.7</td>
<td>100</td>
<td>66.7</td>
<td>77.3</td>
</tr>
<tr>
<td>6</td>
<td>5.5</td>
<td>75</td>
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<tr>
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<td>5.3</td>
<td>100</td>
<td>100</td>
<td>76.5</td>
</tr>
<tr>
<td>8</td>
<td>6.3</td>
<td>16</td>
<td>28.6</td>
<td>17.4</td>
</tr>
<tr>
<td>9</td>
<td>13.5</td>
<td>100</td>
<td>65.9</td>
<td>71.5</td>
</tr>
<tr>
<td>Trolox</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Quercetin</td>
<td>7.28(^c)</td>
<td>20.0(^d)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Catechin</td>
<td>6.76(^e)</td>
<td>44.5(^d)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\) The scavenging activity of hydroxyl and DPPH radicals effect was calculated as follows: \([ (A_0 - A_x)/A_0 ] \times 100\), where \(A_0\) and \(A_x\) 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: \([ (I_{pc\ blank} - I_{pc\ aox})/I_{pc\ blank} ] \times 100\), where \(I_{pc\ blank}\) is cathodic current in the presence of the studied compounds and \(I_{pc\ aox}\) is the cathodic current in the absence of the studied compounds.

\(^c\) Data collected from Ref. 45.

\(^d\) Data collected from Ref. 49.

\(^e\) Data collected from Ref. 46.
tively), in such way that the reaction rate between DPPH and several antioxidants provides a good approximation for scavenging activities with lipid peroxyl radicals.\(^{40,41}\) ESR spectra of DPPH in absence and presence of compounds \(^4\), \(^7\) and \(^9\) are showed in Figure 4.

Superoxide anion radical was generated by one electron reduction of the atmospheric molecular oxygen dissolved in DMSO at room temperature (\(25^\circ\text{C}\)). 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 isozyme 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—peroxyl, 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

![Figure 2](image2.png)

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](image3.png)

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).

![Figure 4](image4.png)

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

![Figure 5](image5.png)

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.
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 logP values compatible with those required to cross membranes. TPSA, described as 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, logP; 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-arylcoumarins 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—peroxyl, 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 9 and 5 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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References and notes