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Hydroxycoumarins as selective MAO-B inhibitors

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ABSTRACT

A series of 3-aryl-4-hydroxycoumarin derivatives was synthesized with the aim to find out the structural features for the MAO inhibitory activity and selectivity. Methoxy and/or chloro substituents were introduced in the 3-phenyl ring, whereas the position 6 in the coumarin moiety was not substituted or substituted with a methyl group or a chloro atom due to their different electronic, steric and/or lipophilic properties. Most of the synthesized compounds presented MAO-B inhibitory activity. The presence of methoxy and chloro groups, respectively in the *para* and *meta* positions of the 3-phenyl ring, have an important influence on the inhibitory activity. Moreover, the presence of a chloro atom in the six position of the moiety (compound **7**) improved the inhibitor activity as well as its selectivity against MAO-B compared with iproniazide, used as reference compound. Docking experiments were carried out to understand which are the most energetically preferred orientations adopted by compounds **5**, **6** and **7** inside the MAO-B binding pocket.

Neurodegenerative diseases (ND) constitute the third most important health problem in developed countries. Alzheimer's disease (AD) is the most prevalent of the ND followed by Parkinson's disease (PD). AD is a neurodegenerative and progressive disorder associated, in the most of the cases, with senile dementia. It seems to have a multiple etiology although the main cause it is thought to be the accumulation of β-amyloid plaques in brain that can provoke a degeneration or atrophy of the cholinergic neurons. PD is also a chronic and progressive neurodegenerative disorder, characterized by a predominant motor symptomatology, usually accompanied by non motor symptoms such as depression and anxiety. It seems to be caused by a decrease in the dopamine levels in neuronal synapses. In the treatment of this disease, dopaminergic agonists are generally employed. However, other therapeutic alternatives can be employed, such as the use of monoamine oxidase B inhibitors (MAOI-B), or the use of antioxidant compounds in order to prevent the oxidative cells damage.

MAO is an important FAD-containing enzyme (flavoenzyme) present in the outer mitochondrial membrane of neuronal, glial and many other cells.[1,2] It exists in two isoforms namely as MAO-A and MAO-B that have been identified based on their amino acid sequences, three-dimensional structure, substrate preference and inhibitor selectivity.[3,4] MAO-A preferentially deaminates serotonin and noradrenaline, whereas MAO-B has a higher affinity to phenylethylamine and benzylamine.[5,6] Therefore, these isoenzymes play a vital role in the monoamines degradation and, as consequence, in the inactivation of neurotransmitters. Their regulation determines the interest of the MAOI compounds as drugs used in the treatment of neurodegenerative and neurological disorders. In particularly, MAO-A inhibitors are effective in the treatment of depression,[7,8] while MAO-B inhibitors are useful in the treatment of PD.[9,10] In the last years, it has been proved that in patients who suffer ND the activity of brain MAO-B is increased. This fact provokes an increase of free radicals that are responsible for the oxidative stress, neuronal cell death and also for the development of the β-amyloid plaques.[11] In this sense, it is an accepted theory that the beneficial effects of the MAO-B inhibitors in the prevention of the neuronal damage is mostly derived to the decrease of hydrogen peroxide generated by the inhibition of this enzyme.

Coumarins are an important group of organic compounds from natural and/or synthetic origin, that show, due to their structural diversity, numerous biological activity.[12–14] There are coumarins described as anticancer, anti-inflammatory, antimicrobial, cardioprotective, vasorelaxant, and antioxidant agents.[15–20] Recently studies pay special attention to the MAO inhibitory properties of the 3-arylcoumarin derivatives.[21–26]

Flavonoids are naturally occurring polyphenolic compounds that are found in diets and medicinal herbs. The physiological benefits of dietary flavonoids (flavones and isoflavones) have been attributed to their antioxidant and free radical-scavenging abilities.[27] Additionally, flavonoids have been revealed to exhibit

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anti-amiloidogenic properties such as inhibitory effects on β-secretase enzimes[28,29] and also MAO-B inhibitory activity.[30]

In previous works, we reported the synthesis and biological evaluation of a series of 3-arylcoumarins as potent and selective MAO-B inhibitors.[23–25] These interesting results prompted us to synthesize and evaluate new 3-arylcoumarin derivatives, in which we introduce a hydroxyl group in the 4 position of coumarin skeleton (Fig. 1).

This modification allows us considering the similarity of the core structure of the 4-hydroxycoumarin (B) comparing with the 2-hydroxyisoflavone (C). Taking into account these data we could establish a potential therapeutic application of these analogues flavonoids/coumarins as a new interesting scaffold with interest against age-related neurodegeneration such as PD or AD.

The synthesis of 3-aryl-4-hydroxycoumarin derivatives (**1**–**7**)[31–33] is outlined in Scheme 1.

The key step for the synthesis of the 3-arylcoumarin skeleton was achieved by a palladium-catalyzed coupling Suzuki reaction between phenyliodonium zwitterions and aryl conveniently substituted phenyl boronic acids. Initially we have synthesized the different phenyliodonium coumarinate species (I–III).[31,32] They are electrophilic molecules with a positive charge at iodine, compensated by a internal negative charge that can be localized formally at the α-carbon or delocalized to a neighboring oxygen. Then we have carried out the palladium-catalyzed coupling reaction using $Pd(OAc)_2$ as catalyst and $P(t\text{-}Bu)_3$ as ligand to afford the compounds (**1–7**) with good yields.

By ^1H and ^{13}C NMR spectroscopy studies we have found out that the synthesized compounds (**1–7**) were presented in the 4-hydroxycoumarin tautomeric form. Therefore we have centered on their analysis at the physiologic pH conditions. In the biological inhibitory assays it was also used the appropriate solvent that guaranties the presence of this structure.

The MAO inhibitory activity of compounds **1–7** was evaluated in vitro by the measurement of the enzymatic activity of human recombinant MAO isoforms expressed in BTI insect cells infected with baculovirus.[34] Subsequently, the IC_{50} values and MAO-B selectivity indexes [IC_{50} (MAO-A)]/[IC_{50} (MAO-B)] for inhibitory effects of both new types of compounds and iproniazide (used as reference inhibitor) were calculated (Table 1).[34,35]

In the present Letter, we have studied the effect in the MAOI activity of the introduction of alkyl, alkoxy or halogen substituents in different positions of the 3-aryl-4-hydroxycoumarin nucleus. We have found that the simple 4-hydroxy-3-phenylcoumarin did not present MAOI activity (compound **1**). The introduction of a methoxy substituent in the *para* position of the 3-phenyl ring led to a compound **2** with inhibitory activity against MAO-B. So we take this compound as a point of reference for the subsequent modifications. Due to the fact that the 6 position of the coumarin

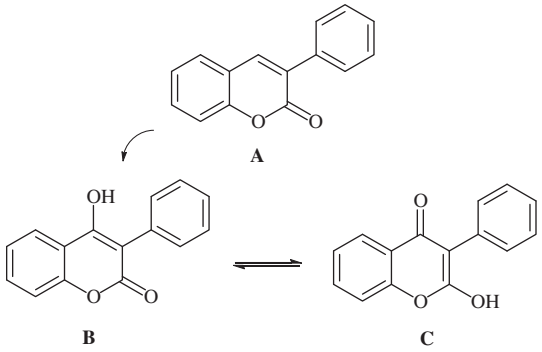
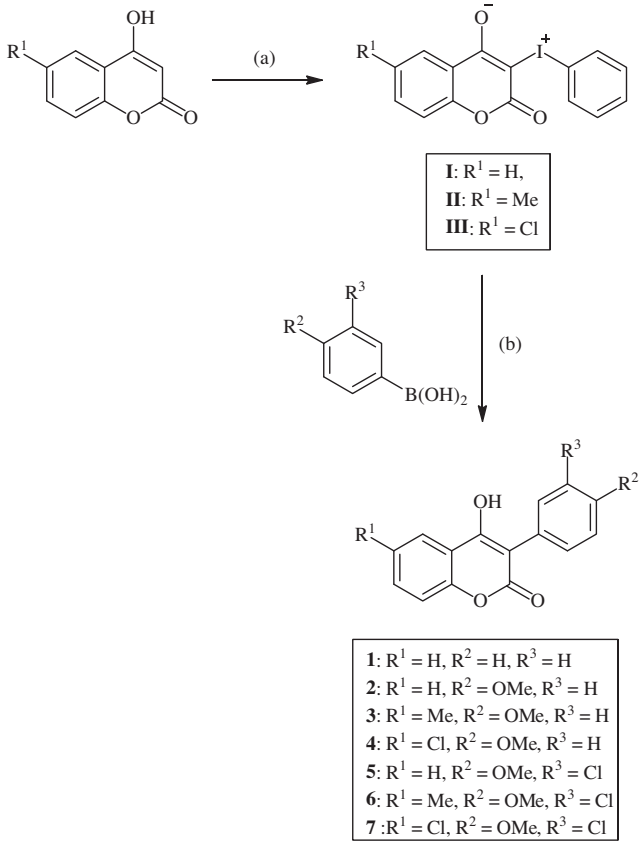


Figure 1. Chemical structures of the 3-phenylcoumarin (A), 4-hydroxy-3-phenylcoumarin (B) and 2-hydroxyisoflavone (C).



Scheme 1. Reagents and conditions: (a) $PhI(OAc)_2$, Na_2CO_3, H_2O, rt, 14 h; (b) $Pd(OAc)_2$, $P(t\text{-}Bu)_3$, LiOH, DME/H_2O, rt, 24–48 h.

Table 1
MAO-A and MAO-B inhibitory activity results for the synthesized compounds **1**–**7** and reference inhibitor

Compounds	MAO-A IC_{50} (μM)	MAO-B IC_{50} (μM)	Selectivity index
1	[a]	[a]	—
2	[a]	69.59 ± 4.70	>1.4[b]
3	[a]	32.04 ± 2.16	>3.1[b]
4	[a]	[a]	—
5	[a]	9.26 ± 0.63	>10[b]
6	[a]	42.68 ± 2.88	>2.3[b]
7	[a]	2.79 ± 0.19	>36[b]
Iproniazide	6.56 ± 0.76	7.54 ± 0.36	0.87
R-(−)-Deprenyl	68.73 ± 4.21	$17x10^{-3} \pm 1.9 \times 10^{-3}$	4.043

All IC_{50} values shown in the table are expressed as means ± SEM from five experiments. Level of statistical significance:
[a] Inactive at 100 μM (highest concentration tested).
[b] Values obtained under the assumption that the corresponding IC_{50} against MAO-A is the highest concentration tested (100 μM).

skeleton is particularly relevant to the MAO-B inhibitor activity and selectivity,[36,37] we have studied the effect of the introduction of a methyl group or a chloro atom in this position. The introduction of a methyl group (compound **3**) slightly improved the activity. However, the replacement for a chloro atom at the same position decreases the activity (compound **4**). In addition, when we analyze the second series (compounds **5**–**7**) where a chloro atom has been introduced at the *meta* position of the *p*-methoxy-3-phenyl ring, the MAO-B inhibitory activity, in most of the cases, improves. In this case, if we introduce a methyl group in the 6 position (compound **6**) the MAOI-B activity did not change, whereas if the substituent is a chloro atom (compound **7**) the MAO-B inhibitory activity considerably improves. Therefore compound **7** is more active against MAO-B isoenzyme than the

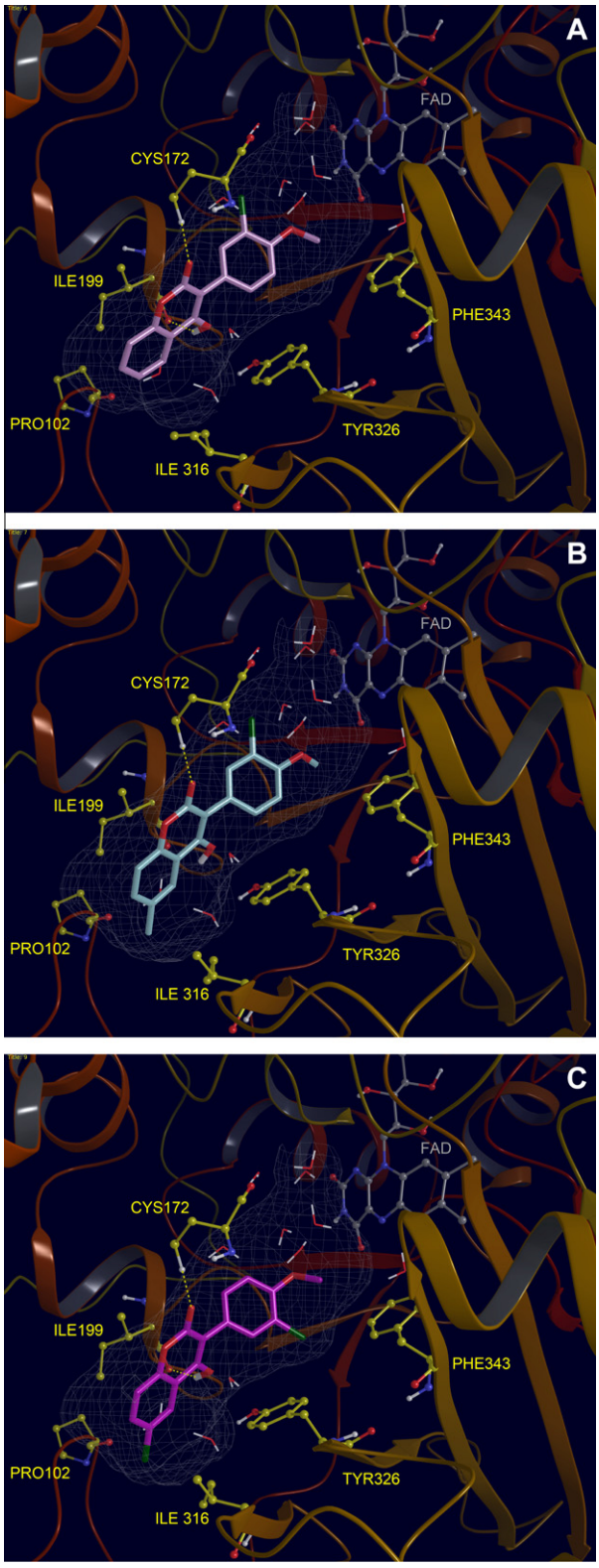


Figure 2. Best docking poses retrieved for compounds **5** (a), **6** (b) and **7** (c) into the MAO-B (PDB code: 2V60). Coumarins are represented in tube with carbon atoms colored in plum for **5**, turquoise for **6** and purple for **7**. Hidden ribbons for a better visualization from residues Glu159 to Glu179 (excluding Cys172). MAO-B binding site displayed as gray mesh style. Interacting residues and FAD cofactor labeled in ball and stick, with carbon atom colored in yellow and gray respectively. Water molecules depicted in wire rendering. H-bonds displayed in yellow dot line. Nonpolar hydrogens are omitted.

iproniazide but not than the *R*-(−)Deprenyl, used as reference compounds. None of the described compounds showed MAO-A inhibitory activity for the highest concentration tested (100 μM).

A computational approach, through docking calculations, was used to better understand which are the most energetically preferred orientations adopted by compounds **5**, **6** and **7** inside the MAO-B binding pocket.

High resolution crystal structure of hMAO-B (PDB code 2V60)[1] was taken as target for docking experiments. Protein Preparation Wizard of Maestro 9.1[38] was used to set-up protein before run calculations. With this helpful tool co-crystallized ligand was removed, water molecules beyond 5 Å from the ligand were deleted, hydrogen atoms were added and then minimized using OPLS_2005 force field. Compounds **5**, **6** and **7** were prepared with LigPrep[39] tool using MMFFs force field. The QM-Polarized Ligand Docking (QPLD)[40] protocol was used for docking calculations. In a first step, ligands were docked into the known[26] MAO-B binding site using Glide[41] standard precision (SP); the initial charges were calculated by semiempirical methods and 8 best poses for each ligand were retained. In the second step the Quantum Mechanical (QM) treatment of charges, calculated by Jaguar[42], allowed to take into consideration the polarization of the charges in the ligand by the protein. The final step consisted to redock the ligand, with improved charges, with Glide extra precision (XP) mode, retaining the 4 best poses for each ligand. With the aim of minimizing the protein–ligand complexes and return the estimate binding free energy for each ligand, the 4 output complexes derived from QPLD were subjected to MacroModel-eMBrAcE[43] minimization. With this flexible-receptor tool all residues with a distance not exceeding 5Å from the ligand were allowed to move freely, while residues outside this shell were frozen. The complexes were minimized using OPLS_2005 force field. Results were retrieved using Energy difference mode which calculates separately the energy associated first on the receptor, than on the ligand and finally on the complex using the follow equation:

$$\Delta E = E_{\text{complex}} - E_{\text{ligand}} - E_{\text{protein}}$$

The most stable binding solution for compounds **5**, **6** and **7** shows the same pattern (Fig. 2).

The entrance cavity of binding site is occupied by the hydroxycoumarin moiety leaving the 3-arylcoumarin rings directed toward FAD cofactor. Cys172 plays a significant role for the complex stabilization, forming an H-bond with carbonyl oxygen of all the three coumarins. The higher activity showed by **5** and **7**, compared to **6**, could be partially due to involvement of another H-bond in the binding mode; in this case only **5** and **7** form an H-bond between the 4-hydroxy group and Ile199. Furthermore, 3-arylcoumarin rings of **5**, **6** and **7** share a π–π stacking interaction with Tyr326. Interestingly, the *p*-methoxy group occupies the same position in the three coumarins, while the position adopted by the chloro atom in the 3-phenyl ring appears to be unimportant for the modulation of MAO-B inhibitor activity, because it assumes an opposite orientation in the two most actives compounds **5** and **7**. Good van der Waals and electrostatic interactions with Pro102, Ile316 and Phe343 were also observed for all the docked molecules.

As conclusion, these preliminary results allow us a better understanding of the molecular fragments that are essential to maintain and/or improve the MAO activity and selectivity. These findings encourage us to continue the efforts towards the optimization of the pharmacological profile of this structural moiety as an important scaffold for the potential treatment of ND.

Acknowledgements

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32. *General procedure for the preparation of 3-phenyliodonium coumarinates*: iodobenzene diacetate (10 mmol) was suspended in a solution of Na_2CO_3 (10 mmol) in water (100 mL) and was stirred for 30 min at room temperature. To this solution was added a mixture of the corresponding 4-hydroxycoumarin (10 mmol) and Na_2CO_3 (10 mmol) in water (100 mL). After the mixture was stirred at room temperature for 14 h, the precipitate was collected by filtration, washed with water (5 × 20 mL) and dried under vacuum. The resulting white solid was used without further purification.
33. *General procedure for the preparation of 3-aryl-4-hydroxycoumarins*: a degassed solution of appropriated phenyl boronic acid (2.2 equiv) and P(*t*-But)$_3$ (27 mL) in DME and H_2O (4:1, 12.5 mL) was added to a mixture of iodonium ylide (0.55 mmol), LiOH/H_2O (3 equiv) and $Pd(OAc)_2$ (6.2 mg) under argon at room temperature. After being stirred at the same temperature for 24–48 h. The resulting mixture was purified by FC (hexane/ethyl acetate, 7:3) to give the desired compound.
3-(3′-Chloro-4′-methoxyphenyl)-4-hydroxycoumarin (**5**). It was obtained with yield 80%. Mp: 298–300 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.94 (s, 3H, OCH_3), 7.21 (s, 1H, H2′), 7.28–7.49 (m, 3H, H6, H8, H5′), 7.56–7.83 (m, 2H, H7, H6′), 7.98 (d, 1H, H5, J = 7.6), 15.7 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 56.2, 105.0, 112.8, 116.6, 116.9, 120.7, 124.2, 124.4, 125.6, 131.5, 132.7, 132.8, 152.7, 154.3, 161.0, 162.3. MS m/z (%): 302 (M^+, 100), 182 (64), 121 (95). Anal. Calcd for $C_{16}H_{11}ClO_4$: C, 63.48; H, 3.66. Found: C, 63.45; H, 3.69.
3-(3′-Chloro-4′-methoxyphenyl)-4-hydroxy-6-methylcoumarin (**6**). It was obtained with yield 80%. Mp: 301–304 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 2.45 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.14–7.24 (m, 1H, H8), 7.25–7.37 (m, 3H, H7, H2′, H5′), 7.40–7.52 (m, 1H, H6′), 7.80 (s, 1H, H5), 15.4 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 20.9, 56.6, 105.0, 112.8, 116.4, 120.8, 123.8, 125.6, 129.0, 131.5, 132.7, 133.6, 133.6, 150.8, 154.3, 160.9, 162.4. MS m/z (%): 316 (M^+, 59), 182 (80), 135 (100), 97 (36), 83 (37), 71 (51), 57 (91). Anal. Calcd for $C_{17}H_{13}ClO_4$: C, 64.46; H, 4.14. Found: C, 64.46; H, 4.09.
6-Chloro-3-(3′-chloro-4′-methoxyphenyl)-4-hydroxycoumarin (**7**). It was obtained with yield 62%. Mp: 321–323 °C. ^{1}H NMR (DMSO-d_6) δ (ppm): 3.90 (s, 3H, OCH_3), 7.0–7.57 (m, 3H, H8, H2′, H5′), 7.60–7.84 (m, 2H, H7, H6′), 8.0 (s, 1H, H5), 15.7 (s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ (ppm): 56.8, 113.0, 119.0, 121.1, 123.6, 125.5, 128.7, 131.7, 132.6, 132.8, 135.2, 136.1, 151.5, 154.6, 160.2, 162.2. MS m/z (%): 338 (M^+, 59), 336 (100), 182 (75), 155 (88), 142 (26), 57 (31). Anal. Calcd for $C_{16}H_{10}Cl_2O_4$: C, 57.0; H, 2.99. Found: C, 57.05; H, 3.05.
34. Determination of human monoamine oxidase (hMAO) isoform activity. The effects of the tested compounds on hMAO isoform enzymatic activity were evaluated by a fluorimetric method. Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing the tested drugs in several concentrations and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain in our experimental conditions the same reaction velocity [165 pmol of *p*-tyramine/min (hMAO-A: 1.1 μg protein; specific activity: 150 nmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min/mg protein; hMAO-B: 7.5 μg protein; specific activity: 22 nmol of *p*-tyramine transformed/min/mg protein)] were placed in the dark fluorimeter chamber and incubated for 15 min at 37 °C. The reaction was started by adding (final concentrations) 200 μM Amplex® Red reagent, 1 U/mL horseradish peroxidase and 1 mM *p*-tyramine. The production of H_2O_2 and, consequently, of resorufin was quantified at 37 °C in a multidetection microplate fluorescence reader (FLX800™, Bio-Tek® Instruments, Inc., Winooski, VT, USA) based on the fluorescence generated (excitation, 545 nm, emission, 590 nm) over a 15 min period, in which the fluorescence increased linearly. Control experiments were carried out simultaneously by replacing the tested drugs with appropriate dilutions of the vehicles. In addition, the possible capacity of the above tested drugs for modifying the fluorescence generated in the reaction mixture due to non-enzymatic inhibition (e.g., for directly reacting with Amplex® Red reagent) was determined by adding these drugs to solutions containing only the Amplex® Red reagent in a sodium phosphate buffer. The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from vials containing all components except the hMAO isoforms, which were replaced by a sodium phosphate buffer solution.
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