Benzopyran-2-ones as attractive scaffold for MAO inhibitors: synthesis, biological evaluation and docking studies

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"Abstract." Neurodegenerative diseases are becoming prevalent pathologies in developed societies due to increasing average of life expectancy of the population. This fact has encouraged an active research in the development of new drugs, since they may represent an important advance in the treatment of complex diseases such as Alzheimer and Parkinson's diseases.

Coumarins are a large family of compounds, of both natural and synthetic origin, important because of the pharmacological activities that this compounds display. Therefore, they occupy an important place in the organic and medicinal chemistry realm. In recent years, coumarins have been attracting interest because of their ability of inhibiting some enzymes. The versatility of the Perkin and Knoevenagel reactions has led to a large family of differently substituted compounds.

In order to find the structural features for the human MAO inhibitory activity and selectivity, in the present communication we report the synthesis, pharmacological evaluation and a comparative study of a new series of 3-phenylcoumarins versus 3-benzoylcoumarins. A bromo atom and a methoxy/hydroxyl substituent were introduced in these scaffolds at different positions of the coumarin moiety. The synthesized compounds were evaluated as MAO-A and B inhibitors using R-(-)-deprenyl and iproniazide as reference compounds. The presence or absence of a carbonyl group between the coumarin and the phenyl substituent in 3 position remarks, respectively, the MAO-A or MAO-B inhibitory activity. Some of the new compounds showed MAO-B inhibitory activities in the low micromolar range.

In addition, docking experiments were carried out on hMAO-A and h-MAO-B structures. This study has provided new information about the enzyme-inhibitor interaction and the potential therapeutic application of this coumarin scaffolds.

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1. Introduction

Coumarins, stilbenes, chalcone and their natural and/or synthetic derivatives (Figure 1) are biologically interesting compounds because of their structural diversity. Due to this variability, these heterocyclic compounds occupy an important role not only in the Organic Chemistry but also in the Medicinal Chemistry realm.[1-6] They are described as anticancer, anti-inflammatory, antimicrobial and antioxidant agents.[7-16] A number of studies pay special attention to coumarin derivatives as monoamine oxidase (MAO)[17-23] inhibitors. Recently, chalcone structure has also been identified as a valid scaffold for monoamine oxidase inhibitors (MAOI).[24] Therefore, recent findings reveal that MAO-A and MAO-B affinity and selectivity can be efficiently modulated by appropriate substitutions at different positions of the coumarin and chalcone moiety.[19, 25-27]

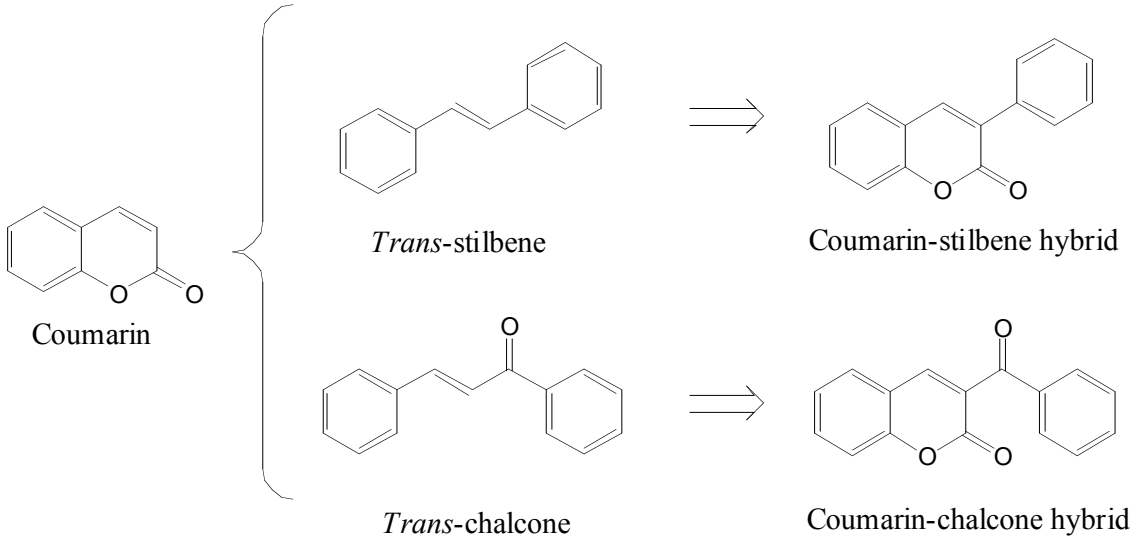


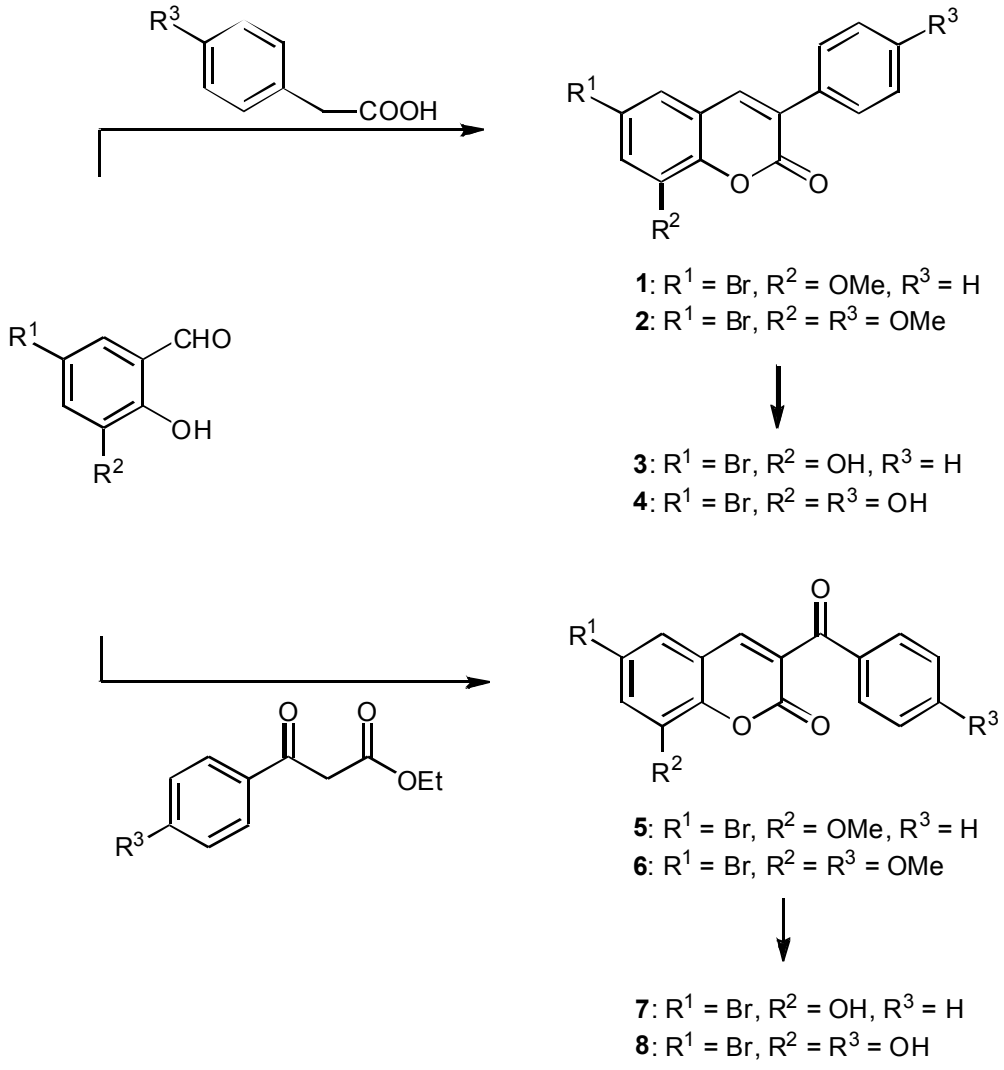
Figure 1. General structures of coumarins, *trans*-stilbenes, *trans*-chalcones, coumarin-stilbene hyvbrids and coumarin-chalcone-hybrids.

MAO is a FAD-containing enzyme (flavoenzyme) bound to the outer mitochondrial membrane in neuronal, glial and many other cells.[28,29] Two isoforms namely as MAO-A and MAO-B have been identified based on their amino acid sequences, three-dimensional structure, substrate preference and inhibitor selectivity.[30,31] These isoenzymes are responsible for the oxidative deamination of neurotransmitters and dietary amines. Therefore, they are responsible for the regulation of intracellular levels of biogenic amines in the brain and the peripheral tissues.[32,33] MAO-B preferentially deaminates phenylethylamine and benzylamine, while MAO-A has a higher affinity for noradrenaline and serotonin.[34,35] Despite of these differences, dopamine and tyramine are common substrates for both isoforms. These properties determine the pharmacological interest of MAOIs. Selective and irreversible MAO-B inhibitors, such as selegiline (R-(-)-deprenyl) and rasagiline are useful compounds for the treatment of Parkinson [36,37] and Alzheimer's diseases.[38,39] Selective MAO-A inhibitors, such as clorgyline (irreversible) and moclobemide (reversible), are useful for the treatment of

neurological disorders, such as depression and anxiety.[40,41] All these findings have led us to an intensive search for novel, selective and efficient MAO inhibitors.

2. Chemistry

With the aim of finding novel and selective MAO-B inhibitors, we have previously synthesized 3-arylcoumarin derivatives in which both the coumarin nucleus and a 3-phenyl ring were differently substituted. The experimental data show that those compounds are potent and selective MAO-B inhibitors.[20-23] In particular, the 6,8-disubstituted coumarins proved to be very interesting derivatives.[22] Based on the previous 3-phenylcoumarins experimental results, in this paper we describe a new project with a comparative study between 3-phenylcoumarins (compounds **1**-**4**) and 3-benzoylcoumarins (compounds **5**-**8**), which are interesting semi-rigid chalcones with the *α,β*-unsaturated double bond included in the coumarin skeleton (Scheme 1).[42]



Scheme 1

The 6-bromo-8-methoxycoumarins were efficiently synthesized by Perkin[43-45] (**1** and **2**) and Knoevenagel[46,47] (**5** and **6**) reactions. The hydroxy derivatives (**3**, **4**, **7** and **8**)[42] were obtained by two different hydrolysis reactions,[48-50] according to the synthetic protocol outlined in scheme 1. Treatment of the corresponding salicylaldehyde and the conveniently substituted phenylacetic acid with *N,N'*-dicyclohexylcarbodiimide (DCC) as dehydrating agent, in dimethyl sulfoxide (DMSO) at 110 ºC during 24 hours, afforded the 3-phenylcoumarins **1** and **2**. The consequent hydrolysis of **1** and **2** in acetic acid and acetic anhydride, with hydriodic acid 57 %, for 4 hours, yielded the hydroxy derivatives **3** and **4**.[42] The synthesis of the 3-benzoylcoumarins **5** and **6** was performed via condensation of the conveniently substituted salicylaldehyde with the corresponding *β*-ketoester, in ethanol at reflux temperature for 5 or 2 hours respectively, using piperidine as basic catalyst. The resulting methoxy derivatives were treated with boron tribromide at 80 ºC for 48 hours, to give the corresponding hydroxy derivatives **7** and **8**. Starting from the same salicylaldehyde, we can afford two series, differing just in the presence (compounds **5**-**8**) or absence (compounds **1**-**4**) of a carbonyl group between the phenyl ring at the 3 position and the coumarin scaffold.

3. Results and discussion

The inhibitory MAO activity of compounds **3**, **4**, **7** and **8** was evaluated *in vitro* by the measurement of the enzymatic activity of human recombinant MAO isoforms expressed in BTI insect cells infected with baculovirus.[52] 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 reference inhibitors were calculated (Table 1).[52]

Table 1. MAO-A and MAO-B inhibitory activity results for the synthesized compounds **1-8** and reference inhibitors.

Compounds	MAO-A IC_{50} (µM)	MAO-B IC_{50} (µM)	Selectivity Index
3	*	30.91±2.09	> 3.2[a]
4	20.74±1.40	16.87±1.14	1.2
7	46.81±3.18	73.92±4.99	0.63
8	19.17±1.29	**	0.19[b]
R-(-)-Deprenyl	67.25±1.02	$19.60 \times 10^{-3} \pm 0.86 \times 10^{-3}$	3,431
Iproniazide	6.56±0.76	7.54±0.36	0.87

*Inactive at 100 µM (highest concentration tested). At higher concentrations compound precipitate.

** 100 µM inhibits enzymatic activity around (by approximately) 45-50 %. At higher concentrations

compound precipitate.

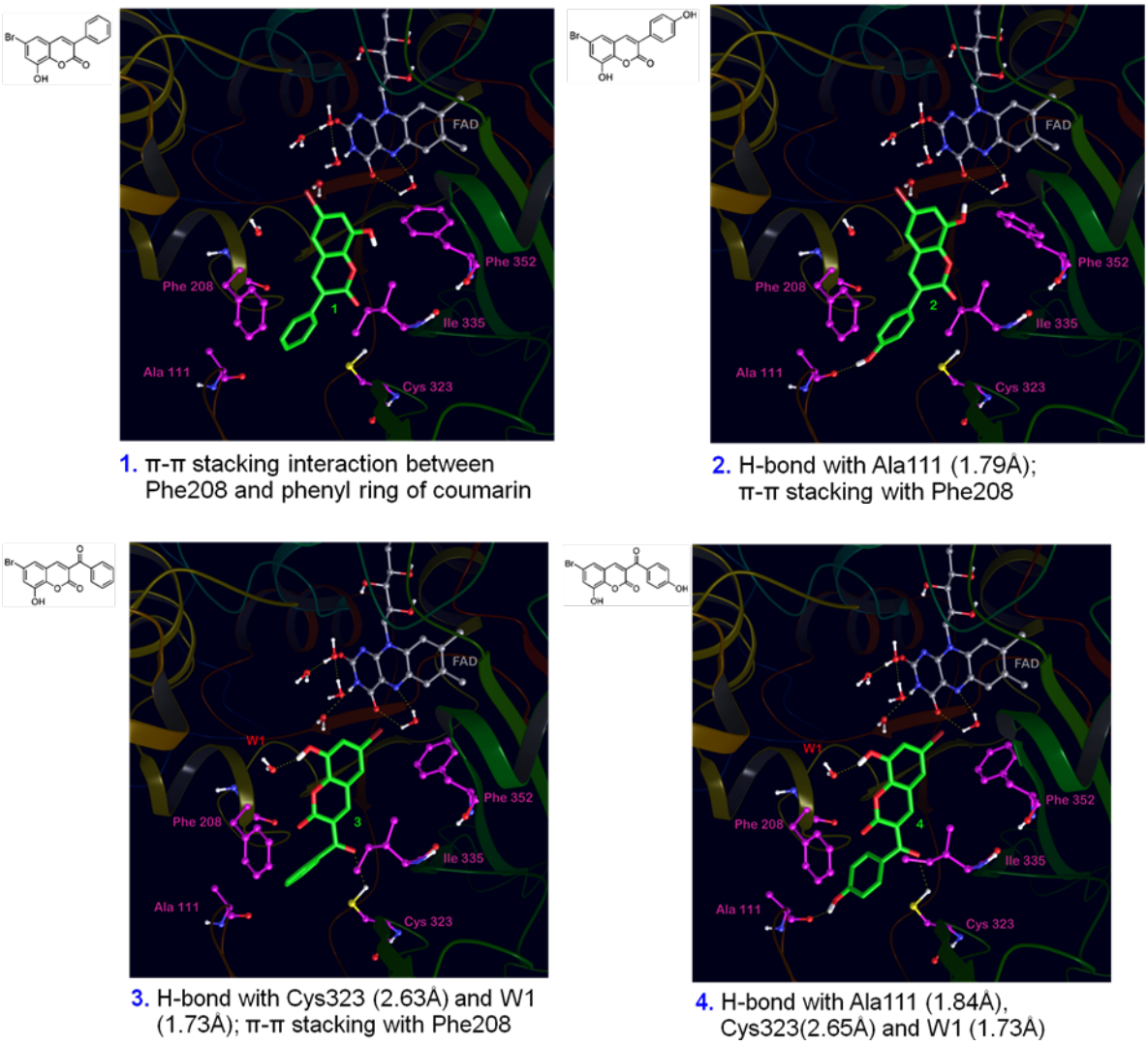
[a]Values obtained under the assumption that the corresponding IC_{50} against MAO-A is the highest concentration tested (100 μM).

[b]Value obtained under the assumption that the corresponding IC50 against MAO-B is the highest tested concentration (100 μM).

In the present communication, the effect of the presence or the absence of a carbonyl group between the coumarin and the 3-phenyl ring is studied. Substituents and their positions in the 3-phenylcoumarin nucleus have been selected based on previous results which showed very high MAOI activity for some derivatives. It was shown that the presence of a bromo atom at position 6 and a hydroxyl group at position 8 of the coumarin nucleus (compound **3**) allows a selective MAO-B compound. When another hydroxyl group is included in *para* position of the 3-aryl ring (compound **4**), the MAO-B selectivity was lost. On the other hand, when we analyze the second series where a 3-benzoyl group has replaced the 3-phenyl substituent, the introduced carbonyl group decreases the MAOI activity against B isoform. Compounds **7** and **8** increase the affinity for the MAO-A receptor, losing the MAO-B selectivity of compound **3**, from the first series. Also, compound **8** is selective against the MAO-A isoenzyme. A small change in the structure causes a big change in the affinity of the molecules for the receptor. These preliminary results allow us to understand slightly better interactions between molecule and receptor and the molecular fragments that are essential to maintain and improve the MAO activity and selectivity.

We try here to rationalize the experimental data by comparing the best docking poses of coumarins derivatives into MAO-A (PDB id: 2Z5X) and MAO-B (PDB id: 2V60) crystal structures. Best docking poses retrieved for molecules **3**, **4**, **7** and **8** into MAO-A and MAO-B isoforms. Three water molecules are found to be conserved in both MAO binding sites. All the water molecules show a H-bonds network with backbone and/or side chain residues of the receptor. H-bonds are displayed in yellow dot line. Hidden residues for a better visualization: from Thr167 to Ser184 in MAO-A, from Glu159 to Val173 in MAO-B.

Structural water molecules are involved (via H-bonds) in ligand-protein binding interactions. The different active site residues in MAO isoforms play a significant role in docking pose orientation of coumarins. Therefore, selectivity of compounds **3** and **8** against MAO-B and MAO-A respectively, might be partially explained taking into account the interactions with key residues at the binding cleft. QM-Polarized Ligand Docking followed by a post-processing analysis with Prime MM-GBSA has proved to be useful to study a congeneric series of compounds. Docking studies with the compounds and both isoforms of the MAO enzyme are shown below:



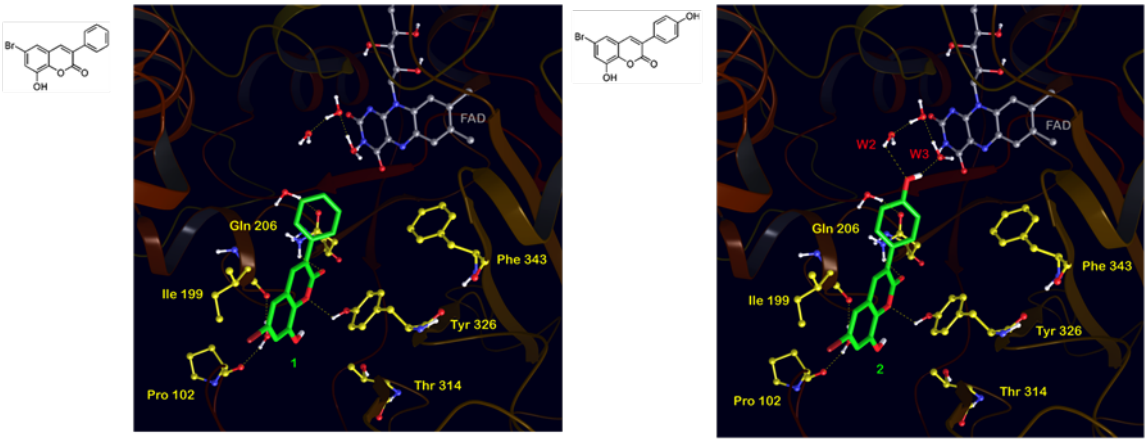
1. π-π stacking interaction between Phe208 and phenyl ring of coumarin

2. H-bond with Ala111 (1.79Å); π-π stacking with Phe208

3. H-bond with Cys323 (2.63Å) and W1 (1.73Å); π-π stacking with Phe208

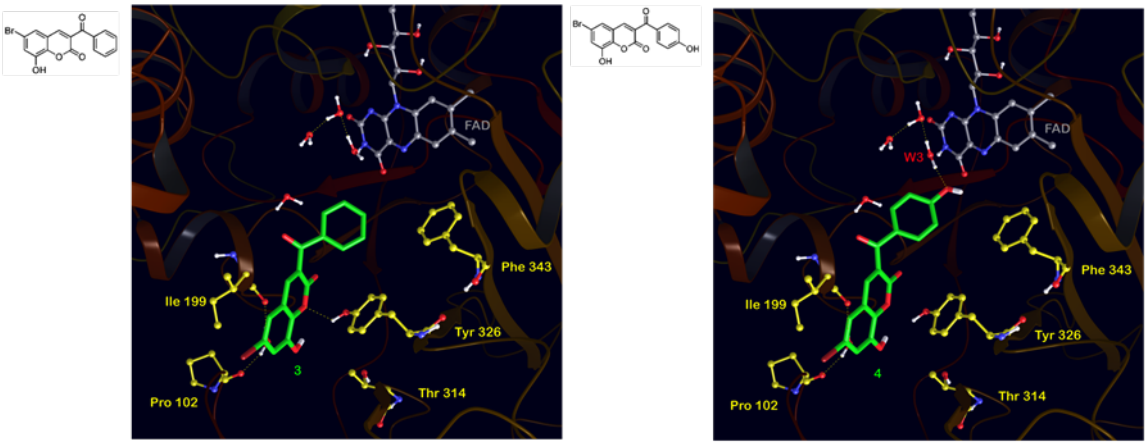
4. H-bond with Ala111 (1.84Å), Cys323(2.65Å) and W1 (1.73Å)

Figure 2. Interaction of compounds **3**, **4**, **7** and **8** with the binding site of MAO-A.



1. H-bond with Tyr326 (2.97Å) and Gln206 (2.58Å)

2. H-bond with Tyr326 (2.89Å), Gln206 (2.20Å), W2 (1.86Å) and W3 (2.49Å)



3. H-bond with Tyr326 (2.91Å) 4. H-bond with W3 (1.80Å)

Figure 3. Interaction of compounds **3**, **4**, **7** and **8** with the binding site of MAO-B.

4. Conclusions

As conclusion, it was verified an important lost of the MAO-B inhibitory activity and selectivity when the 3-phenyl skeleton is substituted for the 3-benzoyl one. However, in some of the 3-benzoyl derivatives it was shown not only inactivity against MAO-B isoenzyme, but showed MAO-A inhibitory activity and selectivity. Therefore, selectivity seems to depend on the nature of the coumarins' substituent. In the present study it was shown that 6-bromo-8-methoxy-3-phenylcoumarins are an interesting scaffold for MAO-B inhibitory studies, whereas the 6-bromo-8-hydroxy-3-benzoylcoumarins are an interesting moiety for MAO-A inhibitory ones. The MAO selectivity is an important factor to discriminate the different potential therapeutic applications of these molecules. These findings encourage us to continue the efforts towards the optimization of the pharmacological profile of these structural types as important scaffolds in the neurodegenerative diseases realm.

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52. All IC_{50} values shown in the table are expressed as means ± SEM from five experiments.