Multi-target Design, Synthesis and Study of a Selected Series of Carbamates and Esters with Coumarin Scaffold

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Abstract

Developed countries' society face, with the increase of the population life expectancy, a major problem related with neurodegenerative diseases (ND), such as Parkinson's and Alzheimer's diseases. Therefore, a need for new drugs targeting these diseases emerges. Some of the few accessible treatments use monoamine oxidases inhibitors (MAOI-A and MAOI-B) and acetylcholinesterase inhibitors (AChEI).

Coumarins are a large family of compounds of natural and/or synthetic origin that proved to have numerous pharmacological properties. In this study, we developed new synthetic methodologies to create novel *multi-target* inhibitors focused on the coumarin scaffold. Following this work, docking and pharmacological studies of the previously prepared compounds are currently in progress. Some preliminary results are presented in this communication.

Keywords Coumarins; carbamate and esters derivatives; neurodegenerative diseases; MAO inhibition; AChEI inhibition; docking studies.

Introduction

Coumarins and their natural and/or synthetic derivatives are pharmacologically interesting compounds due to their structural diversity.[1,2] Due to the synthetic accessibility and substitution variability these heterocyclic compounds play an important role not only in organic chemistry but also in the field of medicinal chemistry.[3-10] In fact, coumarins have been previously described as anticancer, antiviral, anti-inflammatory, antimicrobial, enzymatic inhibitory and antioxidant agents.[3-18]

This study aims to develop new compounds with potential inhibitory activity for MAO-A, MAO-B and/or AChE, based on the coumarinic moiety. Considering that the methodology for the development of new chemical entities based on only one therapeutical target has been less successful in the case of multifactorial diseases, such like cancer and ND, we opted in this project for a broader application of the synthesised compounds, by checking their activity on different targets.

The therapeutical aim when we are dealing with ND is, mainly, to boost the dopamine levels in the brain. For such purpose, there are several strategies such as stimulating the dopaminergic recievers or to inhibit dopamine metabolism, amongst others. In the particular case of Alzheimer's disease (AD), one can observe the formation of β-amiloid plaques in the brain[19] – related to a number of factors including a rise of MAO's activity and consequently originating a large amount of free radicals leading to oxidative stress[20] – wich promote the atrofy of colinergic neurones. Therefore, usually one can rely on AChE inhibitors to rise the acetylcholine levels in the brain. And is for all the reasons cited above that our work is focused on the development of multitarget inhibitors for MAO and AChE.

One of the objectives of this work is the synthesis of new compounds with multitarget activity for MAO and AChE. As it is shown in Figure 1, we identified some pharmacophores from well known inhibitors of both MAO-A and MAO-B and AChE and tried to incorporate such pharmacophores in the coumarin moiety (Figure 2). Some of these inhibitors were prepared by our research group, such as 3-arylcoumarins and 7-oxopropoxycoumarins.[14] This novel coumarins were obtained by using as starting material 3-hydroxycoumarin, 6-hydroxycoumarin, 7-hydroxycoumarin and the 3-aminocoumarin.

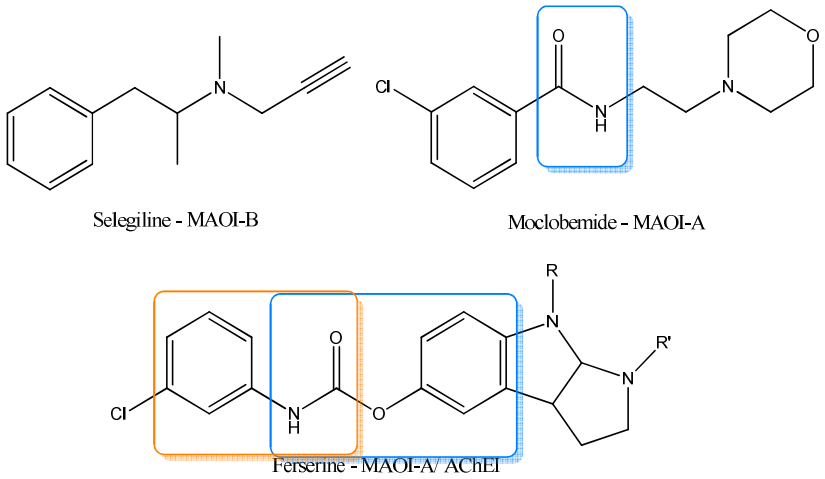


Figure 1 – Some known inhibitors of MAO-A, MAO-B and AChE. In our work we incorporate the highlighted pharmacophores on the coumarin moiety.

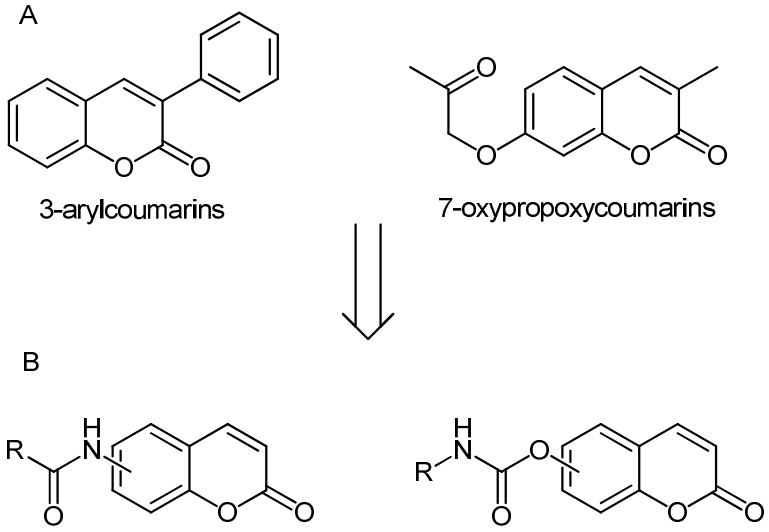


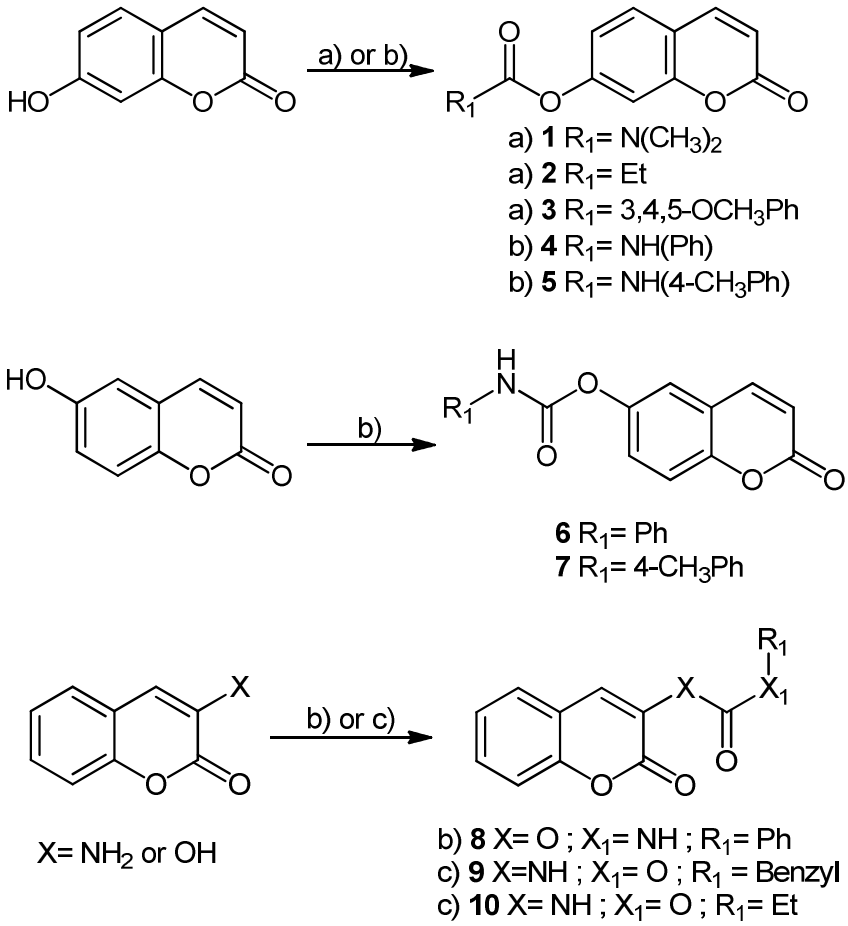
Figure 2 – A- Previously synthesized coumarins within our group with known activity towards MAO. B- New compounds merging the coumarin moiety with the pharmacophores (amide and carbamate) highlighted in figure 1.

Experimental

Chemistry

^{1}H NMR spectra were recorded on a Bruker AMX spectrometer at 250 MHz, using TMS as internal standard (chemical shifts in δ values, J in Hz). 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). The purity of the compounds was found to be higher than 95%.

Synthetic methodologies



Scheme 1 – Synthetic scheme for obtaining the desired coumarins. Reagents: a) 0ºC; Py; acetone; R_1(C=O)Cl b) MeCN; Et_3N; O=C=N-R_1; c) DCM; Py.

General synthetic procedure for compounds 1-3: The method used to prepare **1-3** used 7-hydroxycoumarin (0,96 mmol) as a starting material dissolved in dry acetone (15 mL) at 0ºC and in a nitrogen atmosphere. After dissolution it was added pyridine (92 μL, 1,15 mmol) and 1,10 mmol of the appropriate chloride. The mixture was then stirred at room temperature for about 6 hours. At the end, the solid obtained was filtered under vacuum, rinsed with cold heptane and purified by column chromatography (DCM).

7-(*N*-dimethylcarbamoyl)coumarin (1) White solid, yield: 68%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3,10 (s, 3H, -CH_3); 3,15 (s, 3H, -CH_3); 6,41 (d, 1H, H(3), J=9,6); 7,07-7,15 (m, 2H, H(6), H(8)); 7,48 (d, 1H, H(5), J=8,4); 7,72 (d, 1H, H(4), J=9,5).

7-(Propionyl)coumarin (2) White solid, yield 50%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 1,45 (t, 3H, -CH_3, J=7,1); 4,40 (dd, 2H, -CH_2, J=7,14); 7,21 (m, 2H, H(6), H(8)); 7,53 (d, 1H, H(5), J=8,5); 7,73 (d, 1H, H(4), J=9,6).

7-(3,4,5-Trimethoxybenzoylcarbamoyl)coumarin (3) White solid, yield 70%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3,77 (m, 9H, -OCH_3); 6,18 (d, 1H, H(3), J=9,5); 6,72 (m, 2H, H(6), H(8)); 7,21 (s, 2H, H(2'), H(6')); 7,52 (d, 1H, H(5), J=8,5); 7,92 (d, 1H, H(4), J=9,5).

General synthetic procedure for compounds 4-8: To a suspension of the appropriate hidroxycoumarin (0,99 mmol) and dry MeCN (20 mL), Et_3N (139 μL, 1,00 mmol) was added. After the starting material was completely dissolved it was added the desired isocyanate (1,00 mmol) divided in four equal parts, spaced between them by 30 minutes. The reaction ended 30 minutes after the last aliquot was added.[21] The insoluble product that appeared on the reaction mixture was then filtered and washed with cold hexane and recrystallized with hexane/AcOEt.

7-(*N*-phenylcarbamoyl)coumarin (4) White solid, yield 73%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 6,46 (d, 1H, H(3), J=9,2); 7,05 (t, 1H, H(4'), J=7,7); 7,45-7,52 (m, 6H, H(6), H(8), H(2'), H(3'), H(5'), H(6')); 7,76 (d, 1H, H(5), J=7,9); 8,07 (d, 1H, H(4), J=9,5); 10,37 (s, 1H, -NH).

7-[(4'-Methyl-*N*-phenyl)carbamoyl]coumarin (5) White solid, yield 64%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 2,24 (s, 3H, -CH_3); 6,18 (d, 1H, H(3), J=10,1); 7,12 (m, 4H, H(6), H(8), H(2'), H(6')); 7,41 (d, 2H, H(3'), H(5'), J=6,3); 7,75 (d, 1H, H(5), J=8,5); 8,04 (d, 1H, H(4), J=9,8); 10,30 (s, 1H, -NH).

6-(*N*-phenylcarbamoyl)coumarin (6) White solid, yield 72%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 6,54 (d, 1H, H(3), J=9,6); 6,94-7,06 (m, 2H, H(3'), H(5')); 7,32 (t, 2H, H(2'), H(6'), J=7,5); 7,46-7,52 (m, 3H, H(5), H(7), H(4')); 7,64 (d, 1H, H(4), J=9,5); 8,05 (d, 1H, H(8), J=9,7); 10,34 (s, 1H, -NH).

6-[(4'-Methyl-*N*-phenyl)carbamoyl]coumarin (7) White solid, yield 65%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 2,21 (s, 3H, -CH_3); 6,54 (d, 1H, H(3), J=9,6); 7,09 (d, 2H, H(5), H(7), J=9,4); 7,39 (d, 2H, H(2'), H(6'), J=8,4); 7,46 (d, 2H, H(3'), H(5'), J=2,4); 7,63 (d, 1H, H(4), J=9,4); 8,05 (d, 1H, H(8), J=9,7); 10,22 (s, 1H, -NH).

3-(*N*-phenylcarbamoyl)coumarin (8) White solid, yield 62%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 6,52 (d, 1H, H(3), J=9,2); 7,02 (t, 1H, H(4'), J=7,7); 7,44-7,53 (m, 6H,

H(6), H(8), H(2'), H(3'), H(5'), H(6')); 7,82 (d, 1H, H(5), J=7,9); 8,29 (d, 1H, H(4); J=9,5); 10,48 (s, 1H, -NH).

General synthetic procedure for compounds 9-10: In an ice cold bath the 3-aminecoumarin (0,93 mmol) was dissolved in DCM (10 mL). Then it was added pyridine (1,02 mmol), and the appropriate chloroformate (0,70 mmol) dropwise. The reaction was left to continue overnight at room temperature.[22] The precipitate was filtered and purified by column chromatography (hexane/AcOEt 1:1).

3-(Benzylcarbamoyl)coumarin (9) White solid, yield 52%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 5.21 (s, 2H, -CH_2); 7,23-7,37 (m, 5H, H(2'), H(3'), H(4'), H(5'), H(6')); 7,38-7,44 (m, 4H, H(5), H(6), H(7), H(8)); 7,60 (s, 1H, H(4)); 8,30 (s, 1H, -NH).

3-(Ethylcarbamoyl)coumarin (10) White solid, yield 58%, ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 1,31 (s, 3H, -CH_3); 4,24 (s, 2H, -CH_2); 7,24-7,30 (m, 3H, H(4), H(6), H(8)); 7,37-7,49 (m, 2H, H(5), H(7)); 8,27 (s, 1H, -NH).

Docking Studies

Our work was complemented by molecular docking assays of about 40 compounds, the 10 novel coumarins synthesized plus 30 on progress to be synthesized, using the flexible ligand algorithm from GLIDE[23] and AutoDock[24] against MAO-A and MAO-B. The obtained complexes were minimized energetically using the PRCG method with 5000 iterations and a MMFFs force field with implicit water solvatation implemented on Macromodel.[23]

The primary goal of this part of the work was to find consensual thermodynamical data from the two programs, so that we could infer about the affinity of the compounds with MAO-A and MAO-B. The enzyme models used (hMAO-A and hMAO-B) were obtained from high resolution crystalline structures included on PDB, 2Z5X and 2V5Z, respectively. The co-crystallized ligands, harmine in MAO-A and safinamide in MAO-B, were removed and the bonding sites were defined by the means of a regular "box" of approximately 1000Å^3, centered on the N5 nitrogen of the FAD co-factor. To evaluate the compound stability, a *scoring* function GLIDE XP was used. The same protocol was utilized also to a series of well known MAO-A and MAO-B inhibitors, which are described in literature,[10, 24-26] so that we could obtain a database that would allow us to validate the theoretical results obtained. In Figure 3 we can see the interactions between a 7-(3',4'-dichlorobenzoyl)coumarin currently being synthesized and MAO-B, on 2D

and 3D, which indicates that this structure might be a suitable candidate for a lead compound.

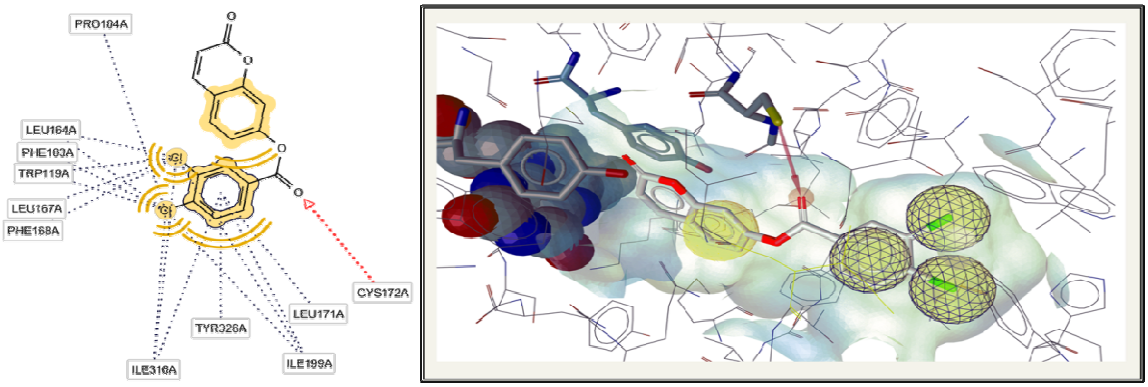
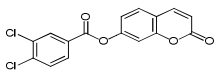
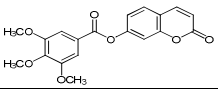
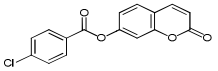
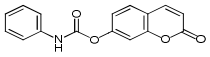
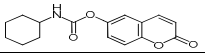


Figure 3 – 2D (left) and 3D (right) representations of a MAO-B complex. The right side also shows some pharmacophores of the 7-(3',4'-dichlorobenzoyl)coumarin.

Preliminary results of *docking score* were obtained and we can see, as shown in the table below, that the studied compounds are promising MAO inhibitors.

Table 1 – *Docking score* of five most promising MAO inhibitors and two known inhibitors of MAO-A (harmine) and MAO-B (safinamide). A low score indicates a stable complex.

	Docking* GLIDE *Score	
Compound	**MAO-A**	**MAO-B**
	-9,219	-12,429
	-8,740	-12,206
	-10,370	-12,168
	-6,369	-12,096
	-11,573	-10,998
Harmine	-11,333	-4,698
Safinamide	-7,658	-10,845

Discussion

In the present work we describe the docking studies of a selected series of differently substituted coumarins. Based in the preliminary results, and on the analysis of the best substitution positions, we started the synthesis of some potential candidates to illustrate the versatility of the synthetic routes and to give some examples of the amide and carbamate derivatives. We are now increasing the series with the best compounds obtained by the docking calculations.

The *in vitro* pharmacological studies are still ongoing, and the preliminary results were promising. As described by us for other coumarin derivatives, these compounds have been showing an interesting profile as MAO-B and AChE inhibitors. Therefore, docking studies are very interesting tools to corroborate the experimental information, helping to understand how the molecules interact with the receptors. Also, these studies are important tools in the design of future molecules. This was the inspiration for the present work.

There were synthesized, purified and characterized ten compounds with different substitution patterns. Positions 3, 6 and 7 were selected according to the previous results obtained by the research group. The synthetic methodologies were versatile and efficient, being the yields between 50-73%.

In this work we present the docking preliminary results, compiled and organized the compounds by their *score,* a function that allows us to predict if the compound-enzyme complex is stable or not. In general, the coumarins substituted at position 6 have more affinity for MAO-A, while the best results for MAO-B came from the coumarins substituted at the position 7. Furthermore, coumarins substituted at the position 3 established selective complexes with MAO-B. With the theoretical study of the described compounds we also found that the presence of an aryl group attached to the amide or the carbamate groups seems to enhance activity, as well as an extra carbon spacing the carbamate function from the aromatic ring. In this paper we showed (table 1) the five best candidates active against MAO according to the predictions which show us that those compounds, substituted at position 6 and 7, were good inhibitors.

Conclusions

The synthesis of a series of new chemical entities by a *one-step* reaction was successful. Theoretically, the most stable complexes with MAO-A were generally the ones that had

coumarin substituted at position 6, whilst for MAO-B the best results were obtained for the positions 3 and 7. Therefore, this study is an important part of a complex work of drug design of *multi-target* drug candidates.

Acknowledgement

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