Synthesis and Study of a Selected Series of Amides with the Coumarin Scaffold for the Treatment of Alzheimer's Disease

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Abstract

Due to the synthetic accessibility of different substituted coumarins and their biological properties, these heterocyclic compounds play an important role in the field of Medicinal Chemistry. In fact, coumarins have been previously described as anticancer, antiviral, anti-inflammatory, antimicrobial, enzymatic inhibitory and antioxidant agents.

Within the field of neurodegenerative diseases are described different types and intervention processes, which include the development of acetylcholinesterase (AChE) inhibitors, which combined with another drugs are used on therapy for Alzheimer's disease (AD). These inhibitors allow acetylcholine levels in the brain to stabilize or even enhance them, since it is established that this enzyme is responsible for the metabolism of this important neurotransmitter.

In our group, we have already synthesized multiple novel compounds incorporating the coumarin moiety with remarkable activity towards MAO and/or AChE. In this work, we continue to exploit this scaffold by the synthesis of novel 3-amidocoumarins for the treatment of neurodegenerative diseases. Following this work, pharmacological studies of the prepared compounds as AChE inhibitors (AChEI) are currently in progress. Some preliminary results are presented in this communication.

Key Words: 3-Amidocoumarins, Acetylcholinesterase inhibitors, Alzheimer's disease.

Introduction

Coumarins and their natural and/or synthetic derivatives are pharmacologically interesting compounds due to their structural diversity and synthetic accessibility.[1,2] In our group, we synthesized previously multiple novel compounds taking advantage of the coumarin moiety to gain remarkable activity against monoamine oxidase B (MAO-B) and/or acetylcholinesterase (AChE).[3-4]

The treatment of neurodegenerative diseases such as Alzheimer's disease (AD) arises as one of the biggest challenges facing the society nowadays. Over 25 million people worldwide are affected by this progressively impairing condition, and so it becomes crucial to develop new therapeutics for AD treatment.[5]

AD is characterized by the presence of beta-amyloid plaques in the brain, neurofibrillary tangles and the atrophy of cholinergic neurons. This loss of cholinergic neurons translates into a decrease of acetylcholine (ACh) levels in the brain synapses which ultimately lead to a cognitive impairment in the individual. The current drugs have a wide range of adverse effects, so the search for new active compounds is still an emergent demand. Very recently we showed that amide substitution at position 3 of coumarin nucleus (Figure 1- A) leads to active compounds against AChE and MAO.[4] According to that and taking in account that some carbamate derivatives are known as AChE inhibitors (AChEI) (Figure 1- B), we focused our work in developing new AChEI. The new compounds (**1-10**) are related to the already mentioned derivatives, incorporating in some cases a quinoline moiety, related to tacrine (Figure 1- C).

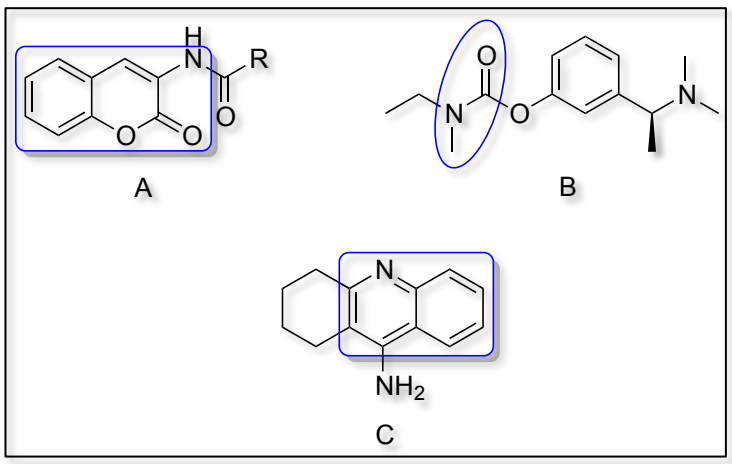


Figure 1 – A. Previously reported AChEI by our group; B. Carbamate derivative rivastigmine, a known AChEI; C. Tacrine, a known AChEI. Highlighted in blue are the structural backbones used to craft compounds **1-10**.

In this paper we report the synthesis and characterization of AChEI and their *in vitro* activity. Docking studies and further completion of the 3-amidocoumarin series is currently in progress.

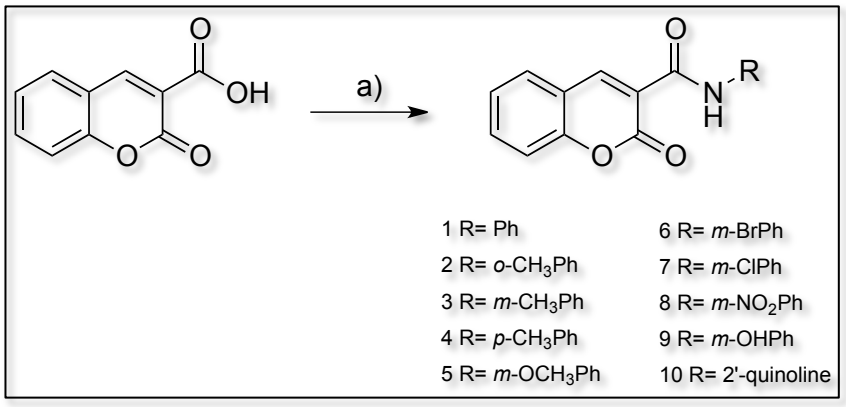
Experimental

Chemistry

The chemicals used in the synthesis were supplied by Aldrich and Merck. Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus and are uncorrected. ^{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).

General Procedure for the preparation of 3-amidocoumarins (1-10)[6]

To a solution of 5 mmol of coumarin-3-carboxylic acid in dichloromethane (DCM), in a round bottom flask with a flux of argon at 0 ºC, 5.5 mmol of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 5.5 mmol of 4-dimethylaminopyridine (DMAP) were added. Short after was added, in small portions, 5 mmol of the corresponding amine, and then the reaction mixture was stirred for 4 h at room temperature. The obtained precipitate was filtered and purified by recrystallization with ethanol or by column chromatography (hexane/ethyl acetate 9:1) to give the desired product (Scheme 1 and Table 1, compounds **1-10**).



Scheme 1 – *Reagents and conditions:* a) EDC, DMAP, 0 ºC to r.t., DCM.

Table 1 – Characterization and yield of the synthesized compounds (**1-10**).

Compounds	^{1}H NMR	m.p. (ºC)	Yield
2-oxo-*N*-phenyl-2*H*-chromene-3-carboxamide (**1**)	7.15 (td, 1H, H-4', *J*=1.1, 7.4), 7.35-7.55 (m, 4H, H-6, H-8, H-3', H-5'), 7.70-7.78 (m, 3H, H-7, H-2', H-6'), 8.00 (dd, 1H, H-5, *J*=1.6, 7.7), 8.90 (s, 1H, H-4), 10.63 (s, 1H, NH).	256-257	78 %
2-oxo-*N*-(*o*-tolyl)-2*H*-chromene-3-carboxamide (**2**)	2.42 (s, 3H, CH_3), 7.10 (td, 1H, H-4', *J*=1.2, 7.4), 7.22-7.29 (m, 2H, H-7, H-8), 7.38-7.47 (m, 2H, H-4', H-5'), 7.67-7.77 (m, 2H, H-5, H-3'), 8.25 (d, 1H, H-6', *J*=8.2), 9.04 (s, 1H, H-4), 10.79 (s, 1H, NH).	212-213	66 %
2-oxo-*N*-(*m*-tolyl)-2*H*-chromene-3-carboxamide (**3**)	2.38 (s, 3H, CH_3), 6.98 (d, 1H, H-4', *J*=7.4), 7.24 (dt, 1H, H-5', *J*=1.3, 7.4), 7.38-7.46 (m, 2H, H-6, H-7), 7.55 (d, 2H, H-6', H-8, *J*=7.2) 7,66-7.75 (m, 2H, H-5, H-2'), 9.02 (s, 1H, H-4), 10.79 (s, 1H, NH).	200-201	55 %
2-oxo-*N*-(*p*-tolyl)-2*H*-chromene-3-carboxamide (**4**)	2.27 (s, 3H, CH_3), 7.16 (d, 2H, H-3', H-5', *J*=8.4), 7.42-7.60 (m, 4H, H-6, H-8, H-2', H-6'), 7.73 (td, 1H, H-7, *J*=1.6, 8.3), 7.98 (dd, 1H, H-5, *J*=1.5, 7.8), 8.89 (s, 1H, H-4), 10.56 (s, 1H, NH).	236-237	69 %
N-(2-methoxyphenyl)-2-oxo-2*H*-chromene-3-carboxamide (**5**)	3.97 (s, 3H, OCH_3), 6.92-7.14 (m, 3H, H-8, H-3', H-6'), 7.41 (t, 2H, H-6, H-4', *J*=7.6), 7.71 (t, 2H, H-7, H-5', *J*=7.6), 8.54 (d, 1H, H-5, *J*=7.6), 9.00 (s, 1H, H-4), 11.28 (s, 1H, NH).	239-240	74 %
N-(2-bromophenyl)-2-oxo-2*H*-chromene-3-carboxamide (**6**)	7.04 (t, 1H, H-4', *J*=7.6), 7.33-7.47 (m, 3H, H-6, H-8, H-5'), 7.60-7.76 (m, 3H, H-5, H-7, H-3'), 8.51 (d, 1H, H-6', *J*=8.0), 9.01 (s, 1H, H-4), 11.21 (s, 1H, NH).	196-197	52 %
N-(2-chlorophenyl)-2-oxo-2*H*-chromene-3-carboxamide (**7**)	7.10 (dt, 1H, H-7, *J*=1.5, 7.7), 7.26-7.47 (m, 4H, H-6, H-4', H-5', H-6'), 7.67-7.76 (m, 2H, H-8, H-3'), 8.56 (dd, 1H, H-5, *J*=1.5, 8.2), 9.02 (s, 1H, H-4), 11.34 (s, 1H, NH).	220-221	68 %
N-(2-nitrophenyl)-2-oxo-2*H*-chromene-3-carboxamide (**8**)	7.22-7.33 (m, 1H, H-4'), 7.37-7.49 (m, 2H, H-8, H-5'), 7.64-7.78 (m, 3H, H-6, H-7, H-6'), 8.21 (dd, 1H, H-5, *J*=1.4, 8.3), 8.75 (d, 1H, H-3', *J*=8.4), 9.00 (s, 1H, H-4), 12.57 (s, 1H, NH).	226-227	62 %
N-(2-hydroxyphenyl)-2-oxo-2*H*-chromene-3-carboxamide (**9**)	6.82-6.92 (m, 3H, H-8, H-4', H-5'), 7.46-7.57 (m, 2H, H-6, H-7), 7.76 (d, 1H, H-5, *J*=7.4), 8.04 (d, 1H, H-3', *J*=7.1), 8.39 (d, 1H, H-6', *J*=7.1), 9.05 (s, 1H, H-4), 10.23 (s, 1H, OH), 11.11 (s, 1H, NH).	269-270	54 %
2-oxo-*N*-(quinolin-8-yl)-2*H*-chromene-3-carboxamide (**10**)	7.38-7.79 (m, 6H, H-5, H-6, H-7, H-8, H-5', H-8'), 8.06 (d, 1H, H-6', *J*=7.64), 8.39 (d, 1H, H-4', *J*=7.64), 8.86 (m, 2H, H-7', H-8'), 9.02 (s, 1H, H-4), 12.54 (s, 1H, NH).	275-276	60 %

Pharmacology: AChE and BuChE inhibition

In 96-well plates, the sample was dissolved in phosphate buffer (8 mM K_2HPO_4, 2.3 mM NaH_2PO_4, 150 mM NaCl, and 0.05% Tween 20 at pH 7.6) and an AChE/BuChE solution (50 µL, 0.25 unit/mL) from *Electroporus electricus* and equine serum,

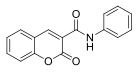
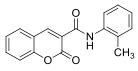
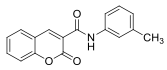
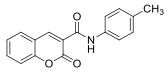
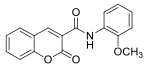
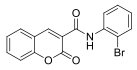
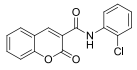
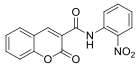
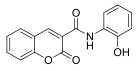
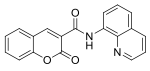
respectively, in the same phosphate buffer, was added. The assay solutions, except substrate, were pre-incubated with the enzyme for 30 min at room temperature. After pre-incubation, the substrate was added. The substrate solution consists of Na_2HPO_4 (40 mM), acetylthiocholine/butyrylthiocholine (0.24 mM) and 5,5′-dithio-bis-(2-nitrobenzoic acid) (0.2 mM, DTNB, Ellman's reagent). Absorbance of the yellow anion product, due to the spontaneous hydrolysis of substrate, was measured at 405 nm for 5 min on a microtiter plate reader (Multiskan EX, Thermo, Vantaa, Finland). The AChE/BuChE inhibition was determined for each compound. The enzyme activity was calculated as a percentage compared to a control using only the buffer and enzyme solution. The compounds were assayed in the dilution interval of 500 to 15 μg/mL, and the alkaloid galanthamine was used as the reference compound. Each assay was run in triplicate and each reaction was repeated at least three independent times. The IC_{50} values were calculated by means of regression analysis.[7]

Results and Discussion

In the present work we continue to exploit the synthesis and AChE inhibition of a series of 3-amidocoumarins (Scheme 1). With the aim to mimic some of the structural functions of already known AChEI (Figure 1), we explored the importance of the nature of different small groups (methyl, methoxy, nitro, hydroxyl, bromo and chloro) attached to the amidophenyl group at position 3. In addition, in order to attest the effects of heteroaryl groups, we incorporated a quinoline moiety in the same position, also with the intent to attain a resemblance with tacrine (Figure 1 – a)).

Our coumarin derivatives **1-10** were efficiently synthesized and characterized (Table 1). We started from the commercially available coumarin-3-carboxylic acid and substituted amines, and we obtained different derivatives in moderate to good yields (52-78 %). Our compounds were tested according the protocol previously reported,[7] and we achieved results in the microMolar range (Table 2).

Table 2 – Activity results of the compounds **1-10**.

Compounds	IC_{50} AChE (μM)	IC_{50} BuChE (μM)
1 	565.93 ± 0.01	N.A.
2 	581.08 ± 0.18	N.A.
3 	N.A.	N.A.
4 	594.54 ± 0.01	N.A.
5 	644.89 ± 0.02	N.A.
6 	N.A.	N.A.
7 	N.A.	N.A.
8 	403.41 ± 0.09	N.A.
9 	284.09 ± 0.13	N.A.
10 	269.91 ± 0.03	N.A.
Tacrine 	0.15 ± 0.01	N.A.

N.A. – not active

All of the tested compounds proved to be inactive against BuChE. Therefore, the compounds achieved one of the goals of the project: the selectivity against AChE. Compound **10** proved to be the most active compound, which leads us to think that further substitution on the coumarin nucleus or the inclusion of the quinoline moiety could improve the activity. The addition of a hydrogen bond acceptor substituent to the *ortho* position seems to affect positively the outcome, as we can see analyzing compound **8** and **9**. The same effect can be seen in compound **10** that has a nitrogen atom in the same area, as an *ortho* substituted compound.

Conclusions

In conclusion, in the present study it was shown that seven out of ten synthesized 3-amidocoumarins presented inhibitory activity against AChE in the microMolar range. A quinoline substituent seems to increase this inhibitory activity, maintaining the desired selectivity. The introduction of halogens ceases the effect, and a substitution of a methyl group by a methoxy one brings little to none advantage.

In general we accomplished to establish a good synthetic route to obtain this kind of compounds in good yields and set the basis of a continuous work towards more active and selective AChEI for the treatment of AD.

Acknowledgements

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