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Study of a series of 8-substitued 7-hydroxy-4-methylcoumarins as AChE and BuChE inhibitors

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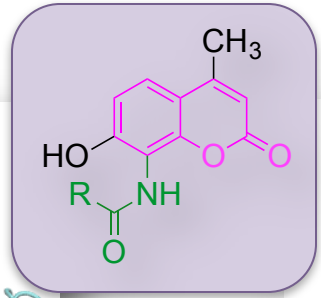
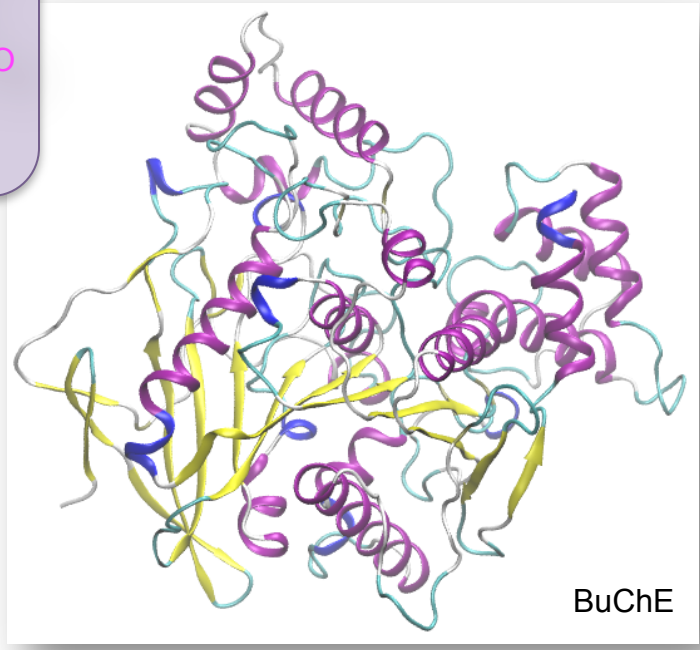




Abstract:

In the current work we studied the interest of a series of 8-substitued 7-hydroxy-4-methylcoumarins as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitors. For the best compounds of the series, the IC_{50} value was determined. This work was based in previous results and is a preliminary screening for further design and synthetize new derivatives as potential compounds that can modulate enzymatic systems involved in the neurodegenerative diseases.

Keywords: 8-Substitued 7-hydroxy-4-methylcoumarins; Acylation reaction; Acetylcholinesterase inhibitors; Butyrylcholinesterase inhibitors; Alzheimer's disease

Introduction

Neurodegenerative disease (ND) is an umbrella term for a range of conditions that primarily affect the neurons in the human brain. ND are still incurable and debilitating conditions that result in progressive degeneration and/or death of nerve cells. Alzheimer's (AD) and Parkinson's (PD) diseases are the most prevalent ND, being considered disorders of multifactorial origin, inevitably progressive and having a long preclinical period. AD is the most prevalent one, causing partially behavioral disturbances related with cholinergic deficiency and is the most common cause of dementia in older adults. This pathology includes symptomatological alterations caused by several factors such as the presence of extracellular β-amyloid plaques and neurofibrillary tangles in the brain. Furthermore, it was proved that acetylcholine (ACh) deficit, which is related to the cholinergic hypothesis, it is involved in this pathology. The decrease of ACh levels is directly connected with AD, and the increasing of cognitive functions improved because of the restoring of the cholinergic neurotransmission.

Introduction

The enzyme responsible for the hydrolysis of ACh into choline and acetic acid is acetylcholinesterase (AChE). This enzyme is also implicated in the amyloid fibril formation. In addition, another cholinesterase (ChE), butyrylcholinesterase (BuChE), is also involved in the cholinergic neurotransmission, but differs from AChE in the kinetics, selectivity and sensitivity to the inhibitors.

Unfortunately, the treatment for AD is still inexistent. However, several cholinergic drugs are used for the treatment of non-severe states of AD, such as tacrine, galantamine, rivastigmine and, more recently, ensaculin (**Figure 1**).

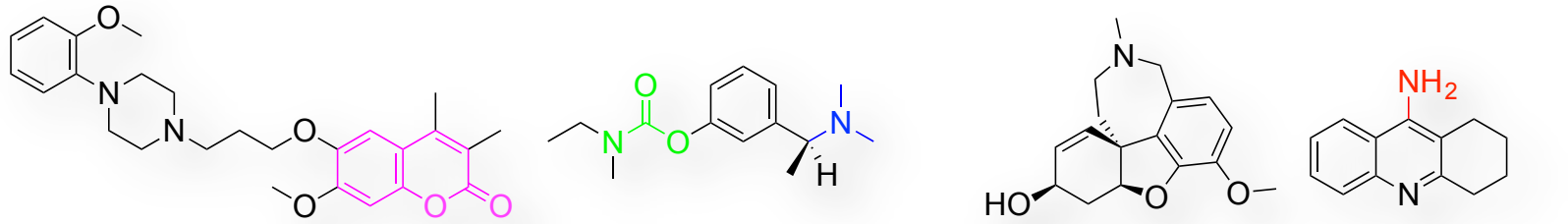


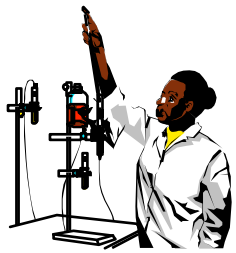
Figure 1. AChE inhibitors chemical structures (some important structural features are highlighted in different colours).

Introduction

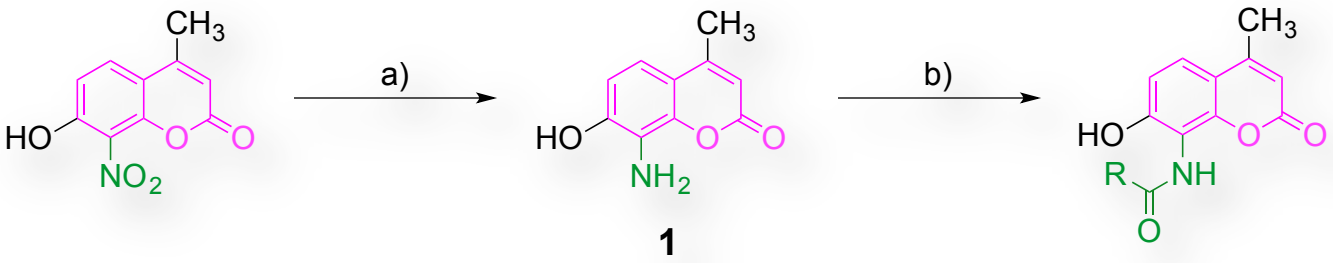
Ensaculin –a coumarin derivative– proved to be helpful to prevent the progressive neurodegeneration. It allowed the increase of ACh levels, helping in the behavioral disturbances and finally, delaying the worsening of symptoms.

In the course of our research, we have synthesized and evaluated series of compounds with very promising activity against some enzymes involved in ND. Some of the studied coumarins, presenting amide groups at position 3, proved to be potent monoamine oxidase (MAO) and/or AChE inhibitors. Inspired by the aforementioned biological significance of the described scaffold, and in order to find more potent, less toxicity and selective compounds against ChE enzymes, in this study, we intended to develop new 8-substitued 7-hydroxy-4-methylcoumarins as AChE and BuChE inhibitors.

Results and discussion – chemistry



With the aim of finding the structural features for the biological activity, we decided to explore the importance of the nature and position of small groups (methyl, hydroxyl, amide, carbamate and urea substituents) into positions 4, 7 and 8 of the coumarin nucleus (**Scheme 1**).



2: R = CH_3
3: R = CH_2CH_3
4: R = $CH(CH_3)_2$
5: R = $N(CH_3)_2$
6: R = $OCH(CH_3)_2$
7: R = OCH_2Cl

Scheme 1. Synthesis of 8-substitued 7-hydroxy-4-methylcoumarins. Reagents and conditions: a) H_2, EtOH, Pd/C, r. t., 3 h (η = 96 %); b) substituted acid chloride, pyridine, DCM, r. t., 3 h (η = 81-92 %).

Results and discussion – biological assays

Table 1. AChE and BuChE inhibitory activity of compounds **1**-**7**.

Compounds	% Inhibition AChE (100 µM)	% Inhibition BuChE (100 µM)	IC_{50} (µM)	
			AChE	BuChE
1	5	28	-	-
2	31	4	-	-
3	2	0	-	-
4	-	-	-	-
5	47	6	58.0	-
6	36	32	194.0	175.0
7	48	-	50.4	-
Galantamine	-	-	0.94	28.29

Results and discussion – enzyme inhibition

In the presence of compound **5**, the Lineweaver–Burk plots (**Figure 2**) showed that this compound was a **mixed-type inhibitor** since increasing the concentration of **5** resulted in a family of straight lines with different slope and intercept, which intersected in the second quadrant. This behavior demonstrated that compound **5** can bind not only with the free enzyme, but also with the enzyme-substrate complex, and their equilibrium constants are different. The equilibrium constants for inhibitor binding with the free enzyme (Ki = 34.8 µM) and with the enzyme-substrate complex (Ki' = 57.3 µM) were obtained from the slope (**Figure 2B**) or the vertical intercept (**Figure 2C**) *versus* inhibitor concentration, respectively.

The plots at different concentrations of compound **7** were linear and parallel, indicating that **7** was a **uncompetitive inhibitor** (**Figure 3**). Ki value of 20.8 µM was calculated from the replot of intercepts (1/Vmax) *versus* concentration of compound **7**.

Results and discussion – enzyme inhibition

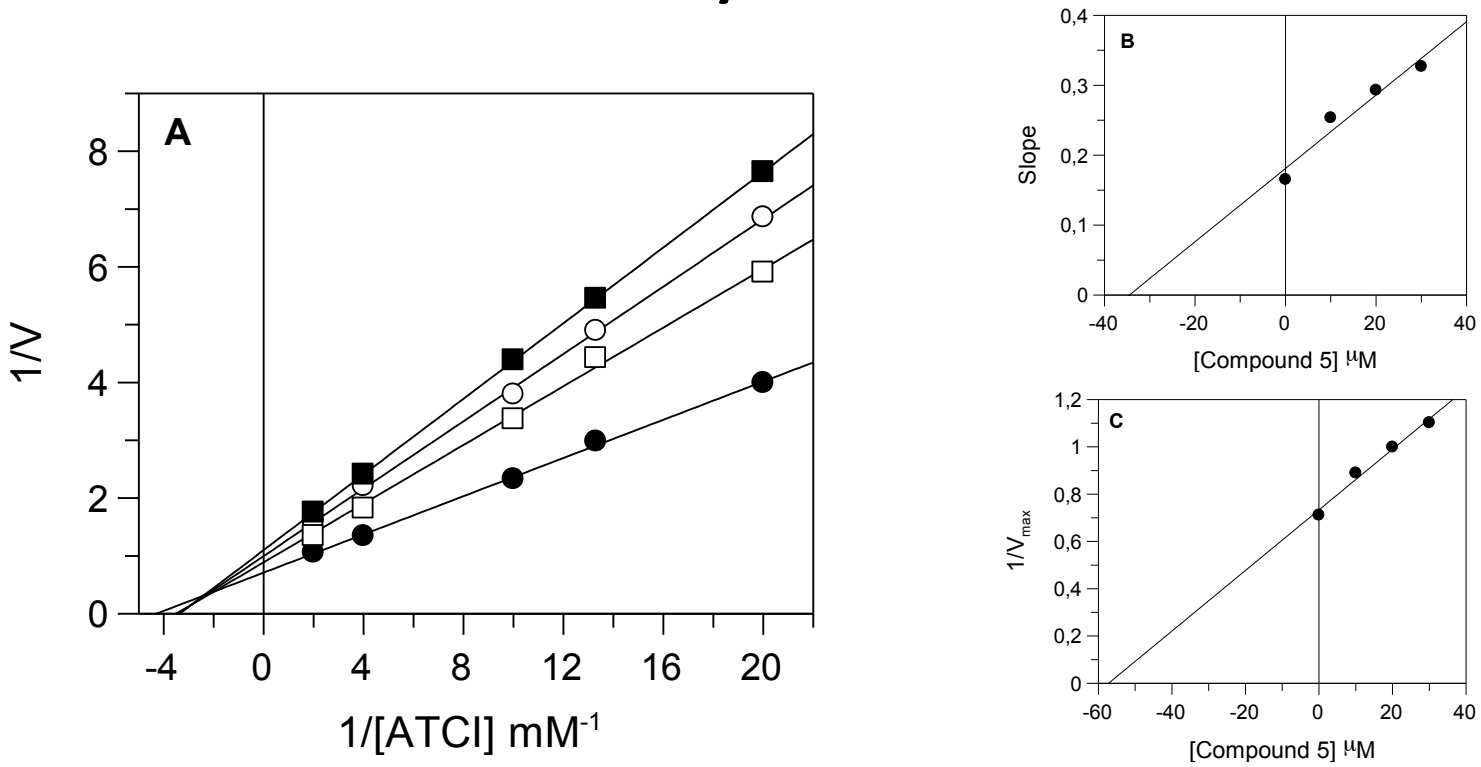


Figure 2. A) Lineweaver–Burk plots for the inhibition of compound **5** on AChE using acetylthiocholine iodide (ATCI) as substrate. The concentrations of inhibitor were 0 (●), 10 (□), 20 (○) and 30 (■) µM. **B**) The secondary plot of slope (Km/Vmax) *versus* concentration of compound **5**, to determine the inhibition constant (Ki). **C**) The secondary plot of 1/Vmax *versus* concentration of compound **5**, to determine the inhibition constant (Ki').

Results and discussion – enzyme inhibition

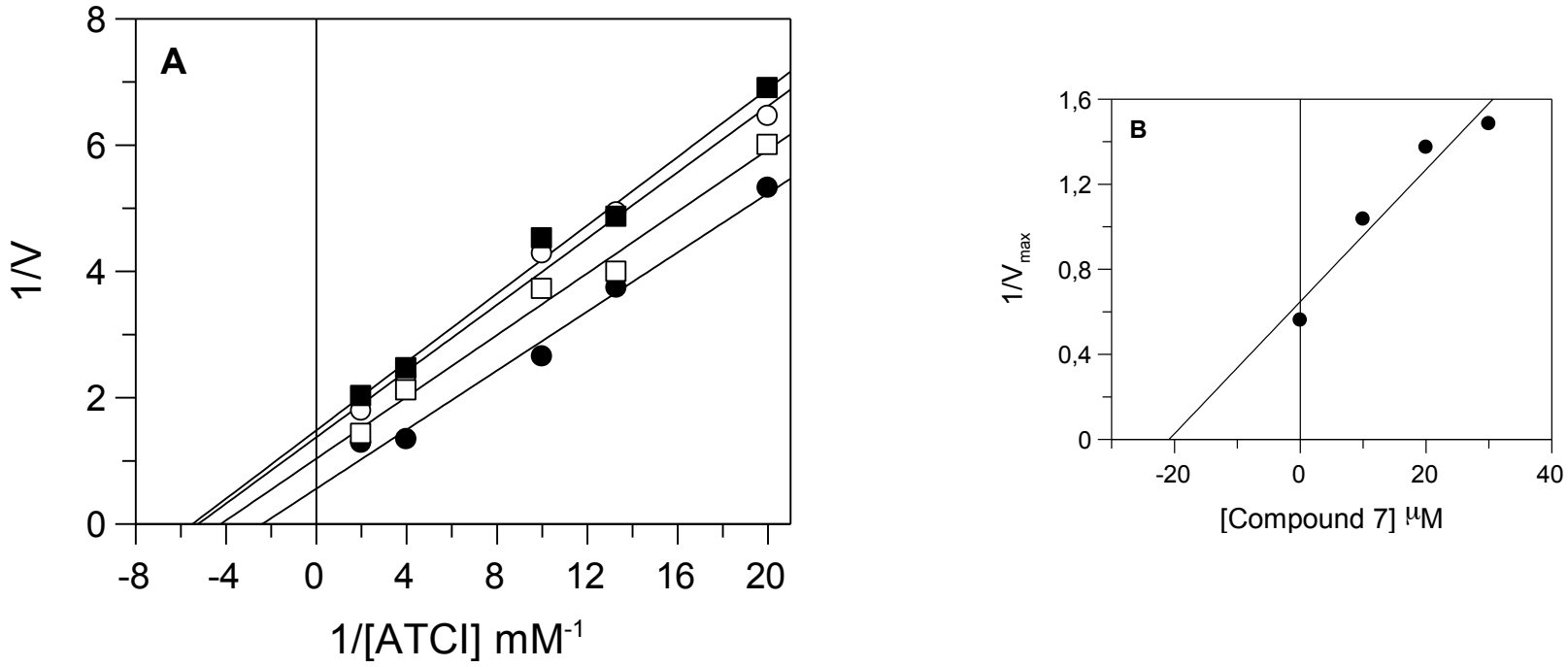


Figure 3. A) Lineweaver–Burk plots for the inhibition of compound **7** on AChE using acetylthiocholine iodide (ATCI) as substrate. The concentrations of inhibitor were 0 (●), 10 (□), 20 (○) and 30 (■) μM. **B**) The secondary plot of 1/Vmax *versus* concentration of compound **7**, to determine the inhibition constant (Ki).

Results and discussion - SAR

In general, the studied 8-substitued 7-hydroxy-4-methylcoumarins presented no significant inhibitory activity against AChE and BuChE (**Table 1**). Compounds **5**-**7** –the urea (**5**) and carbamates (**6** and **7**) derivatives– proved to be the best candidates of the series. Therefore, the IC_{50} values of these compounds were calculated. The best compound of the entire series proved to be compound **7**, chloromethyl-(7-hydroxy-4-methylcoumarin-8-yl)carbamate, with and IC_{50} against AChE of 50.4 µM. Compound **5**, 3-(7-hydroxy-4-methylcoumarin-8-yl)-1,1-dimethylurea, has a similar IC_{50} value (IC_{50} AChE = 58 µM) to compound **7**. Compound **6**, isopropyl(7-hydroxy-4-methylcoumarin-8-yl)carbamate, was active against both ChE (IC_{50} AChE = 194 µM and IC_{50} BuChE = 175 µM). In spite of being less active, this could be also an interesting candidate due to the dual activity.

Experimental methodologies - chemistry

General methodology to prepare the 8-amino-7-hydroxy-4-methylcoumarin (1): the commercially available 7-hydroxy-4-methyl-8-nitrocoumarin (2.5 mmol) was dissolved in ethanol and a catalytic amount of Pd/C was added to the mixture. The solution was stirred, at room temperature, under H_2 atmosphere, for 3 hours. After the completion of the reaction, the mixture was filtered to eliminate the catalyst. The obtained crude was then purified by flash chromatography (hexane/ethyl acetate 9:1) to give the desired coumarins.

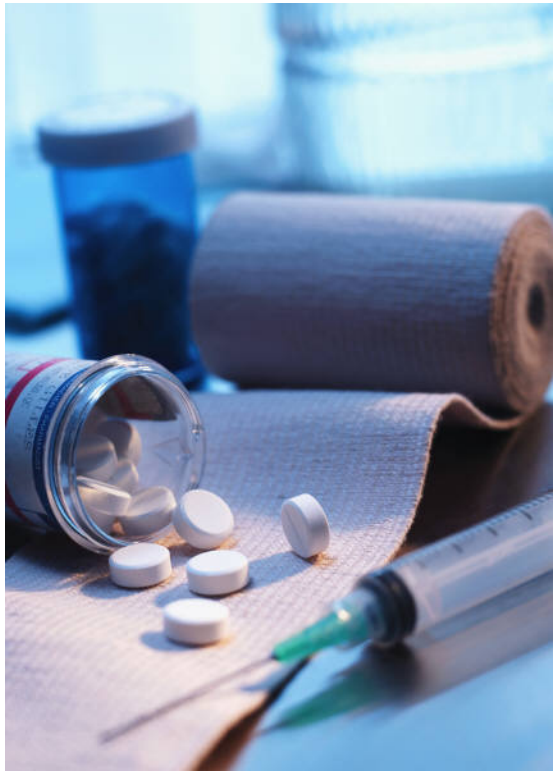
General methodology to prepare 8-substitued 7-hydroxy-4-methylcoumarins (2-7): To a solution of 8-amino-7-hydroxy-4-methylcoumarin (**1**, 1 mmol) and pyridine (1.1 mmol) in dichloromethane (9 mL), the corresponding acid chloride (1.1 mmol) was added dropwise and the reaction was stirred, at room temperature, for 3 hours. The solvent was evaporated under vacuum and the dry residue was purified by FC (hexane/ethyl acetate 9:1) to give the desired coumarins.

Experimental methodologies – biological assays

AChE activity was measured spectrophotometrically using Ellman's reagent according to the method previously reported by the authors. Briefly, the reaction mixture contained 0.1 M phosphate buffer (pH 8.0), 1.5 mM 5,5'-dithiobis(2-nitrobenzoic acid), enzyme and inhibitor dissolved in DMSO at the desired concentrations or only DMSO (control) in a final volume of 1mL. Finally, acetylthiocholine iodide (1.5 mM) was added to the reaction mixture and the absorbance immediately monitored at 405 nm. For the BuChE assay, the same procedure described above was followed, except for the use of enzyme and substrate, which were BuChE from equine serum and *S*-butyrylthiocholine chloride, respectively. Galantamine was used as standard ChE inhibitor. IC_{50} values were calculated as concentration of compounds that produced 50 % of ChE activity inhibition.

Conclusions

Compounds **5** and **7** proved to be the best AChE inhibitors of the studied series. Compound **6** is also an interesting candidate, due to the dual AChE/ BuChE activity. Compound **5** proved to be a mixed-type inhibitor, while compound **7** proved to be a uncompetitive inhibitor. Therefore, they could be the inspiration for the design of further more potent and less toxic AChE and BuChE inhibitors.



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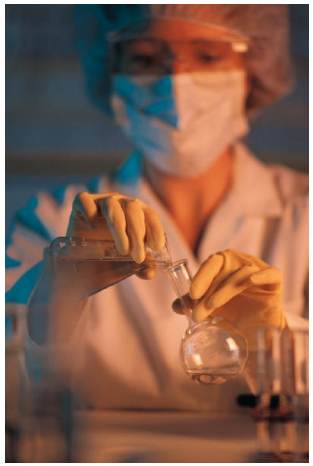

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