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Original article

Novel coumarin-3-carboxamides bearing *N*-benzylpiperidine moiety as potent acetylcholinesterase inhibitors

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ABSTRACT

Some novel coumarin-3-carboxamide derivatives linked to *N*-benzylpiperidine scaffold were synthesized and evaluated as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitors. The screening results showed that most of compounds exhibited potent anti-AChE activity in the range of nM concentrations. Among them, compound **10c** bearing an *N*-ethylcarboxamide linker and a 6-nitro substituent showed the most potent activity (IC_{50} = 0.3 nM) and the highest selectivity (SI = 26,300). Compound **10c** was 46-fold more potent than standard drug donepezil against AChE. The kinetic study revealed that compound **10c** exhibited mixed-type inhibition against AChE. Protein-ligand docking study demonstrated that the target compounds have dual binding site interaction mode and these results are in agreement with kinetic study.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease with symptoms of memory loss, cognition defect and behavioral impairment [1]. AD is mainly characterized by the pre-synaptic decrease of acetylcholine (ACh) due to damage of cholinergic neurons in some especial parts of the brain such as hippocampus and cortex (cholinergic hypothesis) [2,3]. Concerning the cholinergic hypothesis one of the rational and effective approaches to treat the AD's symptoms, is raising the ACh through inhibition of acetylcholinesterase (AChE) that is responsible for hydrolysis of ACh in pre-synaptic areas [4]. Moreover, it has been recently reported that dual inhibition of AChE and butyrylcholinesterase (BuChE) might improve the signs of AD and symptoms owing to the key role of BuChE in hydrolysis of ACh. Indeed in damaged brain, preserving the AChE/BuChE activity ratios is essential for successful treatment of AD [5,6]. Based on these findings, several AChE inhibitors comprising different chemical scaffolds such as donepezil [7], galantamine [8] and ensaculin [9] were synthesized and used clinically to prevent progression of AD in early stages (Fig. 1).

Previously, due to the straightforward functionalization of coumarin ring, several coumarin derivatives have been prepared and evaluated as AChE inhibitors [10]. It has been observed that coumarins such as AP2238 (Fig. 1) encompassing substitution at position 3 or 4 have shown increased activity against AChE rather than 6 or 7 substituted coumarins [10].

Regarding the X-ray crystallographic structure of AChE (PDB ID: 1EVE), three main binding sites are determined: the catalytic triad at the bottom of active site including Ser200, His440, and Glu327, the catalytic anionic site (CAS) at the vicinity of the catalytic triad

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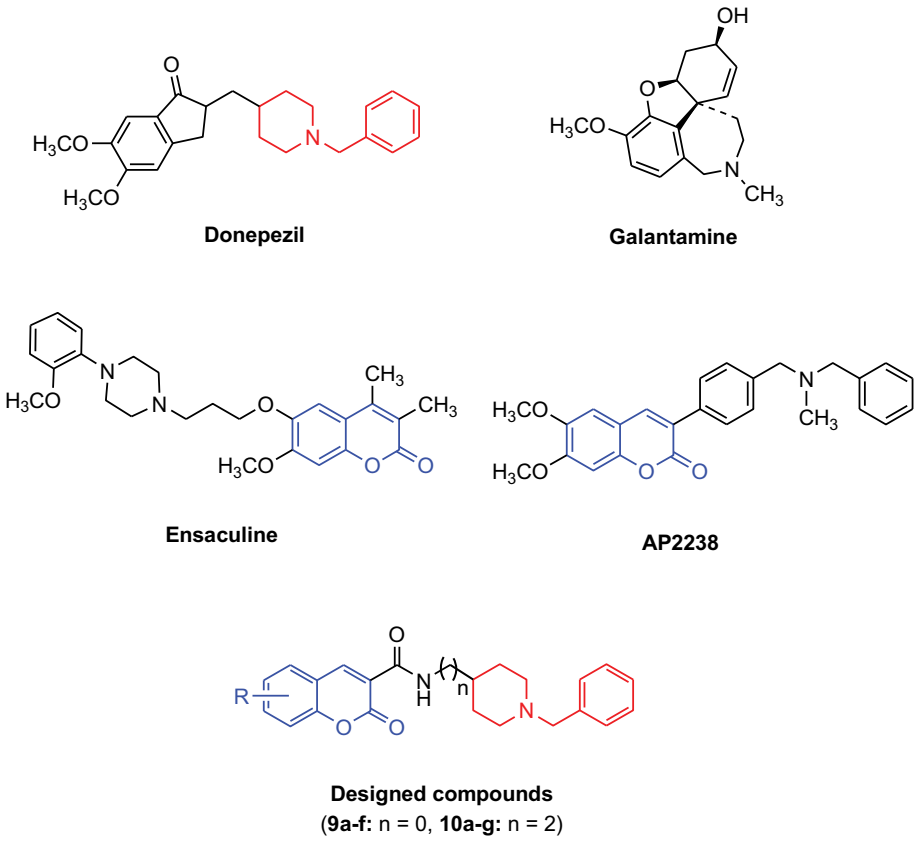


Fig. 1. Structures of well-known cholinesterase inhibitors and designed compounds.

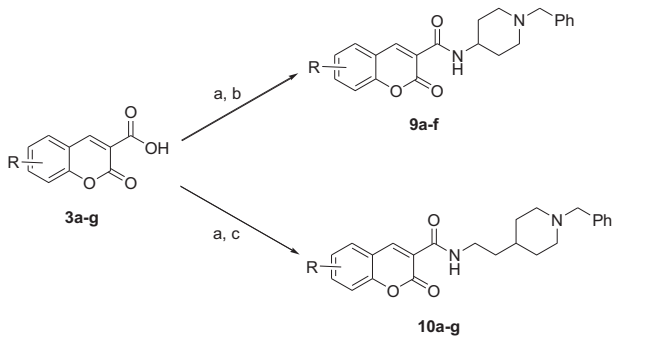
consisting of Trp84, Tyr130, Gly199, His441, His444 and peripheral anionic site (PAS) at the gorge rim comprising Tyr70, Asp72, Tyr121, Trp279 and Tyr334. Furthermore, the key residue Phe330 in the midgorge is also involved in the recognition of ligands [11,12]. It was proved that dual binding site (DBS) AChE inhibitors were more potent inhibitors compared to the compounds that interact with only one site of the enzyme [13]. Several studies have shown that coumarin moiety can bind primarily to PAS of AChE [10]. Accordingly, different groups such as benzylamino, phenylpiperazine, and aniline, which interact with the catalytic site, have been successfully connected to the coumarin scaffold using different spacers to obtain dual binding site inhibitors [14]. In continuation of our previous work on coumarin AChE inhibitors [15], a novel series of coumarins have been designed through hybridization approach in which *N*-benzylpiperidine moiety of donepezil as a CAS binder and the coumarin scaffold as PAS binding core were connected together with amide linker (Fig. 1). Herein, the synthesis, biological evaluation and *in silico* studies of some substituted coumarins **9a**–**f** and **10a**–**g** as novel dual binding site inhibitors of AChE are reported.

2. Results and discussion

2.1. Chemistry

The synthetic routes from key intermediates **3a**–**g** and **8a**,**b** to target compounds **9a**–**f** and **10a**–**g** have been shown in Scheme 1. Initially, several ethyl coumarin-3-carboxylate derivatives **2a**–**g** were synthesized using commercial 2-hydroxybenzaldehydes **1a**–**g** and diethylmalonate in the presence of catalytic amount of piperidine which was then hydrolyzed with aqueous solution of sodium hydroxide to furnish the corresponding coumarin-3-carboxylic acid **3a**–**g** (Supplementary material) [16]. The amine intermediate 2-(1-benzylpiperidin-4-yl)ethanamine (**8b**) was prepared from commercially available 1-benzyl-4-piperidone (**4**) and malonitrile in 60% yield over four steps [17]. Thus, simple condensation of malonitrile with compound **4** provided 2-(1-benzylpiperidin-4-ylidene)malononitrile (**5**). The latter compound was then converted to ethyl cyanoacetate **6** through reduction and simultaneous formation of esteric bond. Then, compound **8b** can be prepared from hydrolysis and decarboxylation of compound **6** followed by reduction with $LiAlH_4$ (Supplementary material).

Finally, coumarin-3-carboxylic acids **3a**–**g**, were chlorinated using thionyl chloride to give the appropriate coumarin-3-carbonyl chloride and then coupled with 1-benzylpiperidin-4-amine **8a** or 2-(1-benzylpiperidin-4-yl)ethanamine **8b** to yield corresponding amides **9** or **10** in moderate yields, respectively (Scheme 1). Activation of the



Scheme 1. Synthesis of target compounds **9** and **10**. Reagents and conditions: (a) thionyl chloride, reflux; (b) 1-benzylpiperidin-4-amine (**8a**), K_2CO_3, toluene, reflux; (c) 2-(1-benzylpiperidin-4-yl) ethanamine (**8b**), K_2CO_3, toluene, reflux.