Bioorganic & Medicinal Chemistry 24 (2016) 1528–1539

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of novel donepezil–coumarin hybrids as multi-target agents for the treatment of Alzheimer's disease

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ARTICLE INFO

Article history:
Received 6 November 2015
Revised 17 February 2016
Accepted 18 February 2016
Available online 20 February 2016

Keywords:
Alzheimer's disease
Coumarin
Donepezil
Cholinesterase
Monoamine oxidase
Docking

ABSTRACT

Combining *N*-benzylpiperidine moiety of donepezil and coumarin into in a single molecule, novel hybrids with ChE and MAO-B inhibitory activity were designed and synthesized. The biological screening results indicated that most of compounds displayed potent inhibitory activity for AChE and BuChE, and clearly selective inhibition to MAO-B. Of these compounds, **5m** was the most potent inhibitor for eeAChE and eqBuChE (0.87 μM and 0.93 μM, respectively), and it was also a good and balanced inhibitor to hChEs and hMAO-B (1.37 μM for hAChE; 1.98 μM for hBuChE; 2.62 μM for hMAO-B). Molecular modeling and kinetic studies revealed that **5m** was a mixed-type inhibitor, which bond simultaneously to CAS, PAS and mid-gorge site of AChE, and it was also a competitive inhibitor, which occupied the active site of MAO-B. In addition, **5m** showed good ability to cross the BBB and had no toxicity on SH-SY5Y neuroblastoma cells. Collectively, all these results suggested that **5m** might be a promising multi-target lead candidate worthy of further pursuit.

1. Introduction

Alzheimer's disease (AD) is the most common type of dementia clinically characterized by progressive loss of memory and deficits in different cognitive domains.[1,2] It has been estimated that there were 36 million people with dementia in 2010, and this number will rise to 42.3 million in 2020 and 81.1 million by 2040.[3,4] Although several factors including low levels of acetylcholine (ACh), the formation of β-amyloid deposits, oxidative stress and dyshomeostasis of biometals have been demonstrated to play significant roles in the pathogenesis of AD, the exact etiology is still not fully understood.[5,6]

The current strategies for AD treatment are mainly based on cholinergic hypothesis, which suggests that a decline of ACh levels in specific brain regions leads to cognitive and memory deficits, and sustaining or recovering cholinergic function can alleviate these symptoms.[7,8] Supporting this notion that four cholinesterase inhibitors (ChEIs), tacrine, rivastigmine, galanthamine and donepezil, have been approved in clinical use.[9] However, due to the complex nature of AD, these drugs can only reverse the symptoms for a short period of time instead of halting or curing the neurodegeneration.[10] Thus, a more appropriate approach termed the Multi-Target Directed Ligands (MTDLs) strategy has been proposed to face this disease.[11–13] MTDLs mean a single compound that can simultaneously modulate different targets involved in the neurodegenerative AD cascade.[14,15] One of the most widely adopted approaches for designing MTDLs is to modify a ChEI and render it exert other biological properties useful for treating AD.[16]

MAOs are flavin adenine dinucleotide (FAD)-containing enzymes responsible for the oxidative deamination of endogenous monoamine neurotransmitters, trace amines, and a number of amine xenobiotics.[17,18] Two isoforms, namely MAO-A and -B, have been identified based on substrate selectivity and inhibitor sensitivity.[19] MAO-A preferentially deaminates serotonin, adrenaline and noradrenaline, and is selectively and irreversibly inhibited by clorgyline, while MAO-B preferentially deaminates β-phenylethylamine and benzylamine, and is irreversibly inhibited by *R*-(−)-deprenyl.[20,21] It was found that MAO-B activity increases with age. Especially in AD, a significant rise in MAO-B activity was found in cerebral spinal fluid (CSF), brain tissue as well as in

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http://dx.doi.org/10.1016/j.bmc.2016.02.023
0968-0896/

platelets.[22,23] The high levels of MAO-B in neuronal tissue could lead to an increase in the level of H_2O_2 and oxidative free radicals, which ultimately contribute to the etiology of AD.[24] Thus, selective inhibition of MAO-B becomes another valuable approach for the treatment of AD.

In recent years, many MTDLs have been designed and synthesized by combining the ChEI and MAO-B inhibitor into one molecule.[25–33] Because studies suggest that simultaneous inhibition of MAO-B and ChE can not only improve the level of ACh and reduce oxidative stress in brain but also can decrease β-amyloid deposition, another hallmark in AD pathogenesis, which will be more effective against AD.[34] Among all these MTDLs, TV2236 (Ladostigil) has been approved for phase IIb clinical trial, which prompt us to search new multi-target compounds with ChE and MAO-B inhibitory activity.

Very recently, we have reported a series of tacrine-coumarin hybrids as multi-target compounds for treatment of AD (Fig. 1).[35] The tacrine was used to inhibit ChEs, and the coumarin moiety was chosen to inhibit MAOs. Among these compounds, compound **1** displayed potent inhibitory activity toward AChE and BuChE, and clearly selective inhibition for MAO-B. However, due to the presence of tacrine moiety,[36] this compound showed severe hepatotoxicity (unpublished work), which hindered its further application.

In order to continue our work to discovery new multi-target compounds with both ChE and MAO-B inhibitory activity and to optimize the tacrine-coumarin hybrids, in this study, we wanted to replace the tacrine with a *N*-benzylpiperidine moiety based on donepezil to design a series of novel donepezil–coumarin hybrids as multi-target compounds for the treatment of AD. Compared to tacrine, donepezil was more potent and had no hepatotoxicity.[37,38] The *N*-benzylpiperidine moiety, like tacrine, could inhibit the ChEs through binding to the catalytic anionic site (CAS) of ChEs. The design strategy of new compounds is shown in Figure 1. Similar to our previous design, the flexible alkyl chain was retained to connect coumarin and *N*-benzylpiperidine moiety, because such linker could be lodged by the AChE cavity, allowing the hybrids simultaneously to interact with PAS and CAS of the enzyme.[39,40] Meanwhile, the position of the linker tethered to coumarin was unchanged in order to better evaluate the effect of this replacement design. In addition, to find the optimal length for ChE inhibition, the linker length was varied in initial step. Once the optimal length was obtained, different substituents were introduced to 3- and/or 4-position of coumarin ring to investigate the possible effects on both ChE and MAO inhibition. All designed compounds were synthesized and evaluated for their ability to inhibit ChEs and MAOs. The kinetic and molecular modeling studies were carried out to investigate interaction mechanism of selected compounds with AChE and MAO-B. The blood–brain barrier (BBB) permeation and the in vitro SH-SY5Y neuroblastoma cell toxicity assays were also performed to test the preliminary drug-like properties of selected compounds. Herein, we report the design, synthesis and evaluation of a series of novel donepezil–couamrin hybrids as multi-target compounds for AD treatment.

2. Result and discussion

2.1. Chemistry

The synthesis of the designed compounds **5a**–**m** is illustrated in Scheme1. The coumarin derivatives **2a**–**k** were obtained according to the previous reported methods.[28,29,35,41–43] Reacting the compounds **2a**–**k** with the corresponding α,ω-dibromoalkanes afforded the key intermediates **3a**–**l**, which was then treated with commercially available compounds **4a**–**b** in the presence of potassium carbonate in acetonitrile to give the target compounds **5a**–**m**.

For compounds **9a**–**b**, they were easily synthesized by the route shown in Scheme 2. Condensation of *m*-cresol with ethyl acetoacetate in the presence of a catalytic amount of concentrated sulfuric acid in 1,4-dioxane provided compound **7**. The obtained **7** was selectively brominated at the 7-methyl group by NBS in dry benzene to give compound **8**.[44] Finally, using same condition for preparation of compounds **5a**–**m**, compound **8** was reacted with **4a**–**b** to furnish the desired compounds **9a**–**b**.

2.2. In vitro inhibition of ChEs

The inhibitory activities of the test compounds **5a**–**m** and **9a**–**b** against eeAChE (from electric eel) and eqBuChE (from equine serum) were determined according to the spectrophotometric method described by Ellman et al.[45] For comparison purpose, tacrine and donepezil were used as reference compounds. The IC_{50} values of all test compounds and their selectivity index for

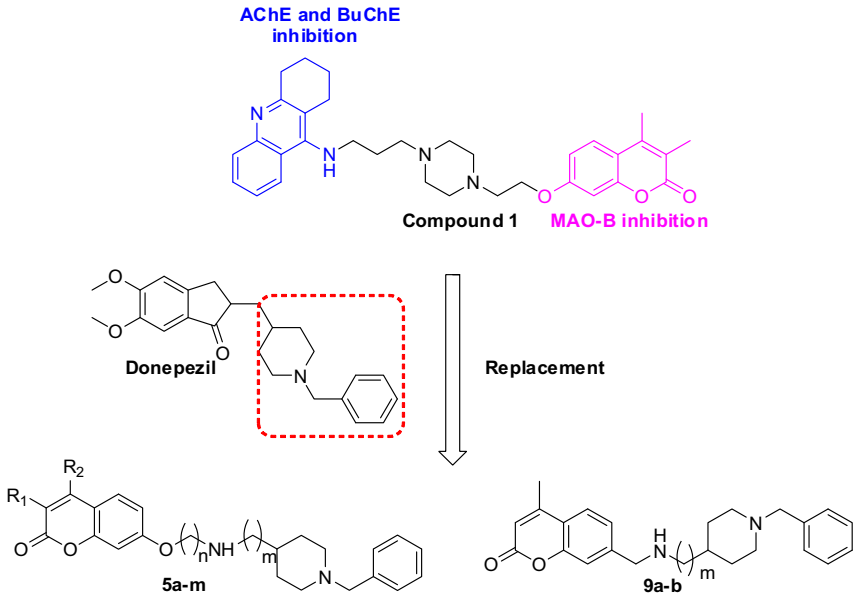

Figure 1. Design strategy for donepezil–coumarin hybrids.