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Original article

Multi-target tacrine-coumarin hybrids: Cholinesterase and monoamine oxidase B inhibition properties against Alzheimer's disease

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ABSTRACT

A series of novel tacrine-coumarin hybrids were designed, synthesized and evaluated as multi-target agents against Alzheimer's disease. The biological assays indicated that most of compounds displayed potent inhibitory activity toward AChE and BuChE, and clearly selective inhibition for MAO-B. Among these compounds, **14c** exhibited strong inhibitory activity for AChE (IC_{50} values of 33.63 nM for *ee*AChE and 16.11 nM for hAChE) and BuChE (IC_{50} values of 80.72 nM for *eq*BuChE and 112.72 nM for hBuChE), and the highest inhibitory activity against hMAO-B (IC_{50} value of 0.24 μM). Kinetic and molecular modeling studies revealed that **14c** was a mixed-type inhibitor, binding simultaneously to catalytic, peripheral and mid-gorge sites of AChE. It was also a competitive inhibitor, which covered the substrate and entrance cavities of MAO-B. Moreover, **14c** could penetrate the CNS and show low cell toxicity. Overall, these results suggested that **14c** might be an excellent multi-target agent for AD treatment.

1. Introduction

Alzheimer's disease (AD) is the most common form of adult onset dementia characterized by progressive loss of learning, memory and other cognitive functions [1]. Although the exact etiology of AD is not completely known, several factors including τ-protein hyperphosphorylation, oxidative stress, amyloid-β (Aβ) deposits and deficit of acetylcholine (ACh) seem to play important roles in the pathophysiology of the disease [2,3]. Despite considerable efforts made in this field, developing an efficient strategy for AD treatment is still needed.

In the past decades, the main strategy for the treatment of AD is to evaluate the level of ACh based on cholinergic hypothesis [4]. This hypothesis suggests that the memory and cognitive decline of AD result from a deficit of ACh in specific brain regions, and inhibiting the cholinesterase (ChE) responsible for the hydrolysis of ACh can alleviate these symptoms [5,6]. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are the two types of ChEs. The AChE consists of two binding sites connected by a nearly 20 Å deep narrow gorge. One is catalytic anionic site (CAS) at the bottom of the gorge and the other is peripheral anionic site (PAS) near the entrance [7,8]. Apart from the function of hydrolysis of ACh, AChE can also induce Aβ aggregation (another hallmark of AD) through the direct interaction of its PAS with Aβ peptide [9–11]. Thus, dual-site inhibitors that interact with both CAS and PAS appear to be more beneficial for AD treatment. Moreover, in healthy brains, AChE hydrolyzes the major ACh while BuChE only play a secondary role. However, in case of AD, the activity of AChE gradually decreases, while that of BuChE significantly increases. In the later stage of the disease, BuChE becomes more important in regulating the ACh level in cholinergic neurons [12,13]. Therefore, both AChE and BuChE are valuable therapeutic targets for the treatment of AD [14].

In addition to the ChEs, monoamine oxidases (MAOs) also receive increasing attention in recent years due to their roles in the treatment of AD. MAOs are flavin adenine dinucleotide (FAD) containing enzymes, which exist as two different isoforms: MAO-A and MAO-B. They were located in the mitochondrial outer membrane of glial, neuronal and other cells, and have been classified

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based on inhibitor sensitivity and substrate specificity [15–17]. Selective inhibitors for MAO-A are used as antidepressants, whereas selective MAO-B inhibitors can be used to treat the neurodegenerative disorders such as AD [18]. MAO-B increases with age and its activity is found elevated in AD patients, leading to an enhanced metabolism of dopamine and to the production of large amount of hydrogen peroxide, which ultimately give rise to neuronal damage [19]. Inhibition of MAO-B can improve the symptoms of AD, and several selective MAO-B inhibitors like selegiline and rasagiline have been demonstrated to retard the further neurodegeneration in AD [20,21]. Therefore, selective inhibition of MAO-B provides another therapeutic approach in treating AD.

Drugs for AD treatment are mainly ChEIs including tacrine, donepezil, galanthamine and rivastigmine [22]. However, these drugs can only provide a temporary symptom alleviation instead of halting or slowing the progression of neurodegeneration [23]. Given that the etiology of AD is complex and the ChEs may not be the only factor resulting in AD, a more appropriate therapeutic strategy based on the 'multi-target-directed ligands' (MTDLs) paradigm has recently been proposed and applied to design multi-target compounds with the activity not just ChE inhibition [24–26]. Considering the importance of MAO-B in AD, multi-target compounds with simultaneous inhibition of ChEs and MAO-B may provide more benefits for the AD treatment. Based on this concept, a series of multi-target compounds possessing inhibition of ChEs and MAO-B have been designed and synthesized recently [19,27–30]. Among them, the compound TV3326 (Ladostigil) was approved for phase IIb clinical trial [31], which encourage us to search new multi-target compounds with ChE and MAO-B inhibitory activity.

Tacrine was the first ChE inhibitor launched on the market for the treatment of AD. Although clinical use of this compound was limited due to the serious hepatotoxicity, the high potency in ChE inhibition and low molecular weight suitable for modification still make it a widely used scaffold for developing multi-target agents [32–34]. A plethora of multi-target molecules designed by connecting tacrine with another fragment having additional activities have been developed, such as tacrine-4-oxo-4*H*-chromene, tacrine–rhein and tacrine-8-hydroxyquinoline hybrids (Fig. 1) [10,13,35]. Among these compounds, most of them exhibit enhanced the biological profile than tacrine, and several compounds can even conquer its side effects [36–39].

Coumarins are an important class of natural occurring compounds, which widely exist in various plant species. In recent years, they have attracted much attention due to a variety of biological activities related to neurological disorders [40,41]. Some natural and synthetic coumarin derivatives have been characterized as MAO inhibitors [42,43]. Especially, 7-substituted coumarin with substituents at position 3 and/or 4 can significantly improve inhibitory activity and selectivity toward MAO-B [44,45]. For example, compound LU 53439 exhibited low nanomolar inhibitory activity toward MAO-B (Fig. 1, $IC_{50} = 0.9$ nM) [46]. In addition, the studies also indicate that coumarin can interact with the PAS of AChE and many compounds containing couamrin moiety exert AChE inhibitory activity, such as AP2238 (Fig. 1) [47,48].

Given the activities of tacrine and coumarin, and in an attempt to obtain new multi-target molecules with both ChE and MAO-B inhibitory activity for the AD treatment, in this study, a series of novel compounds by hybridization of tacrine with coumarin have been designed and synthesized. The tacrine was chosen to inhibit the ChEs through binding to CAS, and the coumarin scaffold was used for MAO-B inhibition. Besides, due to the aromatic character of the coumarin, it might have potential interaction with the PAS of AChE. The piperazine side-armed alkane spacers of different lengths were considered to connect these two heterocyclic fragments. Because our previous work showed that such linkers could be lodged in the narrow enzymatic cavity, allowing the compounds simultaneously binding to the PAS and CAS of AChE and could also give additional contribution to AChE inhibition by binding to mid-gorge site of the enzyme [32]. All designed compounds were synthesized and evaluated for their abilities to inhibit ChEs, MAOs and to penetrate the blood–brain barrier (BBB). Moreover, kinetic and molecular modeling studies were also carried out to investigate interaction mechanism of selected compound with AChE and MAO-B. Herein, we report the design, synthesis and evaluation of a series of novel tacrine-coumarin hybrids as multi-target agents for AD treatment.

2. Result and discussion

2.1. Synthesis of tacrine-coumarin hybrids and in vitro inhibition of ChEs

To get the optimal linker length between coumarin and tacrine scaffolds for ChE inhibition, compounds **9a**–**j** with varied linker length (m + n = 4–9) and no substituents at coumarin moiety were synthesized (Scheme 1). The methods for preparation of intermediates **3a**–**e** and **8a**–**b** have been described in our previous reports [32,35]. Reacting **3a**–**e** with corresponding **8a** or **8b** in the presence of potassium carbonate in acetonitrile afforded compounds **9a**–**j**. After obtaining these compounds, their inhibitory activities against *ee*AChE and *eq*BuChE were determined according

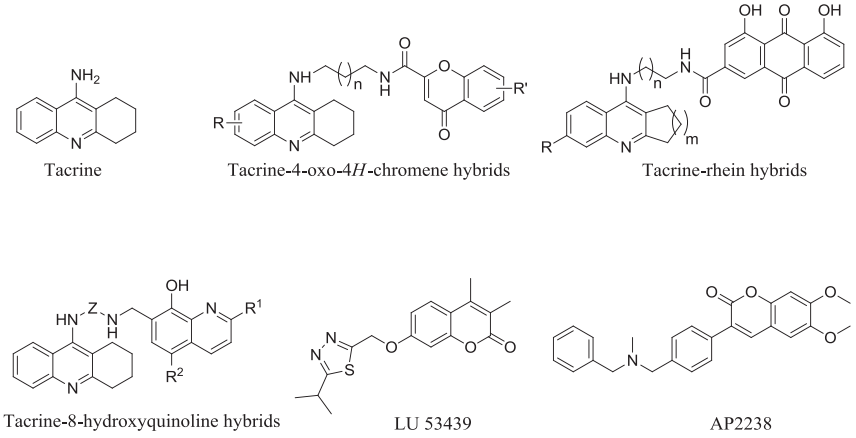

Fig. 1. Structures of tacrine, tacrine-4-oxo-4*H*-chromene hybrids, tacrine-rhein hybrids, tacrine-8-hydroxyquinoline hybrids, LU 53439 and AP2238.