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Multifunctional tacrine–flavonoid hybrids with cholinergic, β-amyloid-reducing, and metal chelating properties for the treatment of Alzheimer's disease

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ABSTRACT

A new series of tacrine–flavonoid hybrids (**13a**–**u**) had been designed, synthesized, and evaluated as multifunctional cholinesterase (ChE) inhibitors against Alzheimer's disease (AD). In vitro studies showed that most of the molecules exhibited a significant ability to inhibit ChE and self-induced amyloid-β ($A\beta_{1-42}$) aggregation. Kinetic and molecular modeling studies also indicated compounds were mixed-type inhibitors, binding simultaneously to active, peripheral and mid-gorge sites of AChE. Particularly, compound **13k** was found to be highly potent and showed a balanced inhibitory profile against ChE and self-induced $A\beta_{1-42}$ aggregation. Moreover, it also showed excellent metal chelating property and low cell toxicity. These results suggested that **13k** might be an excellent multifunctional agent for AD treatment.

1. Introduction

Alzheimer's disease (AD), the most common form of adult onset dementia, is a complex neurodegenerative process occurring in the central nervous system (CNS), characterized by progressive cognitive decline and memory loss [1,2]. Although the etiology of AD is not fully known at present, several factors are considered to play significant roles in the pathophysiology of AD. These include the deposits of aberrant proteins namely β-amyloid (Aβ) and τ-protein, oxidative stress, dyshomeostasis of biometals, and low levels of acetylcholine (ACh) [3,4].

Current clinical therapy for AD patients is mainly based on the cholinergic hypothesis [5,6]. It suggests that, the decline of ACh level leads to cognitive and memory deficits, and sustaining or recovering the cholinergic function is supposed to be clinically beneficial. ACh can be degraded by two types of cholinesterases, namely AChE and butyrylcholinesterase (BuChE) [7]. The crystallographic structure of AChE reveals that it has a narrow 20 Å gorge, containing two binding sites: the catalytic active site (CAS) at the bottom and the peripheral anionic site (PAS) near the entrance of the gorge [8–10]. Hence, inhibitors that bind to either one or two sites could inhibit the AChE. However, current studies indicated that AChE could promote amyloid fibril formation by interaction through the PAS of AChE, giving stable AChE–Aβ complex, which are more toxic than single Aβ peptides alone [11]. For this reason, the dual binding inhibitors, which target both PAS and CAS, have become more promising in AD treatment [12,13]; since they will not only alleviate the cognitive deficit of AD patients by elevating ACh levels but also act as disease-modifying agents delaying amyloid plaque formation [14]. In normal brain, AChE is more active than BuChE and hydrolyzes about 80% of ACh. However, as AD progresses, the activity of AChE decreases, while that of BuChE significantly rises becoming a modulator to regulate ACh levels in cholinergic neurons [15]. Consequently, both enzymes are useful therapeutic targets for AD.

Converging lines of evidence suggest that progressive cerebral deposition of Aβ also plays an important role in the pathogenesis and development of AD, as its accumulation may result in a cascade

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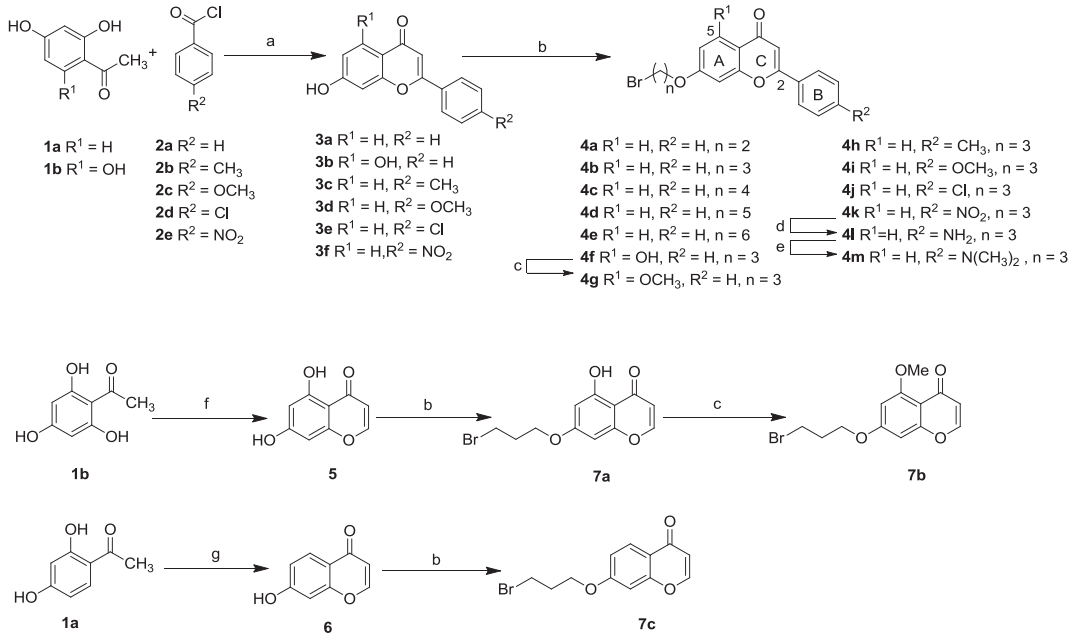

Scheme 1. Synthesis of intermediates **4a**–**m**, **7a**–**c**. Reagents and conditions: (a) LiHMDS, THF, −78 °C to r.t., 4 h; (b) $Br(CH_2)_nBr$ ($n = 2$–6), anhydrous K_2CO_3, acetone, reflux, 4 h; (c) MeI, anhydrous K_2CO_3, acetone, reflux, 2 h; (d) $SnCl_2$, EtOH, reflux, 1 h; (e) $(CH_2O)_n$, $NaCNBH_3$, HOAc, r.t., 3 h; (f) DMF, CH_3SO_2Cl, $BF_3{\cdot}Et_2O$, r.t. to 90 °C; (g) Triethyl orthoformate, 70% $HClO_4$, −20 °C to r.t., 8 h, then, H_2O, 100 °C, 1 h.

of biochemical events leading to neuronal dysfunction [16]. Aβ is a proteolytic fragment derived from the amyloid precursor protein (APP), a transmembrane glycoprotein that is usually processed by the enzyme α-secretase to generate in physiological conditions small and soluble peptides [17]. In AD affected brain, amyloidogenic pathway takes place involving the sequential action of β-secretase followed by γ-secretase to generate two predominant Aβ peptides, either 40 (Aβ40) or 42 (Aβ42) amino acids in length. This longer form is more prone to aggregate into fibrils, and it makes up the major component of amyloid plaques. Therefore, inhibitors of the Aβ42 aggregation may be effective in blocking the progression of the pathology [18].

Recent studies indicate that another hypothesis, called metal hypothesis, may contribute to AD pathology [19]. It is observed that the level of metal ions (Fe, Cu, Zn) in AD patients is 3–7 folds higher than that of healthy individuals [20]. The abnormal accumulation of metals is able to accelerate the formation of Aβ aggregates and neurofibrillary tangles, which promote inflammation and activate neurotoxic pathways, leading to dysfunction and death of brain cells [21,22]. In addition, studies suggest that redox-active ions like Cu^{2+} and Fe^{2+} are involved in the production of reactive oxygen species (ROS) and oxidative stress which are critical for Aβ neurotoxicity [23]. Therefore, modulation of such biometals in the brain has been proposed as a potential therapeutic strategy for the treatment of AD.

Due to the pathological complexity found in AD, a single drug that acts on a specific target to produce the desired clinical effects might not be suitable. Thus, multifunctional molecules with two or more complementary biological activities may represent an important advance for the treatment of the disease [24–26]. Herein, our aim is to obtain new multifunctional cholinesterase inhibitors (ChEIs) endowed with addition properties such as lowering the Aβ aggregation and chelating metals.

Tacrine, the most potent and clinically effective acetylcholinesterase inhibitor (AChEI), was approved by FDA in 1993 [27]. However, it was withdrawn from the market due to its dose–dependent hepatotoxicity [28]. Because of the clinical effectiveness of acetylcholinesterase inhibitors (AChEIs) in general and the high potency of tacrine in particular, this structure has been widely used for application in hybrid or multitarget compounds in order to obtain potent AChEIs with other pharmacological properties [29,30]. For example, tacrine–melatonin hybrids and tacrine–ferulic acid hybrids have been designed as potent ChEIs with antioxidant properties [31], tacrine–4-oxo-4*H*-chromene hybrids have been designed as multifunctional agents capable of inhibiting ChE and β-secretase [32], and tacrine–8-hydroxyquinoline hybrids have been designed to exhibit neuroprotective, cholinergic, antioxidant, and copper-complexing properties [33]. Flavonoids, a group of naturally occurring compounds with low molecular weight, have attracted increasingly widespread attention in present-day society, since they possess a wide range of pharmacological properties related to a variety of neurological disorders, such as AChE and BuChE inhibitory activities [34,35], Aβ fibril formation inhibitory activity [36], free radical scavenging effect [37] and metal-chelating ability [38]. Thus, the design and synthesis of new effective flavonoid derivatives are an interesting strategy for the research on anti-AD drugs.

Very recently, our group has reported the synthesis of tacrine–coumarin hybrids as multifunctional ChEIs against AD [39]. Continuing with our research on various natural products with potential application in the AD field. In this paper, flavonoid was selected to hybridize with tacrine to design a series of new hybrids, exhibiting multifunctional activities. Tacrine for the inhibition of ChE through its binding to the CAS while flavonoid for its metal-chelating, and Aβ-lowering activities, as well as for its potential interaction with the PAS due to its aromatic character. Since the AChE-CAS is located at the bottom of a deep gorge, we considered connecting tacrine and flavonoid fragments by piperazine side-armed-alkane spacer. These flexible linkers could be lodged by the enzyme cavity, allowing simultaneous interaction between the heteroaromatic fragments and both the CAS and PAS of AChE. Moreover, the linker fragment could establish additional contacts by binding to the aromatic residues of the enzyme mild-gorge through cation–π interactions [40,41].