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Design, synthesis and evaluation of novel 5,6,7-trimethoxyflavone–6-chlorotacrine hybrids as potential multifunctional agents for the treatment of Alzheimer's disease

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ABSTRACT

A series of 5,6,7-trimethoxyflavone–6-chlorotacrine hybrids were designed, synthesized and evaluated as multifunctional agents for the treatment of Alzheimer's disease (AD). The results showed that the target compounds exhibited good acetylcholinesterase (AChE) inhibitory potencies, high selectivity toward AChE over butyrylcholinesterase (BuChE), potential antioxidant activities and significant inhibitory potencies of self-induced beta-amyloid peptide (Aβ) aggregation. In particular, compound **14c** had the strongest AChE inhibitory activity with IC_{50} value of 12.8 nM, potent inhibition of self-induced $A\beta_{1-42}$ aggregation with inhibition ratio of 33.8% at 25 μM. Moreover, compound **14c** acted as an antioxidant, as well as a neuroprotectant. Furthermore, **14c** could cross the blood–brain barrier (BBB) in vitro. The results showed that compound **14c** might be a potential multifunctional candidate for the treatment of AD.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by progressive cognitive and memory loss.[1] Although 100 years have passed since its discovery, treating AD remains a challenge for the pharmaceutical community.[2] Several factors including low levels of acetylcholine (ACh), oxidative stress, dyshomeostasis of biometals and β-amyloid (Aβ) deposits have been considered to play definitive roles in AD pathogenesis.[3] Current therapeutic options for the treatment of AD focuses on cholinesterases inhibitors (donepezil, rivastigmine, and galantamine) and one *N*-methyl-D-aspartate receptor antagonist (memantine).[4] These drugs show modest improvement in memory and cognitive function, however, they do not prevent progressive neurodegeneration.[5]

Among the multiple factors of AD, the progressive aggregation and deposition of Aβ peptide in brain is considered to be crucial to the pathogenesis of AD, as they may result in a cascade of biochemical events leading to neuronal dysfunction.[6] Therefore, reducing the aggregation of Aβ in the brain appears to be a rational therapeutic approach for treating AD.[7] On the other hand, oxidative stress is one of the earliest events in AD pathogenesis, which damages biological molecules such as proteins, DNA, and lipids.[8] Recent studies have indicated that oxidative damage could promote the appearance of amyloid plaques and neurofibrillar tangles in AD.[9] Thus, the successful protection of neuronal cells from oxidative damage could effectively prevent AD.

6-Chlorotacrine, a potent acetylcholinesterase inhibitor (AChEI) already used in other dual binding site AChEIs.[10] Its hybrids have been investigated widely at present, Such as the synthesis of pyrano[3,2-*c*]quinoline–6-chlorotacrine hybrids,[10] tetrahydrobenzo[*h*][1,6]naphthyridinee–6-chlorotacrine hybrid,[11] 6-chlorotarine–mefenamic acid hybrids and new types of 6-chlorotacrine derivatives,[12] which possessed two or more complementary biological activities through the strategy of multitarget-directed ligands (MTDLs), act as promising anti-Alzheimer lead compounds. 5,6,7-Trimethoxyflavone, methylations of the hydroxyl groups of oroxylin A or baicalein, has been isolated from many plants including *Callicarpa*

Abbreviations: AD, Alzheimer's disease; AChE, acetylcholinesterase; BuChE, butyrylcholinesterase; Aβ, β-amyloid peptide; BBB, blood–brain barrier; ACh, acetylcholine; MTDL, multitarget-directed ligand; *Ee*AChE, *electric eel* AChE; *Hu*AChE, human erythrocyte AChE; CAS, catalytic anionic site; PAS, peripheral anionic site; ORAC-FL, Oxygen Radicals Absorbance Capacity by Fluorescence; ThT, thioflavin T; PAMPA-BBB, parallel artificial membrane permeation assay of the blood–brain barrier.

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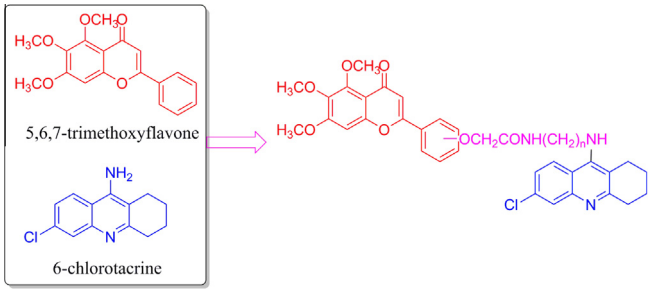


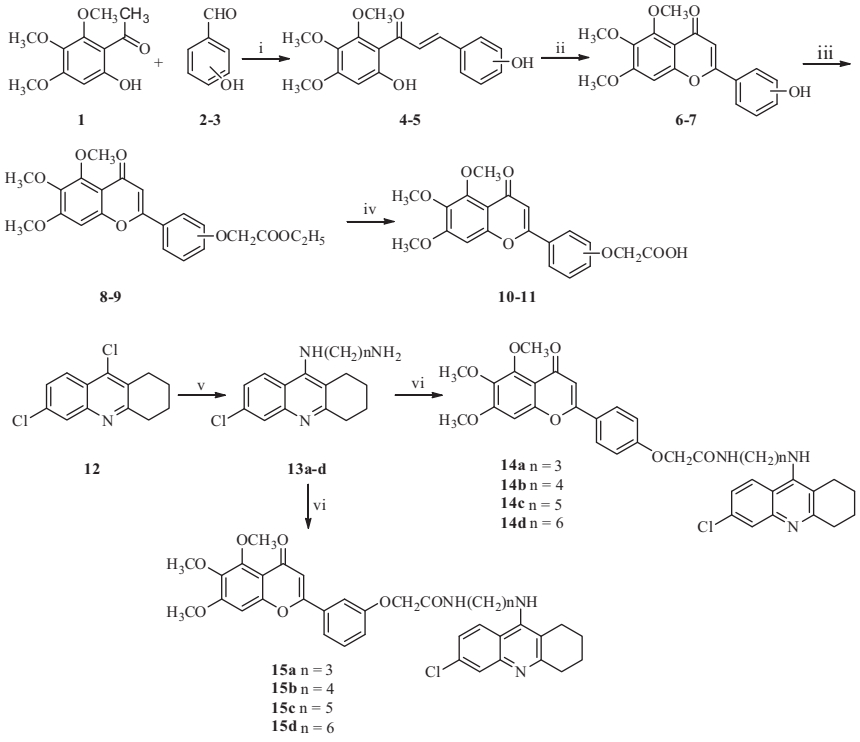
Figure 1. Design strategy for the 5,6,7-trimethoxyflavone–6-chlorotacrine hybrids.

japonica, *Popowia cauliflora*, *Zeyhera tuberculosa* and *Colebrookia oppositifolia*.[13] It and its derivatives possess a wide range of pharmacological properties such as free radical scavenging effect, Aβ fibril inhibition effect, anticancer activity, anti-inflammatory activity and neuroprotective effect.[14–16] The study that tetrahydrobenzo [*h*][1,6]naphthyridinee–6-chlorotacrine hybrids with molecular weights over 500 could be a promising leads for developing anti-Alzheimer drug candidates,[11] which open the way to a new design approach to anti-Alzheimer candidates. Moreover, the important recent works about tacrine–flavonoid hybrids encourage our studies.[17,18] Therefore, we plan to create a series of novel hybrids that combine 5,6,7-trimethoxyflavone with 6-chlorotacrine, although the molecular weight of hybrids is more than 500, we hope that the hybrids possess more complementary biological activities and could cross the blood–brain barrier (Fig. 1).

In this Letter, a series of 5,6,7-trimethoxyflavone–6-chlorotacrine hybrids are designed, synthesized, and evaluated for their biological activities, including inhibitory activities of AChE and BuChE, antioxidant activities, neuroprotective effects, inhibitory effects on self-induced $A\beta_{1-42}$ aggregation and the abilities to cross the blood–brain barrier.

Target compounds **14** and **15** were synthesized as shown in Scheme 1. The starting material compound **1** was treated with *p*-hydroxybenzaldehyde or *m*-hydroxybenzaldehyde by addition of 50% KOH in ethanol to obtain the intermediates **4** and **5**, which were reacted with iodine in DMSO to give compounds **6** and **7**. Subsequently, reaction with bromoacetate in the presence of K_2CO_3 got the intermediates **8** and **9**, and then hydrolysis using 30% LiOH, acidizing with 10% HCl to afford the desired compounds **10** and **11**. On the other hand, reaction of 6,9-dichlorotacrine (**12**) with diaminoalkyl derivatives at reflux in 1-pentanol afforded compounds **13a–d**. Finally, coupling reaction of the intermediates **10** and **11** with the appropriate intermediates **13a–d** in the presence of EDCI and HOBt gave the desired products **14** and **15**.

To evaluate the inhibitory activities of 5,6,7-trimethoxyflavone–6-chlorotacrine hybrids **14a–d** and **15a–d** against AChE and BuChE, the modified Ellman's method was performed using 6-chlorotacrine as reference compound.[5] The precursor compounds **6** and **7** were also evaluated for comparative purpose. AChE was from *electric eel* (*Ee*AChE) and BuChE was from equine serum. The results were summarized in Table 1, as expected, in agreement with our rational design strategy, all the target compounds showed significant ChEs inhibitory activity and high selectivity for BuChE over AChE, which indicated better AChE inhibitory activity than the tacrine–flavonoid hybrids reported previously.[17] The target hybrids exhibited better inhibitory activity toward AChE than 6-chlorotacrine (IC_{50} = 78.5 ± 0.04 nM). Among these compounds, compound **14c**, with five-carbon spacers linking 5,6,7-trimethoxyflavone moiety and 6-chlorotacrine, showed the most inhibitory potency for AChE with IC_{50} value of 12.8 nM. In addition, the hybrids **14a**, **14c**, **15a** and **15c** with odd carbon linkers indicated more potent inhibitory activities than compounds **14b**, **14d**, **15b** and **15d** with even carbon linkers. On the other hand, the com-



Scheme 1. Synthesis of compounds **14** and **15**. Reagents and conditions: (i) 50% KOH, EtOH, room temperature, 3 days. (ii) DMSO, I_2, reflux, 4 h. (iii) $BrCH_2COOC_2H_5$, K_2CO_3, CH_3CN, 65 °C, 6–8 h. (iv) 30% LiOH, THF, 50 °C, 3 h, then 10% HCl. (v) $H_2N(CH_2)nNH_2$, 1-pentanol, 135 °C, 36 h. (vi) Compounds **10** and **11**, EDCI, HOBt, THF, room temperature, overnight.