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Design, synthesis and pharmacological evaluation of chalcone derivatives as acetylcholinesterase inhibitors

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ABSTRACT

A novel series of chalcone derivatives (**4a**–**8d**) were designed, synthesized, and evaluated for the inhibition activity against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The log*P* values of the compounds were shown to range from 1.49 to 2.19, which suggested that they were possible to pass blood brain barriers in vivo. The most promising compound **4a** (IC_{50}: 4.68 μmol/L) was 2-fold more potent than Rivastigmine against AChE (IC_{50}: 10.54 μmol/L) and showed a high selectivity for AChE over BuChE (ratio: 4.35). Enzyme kinetic study suggested that the inhibition mechanism of compound 4a was a mixed-type inhibition. Meanwhile, the result of molecular docking showed its potent inhibition of AChE and high selectivity for AChE over BuChE.

1. Introduction

Alzheimer's disease (AD) is a progressive, chronic, neurodegenerative disorder, characterized by declining in memory and cognitive abilities. It is estimated that about 6% of the population worldwide aged over 65 currently suffer from AD.[1,2] The etiology of AD is still not full known, but many investigations have suggested that reduced level of the neurotransmitter acetylcholine (Ach), formation of β-amyloid peptide (Aβ) plaques, increased oxidative stress, inflammation and Tau-protein aggregation are thought to play significant roles in the process of this disease.[3–5]

Current treatment of AD mainly focuses on the inhibition of AChE activity aimed at rectifying the deficiency of cerebral acetylcholine.[6] Based on the cholinergic hypothesis, the deterioration of memory and cognition in AD patients is mainly caused by the extensive decline of ACh, which is released into presynaptic neuron to transport nervous signal and rapidly hydrolyzed by AChE.[7] Moreover, recent studies have identified that AChE could also play a key role in accelerating the assembly of β-amyloid into amyloid fibrils.[8] Up to now, several AChE inhibitors (such as donepezil, Rivastigmine, galantamine and tacrine) have been approved by European and US regulatory authorities for the clinical treatment of AD in the early to moderate stages.[9] However, these AChE inhibitors are known to have side effects or demerits such as hepatotoxicity, short half life, and gastrointestinal tract excitement.[10] Therefore, the investigation on searching for new and better AChE inhibitors is still of great interest.

Recently, AChE inhibitors from natural products had attracted significant attention because of their fewer side effects. Huperzine A and galantamine are successful examples of AChE inhibitors from natural products.[11] However, it is not so easy to find the potent compounds which could be potential drugs without any structure modifications for many natural products had weak bioactivities yet. In addition, the total chemical synthesis of some complicated natural products is usual difficult and costly. So, it is highly desirable to synthesize some derivates with natural compounds backbone according to the idea of rational molecular design to gain potential drugs for the therapy of AD. For the research and development of AChE inhibitors, many investigations revealed that nitrogen atom was important to the inhibition of AChE.[12] Several flavonoid and coumarin derivatives with terminal amine groups (Fig. 1) have been successfully designed and synthesized and some of them exhibited potent inhibitory activity against AChE.[13–15] Among them, ensaculin, a coumarin derivative, is under clinical investigation for potential AD management.[16]

Chalcones, natural compounds widely existed in fruits and vegetables, belong to the flavonoid family and consist of two aromatic rings connected by an α,β-unsaturated carbonyl group. Possibly due to the flexible structure, chalcones can bind effectively to many kinds of enzymes or receptors and exhibit diverse biological

Abbreviations: AD, Alzheimer's disease; AChE, acetylcholinesterases; ACh, acetylcholine; BuChE, butyrylcholinesterase; PAS, peripheral anionic sites; Log*P*, octanol/water partition; CNS, central nervous system; BBB, blood brain barrier.

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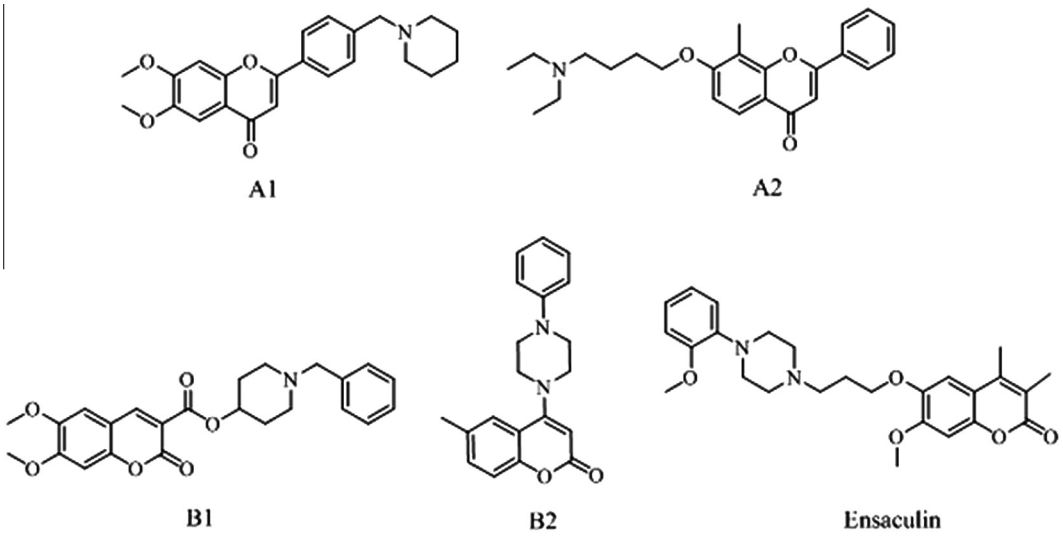


Figure 1. The chemical structure of flavonoid derivatives (A1, A2) and coumarin derivatives (B1, B2, Ensaculin) as the AChE inhibitors.

activities, such as anti-cancer, anti-infective, anti-inflammatory, anti-oxidant and anti-angiogenic effects.[17–20] But few investigations were conducted by pharmaceutical researchers about their biological activities of inhibiting AChE. Thus, based on the design experiences from existing AChE inhibitors, we designed and synthesized a series of chalcones with different basic side chains (n = 2–6) and evaluated their biological activity of inhibiting AchE. Moreover, we explored the binding mode of the compounds to AchE by kinetic experiments and measured the logarithm 1-octanol/water partition coefficient (log$P$ values). The log$P$ value was used to assess the ability of compounds to penetrate blood brain barrier (BBB). Furthermore, molecular docking studies were carried out to study the binding mode and selectivity of these compounds against AChE.

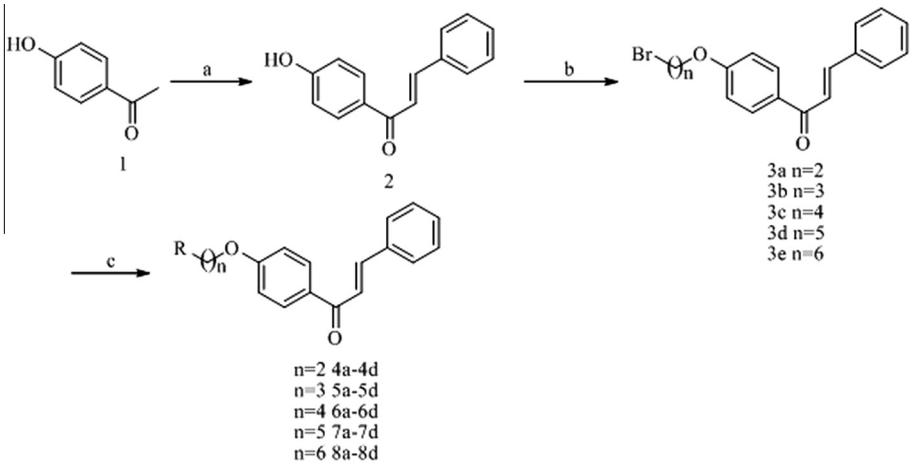
2. Results and discussion

2.1. Chemistry

The synthetic routes to target compounds 4a-8d are outlined in Scheme 1. Reaction of 4-hydroxyacetophenone 1 with benzaldehyde under the catalysis of NaOH/EtOH provided compound **2**.[21] Then, compound **2** was treated with dibromoalkanes bearing two to six carbons and K_2CO_3 in *N*,*N*-dimethylformamide (DMF) at 80 °C to generate compounds **3a**–**3e**. Finally, the target compounds **4a**–**8d** were obtained by refluxing **3a**–**3e** with commercially available secondary amines (dimethylamine, diethylamine, dipropylamine and dibutylamine) in acetone in the presence of K_2CO_3 and NaI. The structures of the designed compounds were characterized by proton nuclear magnetic resonance spectroscopy (^{1}H NMR), infrared spectrum (IR) and mass spectrometry (MS). Besides, the purities of all synthesized compounds were confirmed to be higher than 95% by HPLC.

2.2. Log*P* values

For a drug to treat AD, the ability to penetrate the BBB is vital. Although the factors that affect the penetration of a drug from the systemic circulation into the central nervous system (CNS) were complicated, log$P$ was thought as an important physical chemistry parameter to evaluate or predict the ability to cross BBB, which widely used in medicinal chemistry investigation.[22] Hansch[23] presumed that the log$P$ with optimum CNS penetration was around 2 ± 0.7. Log$P$ values of the synthesized compounds were measured by the classical shake-flask method[24] using RP-HPLC. As shown in Table 1, the log$P$ values of the tested



Scheme 1. Reagents and conditions: (a) benzaldehyde, NaOH, EtOH, rt; (b) Br(CH$_2$)$_n$Br, K_2CO_3, DMF, 80 °C; (c) second amine, K_2CO_3, NaI, acetone, reflux.