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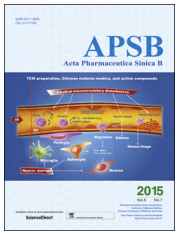

ORIGINAL ARTICLE

Design, synthesis and evaluation of genistein-polyamine conjugates as multi-functional anti-Alzheimer agents

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Inhibition;
Rivastigmine

Abstract A series of genistein-polyamine conjugates (**4a–4h**) were designed, synthesized and evaluated as multi-functional anti-Alzheimer agents. The results showed that these compounds had significant cholinesterases (ChEs) inhibitory activity. Compound **4b** exhibited the strongest inhibition to acetylcholinesterase (AChE) with an IC_{50} value of 2.75 μmol/L, which was better than that of rivastigmine (5.60 μmol/L). Lineweaver–Burk plot and molecular modeling study showed that compound **4b** targeted both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE. Besides, compound **4b** showed potent metal-chelating ability. In addition, it was found that **4a–4h** did not affect HepG-2 cell viability at the concentration of 10 μmol/L.

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1. Introduction

Alzheimer's disease (AD), the most common form of neurodegenerative senile dementia, is associated with selective loss of cholinergic neurons and reduced level of acetylcholine neurotransmitter. It is characterized by memory deficit and progressive impairment of cognitive functions[1]. AD affects millions of elderly people, and the number of patients is expected to increase in the next 20 years. Many factors have been found to be implicated in AD, such as low levels of acetylcholine, β-amyloid deposits, oxidative damage and metal ions, which seem to play significant roles in the disease[2]. Current treatment of AD focuses on increasing cholinergic neurotransmission in the brain by inhibiting cholinesterases (ChEs) with medicines including tacrine, donepezil, rivastigmine and galantamine[3]. Unfortunately, the potential effectiveness offered by the above inhibitors is often limited by their side effects. For example, clinical studies have shown that tacrine has hepatotoxic liability[4]. Due to the multi-pathogenesis of AD, one of the current strategies is to develop novel anti-AD agents with multiple potencies[5].

Genistein is biosynthetically the simplest isoflavonoid compound of the Leguminosae[6]. It expresses a wide range of biological activities, such as antioxidant, anti-cancer and antimicrobial[7–9]. In recent years, it was reported that genistein showed neuroprotective effect and ameliorated learning and memory deficits in the AD rat model[10,11]. Besides, a number of genistein derivatives have been reported as anti-AD agents in the past years (Fig. 1)[12,13]. These results indicate that genistein could be used as leading compound for the treatment of AD.

Polyamines are aliphatic molecules with amine groups distributed along their structures[14]. They have always been the concern of medicinal chemists as a universal template[15]. Our group has been involved in the development of polyamine conjugates as potential drugs for many years[16–19]. It was found that quinoline-polyamine conjugates exhibited potent ChEs inhibition activity and polyamine occupied the gorge of AChE[20]. Therefore, in the present study, in order to enhance the pharmacological potential of genistein, a series of genistein conjugates modified with polyamine were designed, synthesized as anti-Alzheimer agents.

2. Results and discussion

2.1. Synthesis of target compounds *4a–4h*

The synthetic routes to target compounds are summarized in Scheme 1. The starting material genistein **1** was treated with ethyl 2-chloroacetate in acetone to give intermediate **2**, which was heated with K_2CO_3 in water and then mixed with HCl yielding compound **3**. Then compound **3** converted to the intermediates by reaction with amines or Boc protected polyamines in DMF. At last, the Boc groups subsequently were removed using HCl (4 mol/L) at room temperature, producing target compounds **4a–4h** as hydrochloride salts. All the structures of the target compounds were confirmed by ^{1}H NMR, ESI-MS and elemental analysis.

2.2. Enzyme inhibition assays

All the newly synthesized compounds (**4a–4h**) were screened against AChE and BChE *in vitro* according to the modified Ellman method. Rivastigmine was used as control. The ChEs inhibition results were listed as the inhibition ratio at a tested concentration of 50 μmol/L (Table 1). We also tested the IC_{50} value of compounds **4b** and **4h** (Table 2).

The results showed that all of the target compounds possessed ChEs inhibition activity, and compound **4b** exhibited the strongest inhibition to AChE with an IC_{50} value of 2.75 μmol/L which was better than rivastigmine (5.60 μmol/L), compound **4h** also showed good activity with IC_{50} values of 46.59 μmol/L. Genistein, the parent molecule, inhibited the AChE activity to less than 50% at the concentration of 100 μmol/L (Table 2). It indicated that conjugation polyamines with genistein could increase the inhibition activity of AChE. Besides, it seemed that AChE inhibitory potency of conjugates was closely related to the length and the end group of the polyamine chain. Compounds (**4b–4d**) modified by diamine were more active than compounds conjugated with monoamine or triamine.

In the assay of BChE inhibtion studies, compound **4h** showed the most potent inhibition for BChE with an inhibition rate of

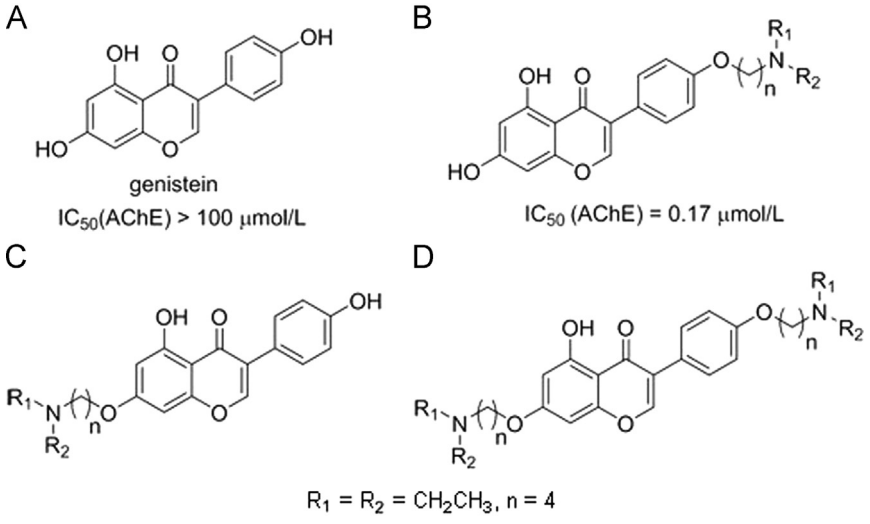

Figure 1 Chemical structures of genistein and genistein derivatives.