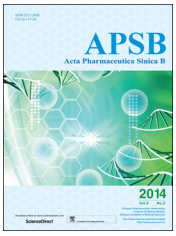
Acta Pharmaceutica Sinica B 2014;4(6):430–437

HOSTED BY

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



ORIGINAL ARTICLE

Inhibition of acetylcholinesterase by two genistein derivatives: kinetic analysis, molecular docking and molecular dynamics simulation

Jiansong Fang[a], Ping Wu[a], Ranyao Yang[a], Li Gao[a], Chao Li[a], Dongmei Wang[a], Song Wu[a,c], Ai-Lin Liu[a,b,c,*], Guan-Hua Du[a,b,c,*]

[a]*Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China*
[b]*Beijing Key Laboratory of Drug Target Research and Drug Screening, Beijing 100050, China*
[c]*State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Beijing 100050, China*

Received 13 July 2014; revised 27 August 2014; accepted 24 September 2014

KEY WORDS

Genistein derivatives;
Acetylcholinesterase (AChE);
Kinetics analysis;
Molecular docking;
Molecular dynamics simulation;
MM/GBSA

Abstract In this study two genistein derivatives (G1 and G2) are reported as inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), and differences in the inhibition of AChE are described. Although they differ in structure by a single methyl group, the inhibitory effect of G1 (IC_{50} = 264 nmol/L) on AChE was 80 times stronger than that of G2 (IC_{50} = 21,210 nmol/L). Enzyme-kinetic analysis, molecular docking and molecular dynamics (MD) simulations were conducted to better understand the molecular basis for this difference. The results obtained by kinetic analysis demonstrated that G1 can interact with both the catalytic active site and peripheral anionic site of AChE. The predicted binding free energies of two complexes calculated by the molecular mechanics/generalized born surface area (MM/GBSA) method were consistent with the experimental data. The analysis of the individual energy terms suggested that a difference between the net electrostatic contributions ($\Delta E_{ele}+\Delta G_{GB}$) was responsible for the binding affinities of these two inhibitors. Additionally, analysis of the molecular mechanics and MM/GBSA free energy decomposition revealed that the

Abbreviations: ACh, acetylcholine; AChEIs, acetylcholinesterase inhibitors; AChE, acetylcholinesterase; AD, Alzheimer's disease; BuChE, butyrylcholinesterase; BuSCh, *S*-butyrylthiocholine chloride; CAS, catalytic active site; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); GAFF, generalized AMBER force field; G1, 3-(4-methoxyphenyl)-7-(2-(piperidin-1-yl)ethoxy)-4*H*-chromen-4-one; G2, (*S*)-3-(4-methoxyphenyl)-7-(2-(2-methylpiperidin-1-yl)ethoxy)-4*H*-chromen-4-one; iso-OMPA, tetraisopropyl pyrophosphoramide; MD, molecular dynamics; MM/GBSA, molecular mechanics/generalized born surface area; PAS, peripheral anionic site; PDB, protein data bank; PME, particle mesh Ewald; RMSD, root-mean-square deviation; *S*-ACh, acetylthiocholine iodide; ΔE_{ele}, electrostatic energy contribution; ΔE_{MM}, gas-phase interaction energy between receptor and ligand; ΔE_{vdw}, van der Waals energy contribution; SASA, solvent accessible surface area; ΔG_{exp}, experimental binding free energy; ΔG_{GB}, polar desolvation energy term; ΔG_{pred}, total binding free energy; ΔG_{SA}, nonpolar desolvation energy term; ΔS, conformational entropy contribution

*Corresponding authors at: Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China. Tel./fax: +86 10 63165184.

E-mail addresses: liuailin@imm.ac.cn (Ai-Lin Liu), dugh@imm.ac.cn (Guan-Hua Du).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2014.10.002

difference between G1 and G2 originated from interactions with Tyr124, Glu292, Val294 and Phe338 of AChE. In conclusion, the results reveal significant differences at the molecular level in the mechanism of inhibition of AChE by these structurally related compounds.

1. Introduction

Alzheimer's disease (AD), a progressively degenerative disorder of the brain, is believed to be a multifactorial syndrome with several target proteins contributing to its etiology[1]. AD is characterized by a loss of basal forebrain neurons and reduced cortical and hippocampal levels of acetylcholine (ACh). The relation between the observed cholinergic dysfunction and AD severity provides a rationale for the therapeutic use of acetylcholinesterase inhibitors (AChEIs)[2].

The inhibition of acetylcholinesterase (AChE, E.C. 3.1.1.7), which is responsible for the breakdown of ACh, has been proven as a successful way to relieve some cognitive and behavioral symptoms of AD[3,4]. So far, several AChE inhibitors (Fig. 1), such as tacrine[5], galanthamine[6], huperzine A[7] and donepezil[8] have been used mainly for the clinical treatment of AD, all of which slow down neurodegeneration in AD patients to some extent.

The three-dimensional structure of AChE, as determined by X-ray crystallography for a large number of enzyme–ligand complexes[9–11], reveals two main binding sites: the catalytic active site (CAS), comprising the Ser-His-Glu catalytic triad, and the peripheral anionic site (PAS), connected by a deep, hydrophobic gorge.

Tacrine is the first AChE inhibitor permitted by the FDA. The co-crystal structure of AChE (protein data bank entry:1ACJ) from *Torpedo californica* complexed with tacrine showed that tacrine only interacted with the CAS of AChE[12]. However, due to its adverse effects such as acute liver toxicity and increased rates of syncope, tacrine has been gradually withdrawn from market. Since then, pharmaceutical chemistry scientists have become interested searching for AChE inhibitors able to simultaneously bind to their CAS and PAS. Several types of dual-binding-site AChE inhibitors have been developed by connecting the two interacting units through a suitable linker, which were generally derived from known AChE inhibitors either commercialized or under development[13–23].

To date, donepezil (PDB entry: 4EY7) is the only dual binding site AChE inhibitor approved for the treatment of AD[24]. The latest X-ray crystallographic structure of the complex between recombinant human AChE and donepezil reveals that the elongated structure of donepezil spans the entire length of the enzyme-active-site gorge[25]. It has a unique orientation along the active-site gorge, extending from the CAS, at the bottom near Trp86, to the PAS at the top near Trp286. This provides a more accurate platform for further design of next-generation derivatives.

In the current work, two genistein derivatives (G1 and G2) have been discovered with strong or moderate activity against both AChE and butyrylcholinesterase (BuChE). Although G1 and G2 (Fig. 1) have quite similar structures, experimental data in this study show that the inhibitory effect of G1 against AChE was almost 80 times greater than that of G2. Since their inhibitory mechanisms against AChE are still unclear, it is of great interest to investigate why these two analogs have differing inhibitory potencies and to reveal the molecular basis for their binding to AChE. Thus, the binding mechanisms of these two inhibitors were studied by multiple approaches consisting of enzyme-kinetic analysis, molecular docking and molecular dynamics (MD) simulation[26–41]. This study provides a molecular basis for understanding how different configurations influence their binding affinities.

2. Materials and methods

2.1. *In vitro* inhibition studies on AChE and BuChE

AChE (E.C. 3.1.1.7) was extracted from rat cortex (Sprague Dawley). BuChE (E.C. 3.1.1.8) was obtained from human plasma (purchased from Beijing Red Cross Blood Center). 5,5′-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine iodide (*S*-ACh), *S*-butyrylthiocholine chloride (BuSCh), donepezil

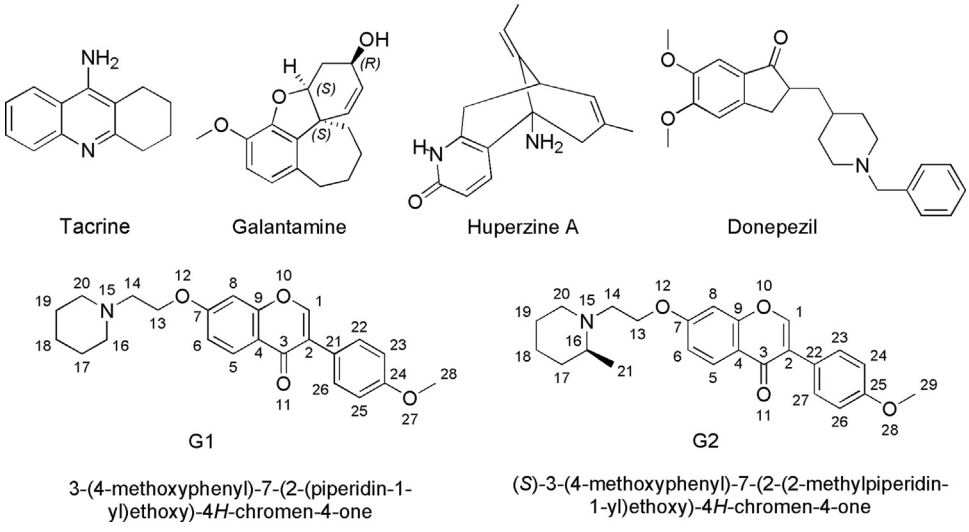


Figure 1 The structures of AChE inhibitors.