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Research paper

Novel series of tacrine-tianeptine hybrids: Synthesis, cholinesterase inhibitory activity, S100B secretion and a molecular modeling approach

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ABSTRACT

Tianeptine was linked to various 9-aminoalkylamino-1,2,3,4-tetrahydroacridines using EDC·HCl/HOBt to afford a series of tacrine-tianeptine hybrids. The hybrids were tested for their ability to inhibit AChE and BuChE and IC_{50} values in the nanomolar concentration scale were obtained. AChE molecular modeling studies of these hybrids indicated that tacrine moiety interacts in the bottom of the gorge with the catalytic active site (CAS) while tianeptine binds to peripheral anionic site (PAS).

Furthermore, the compounds **2g** and **2e** were able to reduce the in vitro basal secretion of S100B, suggesting its therapeutic action in some cases or stages of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD), acknowledged as progressive multifarious neurodegenerative disorder, is the most devastating and loosening of thinking and judging ability disease that occurs in the old age people [1–3]. The average period of survival is about nine years after the onset of clinical symptoms. Its risk increases with the age but it is not a part of normal aging. The AD brain may weigh one third less than the normal brain of a person with the same age [1].

Approximately 200,000 people younger than 65 years with AD comprise the younger onset AD population; 5 million are age 65 years or older. It is expected that by 2050 nearly a million new cases per year [3,4]. Several hypotheses have been put forward on the basis of the various causative factors in order to explain this multifactorial disorder [2–8], such as the cholinergic hypothesis, amyloid cascade hypothesis, also called Aβ hypothesis, tau hypothesis, inflammation hypothesis, metal ion hypothesis, oxidative stress hypothesis and GSK-3 hypothesis [1,2]. Recently it has been shown that the most commonly used Aβ hypothesis, prevailing for the last two decades, does not account for the complex pathophysiology of this incapacitating disease. Recent studies have also highlighted the role of Aβ oligomers in synaptic impairment, suggesting that these are primarily the only one among several other signals that destroy the integrity of brain functions. Also, formation of amyloid plaques that develop in the later age appears to be a rather late event [2,7].

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The cholinergic hypothesis was the first theory proposed to explain AD. This hypothesis suggests that the memory and cognitive decline of AD result from a deficit of acetylcholine (ACh), an important neurotransmitter in specific brain regions, and inhibiting the cholinesterase (ChE) responsible for the hydrolysis of ACh can alleviate these symptoms [8].

The current therapeutic options for the treatment of AD are limited to three Acetylcholinesterase inhibitors (AChEI) [8,9], namely donepezil (Aricept®), rivastigmine (Exelon®) and galantamine (Razadyne®, Reminyl®), and a *N*-methyl-D-aspartate (NMDA) receptor antagonist [10–12], memantine (Namenda®). These compounds have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of AD.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are enzymes which constitute the group of the cholinesterases. In healthy brains, AChE hydrolyzes the majority of acetylcholine while BuChE plays a secondary role. However, as AD progresses, the activity of AChE decreases, while that of BuChE significantly increases and may even surpass the AChE activity [13,14].

The results of pharmaceutical tests have indicated that ChEIs act via multiple pathways of neuronal protection on different targets [15,16]. Accordingly, favorable inhibitory activities have been observed for the following types of receptors/enzymes: *N*-methyl D-aspartate (NMDA), gamma-aminobutyric acid (GABA), the L-type voltage-dependent calcium channel (L-VDCC), nitric oxide synthase (NOS), and β- and γ-secretase. In addition, ChEIs operate by inducing the activity of protein kinase type C (PKC) and consequently α-secretase, resulting in the reduction of harmful amyloid species levels [17]. Thus, ChEIs act as ligands directed to multiple targets that are suitable for treating multifactorial diseases, such as AD.

Acetylcholine binding site of AChE is formed by a catalytic anionic site (CAS) composed by the catalytic triad Ser200, His440 and Glu327 and the anionic subsite which is defined by Trp84, Tyr130, Tyr330, and Phe331 amino acid residues [18]. These binding sites are located at the base of a deep hydrophobic channel measuring approximately 20 Å in length. A specific peripheral anionic site (PAS), located at the entrance of the gorge, is formed by five residues (Tyr70, Asp72, Tyr121, Trp279, Tyr334) and exhibits a large conformational flexibility [19]. Diverse studies indicate that this binding site is a potential target for the development of bivalent and selective inhibitors for AChE over BuChE [20–23].

Recently, it has also been discovered that BuChE plays a key role in the etiology and progression of AD beyond regulation of synaptic ACh levels [24–27]. The correlation between BuChE polymorphisms and the progression of cognitive impairment in dementia with Lewy bodies and AD have suggested that BuChE is a key player in brain areas that influence the aggregation of neuritic Aβ plaques [28–30]. BuChE may be particularly important in individuals with more severe dementia, as BuChE activity increases with disease development. Also, it could be shown that BuChE activity in the brain is exclusive to some neuronal populations. For this reason, BuChE has evolved as a more potent drug target than AChE. Several drugs that are selective BuChE inhibitors have been evaluated for the treatment of AD [31,32]. Therefore, syntheses and evaluation of inhibitors of BuChE with an enhanced specificity can be a promising therapeutic strategy of medicinal and pharmaceutical chemistry to develop new drugs for the treatment of AD. The co-crystallization of BuChE or AChE with transition-state analogs and different inhibitors has provided high impact information about the binding modes of substrates and inhibitors with the possibility to apply docking protocols on the protein–inhibitor complexes [24].

Therefore, the mainstays of current pharmacotherapy of AD are drugs aimed at increasing the acetylcholine level through the inhibition of both enzymes, acetylcholinesterase and butyrylcholinesterase, and the most known AChEIs have been designed to interact with the catalytic site of the enzyme, which is placed at the bottom of a 20 Å deep narrow gorge [33,34].

Tacrine (1,2,3,4-tetrahydroacridin-9-amine, THA, Fig. 1) is one of the most important nonselective inhibitor of both AChE and BuChE [35–38]. Although this lack of selectivity, and hepatotoxicity, has reduced its therapeutic use, it has been one of the most studied compounds and remains a reference structure in the development of new ChEs inhibitors as potential drugs for AD [32,33]. About twenty years ago, with the aim of finding more potent compounds in inhibiting AChE, Pang et al. performed computational studies to test the strategy of a double interaction with the enzyme inhibitor. The authors found that two tacrine units joined by methylene chain spacers allowed a double interaction of the compound with the enzyme by binding simultaneously to the CAS and PAS [38–42]. Based on the good results obtained *in silico*, the authors performed the synthesis of alkylene-linked bis-tacrine compounds (Fig. 1) [42]. Among the compounds obtained, the heptylene-linked bis-tacrine derivative proved to be almost fifteen hundred times more potent in inhibiting AChE than tacrine, confirming the trend observed in studies of computer simulation, subsequently ratified with crystallographic studies [43]. When the dimmer binds to its target, it positions a THA component in the CAS region, close to the enzyme catalytic triad, while the other THA component is positioned at the entrance of the catalytic gorge of PAS [16].

Recently, the interest in these AChEIs has been increased, since some of these compounds have proved to be active in non-amyloidogenic route, decreasing the lag phase of the peptide aggregation and showing that may be involved in APP metabolism and suggesting a role of AChE as a chaperone for Aβ1–40 assembly into oligomers of a high structural complexity. For example, it was suggested that α-secretase is activated by heptylene-linked bis-tacrine [17,44,45].

Among achievements in synthesis and pharmacomodulation, various tacrine-based heterodimers have been designed and studied. These tacrine heterodimers are usually obtained by connecting natural or synthetic compounds by a linker of suitable length. Examples of tacrine-based heterodimer families include the combination of tacrine with structural units such as tacrine-huperzine A [46], tacrine-donepezil [47], tacrine-indole [48], tacrine- 4-oxo-4*H*-chromene [9], tacrine-cysteine [49], tacrine-8-hydroxyquinoline [50], tacrine–ferulic acid [51], tacrine–-flurbiprofen–nitrate [52], tetrahydrobenzo[*h*] [1,6]naphthyridine-6-chlorotacrine [53] and tacrine-lophine hybrids [14].

Tianeptine (7-[(3-chloro-6-methyl-5,5-dioxo-11*H*-benzo[*c*][1,2]benzothiazepin-11-yl)amino]heptanoic acid) is a neuroprotective antidepressant. Its molecular structure (Fig. 1) includes a substituted dibenzothiazepine nucleus with two fused benzene rings and an aminoheptanoic acid side chain that distinguishes it from other tricyclic antidepressants [54,55]. The neurobiological properties of tianeptine involve a dynamic interplay between numerous neurotransmitter systems and the critical ability to restore normal neuroplasticity in circumscribed limbic brain regions and to reverse stress-induced impairments in synaptic glutamate transmission [54–56]. Recent studies revealed tianeptine as an efficacious μ-opioid receptor (MOR) and δ-opioid receptor (DOR) agonist, and it is proposed that MOR-agonism (or combined MOR/DOR-agonism) underlies the clinical, preclinical and in vitro effects of tianeptine [55].

Some experimental data also suggest that tianeptine may prevent the human hippocampus atrophy and dysfunction, the feature related to both ageing and AD [57,58].

As part of our search of novel and improved ChEIs based on tacrine units [14,40,59], we describe in the present study the

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