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Original article

Synthesis, biological evaluation and molecular modeling study of novel tacrine–carbazole hybrids as potential multifunctional agents for the treatment of Alzheimer's disease

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ABSTRACT

New tacrine–carbazole hybrids were developed as potential multifunctional anti-Alzheimer agents for their cholinesterase inhibitory and radical scavenging activities. The developed compounds showed high inhibitory activity on acetylcholinesterase (AChE) with IC_{50} values ranging from 0.48 to 1.03 μM and exhibited good inhibition selectivity against AChE over butyrylcholinesterase (BuChE). Molecular modeling studies revealed that these tacrine–carbazole hybrids interacted simultaneously with the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE. The derivatives containing methoxy group showed potent ABTS radical scavenging activity. Considering their neuroprotection, our results indicate that these derivatives can reduce neuronal death induced by oxidative stress and β-amyloid (Aβ). Moreover, S1, the highest potency for both radical scavenging and AChE inhibitory activity, exhibited an ability to improve both short-term and long-term memory deficit in mice induced by scopolamine. Overall, tacrine–carbazole derivatives can be considered as a candidate with potential impact for further pharmacological development in Alzheimer's therapy.

1. Introduction

Alzheimer's disease (AD) is a multifaceted neurodegenerative disorder characterized by loss of memory, progressive deficits in cognitive functions, and severe behavioral abnormalities [1]. AD, the most common form of dementia in the elderly population, has become the fourth leading cause of death in the developed countries. Currently, AD is increasing in people over 65 years and affects over 35 million people worldwide. The number of the affected population is expected to triple by 2050 if no efficient treatment is discovered [2].

The pathogenesis of AD has been found to be associated with numerous pathways including deficiency in cholinergic neurotransmission [3], defective beta-amyloid protein metabolism and tau protein phosphorylation [4], and the involvement of inflammatory and oxidative pathways [5]. Many rational pharmacological strategies have emerged over the past decades which include cholinergic and noncholinergic interventions [6,7]. However, the only therapeutic strategy which was seen to enhance the central cholinergic function is currently approved for the treatment of AD. The first approved drugs were tacrine, donepezil, rivastigmine and galantamine [8,9]. However, these drugs that modulate such a single target could only enable a palliative treatment instead of curing or preventing AD.

Abbreviations: AD, Alzheimer's disease; BuChE, butyrylcholinesterase; AChE, acetylcholinesterase; AChEI, acetylcholinesterase inhibitor; PAS, peripheral anionic site; CAS, catalytic active site; MAO, monoamine oxidase; ABTS, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid; MTT, methyl thiazolyl tetrazolium; Aβ, beta-amyloid peptide.

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Due to the multi-pathogenesis of AD, the classical approach modulating at one target may be inadequate in this complex disease. Therefore, searching the candidates acting at multiple sites of pathologic cascade has become a new strategy for the design of new drugs for AD. Thus, associations of AChEIs with compounds acting at different sites of pathologic cascade provide additional benefits. The multitarget approach in drug design for the treatment of AD includes novel tacrine–melatonin hybrids [10], dual inhibitors of AChE and monoamine oxidase (MAO) [11], dual AChEI and serotonin transporters [12] and potent AChEI with calcium channel blocker [13].

In recent years, several studies have focused on the role of free radical formation and oxidative cell damage in the pathogenesis of AD. Recent research has demonstrated that oxidative stress plays a key role in initiating the aggregation of Aβ and tau protein hyperphosphorylation, involved in the early stage of the pathologic cascade [14]. Thus, oxidative stress has become the important target for AD treatment. Several antioxidants have been tested in clinical trials [15,16].

Because ChE as well as oxidative stress are important targets for the treatment of AD, some studies have been devoted to searching multifunctional agents that act on both ChE and oxidative stress, such as tacrine–lipoic acid hybrid [17] and tacrine–melatonin hybrid [10]. These findings support that the combination of AChEIs and antioxidant is an effective strategy for developing new multifunctional drugs for AD treatment.

Tacrine was the first ChEI approved by the FDA for the treatment of AD. Its mechanism of action is the inhibition of ChE, to normalize acetylcholine levels in the synaptic cleft [18]. Due to severe liver toxicity, tacrine was withdrawn from the market. However, the tacrine structure is still required as a scaffold for development of multifunctional anti-AD agents because of its high potency and low molecular weight [19].

Carbazoles, naturally occurring phytochemicals, are widely present in many plant species and possess a wide range of biological activities associated with AD. It has been reported that naturally occurring carbazole derivatives are able to directly scavenge a variety of reactive oxygen species and possess strong antioxidant actions [20]. Moreover, a recent study has also shown that carbazole derivatives have the capacity of inhibiting Aβ aggregation [21]. Recently, we have studied a series of carbazole derivatives extracted from root bark of *Clausena Harmandiana* as an antioxidant, and found that 7-methoxyheptaphylline and heptaphylline possess a strong antioxidant effect *in vitro* [22].

Thus, in the present study, we combined the AChEI, tacrine, to the active functions of natural antioxidants, 7-methoxyhepta phylline and heptaphylline, as potential multifunctional agents for the treatment of AD. These compounds were synthesized by connecting the antioxidant moiety to AChEI pharmacophore via the alkylenediamine side chain (Fig. 1). All synthetic derivatives were investigated for their biological activities towards selected targets involved in AD, namely AChE, BuChE, and antioxidant. To investigate their mechanism of interaction with AChE, molecular modeling was also performed. In addition, the neuroprotection effect against oxidative stress and Aβ toxicity of the test compounds was evaluated in a cell culture model. Furthermore, the most potent compound *in vitro* was selected to evaluate for improvement of memory deficit in mice by Morris water maze and Y-maze model.

2. Results and discussion

2.1. Chemistry

The synthetic pathway of tacrine–carbazole hybrids is provided in Scheme 1. The target molecules consist of three parts, therefore, the synthetic strategy is firstly to prepare the imine derivative by reacting an antioxidant moiety with the alkylenediamine side chain, and then connecting it to the AChEI moiety. Firstly, the reaction between 7-methoxyheptaphylline or heptaphylline and 1,3-diaminopropane or 1,5-diaminopentane under reflux for 18 h in methanol affords intermediated imine. Subsequently, the intermediated imine was reacted with 9-chlorotacrine in the presence of pentanol under reflux for 24 h to obtain target products S1–S3, whose structures are shown in Fig. 1. The structures of all synthesized compounds were elucidated by IR spectroscopy, mass spectrometry and ^{1}H NMR and ^{13}C NMR spectrometry.

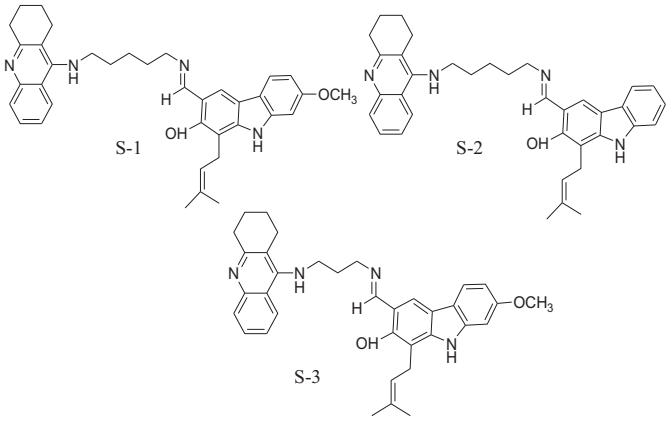

Fig. 1. Chemical structures of tacrine–carbazole hybrids S1–S3.

2.2. In vitro antioxidant activity assays

The reduction of the oxidative stress is another crucial aspect in designing agents for AD treatment. We examined the antioxidant activities of our synthetic derivatives by using the ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) radical scavenging method. Their ability to scavenge radicals was shown as IC_{50}, the test compound's concentration resulting in 50% inhibition of free radical. Trolox, a water-soluble vitamin E analog, was used as a reference standard and tacrine, heptaphylline and 7-methoxyheptaphylline were also determined for comparison. Tacrine showed negligible radical scavenging activity, whereas heptaphylline and 7-methoxyheptaphylline had the ability to scavenge the ABTS radical. Our synthetic derivatives showed potent ABTS radical scavenging capacities with IC_{50} in the range of 8.34–11.24 μM as shown in Table 1. Interestingly, our synthesized compounds showed higher radical scavenging activity than trolox which has IC_{50} of 23.67 μM. The best results were obtained with derivatives bearing a methoxy group at 7-position of the carbazole ring. S1 containing a methoxy group at position 7 of the carbazole ring was more potent than S2 which does not bear any substituent in the carbazole ring. These results corresponded with their starting compounds, heptaphylline and 7-methoxyhepta phylline.

With regard to the length of the linker, S1 and S3 which possess 5-methylene and 3-methylene linkers, respectively, showed identical IC_{50} value for ABTS radical scavenging action. Hence, the length of the side chain did not affect antioxidant activity.

Tacrine, the AChE inhibitor moiety, had no ability to scavenge the ABTS radical. Hence, radical scavenging activity of the synthesized compounds might come from the antioxidant moiety. However, the addition of the tacrine moiety in our synthetic derivative structure improved the ability to scavenge radicals. The effect might be from lipophillic improvement.