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Synthesis and evaluation of multi-target-directed ligands for the treatment of Alzheimer's disease based on the fusion of donepezil and melatonin

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ABSTRACT

A novel series of compounds obtained by fusing the acetylcholinesterase (AChE) inhibitor donepezil and the antioxidant melatonin were designed as multi-target-directed ligands for the treatment of Alzheimer's disease (AD). In vitro assay indicated that most of the target compounds exhibited a significant ability to inhibit acetylcholinesterase (*ee*AChE and *h*AChE), butyrylcholinesterase (*eq*BuChE and *h*BuChE), and β-amyloid (Aβ) aggregation, and to act as potential antioxidants and biometal chelators. Especially, **4u** displayed a good inhibition of AChE (IC_{50} value of 193 nM for *ee*AChE and 273 nM for *h*AChE), strong inhibition of BuChE (IC_{50} value of 73 nM for *eq*BuChE and 56 nM for *h*BuChE), moderate inhibition of Aβ aggregation (56.3% at 20 μM) and good antioxidant activity (3.28 trolox equivalent by ORAC assay). Molecular modeling studies in combination with kinetic analysis revealed that **4u** was a mixed-type inhibitor, binding simultaneously to catalytic anionic site (CAS) and the peripheral anionic site (PAS) of AChE. In addition, **4u** could chelate metal ions, reduce PC12 cells death induced by oxidative stress and penetrate the blood–brain barrier (BBB). Taken together, these results strongly indicated the hybridization approach is an efficient strategy to identify novel scaffolds with desired bioactivities, and further optimization of **4u** may be helpful to develop more potent lead compound for AD treatment.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia among the elderly, is a dreadful neurological illness characterized by loss of brain function, affecting memory, cognition, and behavior.[1] Although the etiology of AD remains elusive, several conditions, such as β-amyloid (Aβ) aggregates, soluble Aβ oligomers (AβOs), oxidative stress, dyshomeostasis of biometals, and levels of acetylcholine, and neuroinflammation, likely play significant roles in the pathogenesis of AD.[2,3] Many different approaches to the treatment of AD have been developed over the past several decades.[4–9] At present, there is no drug that can definitively cure Alzheimer's disease, and the primary therapeutic options currently approved by the U.S. Food and Drug Administration for the treatment of AD are acetylcholinesterase inhibitors (AChEIs), namely, tacrine, donepezil, rivastigmine, and galantamine.[10–12] Among them, donepezil is the most effective pharmacological agent for AD treatment. However, it is effective in reversing the symptoms for only a short period of time.

Current clinical therapy for AD patients is mainly based on the cholinergic hypothesis. This hypothesis asserts that the decline of acetylcholine (ACh) level lead to cognitive and memory deficits, and sustaining or recovering the cholinergic function is supposed to be clinically beneficial.[13] ACh can be degraded by two types of cholinesterases in the central nervous system (CNS), namely acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). The crystallographic structure of AChE reveals that it includes two separate ligand binding sites: a peripheral cationic site (PAS) at the entrance and a catalytic active site (CAS) at the bottom. Hence, inhibitors that bind to either site could inhibit the AChE.[14] In addition, the assumption that AChE promotes amyloid fibril formation by interaction through the PAS of AChE has led to the

Abbreviations: AchE, Acetylcholinesterase; BuChE, Butyrylcholinesterase; AD, Alzheimer's disease; Aβ, β-Amyloid; CAS, Catalytic anionic site; PAS, Peripheral anionic site; BBB, Blood–brain barrier; AβOs, Soluble Aβ oligomers; CNS, Central nervous system; NFTS, Neurofibrillary tangles; ChEs, Cholinesterases; ChEIs, Cholinesterase inhibitors; CDI, 1,1′-Carbonyldiimidazole; ORAC, Oxygen radical absorbance capacity; PAMPA, Parallel artificial membrane permeation assay.

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development of dual binding site inhibitors of both CAS and PAS.[15] For this reason, the dual binding inhibitors may be promising therapeutic strategy.[16,17] Furthermore, in healthy brains, AChE is more active than BuChE and can hydrolyze about 80% of ACh.[18–20] As AD progresses, the ability of BuChE significantly increases, and that of AChE diminishes in the hippocampus and temporal cortex. As a result, both AChE and BuChE are important targets in the therapy of AD.[21]

During aging, the endogenous antioxidant protection system progressively decays and may be further diminished in AD.[22,23] In fact, recent research had demonstrated that oxidative damage in cellular structures is an event that precedes the appearance of other pathological hallmarks of AD, namely, senile plaques and neurofibrillary tangles.[24,25] Many evidences proved that antioxidants could attenuate the syndrome of AD, and prevent the progression of the disease.[26] Thus, drugs with specifically antioxidants activity could be useful for either the prevention or the treatment of AD.[27]

Converging lines of evidences suggest that accumulation of Aβ peptide aggregation in the brain play an important role in the pathogenesis and development of AD, as its accumulation may result in senile plaque, neurofibrillary tangles, neuronal cell death, and ultimately dementia.[28,29] The $A\beta_{1-40}$ and $A\beta_{1-42}$ are the major components of amyloid plaques found in the brain of AD patients. However, $A\beta_{1-42}$ is more prone to self-assembly into fibrils and more neuronal toxicity compared to relatively soluble $A\beta_{1-40}$.[7,30] Therefore, the prevention of $A\beta_{1-42}$ aggregation currently attracts much attention.

Recent studies indicated that another hypothesis, called metal hypothesis, also contribute to AD pathology.[31] It is observed that abnormally high levels of metal ion (Fe, Zn, Cu) in the brain is closely to associated with the formation of Aβ aggregates and neurofibrillary tangles (NFTs), which the promoted inflammation of Aβ and activate neurotoxic pathways, leading to dysfunction and the death of brain cell.[31,32,26,33] Therefore, lowering of concentration of brain metals by chelating metals has been proposed as a rational therapeutic approach for halting AD pathogenesis.

Donepezil (1-benzyl-4-[5,6-dimethoxy-(1-indanone)-2-yl]methyl piperidine, **1**) also known as E2020, was found to be highly selective AChE inhibitor. Donepezil was approved by FDA in 1996 for the treatment of mild to moderate AD.[34,35] Donepezil is recognized by AChE active site by interactions with benzyl moiety (CAS of AChE), the atom of the piperidine (mid-gorge) and dimethoxyindanone moiety (PAS of AChE).[36] Melatonin (*N*-acetyl-5-methoxyltryptamine, **2**) is an endogenous neurohormone whose level decreases during aging, especially in AD patients.[6,7,18,20,37,38] It had been reported to possess strong antioxidant property and is able to directly scavenge a variety of reactive oxygen species.[39] Moreover, it has been demonstrated to be a moderate inhibitor of Aβ aggregation by disrupting Asp^{-}-His^{+} salt bridges of Aβ or affecting the synthesis and maturation of amyloid precursor protein (APP).[40]

Due to the pathological complexity found in AD, multifunctional molecules with two or more complementary biological activities may represent an important advance for the treatment of this disease.[41–44] In recent years, many interesting MTLs have been developed, such as tacrine–melatonin hybrid, melatonin–*N*, *N*-dibenzyl(*N*-methyl)amine hybrid, and curcumin-melatonin hybrid, among others.[45–47] Tacrine–melatonin hybrid is an AChE inhibitor with additional antioxidant and Aβ anti-aggregating properties.[48–50] Melatonin–*N*,*N*-dibenzyl(*N*-methyl)amine hybrid is able to reduce amyloid burden and behavioral deficits in a mouse model of AD.[10] Curcumin–melatonin hybrid show significant neuroprotection in MC65 cells. Our group has also reported several families of MTLs that combined neuroprotective, cholinergic, and antioxidant properties.

Continuing with our interest in the design, synthesis, and biological evaluation of multifunctional molecules, our work is currently focused on taking advantage of the potential neurogenic profile of melatonin-based hybrids, which are endowed with additional anticholinergic properties. Thus, in this work, we have designed new melatonin–donepezil hybrids (**4a**–**u**) by binding two fragments with interesting and complementary properties. The melatonin framework, in addition to its above-mentioned neurogenic profile, could demonstrate antioxidant and neuroprotective features and could also interact with the AChE-PAS via π–π aromatic stacking for its aromatic character, as we observed in the tacrine–melatonin series. The second selected fragment, the protonable *N*-benzyl piperidine which is present in the well-known AChE inhibitor donepezil can interact with AChE-CAS through cation–π interaction. The last feature of the designed compounds is the linker between the groups interact with PAS and CAS site (Fig. 1).

In this study, a series of new multifunction compounds by fusing donepezil with melatonin were designed and synthesized as multifunctional anti-AD agents. The pharmacological evaluation of these new compounds included AChE and BuChE inhibition, the kinetics of enzyme inhibition, self-induced Aβ aggregation, metal chelation, antioxidant, and neuroprotection.

2. Results and discussion

2.1. Chemistry

The synthetic pathways of the target and intermediate compounds were summarized in Scheme 1. The synthesis of target and intermediate compounds applied readily available starting material and was completed using well-established methods. Refluxing 4-piperidyl esters with benzyl bromide derivations (**1a**–**j**) in the presences of MeCN, K_2CO_3 under nitrogen for 8 h at 85 °C gave the respective intermediates **2a**–**j**.[10,51] Intermediates **2a**–**j** were then hydrolyzed to provide the acids **3a**–**j**.

Finally, activation of **3a**–**j** with 1,1′-carbonyldiimidazole (CDI) and subsequent coupling with tryptamine analogues afforded targets compounds **4a**–**s**. On the other hand, activation of 3-Indolepropionic acid (**3**) with 1,1′-carbonyldiimidazole (CDI) and subsequent coupling to **3t**–**u** provided target compound **4t**–**u**.[27]

2.2. Cholinesterase inhibitory activity

The inhibitory activities of hybrids **4a**–**u** against AChE (from electric eel) and BuChE (from equine serum) were measured according to the spectrophotometric method of Ellman et al.[52] For comparison purpose, tacrine and donepezil were used as reference compounds. The IC_{50} values of all tested compounds were summarized in Table 1. As illustrated in Table 1, it could be seen that all new target compounds showed good inhibitory activities to both ChEs with IC_{50} values ranging from sub-micromolar to nanomolar. All new target compounds displayed little fluctuation after the introduction of different substituents in the benzene or indole ring. Most of compounds exhibited higher inhibitory activity for BuChE than for AChE, indicating that these compounds were selective inhibitors for BuChE. According to the above results, substituents with varying electronic properties were introduced to the benzene ring. Compared with **4a**, **4h**, **4j** and **4l** bearing a chlorine group exhibited a decrease in AChE inhibition. However, the compounds **4b**, **4d** and **4f** with a methyl group was a slight increase in AChE inhibition. The compound **4b** bearing a methyl group at the 2-position of the phenyl group is more active than the compound **4d** with a methyl group at the 3-position of the phenyl group and the compound **4f** with a methyl group at the 4-position of the phenyl group. These results implied that electron-donating