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Research paper

Design, synthesis and biological evaluation of new coumarin-dithiocarbamate hybrids as multifunctional agents for the treatment of Alzheimer's disease

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ABSTRACT

A series of new coumarin-dithiocarbamate hybrids were designed, synthesized and evaluated as multifunctional agents for the treatment of Alzheimer's Disease (AD). The biological assays indicated that most of them showed potent inhibition and excellent selectivity towards acetylcholinesterase (AChE), and could inhibit self-induced β-amyloid (Aβ) aggregation. Especially, compound **4n** presented the highest ability to inhibit AChE (IC_{50}, 0.027 μM for hAChE) and good inhibition of Aβ aggregation (40.19% at 25 μM). Kinetic and molecular modeling studies revealed that **4n** was a mixed-type inhibitor, which could interact simultaneously with the catalytic active site (CAS) and peripheral anionic site (PAS) of AChE. In addition, it also possessed specific metal-chelating ability, good BBB permeability and low toxicity on SH-SY5Y neuroblastoma cells. Moreover, compound **4n** did not exhibit any acute toxicity in mice at doses up to 1000 mg/kg, and could reverse the cognitive dysfunction of scopolamine-induced AD mice. As far as we know, **4n** was the first reported dithiocarbamate derivative with multifunctional activity. Its excellent profiles in vitro and effectivity in vivo highlight this structurally distinct compound as a potential lead compound in the research of innovative multifunctional drugs for AD.

1. Introduction

Alzheimer's disease (AD), characterized by memory loss, decline in language skills and many other cognitive impairments, is an age-related neurodegenerative disorder and a global public health issue [1,2]. Today, the dementia number is estimated 46 million people worldwide and that is expected to reach 131.5 million by 2050 [3]. Due to the complexity and unidentified etiopathogenesis, many factors are thought to be related to the initiation and development of AD, including deficits of acetylcholine (ACh), amyloid-β (Aβ) peptide deposits, dyshomeostasis of biometals, oxidative stress and hyperphosphorylated tau protein [4].

Among the various pathogenic factors of AD, current clinical treatment of AD has mainly focused on deficits of acetylcholine (ACh). The palliative drugs approved by the FDA consists of four acetylcholinesterase inhibitors (AChEIs), tacrine (now withdrawn the market due to hepatotoxicity), donepezil, rivastigmine and galantamine (Fig. 1a) [5]. Base on cholinergic hypothesis, the decline levels of ACh leads to memory deficits and cognitive impairments, and reducing the ACh metabolism is beneficial to improvement in memory and cognitive dysfunction [6]. There are two types of cholinesterases that can hydrolyze ACh in the central nervous system (CNS), namely acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) [7]. Although a few studies suggested that BuChE played a significant role in AD pathophysiology, the mechanism of BuChE was not yet completely identified. In

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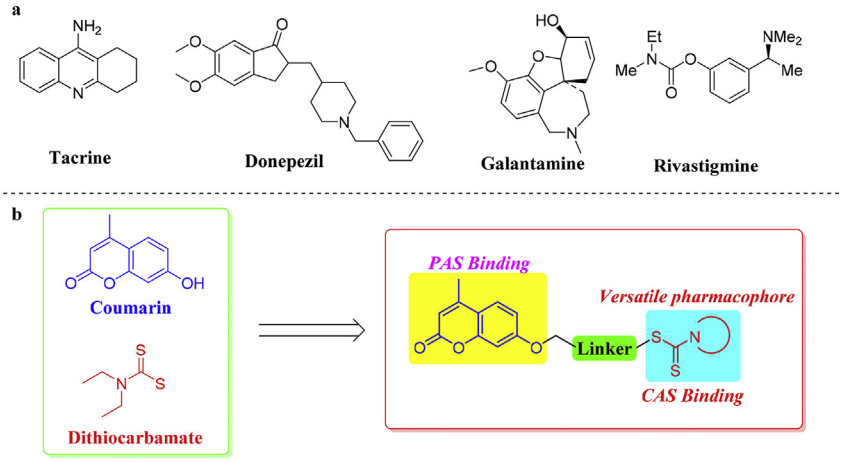

Fig. 1. (a) AChE inhibitors used for the treatment of AD. (b) Design strategy for coumarin-dithiocarbamate hybrids.

addition, BuChE primarily distributes in peripheral system such as plasma, liver and muscle tissues, and accordingly BuChE inhibitor may potentially result in peripheral side effects. For example, tacrine, a dual AChE and BuChE inhibitor, has worst hepatotoxicity and other peripheral adverse reaction [8,9]. Therefore, discovery of effective and selective AChE inhibitors with lower side effects may be more valuable for the treatment of AD. The crystallographic structure of AChE reveals that it has a nearly 20 Å deep narrow gorge which consists of two binding sites: a catalytic active site (CAS) at the bottom of the gorge and a peripheral anionic site (PAS) near the entry of the gorge [10,11]. Research suggest that AChE inhibitors should contain a core ring system that binds to PAS, a basic center that interact with CAS, and a linker, such as, CH_2, −S−, -O-, and CONH(CH_2)n, etc., between the core ring system and the basic center to fulfil the structural requirements [12].

Another significant hypothesis reveals that Aβ plaques in the brain plays a crucial role in AD pathogenesis. The amyloid precursor protein (APP) is hydrolyzed by α, β, γ-secretase enzymes to produce Aβ peptides that can aggregate into monomers, oligomers and large Aβ plaques [13,14]. These aggregates can initiate pathogenic cascade and ultimately lead to the neuronal loss and dementia [15]. The Aβ peptides have two key isoforms: $Aβ_{1-40}$ and $Aβ_{1-42}$. $Aβ_{1-42}$ shows lower solubility and is more prone to aggregate into fibrils than $Aβ_{1-40}$ [16]. The Aβ plaques generated from $Aβ_{1-42}$ lead to severe neuronal toxicity [17]. Hence, the prevention of $Aβ_{1-42}$ aggregation could serves as a rational strategy for the treatment of AD. Studies also reveal that AChE can facilitate amyloid fibril formation through the interaction with the PAS of AChE, giving stable AChE-Aβ complexes, which are more toxic than single Aβ peptides. Dual-site inhibitors that bind simultaneously to the PAS and CAS of AChE can not only stimulate the cholinergic system, but also inhibit the Aβ aggregation promoted by AChE [18]. Therefore, dual-site inhibitors are thought to be more promising anti-AD drug candidates.

Recently, many studies show that there are excessive bio-metal ions (Cu, Zn, Fe) in the brains of AD patients, which is several-fold higher than that of healthy person [19]. The excessive metal ion is able to interact with Aβ peptides and then accelerate the Aβ aggregates and neurofibrillary tangles, which lead to dysfunction and neuron death [20]. On the other hand, excess of these redox-active metals that interact with Aβ is able to contribute to the production of reactive oxygen species (ROS) and cause the oxidative damage of the central nervous systems (CNS) [21,22]. Hence, modulating these biometal ions in the brain may be a potential therapeutic method for the AD treatment.

So far, there are only four AChE inhibitors and one N-methyl-D-aspartate receptor antagonist approved by FDA for clinical treatment of AD. Although these drugs can temporarily improve the cognitive and daily function, they cannot mitigate or halt the progression of AD. Due to the complex etiology disease networks, an efficient therapy is more inclined to discover multifunctional drugs that can simultaneously modulate the complex etiology networks. Therefore, many medicinal chemists have made great efforts to develop multifunctional molecules by incorporating several different active structural fragments into one molecule for the treatment of multi-factor neurodegenerative diseases [23,24].

Coumarins, an important group of naturally occurring compounds with small molecular weight, exist in various plant species. Coumarins have attracted increasingly attention in recent years due to a wide of biological activities related to neurological disorders [24–28], such as AChE inhibition, anti-Aβ aggregation and MAO-B inhibition. Our previous studies demonstrated that coumarin was able to bind to the PAS of AChE *via* aromatic π-π stacking interactions [24,29] and therefore could serve as one part of the dual-binding mode of action. On the other hand, dithiocarbamate is a versatile pharmacophore and has been widely used for drug design [30]. However, to our knowledge, there are few reports about the research on dithiocarbamate derivatives against AD. Recently, we found that dithiocarbamate could serve as the other part that bind to the CAS of AChE. Meanwhile, considering the broad activities of dithiocarbamate, we reasoned that compounds containing this group might exert multifunctional activity for the treatment of AD.

Therefore, to further find new dual-acting AChE inhibitors with potential multifunctional activity, in this study, we attempted to connect coumarins (binding to PAS) with the dithiocarbamate moieties (binding to CAS) to design a series of new coumarin-dithiocarbamate hybrids as multifunctional agents (Fig. 1b). All designed compounds were synthesized and evaluated for ChE inhibition and anti-Aβ aggregation. Besides, the outstanding compound was selected for further evaluation including metal chelation, the ability to cross the blood-brain barrier (BBB) in vitro, acute toxicity and neuroprotective effects in scopolamine-induced cognitive impairment in mice. Furthermore, kinetic and molecular modeling studies were also carried out to investigate the binding mode of compounds with AChE.