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Design, synthesis and biological evaluation of 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives as multifunctional agents for the treatment of Alzheimer's disease

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ABSTRACT

A series of 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives was designed, synthesized and evaluated as multi-function inhibitors for the treatment of Alzheimer's disease (AD). The results revealed that compound **TM-3** indicated selective AChE inhibitory potency (*ee*AChE, IC_{50} = 0.69 μM, selective index (SI) = 32.7). Both kinetic analysis of AChE inhibition and molecular modeling study suggested that **TM-3** could simultaneously bind to the catalytic active site and peripheral anionic site of AChE. And **TM-3** was also a highly selective MAO-B inhibitor (IC_{50} = 6.8 μM). Moreover, **TM-3** could act as antioxidant (ORAC value was 1.5*eq*) and neuroprotectant, as well as a selective metal chelating agent. More interestingly, compound **TM-3** could cross the blood-brain barrier (BBB) *in vitro* and abided by Lipinski's rule of five. Therefore, compound **TM-3**, a promising multi-targeted active molecule, offers an attractive starting point for further lead optimization in the drug-discovery process against AD.

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by deterioration of memory, language skills, and other cognitive impairments in elder people. This situation intensely influences the patient's social life and activity. It is evaluated by World Alzheimer's Report that more than 35 million people suffered from AD in 2015, and this number will be double by 2030 and approximately triple to 131 million by 2050.[1] Current clinical treatment of AD is mainly focused on controlling symptoms by providing temporary improvement. To date, there are four acetylcholinesterase inhibitors (AChEIs), named donepezil, tacrine, rivastigmine, galantamine and one *N*-methyl-D-aspartate (NMDA) receptor antagonist memantine have been approved for the treatment of AD. Unfortunately, these drugs are just palliative treatment and do not address the molecular mechanisms that underlie the pathogenic processes due to the multifactorial nature of AD.[2]

The etiology of AD is not completely known, but several characteristic pathological features such as β-amyloid (Aβ) deposits, tau protein (τ) aggregation, oxidative stress, decreased level of acetylcholine (ACh), neuroinflammation, and dyshomeostasis of biometals have been thought to play significant roles in the pathogenesis of AD.[3] In this regard, one single drug that acts on a specific target to produce the desired clinical effects might not be suitable for the complex nature of AD, the development of multitarget-directed ligands (MTDLs), i.e., single chemical entities able to hit different targets involved in the cascade of AD pathological events, to act as multifunctional agents to treat this disease has been applied by many research groups, and the results obtained have been encouraging and convince researchers that MTDLs might present the best pharmacological option for tackling the multifactorial nature of AD and for halting the progression of the disease.[4–6] Several

Abbreviations: AD, Alzheimer's disease; AChEIs, acetylcholinesterase inhibitors; NMDA, *N*-methyl-D-aspartate; Aβ, β-amyloid; τ, tau protein; ACh, acetylcholine; MTDLs, multi-target-directed ligands; MAO-B, monoamine oxidase B; MAO-A, monoamine oxidase A; BuChE, butyrylcholinesterase; *Ee*AChE, *Electrophorus electricus* AChE; eqBuChE, *equine serum* BuChE; PAS, peripheral anionic site; CAS, catalytic active site; ORAC-FL, oxygen radical absorbance capacity by fluorescein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; PAMPA-BBB, parallel artificial membrane assay for the blood-brain barrier; SD, standard deviation; MW, molecular weight; TPSA, topological polar surface area; ADME, absorption, distribution, metabolism, excretion.

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MTDLs candidate drugs with disease modifying potential are now in the pipeline and have reached testing stage in clinical trials.[7] Therefore, the discovery of a lead compound that can modulate multi-factors simultaneously is a crucial step in the search for a candidate for the clinical treatment of AD.

2-Acetyl-5-alkoxyphenol analog **DDDT-2d**, is a potent and selective MAO-B inhibitor, the IC_{50} values of MAO-B was 2.9 nM, as well as 17,000-flod selective for MAO-B over the MAO-A.[8] Recent studies shows that the selective MAO-B inhibitor has been shown to significantly improve learning and memory deficits in animal models associated with AD and to slow the disease progression in AD patients,[9] and seem to be an important treatment of AD.[10] So, it is appropriate for the design of therapies for neurodegenerative disorders such as Alzheimer's disease. Recently, our group has reported the synthesis of scutellarein—*O*-alkylamine derivatives and discovered representative **EJMC-16d** as a potential multifunctional agent for the treatment, and the alkylamine side chain is required pharmacophore for the AChE inhibition.[11] Therefore, in this paper, 2-acetylphenol was selected to combine with different length alkylamine fragment to design a series of novel 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives, to evaluate whether these novel molecules might possess more potency in various multifunctional activities.

In this study, we report the study of the design, synthesis and evaluation of a series of 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives based on MTDLs (Fig. 1). They were found to show potentially applicable biological activities, including inhibition of ChEs (AChE and BuChE) and MAOs (MAO-A and MAO-B), antioxidant properties, neuroprotective effects, and metal chelation.

Total 20 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives were synthesized in good yields. The synthetic pathway of these target compounds was outlined in Scheme 1. Briefly, the starting material **1** was treated with excessive amounts of 1,3-dibromopropane (**2a**), 1,4-dibromobutane (**2b**), 1,5-dibromopentane (**2c**) or 1,6-dibromohexane (**2d**) in the presence of K_2CO_3 in CH_3CN at 60–65°C to obtain the intermediates **3a**–**3d**. Subsequently, the target compounds **TM-1**–**TM-20** were got by the reaction of intermediates **3a**–**3d** with secondary amines (**A**–**E**) in the presence of K_2CO_3 in anhydrous CH_3CN at 60–65 °C. All target compounds were purified by chromatography, and the analytical and spectroscopic data confirmed their structures, as detailed in the experimental section.

The 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives **TM-1**–**TM-20** were tested *in vitro* for their cholinesterase inhibitory activities were determined by the modified Ellman method using *ee*AChE (from *electric eel*) and *eq*BuChE (from *equine serum*).[12] Donepezil, a well-known cholinesterase inhibitor approved by FDA, was used as the positive control, and the precursor compound 2,4-dihydroxyacetophenone (**1**) was also tested, the results are listed in Table 1. Compared with compound **1** and donepezil, all the target compounds were selective AChE inhibitors, and showed moderate to good *Ee*AChE inhibition potent with IC_{50} values ranging from 0.96 to 57.0 μM. It revealed that the introduction of *O*-alkylamines increased ChEs inhibitory activity and improved the selectivity for *ee*AChE over *eq*BuChE, which was consistent with our previous work.[11,13,14] The selectivity may be helpful to diminish peripheral cholinergic side effects. The results data also displayed that both the structure of terminal groups NR^1R^2 of side chain and the methylene chain length significantly affected the AChE inhibitory potencies. When the terminal groups NR^1R^2 was piperidine and pyrrolidine, the AChE inhibitory activity remarkably enhance as the methylene chain increases, for example, **TM-1** < **TM-6** < **TM-11** < **TM-16**; **TM-2** < **TM-7** < **TM-12** < **TM-17**, while the compounds containing two basic centers (benzylpiperazine, 1-(2-pyridyl)piperazine and 2-(1-piperazinyl)pyrimidine group) demonstrated the opposite result, that is, the inhibitory activity decrease gradually with the increase of methylene chain, such as **TM-3** > **TM-8** > **TM-13** > **TM-18**; **TM-4** > **TM-9** > **TM-14** > **TM-19**; **TM-5** > **TM-10** > **TM-15** > **TM-20**. This phenomenon revealed that the alkylamine side chain can interact with catalytic active site (PAS) of AChE in different ways. In addition, in the same methylene chain length situation, and for the compounds possessing piperazine groups, benzylpiperazine indicated better inhibitory potency than the other groups. From the screening data, all the target compounds showed weak BuChE inhibitory potency, and were significantly selective AChE inhibitors. Especially, compound **TM-3** was the best AChE inhibitor with IC_{50} value was 0.96 μM, and the selective index was 32.7.

The Lineweaver–Burk plots (Fig. 2) showed that both inhibitions had rising slopes and increasing intercepts at higher concen-

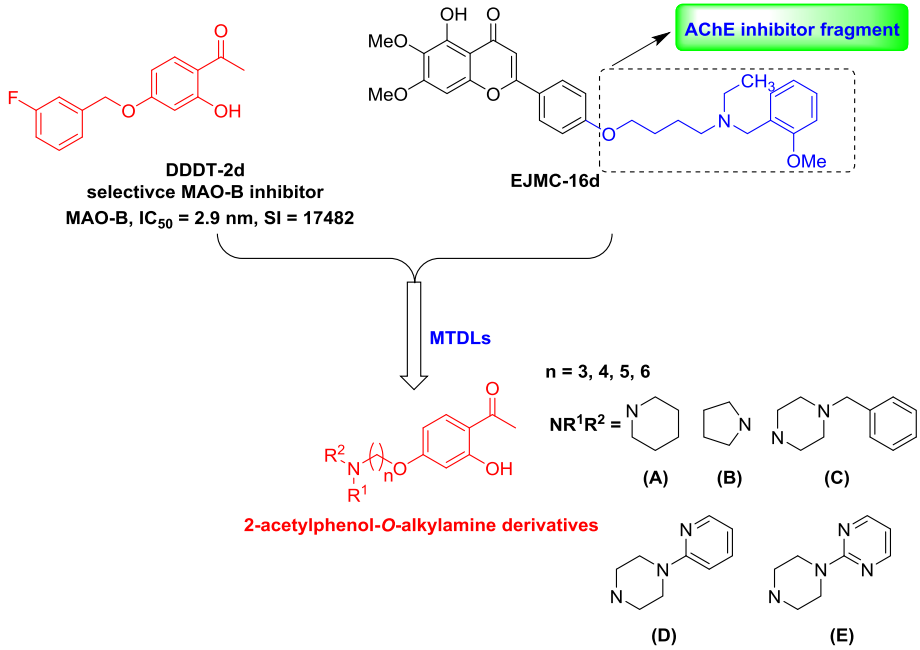

Fig. 1. Design strategy for the 2-acetyl-5-*O*-(amino-alkyl)phenol derivatives.