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Synthesis and evaluation of 4-hydroxyl aurone derivatives as multifunctional agents for the treatment of Alzheimer's disease

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ABSTRACT

A series of 4-hydroxyl aurone derivatives were designed synthesized and evaluated as potential multifunctional agents for the treatment of Alzheimer's disease. The results demonstrated that most of the derivatives exhibited good multifunctional properties. Among them, compound **14e** displayed good inhibitory activities of self- and Cu^{2+}-induced $A\beta_{1-42}$ aggregation with 99.2% and 84.0% at 25 μM, respectively, and high antioxidant activity with a value 1.90-fold of Trolox. In addition, **14e** also showed remarkable inhibitory activities of both monoamine oxidase A and B with IC_{50} values of 0.271 μM and 0.393 μM, respectively. However the 6-methoxyl aurones **15a–c** revealed excellent selectivity toward MAO-B. Furthermore, the representative compounds **14e** and **15b** displayed good metal-chelating abilities and blood–brain barrier (BBB) permeabilities in vitro.

1. Introduction

Alzheimer's disease (AD), an age-related neurodegenerative disorder of the central nervous system (CNS), is characterized by insidious onset of memory loss, progressive cognitive deterioration, impairment of activities of daily living and loss of independent function.[1] In addition, 50–80% of AD patients suffer from behavioral and psychological symptoms such as depression, psychosis and agitation.[2] Although the etiology of AD remains elusive, multiple factors such as β-amyloid (Aβ) deposits, τ-protein aggregation, oxidative stress, dyshomeostasis of biometals and decreased levels of acetylcholine (ACh) are considered to play vital roles in the pathophysiology of AD.[3] According to the amyloid cascade hypothesis, the increased production and accumulation of Aβ oligomeric aggregates in the brain are considered as a central event in the pathogenesis of AD, initiating the pathogenic cascade and ultimately leading to neuronal loss and dementia.[4] The $A\beta_{1-40}$ and $A\beta_{1-42}$ are the major isoforms of Aβ peptides. $A\beta_{1-40}$ is expressed in larger amounts in the brain, yet $A\beta_{1-42}$ displays lower solubility and more neurotoxic and has a higher tendency to aggregate.[5,6] Therefore, preventing the $A\beta_{1-42}$ aggregation is a potent therapeutic strategy for AD treatment.

In addition, the dyshomeostasis of metal ions such as Cu^{2+}, Fe^{2+}, Zn^{2+} and Al^{3+} clearly occurs in AD brains.[7] Studies suggest that excess of these metal ions have been found in Aβ plaques and they are closely associated with the formation of Aβ plaques and neurofibrillary tangles.[8] Moreover, the abnormally high levels of these redox-active metals could promote the production of reactive oxygen species (ROS) and oxidative stress.[9] However, oxidative stress is one of the earliest events in the pathogenesis of AD and may occur before the onset of memory loss, the appearance of senile plaques and neurofibrillary tangles.[10] And oxidative damage plays a crucial role in neuronal degeneration, which can harm biological molecules such as proteins, DNA, and lipids.[11,12] It is demonstrated that Aβ catalyses the reduction of Cu^{2+} and Fe^{3+}, which could lead to the production of ROS. In turn, oxidative stress may promote Aβ accumulation by generating the modified Aβ species which are prone to aggregate and resistant to clearance.[13] Thus, biometals chelators and antioxidants have been proposed as potential therapeutic agents against AD.

Monoamine oxidase A and B (MAO-A and -B) are flavin adenine dinucleotide (FAD)-containing enzymes, bound to the membrane surface of mitochondria and involved in the degradation of neurotransmitters and xenobiotic amines. These enzymes are responsible for regulation and metabolism of major monoamine neurotransmitters such as serotonin, dopamine and noradrenaline.[14] Selective MAO-A inhibitors are used in clinical practice for the treatment of depression and anxiety, while MAO-B inhibitors are used to slow down the progression of Parkinson's disease

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(PD) and symptoms associated with AD. However, the biochemical activity of MAO generates hydroxyl radicals, which are harmful members of the oxygen free radical group involved in many neurodegenerative disorders like AD.[15] Recently, MAO inhibitors have been re-evaluated as potential therapeutics against many age-related pathologies, for example AD and PD. And in these diseases, neurotoxicity, protein misfolding and/or aggregation, iron accumulation, mitochondrial damage, and oxidative stress have been considered as major downstream causes.[16,17] As mentioned above, MAO could be a potential therapeutic target of AD and other psychiatric and neurological diseases. In fact, selective MAO-B inhibitors such as selegiline and rasagiline are beneficial for the treatment of PD and AD, and selective MAO-A inhibitors such as clorgyline and moclobemide are useful for the treatment of neurological disorders, for example, depression and anxiety.[15]

Because of the complicated and multifactorial etiology, the 'one-molecule, one-target' paradigm seems not so effective in treating complex diseases like AD. It can only improve clinical symptoms but cannot mitigate progression of the disease. Therefore, with the development of multi-target-directed ligands (MTDLs), it is significant to develop novel multifunctional drugs with two or more complementary bioactivities for the treatment of complex diseases as AD.[18–20]

Aurones, 2-benzylidenebenzofuran-3(2*H*)-ones, which are structural isomerides of flavones, are present in vegetables and flowers (Fig. 1, **1**).[21] Aurones have attracted considerable attentions in recent years because they possess a wide range of bioactivities associated with neurological diseases especially for AD. Many studies have shown that naturally occurring aurones as well as the chemically synthesized analogs exhibited high affinities toward Aβ aggregates[22–24] and good MAO inhibitory activities.[25,26] These results suggest that aurone could be an excellent leading scaffold to design multifunctional drugs against AD. Recently, Masahiro Ono et al. reported an aurone analog, which possess a radioiodine at 5-position and a dimethylamino group 4′-position (Fig. 1, **2**). It could serve as a probe of Aβ plaques in AD, with high binding affinity (K_i = 6.82 nM).[23] In addition, some studies have shown that electron-donating groups like dimethylamino group are closely associated with the binding affinity to Aβ aggregates.[27] Collectively, we designed a series of aurone derivatives that are expected to be multifunctional agents with anti-Aβ aggregation, MAO inhibition, antioxidant and biometal chelating properties (Fig. 2). Our derivatives reported in this study are either novel compounds, or although known substances[28–31] their testing as multifunctional anti-AD agents has not been reported.

2. Results and discussion

2.1. Chemistry

The synthetic pathways of target derivatives were summarized in Schemes 1 and 2. Substituted benzaldehydes **8b**–**h** were synthesized according to well-established method.[32] The key intermediate **5** was produced from phloroglucinol **3** after Fries rearrangement and cyclization reaction. Compound **7** was obtained by the dimethylation of **5** and subsequent selective deprotection due to the difficulty of direct transformation from **5** to **7**.[33] Target compounds **14a**–**h** and **15a**–**c** were afforded by the condensation of **5** or **7** with corresponding substituted benzaldehydes **8a**–**h** in ethanolic 50% KOH solution. As reported in many literatures, only *Z* stereoisomers were isolated. The stereochemistry of the aurone diastereomers has been elucidated by NMR spectroscopic measurements and by X-ray diffraction analysis.[29,34–36] Compound **16a** and **16b** were obtained through catalytic hydrogenation of **14a** and **14f** with 10% Pd/C as catalyst in THF. The synthesis of **11** was reported by our group previously.[6] The condensation of **11** with *p*-dimethylaminobenzaldehyde (**8a**) in ethanolic 50% KOH solution afforded chalcone **12**. Subsequently, cyclization of **12** with mercuric acetate in pyridine produced compound **13**, deprotection of which afforded the target compound **17**. All the aurone derivatives were characterized by ^{1}H NMR and HR-ESI-MS, and parts of them were further characterized by ^{13}C NMR.

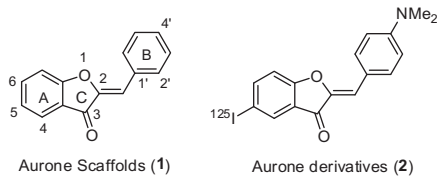

Figure 1. Aurone scaffolds (**1**) and aurone derivative (**2**) as Aβ plaques probes.

2.2. Pharmacology

2.2.1. Inhibition of self- and Cu^{2+}-induced Aβ_{1-42} aggregation

The inhibition of self- and Cu^{2+}-induced Aβ_{1-42} aggregation by our synthetic derivatives was determined by using thioflavin T (ThT) assay, with curcumin as a reference compound.[37,38] The results summarized in Table 1 indicated that these aurone derivatives exhibited moderate to good inhibitory activities of self- and Cu^{2+}-induced Aβ_{1-42} aggregation (22.3–99.8% and 20.9–90.1% at 25 μM, respectively) compared with that of curcumin (41.3 ± 0.9% and 67.2 ± 1.3%, respectively). Noticeably, the potencies of dihydroxy aurones **14a**–**h** were much higher than those of corresponding monohydroxy aurones and the reduction products. It revealed that 6-hydroxy group in aurone may strengthen the interaction between the derivatives and Aβ_{1-42} protein and the α, β-unsaturated ketone skeleton of aurone was essential for the potency. From the comparison between the potencies of **14a** and **14h**, we could find that the substitution position of amino groups may have no obvious impact on the inhibitory activities of Aβ_{1-42} aggregation. The results of **14a**–**h** demonstrated that, unlike hydroxyl group, diverse amino groups showed no significant difference between the effects on potencies (except for **14f** and **14g**). Compared the potencies of **14a** and **17**, we could find that the transformation of 6-OH to 6-N$(CH_3)_2$ lowered the inhibitory activity, further indicating the importance of 6-OH group.

2.2.2. Recombinant human MAO-A and –B inhibition studies

Subsequently, we evaluated these compounds for their potential MAO-A and -B inhibitory properties. Recombinant human MAO-A and -B were employed as enzyme sources, with clorgyline, rasagiline and iproniazid as reference compounds. Kynuramine, the common substrate for MAO-A and -B, was used for the enzyme activity measurement of both isozymes. Kynuramine is oxidized by the isozymes to yield 4-hydroxyquinoline which fluoresces in the alkaline medium used to terminate the enzymatic reactions.[39–41] The concentrations of 4-hydroxyquinoline in the reactions were measured using fluorescence spectrophotometry without interference from kynuramine or test compounds. We first tested our derivatives at the concentration of 10 μM for preliminary screening. The samples with the inhibition ratios greater than 50% would be retested for the IC_{50} values at various concentrations. All determinations were carried out in triplicate. The results were summarized in Table 1. As it shown, most of the aurone derivatives were effective in inhibiting MAO in submicromolar range. Compound **14b** was the most potent MAO-A inhibitor with an IC_{50} of 0.0279 ± 0.004 μM. And the most potent MAO-B inhibitor was **15b** (IC_{50} = 0.226 ± 0.017 μM). From the results of **14a**–**g**, we could find that cyclic amine substituted aurones exhibited MAO-B selective inhibitory activities, while the non-cyclic amine substituted