Bioorganic & Medicinal Chemistry 25 (2017) 5917–5928

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis, and evaluation of salicyladimine derivatives as multitarget-directed ligands against Alzheimer's disease

Hua-Li Yang [a,b], Pei Cai [b], Qiao-Hong Liu [b], Xue-Lian Yang [b], Si-Qiang Fang [b], Yan-Wei Tang [b], Cheng Wang [b], Xiao-Bing Wang [b,*], Ling-Yi Kong [a,b,*]

[a] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] Jiangsu Key Laboratory of Bioactive Natural Product Research and State Key Laboratory of Natural Medicines, Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 18 July 2017
Revised 24 August 2017
Accepted 29 August 2017
Available online 27 September 2017

Keywords:
Alzheimer's disease
Monoamine oxidases
$A\beta_{1-42}$ aggregation
Antioxidant
Metal chelation

ABSTRACT

A series of salicyladimine derivatives were designed, synthesized and evaluated as multi-target-directed ligands for the treatment of Alzheimer's disease (AD). Biological activity results demonstrated that some derivatives possessed significant inhibitory activities against amyloid-β (Aβ) aggregation and human monoamine oxidase B (*h*MAO-B) as well as remarkable antioxidant effects and low cell toxicity. The optimal compound, **5**, exhibited excellent potency for inhibition of self-induced $A\beta_{1-42}$ aggregation (91.3 ± 2.1%, 25 μM), inhibition of *h*MAO-B (IC_{50}, 1.73 ± 0.39 μM), antioxidant effects (43.4 ± 2.6 μM of IC_{50} by DPPH method, 0.67 ± 0.06 trolox equivalent by ABTS method), metal chelation and BBB penetration. Furthermore, compound **5** had neuroprotective effects against ROS generation, H_2O_2-induced apoptosis, 6-OHDA-induced cell injury, and a significant *in vitro* anti-inflammatory activity. Collectively, these findings highlighted that compound **5** was a potential balanced multifunctional neuroprotective agent for the development of anti-AD drugs.

1. Introduction

Alzheimer's disease (AD), an age-related progressive neurodegenerative disease and complicated multi-factorial disorder, is one of the most common forms of dementia.[1] It is characterized by memory loss, decline in language skills and other cognitive impairments in performing daily activities along with depression.[2] The disorder has shown a surge in the overall mortality rate by over 66%.[3] Increased oxidative stress, dyshomeostasis of biometals, and the formation of toxic Aβ peptide oligomers are all considered to contribute to the etiology of AD.[4,5]

One of the main pathological features of AD is the formation of senile plaques (SP), which caused by amyloid-β (Aβ) deposition.[6] The accumulation of toxic Aβ (especially $A\beta_{1-42}$) peptide oligomers results in a cascade of biochemical processes mediating the formation of reactive oxygen species (ROS), calcium dysregulation, and neuronal cell membrane damage leading to neuronal dysfunction.[7] Therefore, prevention and clearance of Aβ aggregation have been considered as the primary therapeutic strategy for the neurodegenerative disease.[8]

Oxidative stress is one of the earliest events in AD pathogenesis.[9] As a factor involved in oxidative stress, monoamine oxidases (MAOs) have drawn much attention in recent years.[10,11] High expression level of MAOs in neuronal tissue relates to the production of hydrogen peroxide (H_2O_2), resulting in oxidative damage and apoptotic signaling events.[12,13] MAOs exist as two isoforms, MAO-A and MAO-B, which exhibit different substrates and inhibitor specificities.[14] Human MAO-A (*h*MAO-A), preferentially degrading serotonin, adrenaline and noradrenaline, has a close correlation with depression, whereas human MAO-B (*h*MAO-B) is specifically responsible for the neurodegenerative disease such as AD.[15] Thus, selective *h*MAO-B inhibitors are considered as potential candidates for anti-AD drugs.

In addition, dyshomeostasis of biometals is suggested to play key roles in the pathogenesis of AD. High levels and miscompartmentalization of metal ions (iron, copper, zinc, etc.) in the brain can affect the oxidative stress response of mitochondria and protein misfolding, and ultimately lead to neurodegeneration.[16,17] Therefore, metal chelators have shown promise in the treatment of AD owing to the increased level of metal ions.

* Corresponding authors at: School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China (L.-Y. Kong).
E-mail addresses: xbwang@cpu.edu.cn (X.-B. Wang), cpu_lykong@126.com (L.-Y. Kong).

https://doi.org/10.1016/j.bmc.2017.08.048
0968-0896/

The poor clinical efficacy of single-target drugs raised the possibility that AD treatments may have to interfere concurrently with more than one neuropathological mechanism to exert disease modifying benefits. Thus, the multi-target-directed ligand (MTDL) approach, aiming for this multifaceted disease, has emerged as a new paradigm in drug discovery.[18,19]

Resveratrol (*trans*-3,4,5-trihydroxylstilbene), mainly founded in grapes and red wine, has shown beneficial effects against neurodegeneration thanks to its antioxidant and antiinflammatory properties.[20] Meanwhile, resveratrol has been extensively investigated as an inhibitor of Aβ_{1-42} aggregation.[21] 7-(3-chlorobenzyloxy)-4-formylcoumarin (**L1**), the cocrystallized ligand in complex with the human MAO-B active site (PDB: 2V60), has been identified as a new type of excellent MAO-B inhibitors.[22] The benzyloxy group was considered important for MAO-B inhibitory properties.[23,24] The metal-chelating agent, clioquinol (CQ, 5-chloro-7-iodo-8-hydroxyquinoline), demonstrated a significant improvement in cognition and memory by redistributing metal ions and decreasing plasma Aβ levels in Phase II clinical trials.[25–27]

Previously, our group developed several multifunctional frameworks based on coumarin-benzyloxy and resveratrol-clioquinol scaffolds for the treatment of neurodegenerative disease.[24,28–30] On these bases, we report herein a series of salicyladimine derivatives as multifunctional anti-AD agents by fusing the structures of resveratrol, benzyloxy and clioquinol (Fig. 1). These ligands exhibited excellent biological activities, including the inhibition of self-induced Aβ_{1-42} aggregation, inhibition of *h*MAO-B activitity, metal chelation, antioxidant, neuroprotection and ability to cross the blood-brain barrier (BBB).

2. Results and discussion

2.1. Chemistry

The synthetic convergent route for accessing the desired compounds **1**–**18** is shown in Scheme 1. *p*-Nitrophenol **1a** was used as starting material for the preparation of the key intermediate **2a**, by a *O*-alkylation reaction with benzyl bromide **1b** in the presence of anhydrous K_2CO_3. The aromatic amine **3a** was obtained by treating **2a** with a catalytic amount of 5% Pd/C under hydrogen atmosphere.[31] The target compounds **1**–**18** were synthesized by the classical method of imine formation involving condensation between **3a** with a variety of salicylaldehyde derivatives in ethanol under room temperature.[29,30,32] All target compounds were elucidated by spectroscopic measurements (IR, HRMS, ^{1}H NMR and ^{13}C NMR).

2.2. Biological assays

2.2.1. Inhibition of self-induced Aβ_{1-42} aggregation

The inhibitory activities of the target compounds **1**–**18** on Aβ_{1-42} self-induced aggregation were first determined by a thioflavin T (ThT) fluorescence assay using resveratrol as reference compounds (Table 1).[27,33] Most of the target compounds significantly inhibited self-induced Aβ_{1-42} aggregation at the tested concentrations. A structure-activity relationship analysis indicated that the property of substituents in salicyladimine derivatives had a significant impact on Aβ_{1-42} inhibitory activity. Compounds **13**, and **17**, which have a strong electron-withdrawing group at the R^3 position, exhibited much higher inhibitory activities (84.4 ± 2.0%, 70.2 ± 1.6% at 25 μM, respectively) than resveratrol (65.1 ± 1.3%). The number of phenolic hydroxyl group was also crucial for proper biological activity. Compounds **5** and **6** (91.3 ± 2.1%, 88.5 ± 1.8% at 25 μM, respectively) with three phenolic hydroxyl groups had more potent inhibition of self-induced Aβ_{1-42} aggregation than compounds **2**–**4** (72.5 ± 1.3%, 59.8 ± 0.8% and 49.2 ± 1.1% at 25 μM, respectively) with two phenolic hydroxyl groups.

The potent inhibitor **5** was selected with X-ray crystal structure of the protein Aβ_{1-42} structure (PDB code 1IYT) for molecular docking studies because of its remarkable performance regarding self-induced Aβ_{1-42} aggregation inhibition. As shown in Fig. 2A and B, the benzene ring of compound **5** interacted with the residue Phe19 via π-π stacking interaction. Hydrogen bond interactions were formed between phenolic hydroxyl groups of compound **5** and the residues Asp23, Ser26. These results indicated that the π-π stacking interaction and hydrogen bond interactions played important roles in the inhibitory activity of compound 5 on Aβ_{1-42} self-induced aggregation.

2.2.2. Inhibition of MAOs in vitro

The *h*MAO-A and *h*MAO-B inhibitory activities of all target compounds **1**–**18** were measured by a previously described fluorescence-based Amplex Red assay using pargyline as reference compound (Table 1).[34] The result showed that compounds **2**–**5** and **10** exhibit good activities against *h*MAO-B with IC_{50} in the micromolar range. Among them, compound **5** (*h*MAO-B,

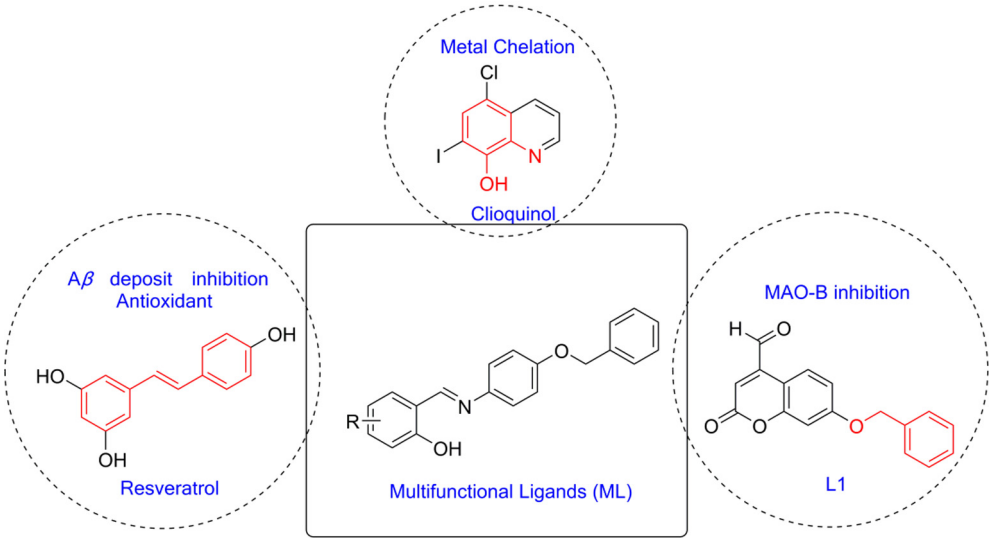

Fig. 1. Design strategy for the multifunctional salicyladimine derivatives.