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Anti-neurotoxicity effects of oxoisoaporphine-lipoic acid hybrids

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ABSTRACT

Four oxoisoaporphine-lipoic acid hybrids were designed, synthesized, and investigated in this study. To develop the hybrids, the oxoisoaporphine fragment was used for its inhibition of cholinesterases and β-amyloid (Aβ) aggregation, while the unit of lipoic acid was used for its radical-capturing and neuroprotective effects. The hybrids exhibited moderate inhibitory effects on the activity of acetylcholinesterase (AChE), with IC_{50} values in the micromolar range and low toxicity in SH-SY5Y cells. Moreover, the learning and memory abilities, climbing capability, and average life expectancy of the Aβ42 transgenic *Drosophila* were all significantly improved by the hybrids. They also enhanced the intracephalic antioxidant activity, the metabolism, and the activity cholinesterase in the flies. More strikingly, Aβ42 aggregation in the hybrids-treated *Drosophila* was attenuated with effective neuroprotection. Our results indicate the potential of using these oxoisoaporphine-lipoic acid hybrids in AD treatments.

1. Introduction

As the common onset of dementia, Alzheimer's disease (AD) is a neurodegenerative disease with memory impairment as the main manifestation [1–3]. Accumulating evidence has indicated that Aβ42 is a key element in the formation and development of AD, and it is induced by a variety of common factors of AD [4]. AD-related pathogenesis has also been reported to be involved in the cholinergic hypothesis in CNS [5]. Acetylcholinesterase (AChE), a serine protease that hydrolyzes the neurotransmitter acetylcholine, contains a peripheral anionic site (PAS) that may mediate the deposition of amyloid-β protein in the brain [6–8]. The deposited β-amyloid protein gradually invades the mitochondria to cause mitochondrial dysfunction [9]. This undermining process inhibits the metabolism of intracephalic ATP, thereby inducing cytotoxicity and reactive oxygen species (ROS) damage [10]. Therefore, developing inhibitors targeting both the active center and PAS of AChE have been widely regarded as a promising anti-Aβ deposition strategy. Many single inhibitors targeting competitive cholinesterase, such as tacrine, stone shirt alkali A, galantamine, have shown some efficacy treating dementia [11]. However, these inhibitors fail to block Aβ deposition due to their low therapeutical potency. Therefore, we have synthesized a series of oxoisoaporphine derivates [12–15], which specifically bind to the active center and PAS of AChE. In this study, we aimed to investigate the potential anti-neurotoxicity effects of these derivates both *in vitro* and *in vivo* and explore the underlying mechanisms of such effects.

2. Materials and methods

2.1. Chemicals and reagents

The bivalent ligand strategy involves the synthesis of drugs in which identical or different pharmacophores are linked via a suitable linker. Hybrids of oxoisoaporphine-lipoic acids were synthesized for the key intermediate compounds 5–7 (Fig. 1).

The preparation of the key intermediate compounds 5–7 is shown in Fig. 1. Synthesis of 9-amino-1-azabenzanthrone 1 was carried out by a previously described reported method [16,17]. Compounds 2–4 were synthesized from compound 1 and

Abbreviations: AChE, acetylcholinesterase; BChE, butyrocholinesterase; Aβ, β-amyloid; AD, Alzheimer's disease; CNS, central nervous system; ROS, reactive oxygen species; PAS, peripheral anionic site; CAS, catalytic active site; EDCI·HCl, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; DCM, dichloromethane; EGCG, (−)-Epigallocatechin gallate; MDA, malondialdehyde; DMA, dimethylacetamide; DMF, dimethylformamide; HE, hematoxylin and eosin; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide; DMSO, dimethylsulfoxide; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium.

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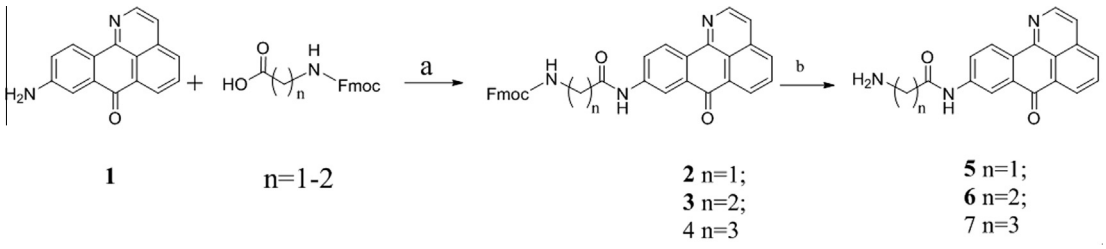

Fig. 1. Preparation of compounds 5–7. Reagents and conditions: (a) EDCI·HCl/DMA, 70 °C, 6 h, (b) piperidine-DCM (V:V = 1:1).

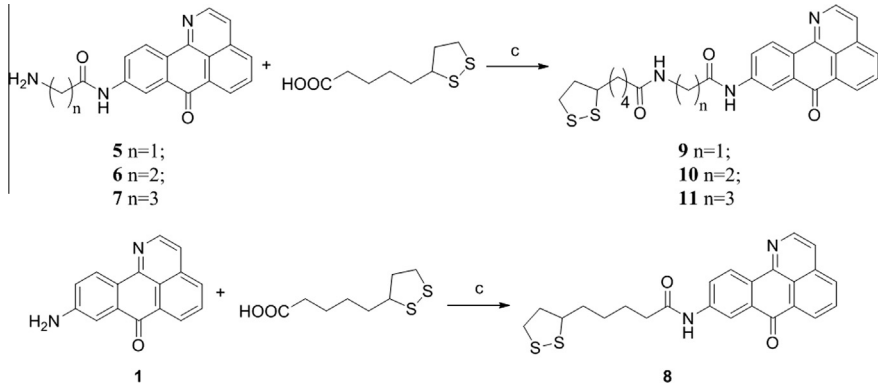

Fig. 2. Preparation of hybrids of oxoisoaporphine-lipoic acids 8–11. Reagents and conditions: (c) EDCI·HCl/DMA/N_2, 60 °C, 5 h.

corresponding Fmoc-amino acids, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI·HCl) as a coupling reagent. The Fmoc groups of compounds 2–4 were removed using piperidine-DCM (V:V = 1:1) to obtain the key intermediate compounds 5–7.

Finally, the heterodimers 8–11 were prepared by the acylation of intermediate amines 1, 5, 6 and 7, with lipoic acid using EDCI·HCl as coupling reagent (Fig. 2). The synthesis details of all the compounds and their ^{1}H NMR and ESI-MS data are included in Supplementary data.

2.2. In vitro studies on AChE and BChE inhibition

All the assays were carried out under 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 8.0, using a Shimadzu 2450 Spectrophotometer. AChE from *Electrophorus electricus* (Sigma) was prepared to give 2.0 units/mL in 2 mL aliquots. The assay medium contained 1 mL of phosphate buffer, (pH 8.0) (1 mL), 50 mL of 0.01 M DTNB, 10 mL of enzyme, and 50 mL of 0.01 M substrate (acetylthiocholine chloride), and inhibitors. The substrate was added to the assay medium containing enzyme, buffer, and DTNB with inhibitor after 15 min of incubation time. The activity was determined by measuring the increases in absorbance at 412 nm with 1 min intervals at 37 °C and calculations were performed using the equation described by Ellman et al. [24].

In vitro assay of BChE (from *equine serum*, Sigma) was carried out using a similar method as described above.

2.3. Culture of SH-SY5Y cells and MTT assay

SH-SY5Y cells at passages 3–16 were cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO) containing 15 nonessential amino acids, 10% fetal calf serum (FCS) (GIBCO), 1 mM glutamine, 50 mg/mL penicillin, and 50 mg/mL streptomycin, in a humidified 37 °C, 5% CO_2 incubator. For MTT assay, SH-SY5Y cells were seeded onto 96-well plates at 10,000 cells/well. After 24 h, the compounds were added to the medium, and after 48 h, the survival of cells was determined with MTT assay that was carried out by incubating with 20 mL MTT (2.5 mg/mL) at 37 °C for 4 h, solubilizing cells in 100 mL dimethyl sulfoxide (DMSO), and measuring the absorbance of cells with a micro culture plate reader at the wavelength of 570 nm.

2.4. Neuroprotection assay: cell viability assessment after H_2O_2 treatments

Several concentrations (20–150 mM) were used to investigate the neuroprotective effects of EGCG and compounds 8–11. Neuroprotection was determined by measuring increases in the viability of neuronal cells cultured in 96-well plates (10,000 cells per well) after 24 h treatment of 100 M hydrogen peroxide by MTT assay.

2.5. Animals and delivery

Drosophila melanogaster expressing Arctic Aβ42 was kindly provided by Prof. Zhong from Life Sciences College of Tsinghua University. Elav-GAL4 *Drosophila* was obtained from the Bloomington stock center. The Elav-GAL4/UAS-Aβ42 hybrid *Drosophila* was identified through RT-PCR that determined the mRNA expression of intracephalic Aβ42. Subsequently, the hybrid *Drosophila* was randomly assigned into five groups (n = 100 per group): normal control group, model control group, DMSO solvent control group, and oxoisoaporphine-lipoic acid hybrids (OIAD-08) administrated groups (0.1, 10 μmol/L). 100 normal *Drosophila* was used as normal control. OIAD-08 mixed into food was administered to the intervention group flies daily until their death.

2.5.1. Measurements of intracephalic superoxide dismutase (SOD) activity and thiobarbituric acid-reactive substances (TBARS) contents in Drosophila

Fresh fly brain samples from each group (n = 100 per group) were collected, and cerebral homogenates were mulled immediately in liquid nitrogen. The homogenates were centrifuged (Thermo Scientific, USA) at 6000 rpm for 10 min at 4 °C and col-