Bioorganic & Medicinal Chemistry 21 (2013) 2462–2470

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Design, synthesis and evaluation of tacrine–flurbiprofen–nitrate trihybrids as novel anti-Alzheimer's disease agents

Yao Chen [a,b,c], Jianfei Sun [d], Zhangjian Huang [a,b], Hong Liao [d,*], Sixun Peng [a,b], Jochen Lehmann [c,e,*], Yihua Zhang [a,b,*]

[a] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China
[b] Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, PR China
[c] Lehrstuhl für Pharmazeutische/Medizinische Chemie, Institut für Pharmazie, Friedrich-Schiller-Universität Jena, Philosophenweg 14, D-07743 Jena, Germany
[d] Neurobiology Lab, New Drug Screening Center, China Pharmaceutical University, Nanjing 210009, PR China
[e] College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:
Received 18 January 2013
Revised 4 March 2013
Accepted 6 March 2013
Available online 13 March 2013

Keywords:
Tacrine
Flurbiprofen
Nitrate
Cholinesterase
Amyloid-β

ABSTRACT

To search for multifunctional anti-Alzheimer's disease (AD) agents with good safety, the previously synthesized tacrine–flurbiprofen hybrids **1a** and **1b** were modified into tacrine–flurbiprofen–nitrate trihybrids **3a–h**. These compounds displayed comparable or higher cholinesterase inhibitory activity relative to the bivalent hybrids. Compound **3a** was the most potent, which released moderate NO, exerted blood vessel relaxative activity, and showed significant Aβ inhibitory effects whereas tacrine and flurbiprofen did not exhibit any Aβ inhibitory activity at the same dose. In addition, **3a** was active in improving memory impairment in vivo. More importantly, the hepatotoxicity study showed that **3a** was much safer than tacrine, suggesting it might be a promising anti-AD agent for further investigation.

1. Introduction

Alzheimer's disease (AD) is a progressive neurological disease leading to impairment in memory, language skills, judgment and orientation.[1] This disease is multifaceted and many factors such as cholinergic dysfunction, deposits of amyloid-β (Aβ) and τ-protein and poor blood supply in brain are believed to play important roles in the development of AD.[2] To our knowledge, most currently prescribed anti-AD drugs are cholinesterase inhibitors (ChEI), for example, tactine and rivastigmine, which act by increasing the acetylcholine (ACh) level in the brain via inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).[3,4] Unfortunately, ChEIs have been proved to be therapeutically limited because ChEIs are not able to delay or prevent the progression of AD.[5,6] Given that the pathogenesis of AD is complicated and cholinesterase may not be the only focused factor,[7–11] an innovative therapeutic approach based on the 'multi-target-directed ligands' (MTDLs) paradigm[12] has recently been applied in the research of ChEIs.[13,14] The most common strategy to rationally design MTDLs is to connect two distinct pharmacological classes of compounds in one molecule via a proper spacer.[15] Since the cooperation of both β- and γ-secretase can cleave amyloid precursor protein (APP) leading to the accumulation of β-amyloid (Aβ) peptides, the inhibitors of β- and γ-secretase are therefore believed to prevent or slow AD development.[16–22] We previously synthesized and evaluated hybrids **1a** and **1b** (Fig. 1) from tacrine and racemic flurbiprofen, a γ-secretase inhibitor, as highly potent anti-AD agents.[23] Kinetic studies revealed that they can block both the catalytic active site (CAS)[24,25] and the peripheral anionic site (PAS),[26–28] which are closely associated with hydrolysis of ACh. Moreover, **1a** inhibited the formation of Aβ in vitro, probably due to the ability of flurbiprofen moiety to lower Aβ40/42 peptide production by an allosteric modulation of γ-secretase.[29]

In continuation of our ongoing studies on novel multifunctional ChEIs, we attempted to extend the MTDL strategy by conjugating a third pharmacophore to the bivalent hybrids.[30] Recently, increasing evidences suggest that nitric oxide (NO), a free radical gas, may be beneficial for the treatment of AD by increasing blood supply[31] and regulating the cerebral circulation.[32] Additionally, the tacrine–ferulic acid–nitrate trihybrids **2a–e** (Fig. 1) from our group exhibited lower hepatotoxicity compared to the dual-acting tacrine–ferulic acid hybrid, which could be beneficial for the treatment of AD.[33] Based on these investigations, we introduced a NO

* Corresponding authors. Tel.: +86 25 83271043; fax: +86 25 83271142 (H.L.); tel.: +49 3641949803; fax: +49 3641949802 (J.L.); tel./fax:+86 25 83271015 (Y.Z.).
E-mail addresses: hliao@cpu.edu.cn (H. Liao), j.lehmann@uni-jena.de (J. Lehmann), zyhtgd@163.com (Y. Zhang).

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.03.005

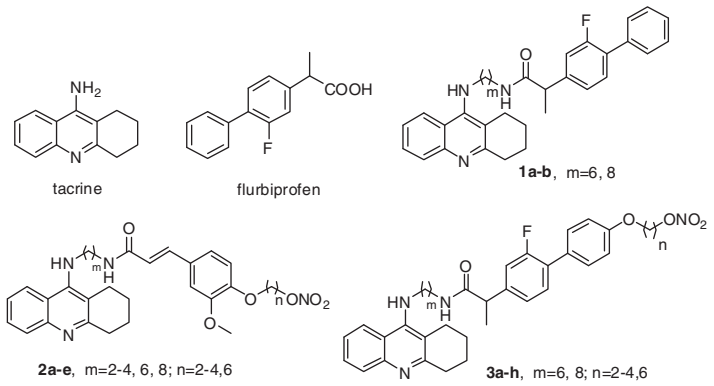


Figure 1. Structures of tacrine, flurbiprofen, tacrine–flurbiprofen hybrids **1a** and **1b**, tacrine–ferulic acid–nitrate trihybrids **2a–e** and tacrine–flurbiprofen–nitrate trihybrids **3a–h**.

donor moiety (nitrate group) into the bivalent hybrids **1a** and **1b** to generate a group of novel tacrine–flurbiprofen–nitrate trihybrids **3a–h** (Scheme 1). Herein, synthesis and biological evaluations including inhibition of AChE and BuChE, kinetics of enzyme inhibition, NO releasing, vascular relaxation, cell-based Aβ inhibition as well as in vivo behavioral and hepatotoxicity studies are described.

2. Results and discussion

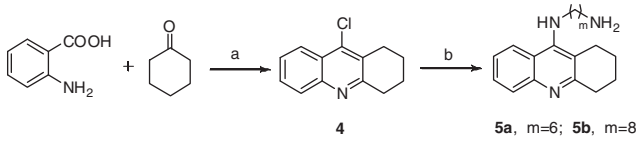
2.1. Chemistry

Scheme 1 depicts that the condensation reaction of anthranilic acid with cyclohexanone furnished 9-chloro-1,2,3,4-tetrahydroacridine **4**,[34] which was subsequently treated with different alkylenediamines to produce the intermediates 9-aminoalkylamino-1,2,3,4-tetrahydroacridines **5a** and **5b**.

To synthesize the other key intermediates NO-donating flurbiprofen derivatives **13a–d**, the carboxyl group of racemic flurbiprofen was protected to form a methyl ester **6**. Friedel–Crafts reaction of **6** yielded acylated compound **7**, which underwent the Baeyer–Villiger oxidation rearrangement to furnish acetyl ester **8**, followed by hydrolysis to give 4′-OH flubiprofen **9**. Compound **9** was esterified to give methyl ester **10**, which was treated with the corresponding dibromoalkanes to give the halogenated intermediates **11a–d**. Subsequent treatment of **11a–d** with $AgNO_3$ in dry CH_3CN offered nitrates **12a–d**. The nitrates were then de-protected under basic conditions to generate acids **13a–d**, which were coupled with **5a** and **5b**, respectively, in the presence of DCC/DMAP to provide the target compounds **3a–h** (Scheme 2). These target compounds were purified by column chromatography and characterized by IR, ESI-MS, ^{1}H NMR, ^{13}C NMR, and elementary analysis, and individual compounds with chemical purity of >95% for subsequent experiments.

2.2. Pharmacology: AChE and BuChE inhibition

Target compounds **3a–h** were tested in vitro for inhibition of AChE from Electrophorus electricus (eeAChE) and of BuChE from equine serum, using the Ellman assay[35] (Table 1). The inhibitory activities of the hybrids were compared to those of tacrine, parent compounds **1a** and **1b**. All target compounds (except **3h**) exhibit similar or even higher inhibitory activities compared to tacrine, **1a** and **1b**. The results revealed that the length of the alkyl, which was connected to nitrate moiety of the target compounds, could significantly influence the AChE inhibitory activity. When the length increased, the activity decreased. The optimal spacer length was two carbon atoms. It was observed that **3a–h** were more active against BuChE (IC_{50}s = 0.6–2.5 nM) than tacrine (IC_{50} = 10.6 nM), **1a** and **1b** (IC_{50}s = 2.1–3.7 nM). The most potent compounds were



Scheme 1. The synthetic route of **5a** and **5b**. Reagents: (a) $POCl_3$, reflux, 3 h; (b) pentanol, $NH_2(CH_2)_mNH_2$, reflux, 18 h.

3a and **3e**, which were characterized by IC_{50} values of 9.1 and 12.5 nM on AChE and IC_{50} values of 2.5 and 1.0 nM against BuChE. Interestingly, **3g** and **3h** showed much higher inhibitory activity against BuChE (IC_{50} = 1.3 and 1.5 nM) relative to AChE (IC_{50} = 70.9 and 255.6 nM), indicating that **3g** and **3h** were selective BuChE inhibitors with selectivity indexes of 54.5 and 150.4, respectively.

2.3. Kinetic study of ChE inhibition

To get insight into the mechanism of action of this new family of compounds on both AChE and BuChE, a kinetic study was conducted for the most potent compound **3a**. Graphical analysis of the Lineweaver–Burk reciprocal plot of AChE inhibition (Fig. 2) showed both increased slopes (decreased V_{max}) and intercepts (higher K_m) with increased concentration of **3a**. This pattern suggested a mixed-type inhibition and accordingly supported the dual site (CAS and PAS) binding of these compounds. A K_i value of 16.1 nM was derived from the plots of the slop versus the concentration of **3a**. As for BuChE, a different plot was observed, with different K_m and constant V_{max} in varied inhibitor concentrations (Fig. 3), suggesting a competitive inhibition, in which **3a** competes with the substrate acetylcholine for the same binding site (CAS). Replots of the slope versus concentration of **3a** give a competitive inhibition constant, K_i of 1.7 nM.

2.4. Detection of nitrite (Griess reaction)

The NO-releasing ability of compounds **3a–h** was measured using the Griess reaction[36] by quantifying the nitrite produced from the oxidative reaction of NO, oxygen and water (Table 1). The results showed that all the target compounds released moderate NO (0.283–0.565 μg/mL), more than tacrine, and comparable to isosorbide mononitrate (ISMN, 0.412 μg/mL), a known NO donor.

2.5. Vascular relaxation study

Previous study have suggested that NO might have a positive correlation with the vasorelaxation effect.[33] Therefore, NO-donating compounds **3a** and **3e** were tested in an ex vivo organs bath