International Journal of Pharmaceutics 477 (2014) 442–453

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Synthesis, biological activity, and biopharmaceutical characterization of tacrine dimers as acetylcholinesterase inhibitors

Shuai Qian [a,b], Lisi He [c], Marvin Mak [d], Yifan Han [d], Chun-Yu Ho [c,e,**], Zhong Zuo [a,*]

[a] School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong Special Administrative Region
[b] School of Traditional Chinese Medicine, China Pharmaceutical University, Nanjing, PR China
[c] Department of Chemistry, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong Special Administrative Region
[d] Department of Applied Biology and Chemical Technology, Institute of Modern Chinese Medicine, The Hong Kong Polytechnic University, Hung Hom, Hong Kong Special Administrative Region
[e] Department of Chemistry, South University of Science and Technology of China, Shenzhen, PR China

ARTICLE INFO

Article history:
Received 20 May 2014
Received in revised form 19 September 2014
Accepted 26 October 2014
Available online 31 October 2014

Keywords:
Tacrine
Dimers
Acetylcholinesterase
Cytotoxicity
Permeability

ABSTRACT

Tacrine (THA), as the first approved acetylcholinesterase (AChE) inhibitors for the treatment of Alzheimer's disease (AD), has been extensively investigated in last seven decades. After dimerization of THA via a 7-carbon alkyl spacer, bis(7)-tacrine (B7T) showed much potent anti-AChE activity than THA. We here report synthesis, biological evaluation and biopharmaceutical characterization of six THA dimers referable to B7T. According to IC_{50} values, the *in vitro* anti-AChE activities of THA dimers were up to 300-fold more potent and 200-fold more selective than that of THA. In addition, the anti-AChE activities of THA dimers were found to be associated with the type and length of the linkage. All studied THA dimers showed much lower cytotoxicity than B7T, but like B7T, they demonstrated much lower absorptive permeabilities than that of THA on Caco-2 monolayer model. In addition, all THA dimers demonstrated significant efflux transport (efflux ratio >4), indicating that the limited permeability could be associated with the efflux transport during absorption process. Moreover, the dimer with higher Log *P* value was accompanied with higher permeability but lower aqueous solubility. A balanced consideration of activity, solubility, cytotoxicity and permeability should be conducted in selection of the potential candidates for further *in vivo* investigation.

1. Introduction

Alzheimer's disease (AD), as the most common form of dementia, is a chronic and neurodegenerative disease that attacks the brain and leads to the impaired memory, thinking and behavior in the elderly. AD has been proven to be a multifactorial disease associated with several aspects including cholinergic deficiency, glutamate induced excitotoxicity, formation of β-amyloid (Aβ) precipitates, oxidative stress, tau hyperphosphorylation and so on (Goedert and Spillantini, 2006; Heppner et al., 2004). The neuropathological alterations described above suggest the possible treatment of AD could come from drugs that can target at these factors. For the past decade the cholinergic approach has been the first and the most frequently used therapeutic strategy for the treatment of mild to moderate AD. Levels of acetylcholine can be enhanced by inhibiting acetylcholinesterase (AChE) with reversible inhibitors. The AChE inhibitors (AChEIs) were found to be the only ones that could produce significant and reproducible therapeutic effects (Standridge, 2004). Since mid-1990s, only four AChEIs, namely tacrine (THA), donepezil, rivastigmine and galantamine, have been approved by the FDA in US for the treatment of AD (Standridge, 2004). Huperzine, a novel *Lycopodium* alkaloid chemically unique from other known AChEIs for AD, was first discovered in a Chinese medicinal herb *Huperzia serrate*. The agent has been approved for AD in China because of its potent memory-enhancing effects, specific anti-AChE activity, and fewer side effects (Tang and Han, 1999). In addition to targeting cholinergic deficiency, reducing glutamate induced excitotoxicity by *N*-methyl-D-aspartate receptor antagonists (*e.g.*, FDA approved memantine) (Reisberg et al., 2003), and preventing the build-up of Aβ by β-secretase 1 (BACE 1) inhibitors (*e.g.*, MK-8931, LY2886721 in clinical trials) also severed two kinds of treatments

* Corresponding author at: School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong Special Administrative Region. Tel.: +852 3943 6832; fax: +852 2603 5295.
** Corresponding author at: Department of Chemistry, South University of Science and Technology of China, Shenzhen, PR China. Tel.: +86 755 8801 8318.
E-mail addresses: jasonhcy@sustc.edu.cn (C.-Y. Ho), joanzuo@cuhk.edu.hk (Z. Zuo).

http://dx.doi.org/10.1016/j.ijpharm.2014.10.058
0378-5173/

of AD. However, none of above strategies could completely prevent the neurodegeneration and cure the AD till now (Evin et al., 2011; Robinson and Keating, 2006; Terry and Buccafusco, 2003), which may be due to that they only focused on one targeting site of drug action. As a consequence, drug discovery in AD is gradually moving from the development of molecules with a single target to the "multi-target-directed ligands" (MTDLs), which is capable to simultaneously address several key pathophysiological processes as described above (Bajda et al., 2011; Cavalli et al., 2008). Recently, Voisin et al. reported a well-controlled clinical trial of memantine (NMDA receptor antagonist)/donepezil (AChEI) dual therapy. They found that combination of donepezil and memantine has superior efficacy than donepezil alone in the severe AD subgroup, potentially supporting a role for dual treatment in more advanced AD patients (Voisin et al., 2004). Such clinical findings also support the multi-target strategy for the treatment of AD.

Accordingly, commercial AChEIs have been widely investigated and modified with additional features, in order to solve the issue of both cholinergic deficiency and other above-mentioned targets in AD. As the first approved AChEI drug, THA was the most extensively investigated drug. Many efforts have been concentrated on the synthesis of its derivatives as MTDLs for AD treatment (Bajda et al., 2011) due to its high anti-AChE activity, much low molecular weight (MW: 198, lower than other approved AChEIs) and potential attenuating Aβ-induced neurotoxicity (Svensson and Nordberg, 1998). Over the past decade, various THA derivatives have been developed and indeed demonstrated multi-target properties *in vitro* and in animal models, including inhibition of AChE, BACE1, Aβ aggregation and reactive oxygen species as well as blockade of NMDA receptor and nitric oxide synthase, etc. (Alcala Mdel et al., 2003; Alonso et al., 2005; Chao et al., 2012; Fu et al., 2007, 2008; Li et al., 2006; Minarini et al., 2012; Pi et al., 2012).

Bis(7)-tacrine (B7T), a dimeric THA analog designed with the aid of computer docking program and synthesized by our research group (Fig. 1b), is a promising anti-AD candidate with multi-potencies including anti-NMDA receptors, anti-nitric oxide synthase signaling, and the reduction of Aβ neurotoxicity in addition to high potency and selectivity on AChE inhibition (Fu et al., 2007; Li et al., 2005, 2006, 2007, 2009). Following a single oral administration to rats, B7T showed significantly improved *in vivo* brain AChE inhibition, reduced *in vivo* serum BChE inhibition, and fewer peripheral cholinergic side effects than that of THA (Wang et al., 1999b). In addition, B7T could ameliorate learning and memory impairment induced by scopolamine (Wang et al., 1999a), AF64A (Liu et al., 2000), or permanent ligation of the bilateral common carotid arteries (Shu et al., 2012) in the Morris Water maze. However, the oral bioavailability of B7T was very low (~10%) in rats mainly due to its high lipophilicity (Log P = 8.2 (Yu et al., 2008)), poor intestinal permeability and significant tissue binding (Zhang et al., 2008). Similar to B7T, HLS-1 and HLS-2 (Fig. 1), designed by Bolognesi et al., also demonstrated multi-target properties via inhibiting AChE, reversing AChE-induced amyloid fibrillogenesis and acting as metal chelators (Bolognesi et al., 2007).

Currently, hundreds of novel AChEIs have been synthesized and investigated (Bajda et al., 2011; Mehta et al., 2012; Tumiatti et al.,

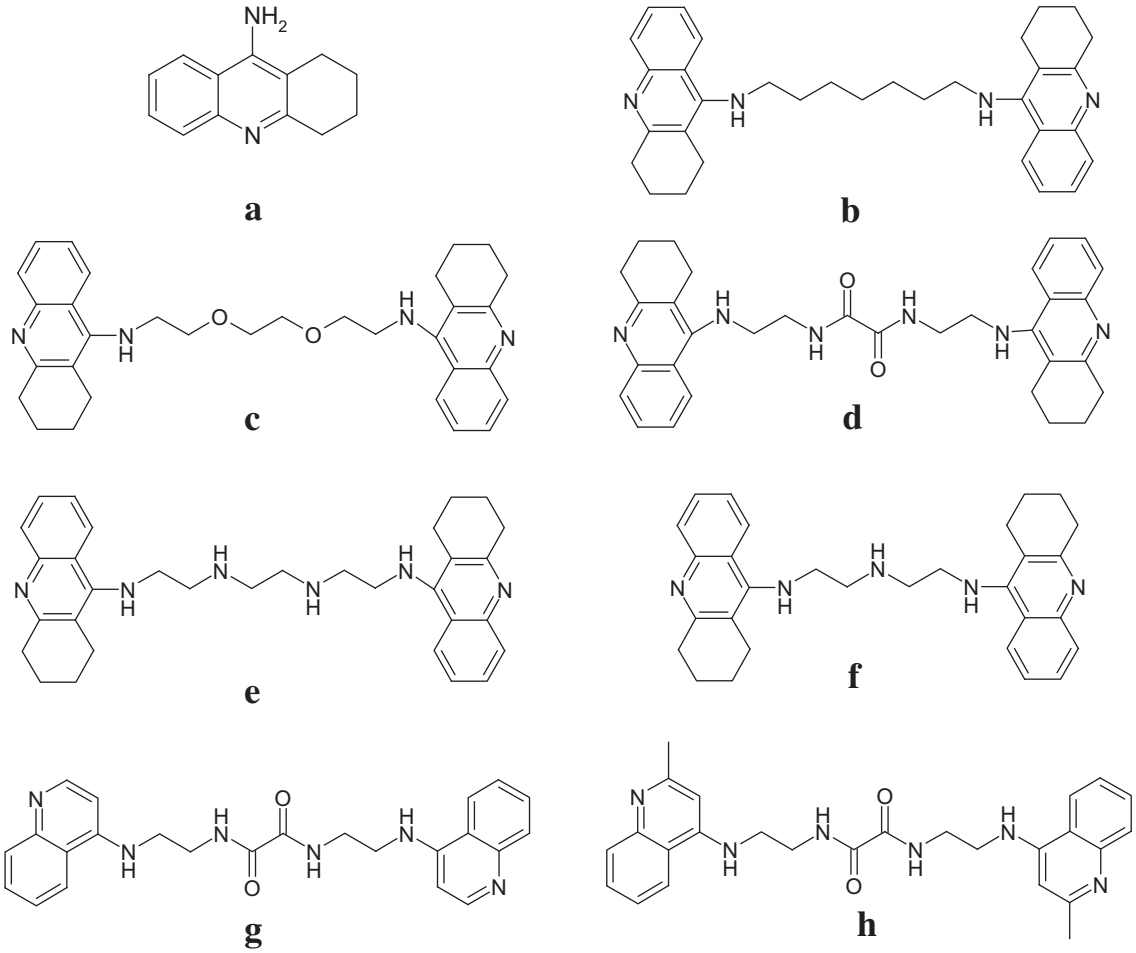

Fig. 1. Chemical structures of THA (a), B7T (b), HLS-1 (c), HLS-2 (d), HLS-3 (e), HLS-4 (f), HLS-5 (g) and HLS-6 (h).