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The physicochemical properties and the *in vivo* AChE inhibition of two potential anti-Alzheimer agents, bis(12)-hupyridone and bis(7)-tacrine

Hua Yu [a,b], Wen-Ming Li [b], Kelvin K.W. Kan [b], Jason M.K. Ho [b], Paul R. Carlier [c], Yuan-Ping Pang [d], Zhe-Ming Gu [e], Zuo Zhong [f], Kelvin Chan [g], Yi-Tao Wang [a,**], Yi-Fan Han [b,*]

[a] *Institute of Chinese Medical Sciences, University of Macau, Macau SAR, PR China*
[b] *Department of Biochemistry, Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong SAR, PR China*
[c] *Department of Chemistry, Virginia Tech, Blacksburg, VA 24061, USA*
[d] *Computer-Aided Molecular Design Laboratory, Mayo Clinic College of Medicine, Rochester, MN, USA*
[e] *XenoBiotic Laboratories, Inc., Plainsboro, NJ, USA*
[f] *School of Pharmacy, Faculty of Medicine, Chinese University of Hong Kong, Shatin, N.T., Hong Kong, SAR, PR China*
[g] *School of Applied Sciences, University of Wolverhampton, United Kingdom*

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Abstract

The lipophilicity and solubility profiles of bis(12)-hupyridone (B12H) and bis(7)-tacrine (B7T), two novel acetylcholinesterase inhibitors dimerized from huperzine A fragments and tacrine, respectively, were investigated over a broad pH range. Lipophilicity was assessed by both shake flask method with 1-octanol–water system and a reverse-phase HPLC system with methanol–water as mobile phase. The former method was used for determining the lipophilicities of the ionized forms ($\log D$) of the dimers while the latter method was used for that of the neutral forms ($\log P$). The $\log P$ values for B12H and B7T were found to be 5.4 and 8.2, respectively, indicating that the two dimers are highly lipophilic. The solubilities of both dimers were found to be affected by pH. The solubility of B12H was >1.41 mg/ml when the pH was <7, but <0.06 mg/ml when the pH was >8. The solubility of B7T was >0.26 mg/ml when the pH was <9, but <0.005 mg/ml when the pH was >12. The ionic strength of a solution could affect the solubilities considerably (11.16 mg/ml for B12H and 12.71 mg/ml for B7T in water; 2.07 mg/ml for B12H and 0.36 mg/ml for B7T in saline). The ionization constants (pK_a) of the two dimers were determined by UV spectrophotometry. Both dimers were found to have two pK_a values: 7.5 ± 0.1 (pK_{a1}) and 10.0 ± 0.2 (pK_{a2}) for B12H; and 8.7 ± 0.1 (pK_{a1}) and 10.7 ± 0.4 (pK_{a2}) for B7T. Furthermore, an *in vivo* pharmacological assay conducted in mice showed that a maximum AChE inhibition occurred 15 min after the single-dose and intraperitoneal administration of either dimer. This indicates that the two dimers may easily cross the blood–brain barrier. In summary, these physiochemical characteristics suggest that the two dimers may be promising candidates for the development of better drugs for Alzheimer's disease.

Keywords: Solubility; Ionization constant; Lipophilicity; AChE inhibition; Bis(12)-hupyridone; Bis(7)-tacrine; Dimer

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disease that attacks the brain and results in impaired memory, thinking and behaviour in the elderly [1]. There are many different theories on the causes of AD and one of the well-established theories suggests that the neurotransmitter acetylcholine levels are too low in the brains of AD patients [2,3]. Although several acetylcholinesterase (AChE) inhibitors have been developed, they are only useful for treating patients with mild to moderate AD [4,5]. Tacrine (TAC, Fig. 1A), the first approved AChE inhibitor for AD, has been found to have potential hepatotoxicity [6] and is therefore seldom used nowadays. As a result, the development of more effective anti-AD agents is still an important area of AD-associated research. With the identification of the three-dimensional structure of AChE and the assistance of computer docking programs, a series of new dimers has been designed based on the structures of tacrine [7,8] and huperzine A (Fig. 1B, extracted from the Chinese

* Corresponding author. Tel.: +86 852 23587293; fax: +86 852 23581552.
** Co-corresponding author. Tel.: +86 853 3974691; fax: +86 853 28841358.
E-mail addresses: ytwang@umac.mo (Y.-T. Wang), bcyfhan@ust.hk (Y.-F. Han).

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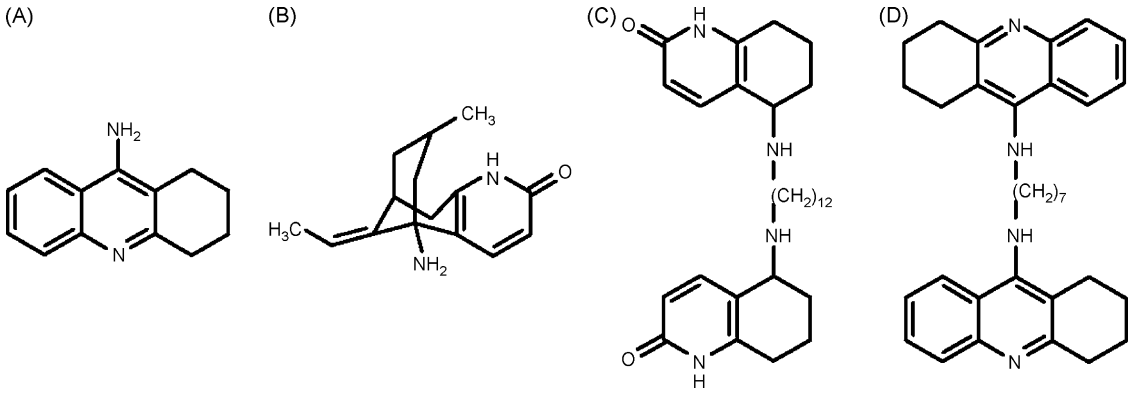

Fig. 1. Structures of tacrine (A), huperzine A (B), bis(12)-hupyridone (C) and bis(7)-tacrine (D).

medicinal plant *Huperzia serrata* and is now in Phase II clinical trial for AD treatment in the USA [9]). Of the newer drugs, bis(12)-hupyridone (B12H, Fig. 1C) is a selective and potent AChE inhibitor *in vitro* while bis(7)-tacrine (B7T, Fig. 1D) has been demonstrated to have similar effects both *in vitro* and *in vivo* [10–14]. B7T has also been shown to have multiple actions such as antagonizing *N*-methyl-D-aspartate (NMDA) receptors [15] and gamma-aminobutyric acid ($GABA_A$) receptors [16] and inhibiting nitric oxide synthase [17], which may play synergistical roles in the treatment of AD [17,18].

Although these new dimers may offer superior therapeutic potentials, their physicochemical properties such as lipophilicities (log *P* or log *D*), ionization constants (pK_a) and solubilities remain unknown. Determination of aqueous solubility as a function of pH along with pK_a is important to the understanding of the dissolution abilities of the dimers (for solid dosage forms) and their subsequent permeability through cell membrane [19]. Further, the 1-octanol/water partition coefficients may help understand the lipophilicity of the compounds, thus predicting their absorption pathways and even their sites of action [20]. An investigation of the physicochemical properties of the dimers is also an essential step in understanding the pharmacokinetic profiles *in vivo*. In this study, we determined the various physicochemical properties of B12H and B7T-partition coefficients, ionization constants and solubilities. In addition, we also examined the *in vivo* AChE inhibitory ability by B12H and compared that with B7T.

2. Materials and methods

2.1. Chemicals

Bis(12)-hupyridone dihydrochloride (B12H, Fig. 1B) and bis(7)-tacrine dihydrochloride (B7T, Fig. 1C) were synthesized as described previously [21,22] and their purities were up to 99.9% (detected by HPLC analysis). Tacrine hydrochloride (TAC, Fig. 1A) and other chemicals (all in analytical-grade) were purchased from Sigma Chemicals Ltd. (St. Louis, MO, USA). Water was prepared with an EASYpure UV system (model D7401; Barnstead Thermolyne Co. Dubuque, IA, USA).

2.2. Animals

Male ICR mice (25–35 g, 6 weeks of age) supplied by the Animal and Plant Care Facility of the Hong Kong University of Science and Technology were fed on a standard laboratory diet with free access to water at a controlled temperature of 20–22 °C and relative humidity of 50% with a 12 h light/dark cycle prior to the study. Before experiments, all the mice were fasted but were allowed to have free access to water overnight.

2.3. Analytical method

About 1.0 mg, 1.5 mg and 1.0 mg of TAC, B12H and B7T, respectively, were accurately weighed and dissolved in water in 25 ml volumetric flasks. 10 ml of each solution was transferred to a 50 ml volumetric flask and then diluted to the mark with water. Certain volumes of the stock solutions (0.1–0.7 ml) were then transferred into 1 ml volumetric flasks and diluted to the marks with water. The final concentrations of the solutions were analyzed in triplicate using a DU 640 UV/Vis Spectrophotometer (Beckman Coulter, Fullerton, CA, USA) at wavelengths of 240, 229 and 244 nm for TAC, B12H and B7T, respectively. Calibration curves were constructed by plotting the concentrations as a function of UV absorbance values.

For method validation, intra-day precision was determined by analyzing six replicates of control samples at low, medium and high concentrations of the calibration range within the same day. The inter-day precision was determined by using samples prepared in a similar manner on five separate days. Precisions were reported as the relative standard deviations (R.S.D.) and accuracies expressed as [(1 − (mean concentration measured − concentration added)/concentration added)] × 100%.

2.4. Measurement of partition/distribution coefficients

The lipophilicities for neutral forms of TAC, B12H and B7T were measured by an HPLC method recommended by the "*Organization for Economic Co-operation and Development* (OECD)" [23]. The analytical process was carried out with an Agilent HP1100 system (Hewlett-Packard, Palo Alto, CA, USA) equipped with an Alltech 2000 Evaporative Light Scattering