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# Letters

## Bis-huperzine B: Highly Potent and Selective Acetylcholinesterase Inhibitors

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**Abstract:** By targeting dual active sites of AChE, a series of bis-huperzine B analogues with various lengths of the tether were designed, synthesized, and tested for their inhibition and selectivity. The most potent bis-huperzine B (**5g**) exhibited 3900-fold increase in AChE inhibition and 930-fold greater in selectivity for AChE vs BuChE than its parent huperzine B.

In the literature there are a number of so-called bis-pharmacophore or bivalent ligands and drugs, in which two of the same pharmacophoric moieties are linked together via a carbon or heterocarbon chain as the tether. Bivalency or homodimer strategy in drug design has proved to be useful to enhance the potency and selectivity relative to its monomeric lead in many cases, particularly in the neuromuscular blockers.[1] Pang et al. reported that bis(7)-tacrine (**1**), the heptylene-linked tacrine dimer, possessed both better acetylcholinesterase (AChE) inhibitory potency and better BuChE/AChE selectivity than tacrine itself.[2] Recently, bis-huperzine A (**2**)[3] and bis-5-amino-5,6,7,8-tetrahydroquinolinone (**3**)[4,5] have been also reported. The crystal structures of *Tc*AChE-bis(7)-tacrine (*Tc: Torpedo californica*) and *Tc*AChE-bis-5-amino-5,6,7,8-tetrahydroquinolinone complexes showed that they were bound within the active gorge of *Tc*AChE in a bivalent fashion, with one unit in the catalytic anionic site and the other in the peripheral anionic site. The increased affinity of **1** and **3** to AChE is conferred by binding to the central active site and peripheral active site of the enzyme.

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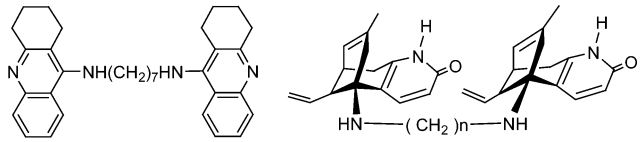
To date, AChE inhibitors are the major drugs approved for the symptomatic treatment of Alzheimer's disease. It has also been demonstrated that AChE could play a key role during the early stage in the development of the senile plaques by accelerating $\beta$-amyloid peptide deposition. Inhibition of the peripheral binding site of AChE might prevent the deposition of $\beta$-amyloid peptide induced by AChE.[6]




bis(7)-tacrine **1** bis-huperzine A **2** (n=7-12)

(−)-Huperzine B (HupB, **4**) is a *Lycopodium* alkaloid isolated from the clubmoss *Huperzia serrata*. It is less potent and selective in the inhibition of AChE than huperzine A (HupA)[7] which is also isolated from the same herb and has been approved in China as a drug for the treatment of AD.[8] However, HupB exhibited a higher therapeutic index in comparison with HupA.[9] In continuation of our studies on the homodimer AChE inhibitors, which are able to bind simultaneously to both catalytic and peripheral binding sites of AChE, a series of bis-HupB derivatives **5** were designed, synthesized, and tested for their inhibitory activity of AChE.

It was also presumed that in vivo two positively charged nitrogen atoms in the long tether of bis-HupB compounds **5** could interact via hydrogen bonds or cation−$\pi$ interaction with residues along the active site gorge of AChE. Additionally, the alkylene tether in **5** could also improve the potency through hydrophobic effects.[10]

Herein we would like to report the synthesis and preliminary data of AChE inhibition with bis-HupB compounds **5**, in which two HupB molecules are linked together by a carbon−nitrogen chain through their secondary amino group of HupB. The bioassay data indicated that most of the bis-HupB compounds **5a**−**h**

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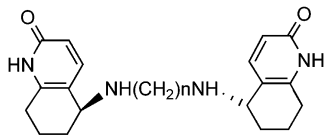
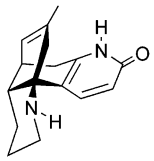


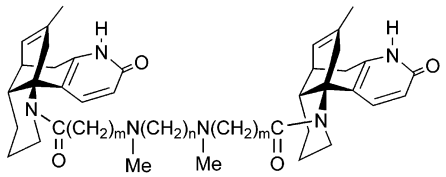
bis-5-amino-5,6,7,8-tetra-hydroquinolinone

**3** (n=5-14)



huperzine B

**4**



bis-huperzine B

**5** (m=1, 2; n=2, 3, 6, 8, 10, 12)

are much more potent than HupB in the inhibition of AChE. The inhibitory potency and selectivity of bis-HupB **5** are dependent on the length of the tether, suggesting a dual-site binding of bis-HupB compounds to AChE. The optimal compound is bis(18)-HupB (**5g**), which showed significantly higher potency (3915-fold) and selectivity (932-fold) for the inhibition of AChE in comparison with natural (−)-HupB (Table 1). It is suggested that bis(18)-HupB (**5g**) could simultaneously interact with both the central catalytic and the peripheral sites in the active site gorge of AChE and thus maximized the inhibitory potency.

For the preparation of bis-HupB compounds, a few methods were tested including the reaction of HupB with α,ω-dihaloalkanes in the presence of silver carbonate or with α,ω-alkanediacyl dihalides in the presence of triethylamine. However, the yields of the corresponding desired products were very low. Reductive-amination of α,ω-alkanedialdehydes with HupB, which successfully delivered six dimers of huperzine A [3], also failed in furnishing the HupB dimers. Considering the steric hindrance of the amino group, HupB was thus first acylated with chloroacetyl chloride and acryloyl chloride to afford chloroacetyl HupB **6** (96% yield) and acryloyl HupB **7** (95% yield), respectively. The reaction of **6** or **7** with α,ω-alkanediamines was complicated; therefore, α,ω-*N*,*N*′-dimethylalkanediamines were used to couple two acylated HupB monomers. Reaction of α,ω-*N*,*N*′-dimethylalkanediamines (1 equiv) with chloroacetyl HupB **6** (2 equiv) proceeded smoothly in acetonitrile at 70 °C in the presence of potassium carbonate and potassium iodide, affording bis-HupB **5a**−**d** in 70−80% yields. Michael addition of acryloyl HupB **7** (2 equiv) and α,ω-*N*,*N*′-dimethylalkanediamines (1 equiv) was conducted in acetonitrile at reflux, catalyzed by silica gel, furnishing bis-HupB **5e**−**h** in 80−98% yields (Scheme 1).

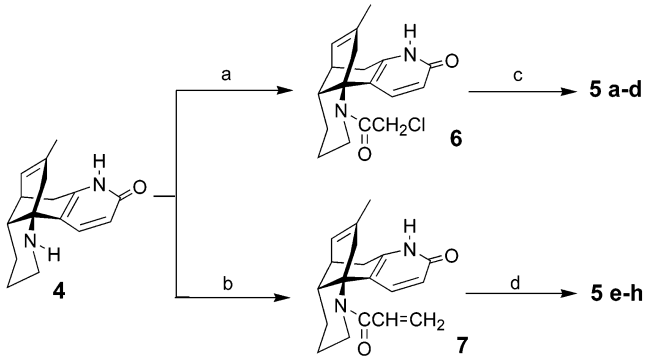
To explore the possible binding conformation and interaction mode of the bis-HupB compounds with AChE, a molecular modeling study was performed employing the docking program DOCK4.0[12,13] based on the structure of the complex of *Tc*AChE with HupB (PDB entry 1GPN[14]). Since bis(18)-HupB (**5g**) afforded

**Table 1.** Cholinesterase Inhibition and Selectivity by Bis-HupB[a]

| compound | *m* | *n* | AChE ($IC_{50}$,nM)[b] | BuChE ($IC_{50}$,nM)[c] | selectivity for AChE[d] |
|---|---|---|---|---|---|
| (−)-HupB | − | − | 19 300 ± 200 | 227000 ± 600 | 11.8 |
| (−)-Hup A | − | − | 72.4 ± 3.8 | 70200 ± 800 | 970 |
| bis(8)-hupB[e], **5a** | 1 | 2 | 1020 ± 390 | − | − |
| bis(9)-HupB,**5b** | 1 | 3 | 471 ± 52 | 60000 ± 3000 | 127 |
| bis(12)-HupB, **5c** | 1 | 6 | 76.2 ± 4.3 | 64500 ± 4500 | 858 |
| bis(14)-HupB, **5d** | 1 | 8 | 50.8 ± 18.7 | 65400 ± 2400 | 1290 |
| bis(14)-HupB, **5e** | 2 | 6 | 35.9 ± 7.5 | 67900 ± 3800 | 1890 |
| bis(16)-HupB, **5f** | 2 | 8 | 18.5 ± 6.2 | 63000 ± 2500 | 3400 |
| bis(18)-HupB, **5g** | 2 | 10 | 4.93 ± 0.23 | 54300 ± 2700 | 11000 |
| bis(20)-HupB, **5h** | 2 | 12 | 25.2 ± 4.6 | 88400 ± 3500 | 3830 |

[a] Assay performed by the modified Ellman method at pH = 7.4.[11] [b] Assay performed using rat cortex homogenate. Results are the mean ± SD. [c] Assay performed using rat serum. [d] Selectivity for AChE is defined as $IC_{50}$ (BuChE)/$IC_{50}$ (AChE). [e] The numbers refer to the atom numbers of the tether.

**Scheme 1.** Synthesis of Bis-HupB[a]



[a] Reagents and conditions: (a) chloroacetyl chloride, $Et_3N$, $CH_2Cl_2$, 0 °C, 1 h, 96%;_(b) acryloyl chloride, $Et_3N$, $CH_2Cl_2$, 0 °C, 0.5h; $NH_3$−$CH_3OH$, rt, 12 h, 95%; (c) α,ω-*N*,*N*′-dimethylalkanediamine, $K_2CO_3$, KI, $CH_3CN$, 70 °C, 8 h, 70−80%; (d) α,ω-*N*,*N*′-dimethylalkanediamine, silica gel (200−300 mesh), $CH_3CN$, reflux, 80−98%.

maximum inhibitory potency, it was the only compound chosen for docking simulations. Two $sp^3$ N atoms in bis-(18)-HupB (**5g**) were both protonated. The central catalytic pocket, peripheral pocket and the binding gorge were taken as the target in the docking simulation. In the search for multiple anchors the *Tc*AChE structure was kept fixed and the bis(18)-HupB(**5g**) flexible while the maximum-orientations and configurations-per-cycle parameters were set to 600 and 150, respectively. The docking results demonstrated that one HupB moiety in bis(18)-HupB (**5g**) interacted with *Tc*AChE in the central catalytic pocket near the residue Trp84, and the another HupB moiety interacted in the peripheral pocket near the residue Trp279, as shown in Figure 1.[15] The binding position and mode of the central catalytic HupB moiety is similar to HupB in the crystal structure (1GPN), having two hydrogen bonds with Tyr130 and hydrophobic interaction with Trp84, Gly118, Gly119, Phe290, Phe330, Phe331, and His440. The another HupB moiety in the peripheral pocket interacted with Asn280 through a hydrogen bond, and with Trp279 and Leu282 via hydrophobic interaction. The long tether could fold with a proper conformation in the gorge that might favorably interact with Tyr70, Asp72, Tyr121, Trp279, Ile287, Phe330, and Tyr334 through hydrophobic interaction. The simultaneous interactions of bis-(18)-HupB (**5g**) in the central pocket, gorge, and periph-

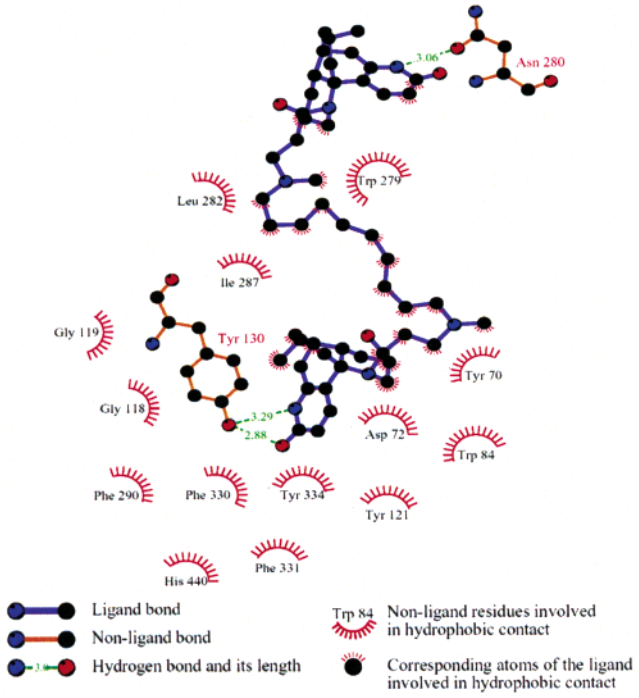



**Figure 1.** Interaction between bis(18)-HupB (**5g**) and *Tc*AChE.

eral pocket of *Tc*AChE suggests the reason for the high inhibitory potency of AChE.

On the basis of the homodimer strategy in rational drug design, natural (−)-HupB was used as the parent monomer to prepare a number of bis-HupB compounds with various lengths of the tether. Most of them exhibited potent AChE inhibition and selectivity. The results provide new insights into the factors affecting AChE−ligand interaction in the active gorge, which is beneficial to the further development of novel AChE inhibitors.

**Supporting Information Available:** Experimental procedures and characterization of compounds **6**, **7**, and **5a**−**h**. This material is available free of charge via the Internet at http://pubs.acs.org.

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