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Synthesis and acetylcholinesterase inhibition of derivatives of huperzine B

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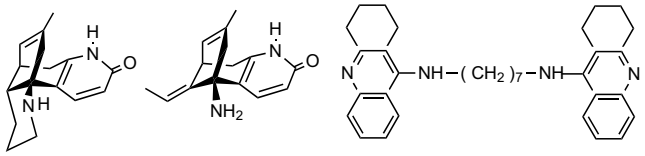
Abstract—By targeting dual active sites of AChE, a number of new derivatives of HupB have been synthesized and tested as acetylcholinesterase inhibitors. The most potent compound, bis-HupB **5b** is 72-fold more potent in AChE inhibition and 79-fold more selective for AChE versus BChE than HupB.

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 β-amyloid peptide deposition.[1]

Natural (−)-huperzine B (HupB, **1**), a *Lycopodium* alkaloid from *Huperzia serrata*, is demonstrated as a potent and reversible inhibitor of AChE.[2,3] In experiments performed on unanaesthetized rabbits,[4] HupB exhibited a higher therapeutic index in comparison with (−)-huperzine A (HupA, **2**), which is more potent than HupB in AChE inhibition. (−)-HupA is the major *Lycopodium* alkaloid and has now been approved in China as a drug for the treatment of AD.[5]

However, the studies on chemical modification and structure–activity relationships of HupB are very limited, only a few simple N-methylated and hydrogenated derivatives of HupB were prepared and tested for their inhibitory activity of AChE.[6]



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Based on the hypothesis with respect to two binding sites in the active-site gorge of AChE and the good example of bis-tacrine (**3**)[7] as well as the crystallographic structure of the complex of AChE with bivalent ligands related to HupA,[8] a series of new derivatives of HupB with two pharmacophoric moieties, which may interact with both central and peripheral binding sites in the active-site gorge of AChE, respectively, were synthesized and tested for their inhibitory activity of AChE. For the preparation of bis-HupB derivatives **5**, a few methods were tested including the reaction of HupB with α,ω-dihaloalkane in the presence of silver carbonate or with α,ω-diacylalkane dihalides in the presence of triethylamine, but no desired products were obtained. Furthermore, reductive amination of α,ω-alkylenedial with HupB, which successfully delivered six dimers of HupA,[9] also failed in furnishing bis-HupB.

In this paper, we would like to describe the synthesis and antiAChE activity of new derivatives of HupB.

Because of the steric hindrance of the amino group, the direct dimerization of HupB via a tether linked to the amino group is very difficult. HupB was thus first acylated by chloroacetyl chloride, obtaining chloroacetyl HupB **4** with a good yield of 96%. When 2 equiv of chloroacylated HupB **4** and 1 equiv of piperazine or

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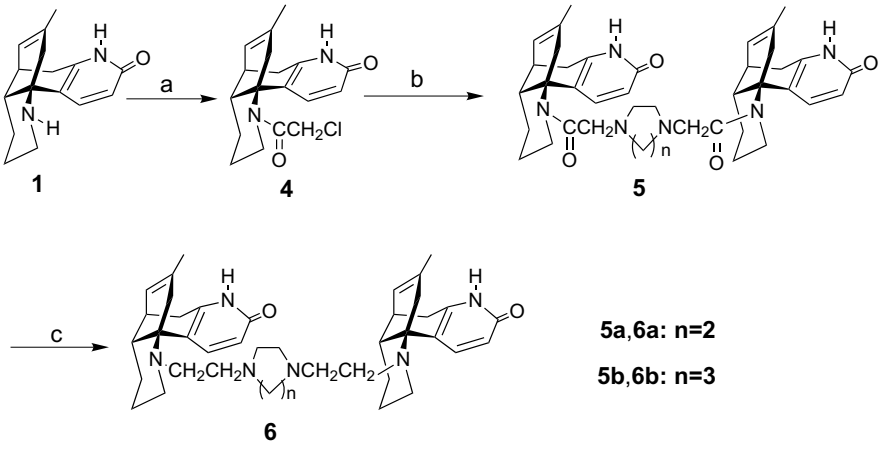
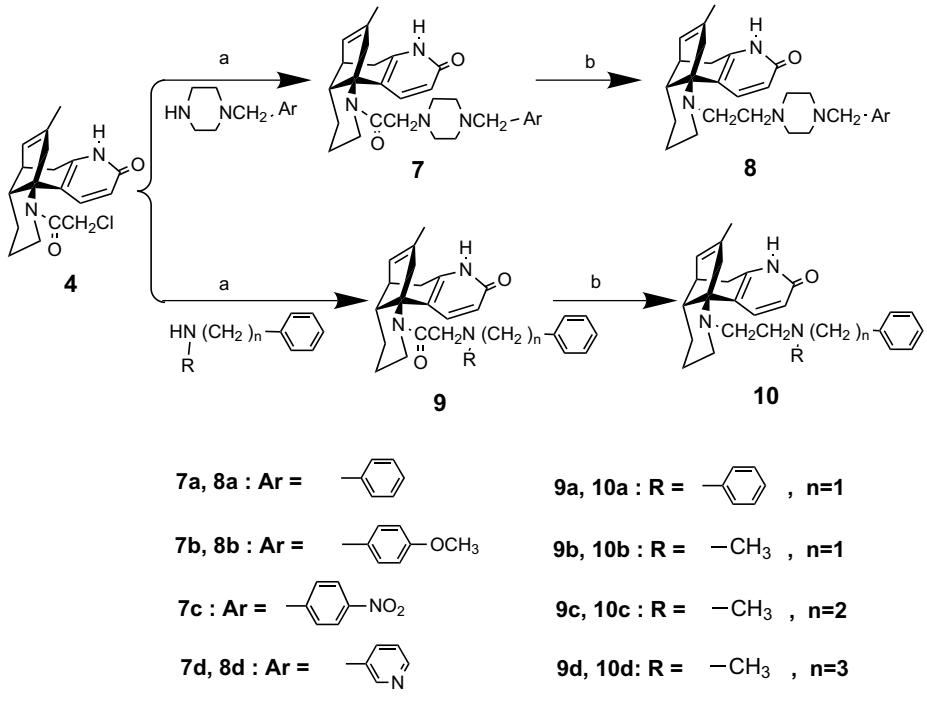
homopiperazine reacted in acetonitrile at 70 °C in the presence of potassium carbonate and potassium iodide, the HupB dimers **5a** and **5b** were afforded, which were further reduced by $LiAlH_4$ to produce **6a** and **6b**, respectively (Scheme 1). The overall yields of **6a** and **6b** are 45–50%.

Considering that an aromatic ring in a bivalent anti-AChE ligand is the important feature for the binding of the ligand to the peripheral site of AChE,[10] we also designed a series of HupB hetero-dimers **7**, **8**, **9** and **10**, in which HupB moiety was combined with an aromatic moiety via a nitrogen-containing tether. The synthetic approach to these hetero-dimers is quite similar to the homo-dimers **5** and **6** (Scheme 2).

The antiAChE activity of the HupB derivatives were measured in vitro according to the modified Ellman method.[11] The results in Table 1 reveal that the homo-dimeric bis-HupB **5** and **6** show much higher potency and selectivity, however, the hetero-dimers **7**, **8**, **9** and **10** exhibit equal or lower activity in AChE inhibition than their parent HupB. The homo-dimer **5b** is 72-fold more potent in inhibiting AChE and 79-fold more selective for AChE versus butyrocholinesterase (BChE) than HupB. The length of the side chain in hetero-dimers **9** and **10** is crucial to the inhibitory activity of AChE. Compounds **9d** and **10d** ($n = 3$) exhibit 7–8-fold increase in AChE inhibition than **9b** and **10b** ($n = 1$). The inhibitory activities of the amides **5**, **7** and **9** are nearly the same as the corresponding amines **6**, **8** and **10**. The pre-



Scheme 1. Synthesis of bis-HupB. Reagents and conditions: (a) chloroacetyl chloride, Et_3N, CH_2Cl_2, 0 °C, 1 h, 96%; (b) piperazine or homopiperazine, K_2CO_3, KI, CH_3CN, 70 °C, 8 h, 70–80%; (c) $LiAlH_4$, THF, reflux 2 h, 60–70%.



Scheme 2. Synthesis of HupB hetero-dimer derivatives. Reagents and conditions: (a) K_2CO_3, KI, $CHCl_3$, reflux, 24 h, 40–90%; (b) $LiAlH_4$, THF, reflux 2 h, 60–80%.

Table 1. Physical properties and AChE inhibition of derivatives of (−)-HupB

Compd[13]	$[\alpha]_D^{25}$ (c = 0.1, $CHCl_3$)	Mp (°C)	AChE IC_{50} (μM)[a]	BChE IC_{50} (μM)[b]	Selectivity for AChE[c]
HupB		270–271	8.202	157	19
5a	107.3	>230 dec	1.173	>184	>157
6a	52.4	>230 dec	0.407	192	472
5b	94.8	>230 dec	0.114	171	1500
6b	−3.5	202–204	0.218	125	573
7a	65.4	98–100	33.30	—	
8a	−6.4	115–117	31.60	—	
7b	81.2	145–147	5.747	—	
8b	−8.4	112–114	10.297	—	
7c	77.1	>140 dec	5.58	—	
7d	88.4	132–134	38.10	—	
8d	4.6	114–116	26.70	—	
9a	127.5	138–140	>52	—	
10a	−26.8	116–118	>54	—	
9b	104.8	112–114	50.70	—	
10b	−8.7	84–86	66.45	—	
9c	100.9	68–70	23.50	—	
10c	−13.9	75–77	20.70	—	
9d	121.7	103–105	6.19	—	
10d	−9.4	69–70	9.531	—	

[a] Assay performed by the modified Ellman method[12] using rat cortex homogenate. Values are means of three different experiments.
[b] Assay performed using rat serum.
[c] Selectivity for AChE is defined as IC_{50} (BChE)/IC_{50} (AChE).

liminary docking (DOCK 4.0) studies of **5b** based on the structure of the complex of *Tc*AChE with HupB,[12] revealed that one HupB moiety bound to central catalytic site and another one bound to the opening of the active-site gorge, with the homopiperazinyl group interacting with the middle part of the gorge. The further molecular modeling studies are now in progress.

In summary, we have succeeded in the synthesis of 19 new HupB derivatives, in which bis-HupB **5a**, **5b**, **6a** and **6b**, are much more potent and selective in AChE inhibition. The obviously increased AChE inhibition and selectivity of bis-HupB are presumed to be related with interaction with both central and peripheral active sites of AChE. Further studies on bis-HupB derivatives are in progress in our laboratory.

Acknowledgements

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13. All new compounds showed satisfactory spectroscopic data.
Selected analytical data:
5b: IR (KBr): 3427, 2928, 1655, 1556, 1406, 1186, 1107, 833, 752 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): 12.90 (br s, 2H), 75.6 (d, 2H, J = 9.47 Hz), 6.41 (d, 2H, J = 9.5 Hz), 5.42 (d, 2H, J = 4.3 Hz), 3.91 (d, 2H, J = 11.4 Hz), 3.42 (m, 4H, J = 14.7 Hz, 17.9 Hz), 3.28 (d, 2H, J = 14.1 Hz), 2.79–2.92 (m, 8H), 2.39–2.58 (m, 8H), 1.91 (m, 4H), 1.57–1.66 (m, 10H), 1.21–1.39 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): 173.4 × 2, 165.3 × 2, 142.8 × 2, 142.2 × 2, 133.4 × 2, 123.8 × 2, 118.2 × 2, 117.3 × 2, 64.6 × 2, 61.4 × 2, 55.3 × 2, 54.1 × 2, 45.5 × 2, 44.7 × 2, 40.7 × 2, 34.5 × 2, 29.6 × 1, 28.9 × 1, 27.9 × 1, 26.2 × 2, 25.6 × 2, 23.0 × 2; ESIMS (m/z): 693.5 ($(M+H)^+$, 100), 409.8 (11), 283.4.
6a: IR (KBr): 3419, 2925, 1658, 1604, 1552, 1457, 1299, 1112, 833, 638 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 11.95 (br s, 2H), 7.63 (d, 2H, J = 9.3 Hz), 6.42 (d, 2H, J = 9.6 Hz), 5.47 (d, 2H, J = 4.1 Hz), 2.84 (dd, 2H, J = 17.6 Hz, 4.9 Hz), 2.46–2.72 (m, 8H), 2.38–2.52 (m, 8H), 2.28–2.36

(m, 8H), 2.05 (d, 2H, J = 16.5 Hz), 1.81 (m, 2H), 1.58 (s, 6H), 1.48–1.72 (m, 4H), 1.42 (m, 2H), 1.28 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 163.8 × 2, 142.5 × 2, 141.2 × 2, 131.5 × 2, 124.8 × 2, 120.4 × 2, 116.7 × 2, 59.9 × 2, 57.0 × 2, 52.6 × 4, 47.2 × 2, 46.8 × 2, 43.5 × 2, 36.9 × 2, 33.6 × 2, 28.7 × 2, 25.1 × 2, 23.5 × 2, 22.1 × 2; ESIMS (m/z): 651.4 ($(M+H)^+$, 100), 395.2 (25), 283.2, 240.2, 226.2.

6b: IR: 3423, 2928, 2794, 1657, 1605, 1552, 1458, 1113, 752 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.63 (2H, br, s), 7.62 (2H, d, J = 9.5), 6.39 (2H, d, J = 9.3), 5.45 (2H, d, J = 5.1), 3.28 (2H, m), 2.84 (4H, d.d, J = 5.4, 17.4), 2.62 (2H, J = 12.4), 2.26–2.47 (16H, m), 1.99 (2H, d, J = 16.35), 1.65–1.79 (12H, m), 1.49–1.58 (8H, m), 1.39 (2H, d, J = 12.1), 1.17–1.25 (4H, m); ESIMS (m/z): 667.5 ($(M+H)^+$, 100), 689.6 ($(M+Na)^+$, 3), 411.4 (5), 354.3 (2).

8a: IR (KBr): 3419, 2931, 2808, 1657, 1552, 1456, 1300, 1112, 833, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.64 (1H, br s), 7.63 (1H, d, J = 9.6 Hz), 7.24–7.32 (5H, m), 6.40 (1H, d, J = 9.5 Hz), 5.43 (1H, d, J = 5.2 Hz), 3.52 (2H, s), 3.25–3.31 (1H, m), 2.85 (1H, dd, J = 16.8, 5.5 Hz), 2.26–2.67 (15H, m), 1.98 (1H, dd, J = 16.5 Hz), 1.74 (1H, m), 1.59 (1H, m), 1.54 (3H, s), 1.48 (1H, m), 1.38 (1H, m), 1.14–1.27 (1H, m); EIMS (m/z): 458 (M^+, 8), 285 (15), 269 (100), 84 (47).

10d: IR (KBr): 3431, 2933, 1660, 1612, 1462, 1404, 1186, 1107, 833, 700, 534 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.20 (1H, br s), 7.56 (1H, d, J = 9.3 Hz), 7.15–7.28 (5H, m), 6.40 (1H, d, J = 9.3 Hz), 5.40 (1H, d, J = 5.5 Hz), 3.98 (1H, m), 3.46 (1H, d, J = 14.3 Hz), 3.38 (1H, d, J = 18.1 Hz), 3.01 (1H, d, J = 14.3 Hz), 2.89 (1H, dd, J = 18.1, 5.5 Hz), 2.56–2.72 (2H, m), 2.34–2.52 (6H, m), 2.29 (3H, s), 1.73–2.00 (4H, m), 1.67 (2H, m), 1.57 (3H, s), 1.22–1.35 (1H, m); EIMS (m/z): 445 (M^+, 2), 255 (20), 162 (100), 91 (15).