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Synthesis of More Potent Analogues of the Acetylcholinesterase Inhibitor, Huperzine B

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Abstract—The synthesis and acetylcholinesterase inhibition activity of analogues of huperzine B are reported. These new racemic analogues show a better AChE inhibitory activity than the natural product huperzine B. © 2002 Elsevier Science Ltd. All rights reserved.

Alzheimer's disease (AD) is a neurodegenerative process associated with the deposition of amyloid plaques and fibrillary tangles, as well as with neurotransmitter alterations in the brain.[1] One of the main functional deficits in AD involves cholinergic neurons, which provided the rationale for a potential therapeutic approach to the treatment of AD.[2] Several therapeutic strategies have been explored to enhance cholinergic neurotransmission in order to alleviate some of the symptoms of AD. These include inter alia the use of acetylcholinesterase (AChE) inhibitors, the administration of acetylcholine precursors, the investigation of acetylcholine releasers and direct acetylcholine receptor agonists (both muscarinic and nicotinic).[3] Among these strategies, inhibition of AChE has proven to be the most successful means to balance the cholinergic deficit and to stabilize the symptoms.[4] Because of the partial success that has been achieved with the use of AChE inhibitors, considerable effort has been expended in identifying potent and selective compounds, which has resulted in the discovery and development of several AChE inhibitors.[5] Among them, huperzine A (**1**, HA), a Lycopodium alkaloid isolated from the clubmoss *Huperzia serrata* (Thunb.), has attracted considerable attention for its possible use in the treatment of AD.[6] This natural product is a very potent and selective reversible inhibitor of AChE with almost no action on butyrylcholinesterase. The superior inhibition properties of HA have been attributed to its very slow dissociation ($t_{0.5} = 35\,\text{min}$) from the enzyme and its long duration of action.[7] Huperzine B (**2**, HB), a congener of HA, also functions as a reversible inhibitor of AChE with a considerable potency ($K_I = 0.79\,\mu\text{M}$).[8–10] Interestingly, when experiments were performed on unanesthetized rabbits, HB exhibited a higher therapeutic index due to its longer duration of action in comparison with HA.[11]

Recently, a new compound **3** was synthesized and tested for AChE inhibition.[12] Although compound **3** incorporated structural features of both HA and HB, it was less active than the parent molecules. A preliminary molecular modeling study of this hybrid analogue in complex with AChE revealed that in comparison to the HA complex, the cation-π interaction between the ammonium group and Trp 84 and Phe 330 is altered slightly by the presence of the piperidine ring methylene groups. Based on these findings, we decided to prepare analogues **4** and **5** (see Fig. 1) to explore the effects of the size of the heterocyclic ring and its substituents on the AChE activity.

The synthesis of analogues **4** and **5** was achieved following Scheme 1. The key intermediate **13** was obtained using our previously established protocol.[12] Acylation of the amino alcohol **13** with either carboxylic acid **14** or **15** in the presence of DCC and HOBt in CH_2Cl_2 provided the amides **16** and **17** in 80–85% yield. Oxidation of alcohols **16** and **17** with $n\text{-}Pr_4NRuO_4$/NMO provided the corresponding ketones **18** and **19** in 90 and 92% yield, respectively. The monoketone **18** was then successively subjected to TBS ether cleavage and oxidation to afford

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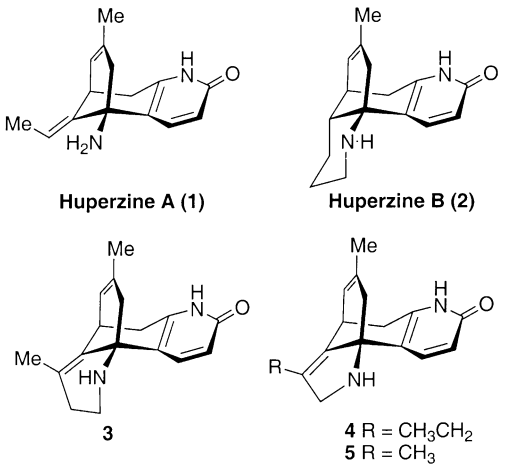


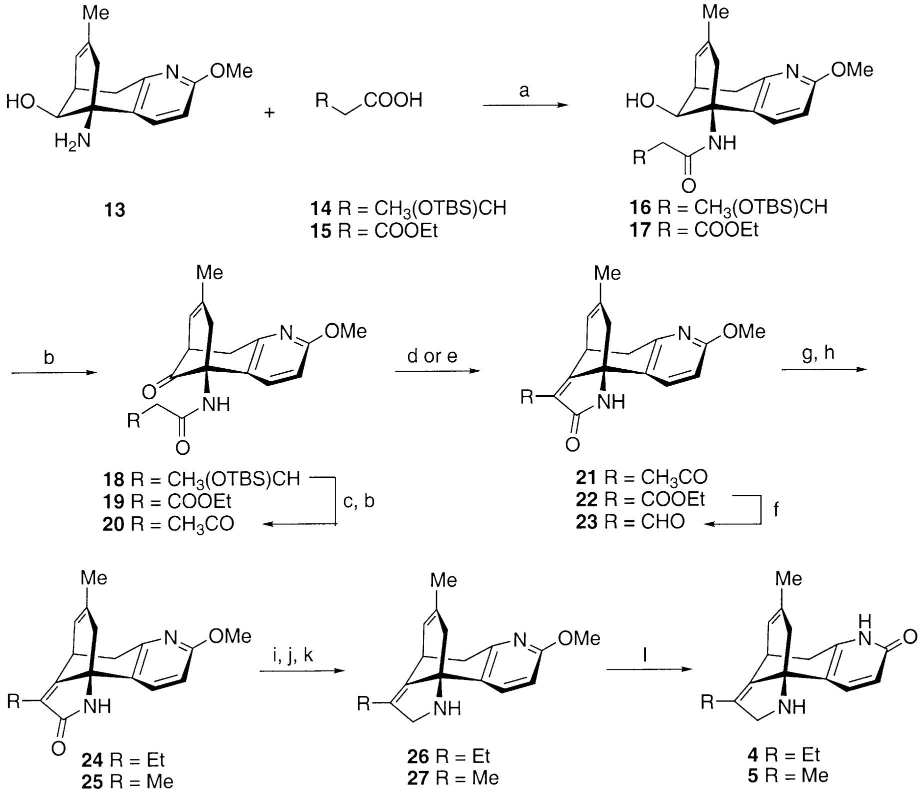
Figure 1. Structures of huperzine A, huperzine B, and the analogues.

diketone **20** in 87% yield. Treatment of diketone **20** with silica gel at room temperature gave the intramolecular aldol product **21** in 87% yield, whereas compound **19** underwent aldol reaction only in the presence of K_2CO_3 in ethanol to give the cyclic compound **22** in 76% yield. Reduction of compound **22** with DIBAL-H at −78 °C gave aldehyde **23** in 72% yield. Direct deoxygenation of **21** to **24** failed, whereas conversion to a thioketal and subsequent Ra-Ni treatment gave **24** in 76% yield. Next, the lactam **24** was treated with Lawesson's reagent, and the resulting thiolactam was successively reduced with Ra-Ni and $NaBH_3CN$ to afford **26** in 67% yield from **24**. Finally, deprotection of **26** with Me_3SiI led to the desired analogue **4** in 83% yield.[13] In a similar fashion, the compound **23** was converted into analogue **5** in good yield.[13]

Table 1. K_I values for the inhibition of fetal bovine serum (FBS) AChE by HB and analogues

Compd	K_I (μM)
(−)-HB	0.794[8]
(±)-HA	0.024[16]
3	2.05±0.11
4	0.40±0.03
5	0.22±0.01

The AChE inhibitory data for the new huperzine B analogues are provided in Table 1 along with the K_I for huperzine B and (±)-huperzine A.[14,15] As is apparent, compound **3** is less active than HA and HB in spite of the fact that it is more closely related to both HA and HB than the remaining two analogues. Although analogues **4** and **5** are racemic, they are 2- and 4-fold more potent than natural huperzine B, respectively. Thus, if either the size of the substituent (methyl to ethyl) or the heterocyclic ring size (five- to six-membered) is increased, activity decreases, possibly indicating a steric interaction with the walls of the active site gorge. Compound **5** thus represents a lead compound for further studies, and the present synthetic work provides a convenient method for preparing analogues of huperzine B.



Scheme 1. Reagents and conditions: (a) DCC, HOBt, CH_2Cl_2, rt, 7 h, 80–85%; (b) n-Pr_4NRuO_4, NMO, CH_2Cl_2, rt, 0.5 h, 90–92%; (c) TBAF, THF, 0 °C, 0.5 h, 87%; (d) SiO_2, rt, 5 h, 86%; (e) cat. K_2CO_3, EtOH, rt, 5 h, 76%; (f) DIBAL-H, CH_2Cl_2, −78 °C, 2 h, 72%; (g) $HS(CH_2)_2SH$, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C to rt, 1 h; (h) Ra-Ni, EtOH, rt, 2 h, 76% (2 steps); (i) Lawesson's reagent, CH_2Cl_2, rt, 12 h; (j) Ra-Ni, THF, rt, 2 h; (k) $NaBH_3CN$, THF, rt, 0.5 h, 67% (3 steps); (l) Me_3SiI, $CHCl_3$, reflux, 4 h, then MeOH, reflux, 12 h, 83%.

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13. Spectral data for compounds **4** and **5**: (**4**) ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.48 (d, 1H, $J = 9.3$ Hz), 6.43 (d, 1H, $J = 9.3$ Hz), 5.46 (d, 1H, $J = 6.0$ Hz), 3.88, 3.80 (ABq, 2H, $J = 13.9$ Hz), 3.40 (s, 1H), 2.87 (dd, 1H, $J = 5.1$, 17.1 Hz), 2.78 (dd, 1H, $J = 1.5$, 16.8 Hz), 2.47 (d, 1H, $J = 16.8$ Hz), 2.22–2.00 (m, 3H), 1.56 (s, 3H), 1.25 (s, 1H), 1.0 (t, 3H, $J = 5.7$ Hz); ^{13}C NMR (75 MHz, CDCl$_3$) δ 165.21, 141.61, 139.74, 134.57, 132.79, 128.25, 124.30, 123.13, 117.85, 67.07, 55.55, 46.94, 35.44, 30.91, 29.68, 23.01, 19.35; MS (EI) m/z 268 (M$^+$, 25%), 253, 239 (100%), 213, 197, 184, 156, 105, 84. (**5**) ^{1}H NMR (300 MHz, CDCl$_3$) δ 7.49 (d, 1H, $J = 9.3$ Hz), 6.43 (d, 1H, $J = 9.3$ Hz), 5.48 (br d, 1H, $J = 5.4$ Hz), 3.88, 3.73 (ABq, 2H, $J = 13.9$ Hz), 3.38 (br s, 1H), 2.91 (dd, 1H, $J = 4.9$, 16.9 Hz), 2.78 (br d, 1H, $J = 16.3$ Hz), 2.47 (d, 1H, $J = 15.9$ Hz), 2.19 (d, 1H, $J = 16.3$ Hz), 1.67 (s, 3H), 1.57 (s, 3H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 165.04, 141.53, 139.75, 134.63, 133.38, 124.29, 122.98, 117.94, 67.00, 57.79, 46.92, 35.23, 30.76, 29.68, 23.00; MS (EI) m/z 254 (M$^+$, 95%), 239 (100%), 211, 199, 184, 83.
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