Synthesis and biological activity of an optically pure 10-spirocyclopropyl analog of huperzine A

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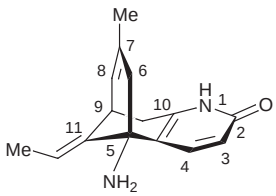
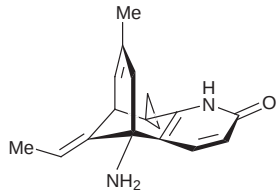
The synthesis of a spirocyclic analog of huperzine A that bears a cyclopropane ring at its 10-position has been carried out in an enantioselective manner using a diastereoselective Michael–aldol reaction; in assays of AChE inhibition, this compound was found to be nearly as active as huperzine A itself, with comparable on and off rates from the enzyme.

Huperzine A (HA), a potent reversible inhibitor of acetylcholinesterase (AChE), is an important psychotherapeutic agent for improving cognitive function in Alzheimer's patients by enhancement of central cholinergic tone.[1] Because of the tremendous promise this alkaloid holds for the palliative treatment of a disease that afflicts millions of individuals worldwide, we and others have been engaged in an intensive effort to explore the structure–activity relationships of this alkaloid.[2] From biological studies with mammalian AChE, Torpedo AChE, mammalian BChEs (butyrylcholinesterases) and mouse AChE mutants, together with molecular modeling studies, mammalian Tyr337(330) and Trp86(84) have been implicated in the binding of HA to AChE.[3] This particular interaction is of the cation (NH_3^+)–π type,[4] while other amino acid residues appear to participate in hydrogen bonding to the pyridone NH and the carbonyl group.[5] The superior inhibition properties of HA have been attributed to the very slow dissociation ($t_{0.5}$ = 35 min) of the AChE–huperzine A complex in solution.[6] To date, we have identified several analogs of HA that have comparable or better activity than the parent structure. In particular, the C-10 axial methyl analog of HA was found to be about 8-fold more potent than HA, whereas the 10,10-dimethyl analog was found to possess comparable activity. In exploring further modifications to this region of the molecule, we felt that it would be of interest to examine a spirocyclic analog bearing a cyclopropyl group at C-10. Such an analog can be viewed as a ring constrained version of the dimethyl derivative. The possibility exists, however, that its activity might be improved due both to the smaller size of the cyclopropyl group and possible electronic effects which may be capable of enhancing interactions with the enzyme.



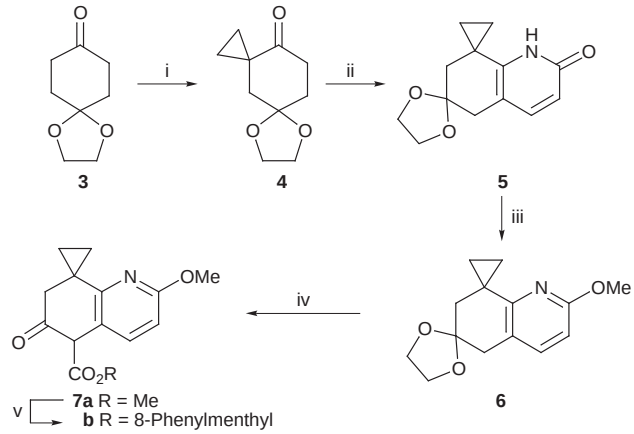
Huperzine A (HA) **1**



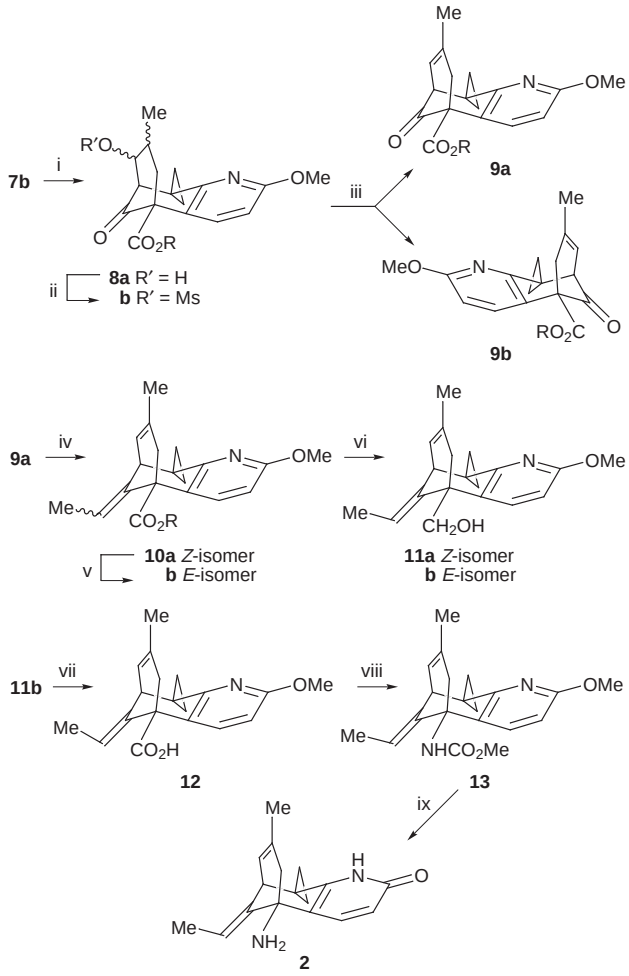
Spirocyclopropyl Analog **2**

The chemical pathway that was followed to assemble the cyclopropyl analog **2** is shown in Schemes 1 and 2. The key intermediate **7b** was obtained in good yield through a sequence of steps starting from the reaction of cyclohexanedione monoethylene ketal **3** with (2-chloroethyl)dimethylsulfonium iodide/Bu^{t}OK in Bu^{t}OH at room temperature to afford the spirocyclohexanone **4** in 60% yield (Scheme 1).[7] The spirocyclohexanone **4** was transformed to the β-keto ester **7a** employing conditions identical to those reported previously.[1,2] Transesterification of **7a** with (−)-8-phenylmenthol gave the desired ester derivative **7b** in 75% overall yield.[8] Next, a diastereoselective Michael–aldol reaction of **7b** with methacrolein was carried out. Under optimal conditions, the reaction was run at −20 °C over a two day period. The alcohol mixture **8a** that formed was converted to a 12.5 : 1 mixture (ratio from ^{1}H NMR analysis) of the olefins **9a** and **9b**, respectively, in 70% yield by NaOAc–HOAc induced elimination of the derived mesylate **8b**. These olefins were readily separable by column chromatography on silica gel using 1 : 9 ethyl acetate–hexanes as eluent. The major isomer **9a** was subjected to a Wittig olefination reaction to afford the *Z*-isomer **10a**. Isomerization of the double bond using thiophenol–AIBN gave a mixture of the *E*- and *Z*-isomers **10b** and **10a** in a 6 : 1 ratio, respectively. The ester group of this mixture was reduced to alcohols **11a** and **11b** by treatment with LAH in THF, and these isomers were separated by column chromatography. The enantiomeric purity of the major isomer **11b** was confirmed at this stage by transforming it to its Mosher ester derivative. ^{1}H and ^{19}F NMR spectroscopy revealed the compound to be of at least 98% optical purity. Jones oxidation of the alcohol **11b** afforded the acid **12**, which was converted to urethane **13** by Curtius rearrangement. Lastly, the required compound **2** was obtained in 75% yield by the removal of the protecting groups from **13** using TMSI in $CHCl_3$ at reflux. The optical rotation of crystalline **2** (mp 235 °C) was found to be −73 (*c* 0.83, $CHCl_3$). For purposes of biological comparison, we also prepared the spirocyclopropyl analog in racemic form, using basically the same approach as above but omitting the transesterification reaction with 8-phenylmenthol.

The biological activity of the analogs of huperzine A was evaluated using AChE purified from fetal bovine serum.[9] AChE



Scheme 1 *Reagents and conditions*: i, $Me_2S^+CH_2CH_2Cl$ I^-, KI, KOBu^t, Bu^{t}OH, room temp.; ii, ethyl propiolate, MeOH–NH_3, 100 °C, 300 psi; iii, MeI, Ag_2CO_3, $CHCl_3$, reflux; iv, MeOH–HCl, reflux, then $(MeO)_2CO$, NaH, THF, reflux; v, (−)-8-phenylmenthol, TsOH, benzene, reflux



Scheme 2 *Reagents and conditions*: i, CH_2=CMeCHO, $(Me_2N)_2C$=NH, CH_2Cl_2, −20 °C; ii, $MeSO_2Cl$, Et_3N, DMAP, CH_2Cl_2, room temp.; iii, NaOAc, AcOH, reflux; iv, $Ph_3P^+Et\ Br^-$, KOBu^t, THF, room temp.; v, PhSH, AlBN, toluene, reflux; vi, LAH, THF, reflux; vii, Jones reagent, acetone, room temp.; viii, $(PhO)_2PON_3$, Et_3N, toluene, reflux, 2 h, then MeOH, reflux; ix, TMSI, $CHCl_3$, reflux, 6 h, then MeOH, reflux

activity was measured in 50 mM sodium phosphate, pH 8.0, at 22 °C as described previously using acetylthiocholine as the substrate.[10] The interaction of HA and its analogs with AChE can be described by eqn. (1):[6]

$$E + HUP\text{-}A \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E \cdot HUP\text{-}A$$

The ratio k_{off}/k_{on} is the dissociation constant (K_I). The K_I values for the inhibition of FBS AChE with analogs of HA were determined by equilibrating a known amount of enzyme (1–2 units ml^{-1}) with various concentrations of the analog. Plots of percent residual activity *versus* [analog] were used to calculate K_I by the steady state method. The rate constant for the inhibition of AChE was determined by diluting an appropriate volume of stock solutions (1–2 μM) of each analog of huperzine A into the enzyme solution (5–10 units ml^{-1} in 50 mM sodium phosphate, pH 8.0, containing 0.05% BSA) and measuring the residual enzyme activity at various time intervals. Plots of percent residual activity *versus* time at each concentration were used to calculate the rate of inhibition (k_{on}). Direct measurement of the rate constant of regeneration of enzyme activity (k_{off}) was initiated by > 10 000-fold dilution of HA-inhibited AChE (2–4 μM) to ascertain that the rate of inhibition by residual inhibitor was negligible in the reactivation medium.

As is apparent from an examination of k_{on}, k_{off} and the K_Is reported in Table 1, the optically pure 10-spirocyclopropyl analog of HA is comparable in activity to HA itself in both its inhibition constants and kinetic parameters, and as expected, it is slightly more active than the 10,10-dimethyl analog (comparison of racemic materials). Further studies are now underway to examine whether these 10-substituted analogs show greater elements of neuroprotection from glutamate toxicity than does HA itself. The neuroprotective aspect of HA represents a newly discovered property of this molecule.[11] This pharmacological property together with the AChE inhibitory activity further enhances the value of huperzine A and its analogs as therapeutic agents for the treatment of Alzheimer's disease.[11]

Table 1 Kinetic and inhibition parameters for huperzine A, 10,10-dimethylhuperzine A and 10-spirocyclopropylhuperzine A

Inhibitor	$k_{on}/10^{-6}$ M^{-1} min^{-1}	k_{off}/ min^{-1}	K_I[a]/ nM	K_I[b]/ nM
(−)-Huperzine A	4.2	0.016	3.9	5.6
(±)-10,10-Dimethylhuperzine A	0.76	0.01	13.2	17.0
(±)-10-Spirocyclopropylhuperzine A	1.0	0.014	14.0	12.4
(−)-10-Spirocyclopropylhuperzine A	2.4	0.015	6.4	8.8

[a] $K_I = K_{off}/K_{on}$. [b] Determined by the steady state method.

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Notes and References

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