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Synthesis and Biological Evaluation of (±)-Z-Huperzine A

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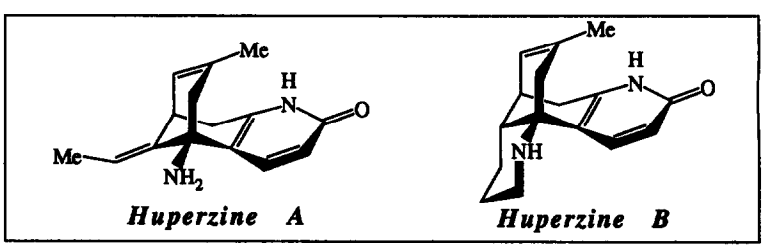
Summary: The synthesis of (±)-Z-huperzine A has been accomplished and the ability of this agent to inhibit acetylcholinesterase has been measured. The compound has an IC_{50} of 6 x 10^{-6} M, which is comparable to that of huperzine B.

As described previously, huperzine A appears to represent a valuable nootropic agent which is capable of alleviating some of the memory deficits which accompany Alzheimer's dementia.[1] While we have synthesized a number of simplified analogues of huperzine A to date, none of these compounds have shown acetylcholinesterase (AChE) inhibitory activity comparable to that of huperzine A.[2]

In selecting additional analogues for biological evaluation, we felt that Z-huperzine A represented a particularly attractive target for synthesis and biological study. This molecule would answer the question as to whether the *E*-stereochemistry of huperzine A is essential to its recognition by and interaction with AChE. In view of the fact that huperzine B contains a substituent, albeit sp^3 hybridized, directed by necessity toward the aliphatic amino group, we anticipated that Z-huperzine A should still retain some anti-AChE activity. In a sense, Z-huperzine A represents a hybrid of the huperzine A and huperzine B structures, and thus might combine the former's superior AChE inhibitory activity with the latter's improved toxicity profile.[3]



The synthesis of Z-huperzine A proceeded by methods similar to those developed in the synthesis of huperzine A.[1] The olefin **2**, available as the major product of the Wittig reaction of the β-keto ester **1** with ethylidenetriphenylphosphorane, was converted to its acid **3** by an S_N2 type dealkylation reaction using lithium *n*-propylmercaptide in HMPA (Scheme 1).[4] Attempts to bring about this transformation with aqueous NaOH in THF/MeOH, conditions which were found effective in the saponification of the *E*-ester, were unsuccessful. Next, the acid was converted by a three step protocol to the urethane **4** in 71% overall yield. Lastly, deprotection with Me_3SiI in $CHCl_3$ at reflux followed by heating in MeOH for 13 h gave crystalline (±)-Z-huperzine A as a white solid: mp 249-250 °C (dec.).

For the assessment of Z-huperzine A's AChE inhibitory activity, the compound was tested over a concentration range of 10^{-11} M to 10^{-3} M. AChE activity was measured by the procedure of Mantione *et al.* using rat brain hippocampal crude homogenates.[5] As is apparent from Figure 1 which compares the inhibitory properties of natural (-)-huperzine A, natural (-)-huperzine B, and (±)-Z-huperzine A, (±)-Z-huperzine A has an IC_{50} = 6 x 10^{-6} M, which is comparable to that of natural huperzine B (IC_{50} = 8 x 10^{-6} M). Of course, the IC_{50} of Z-huperzine A possessing the same absolute stereochemistry as (-)-huperzine A should be ~3 x 10^{-6} M.[6]

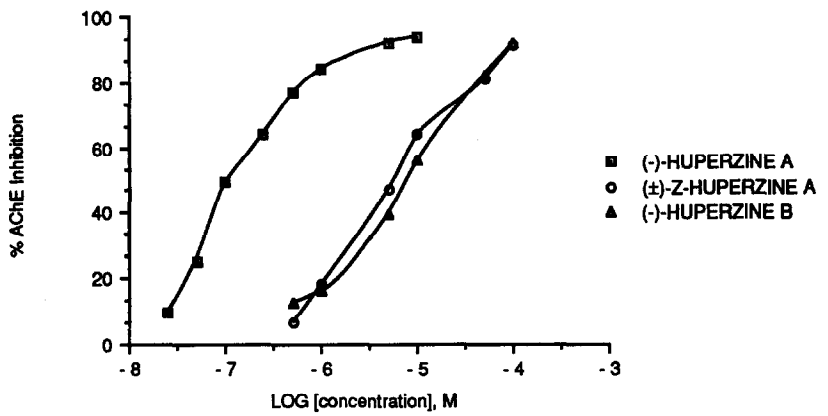
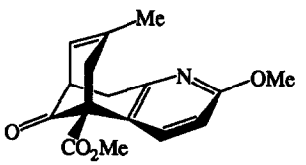


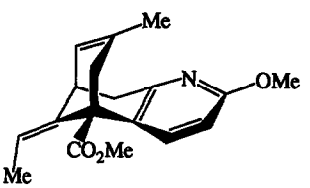
Figure 1. Effects of increasing concentrations of compounds *in vitro*, on AChE activity in rat hippocampal crude homogenates. Each value represents a mean of triplicates from 2-3 different experiments; IC_{50} (-)-Huperzine A = 10^{-7} M; IC_{50} (±)-Z-Huperzine A = 6 x 10^{-6} M; IC_{50} (-)-Huperzine B = 8 x 10^{-6} M.

Scheme 1. Synthesis of (±)-*Z*-Huperzine A.



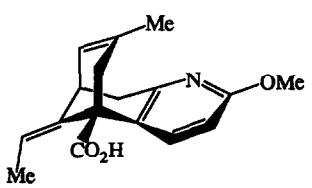
1

$CH_3CH{=}PPh_3$



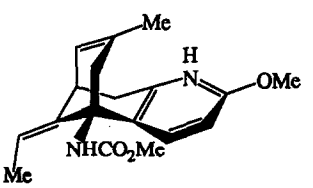
2 (separated from 10% of the *E*-isomer)

n-PrSLi, HMPA, rt, 18 h (80%)



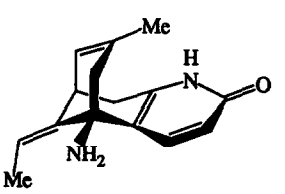
3 (mp 186-187 °C)

1. $SOCl_2$, toluene, 80 °C, 2 h
2. NaN_3, 80 °C, 14h
3. MeOH, ↑↓, 2 h (71% overall)



4 (mp 152-154 °C)

1. Me_3SiI, $CHCl_3$, ↑↓, 8 h
2. MeOH, ↑↓, 13 h (72%)



(±)-Z-huperzine A
mp 249-250 °C (dec.)

Thus, it would appear that the *E*-stereochemistry of huperzine A does, indeed, contribute to its potency as an AChE inhibitor. It would now be of interest to determine the biological properties of *Z*-huperzine A *in vivo*. While its IC_{50} indicates that it is not as potent as either huperzine A or as physostigmine, it may have a longer duration of action and a wider margin of safety, in terms of its physiological side effects (*vide supra*). If that turns out to be the case, then *Z*-huperzine A might be a much more efficacious candidate for use in the clinic than the parent compound, huperzine A.[7]

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References

1. A. P. Kozikowski and Y. Xia, *J. Am. Chem. Soc.*, **111**, 4116, (1989).

2. A. P. Kozikowski, Y. Xia, E. R. Reddy, I. Hanin, and X. C. Tang, *J. Med. Chem.*, submitted.

3. J.-S. Liu, C.-M. Yu, Y.-Z. Zhou, Y.-Y. Han, F.-W. Wu, B.-F. Qi, and Y.-L. Zhu, *Acta Chim. Sin.*, **44**, 1035 (1986). Also, see: X.-C. Tang, P. De Sarno, K. Sugaya, and E. Giacobini, *J. Neurosci. Res.*, **24**, 276 (1989).

4. P. A. Bartlett and W. S. Johnson, *Tetrahedron Lett.*, 4459 (1970).

5. C. R. Mantione, M. J. Zigmond, A. Fisher, and I. Hanin, *J. Neurochem.*, **41**, 251 (1983).

6. The synthesis of huperzine A in optically pure form has been accomplished in our laboratories and will be reported in due course. A. P. Kozikowski, F. Yamada, E. R. Reddy, Y. P. Pang, X.-C. Tang, and I. Hanin, manuscript in preparation.

7. Satisfactory NMR, IR, and mass spectral data were obtained for all new compounds. The spectral data for (±)-*Z*-Huperzine A follow: Colorless prisms from acetone, mp 249-250 °C (dec.); R_f = 0.53 (SiO_2, $CHCl_3$ - MeOH - NH_4OH = 46:5:0.5); IR (film) 2965, 2927, 2907, 2872, 1657, 1613, 1552, 1461, 832, 754, 650, 624 cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.53 (br s, 1 H, -NH-C-), 7.86 (d, 1 H, *J* = 9.5 Hz), 6.43 (d, 1 H, *J* = 9.5 Hz), 5.41 (q, 1 H, *J* = 7.4 Hz), 5.38 (br s, 1 H), 2.97 (br s, 1 H), 2.93 (dd, 1 H, *J* = 15.4 and 5.2 Hz), 2.68 (d, 1 H, *J* = 15.4 Hz), 2.39 (d, 1 H, *J* = 16.7 Hz), 2.05 (d, 1 H, *J* = 16.7 Hz), 1.93 (d, 3 H, *J* = 7.4 Hz), 1.69 (br s, 2 H, $-NH_2$), 1.54 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 165.4, 143.2, 140.2, 139.8, 133.5, 125.3, 123.0, 117.0, 115.5, 56.4, 49.8, 43.8, 36.1, 22.5, 13.8; MS *m/z* 242 (M^+), 227, 187. Exact mass calcd for $C_{15}H_{18}N_2O$ 242.1419; Found 242.1419.

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