Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 3, pp. 259-262, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00012-1

SYNTHESIS of (±)-10,10-DIMETHYLHUPERZINE A - A HUPERZINE ANALOGUE POSSESSING A SLOWER ENZYME OFF-RATE

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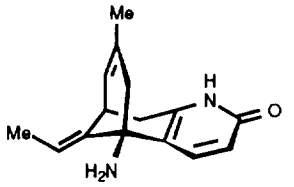
Abstract: The synthesis of (±)-10,10-dimethylhuperzine A is reported together with its kinetic (k_{on} and k_{off}) and dissociation constants for the inhibition of fetal bovine serum acetylcholinesterase. While somewhat more potent than (±)-huperzine A, the dimethyl analogue shows a slower off-rate from AChE, a result that may be of potential therapeutic value.

Alzheimer's disease (AD) is one of the major diseases affecting the elderly population in this country. It has been estimated that this disease now affects 5-15% of the population of the US over the age of 65. Despite tremendous research efforts, no truly effective drug has as yet been found to treat this progressive loss of cognitive function. While a number of avenues of therapeutic intervention are under active investigation, the only approved therapy involves the administration of THA, a compound exhibiting a number of pharmacological actions, foremost of which is its ability to act as an inhibitor of brain acetylcholinesterase.[1] The use of THA in the treatment of AD is supported in part by data from a number of laboratories which indicate a disorder of cortical cholinergic systems in Alzheimer's disease (AD), including the loss of acetylcholinesterase and choline acetyltransferase activity, and loss or abnormalities of the cholinergic neurons in the basal forebrain.[2] To the extent that AChE inhibitors can serve as useful adjuncts in the treatment of AD, the lycopodium alkaloid huperzine A, isolated from *Huperzia serrata* (Thunb.) Trev., appears superior to THA and physostigmine.[3] Huperzine A has been evaluated in random, match and double-blind controlled studies conducted in China on patients suffering from senile and presenile memory disorders. The "curative" effects of huperzine A were reported to be significant.[4] Because of its selective pharmacological properties, huperzine A has been classified as a second generation cholinesterase inhibitor.[5]

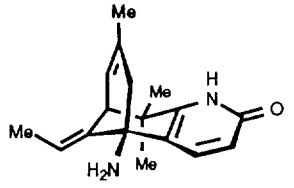
This laboratory has reported previously the first total syntheses of (±)-huperzine A, (-)-huperzine A, and a number of selected analogue structures.[6-9] We have also reported on the identification of the possible binding site of huperzine A within Torpedo acetylcholinesterase using computational methods including the Sysdoc program.[10] As part of a larger program aimed at the discovery of cognitive enhancing agents, we have attempted to discover huperzine analogues of increased potency, longer duration of action, and reduced toxicity. Herein we disclose the synthesis of a huperzine analogue which while comparable to (±)-huperzine A in its AChE inhibitory activity was found to possess a slower off-rate from the enyzme. While the discovery of this new structure will be valuable to modeling studies aimed at furthering our understanding of the huperzine A

binding site, this compound may also offer some therapeutic advantage over the parent structure.

Many of the structural alterations to huperzine A that we had explored previously required that simple modifications be made at later stages of the synthesis, e.g., the use of a longer chain Wittig reagent in place of ethylidenetriphenylphosphorane in order to explore the importance of the size of the exocyclic olefinic appendage to activity.[9] This time we chose to explore modification to huperzine A's C-10 position, which required that we modify the 1,4-cyclohexanedione monoethylene ketal at the very beginning of our synthesis. Specifically, we chose to examine the activity of the analogue **2** having two methyl groups situated at the C-10 position.



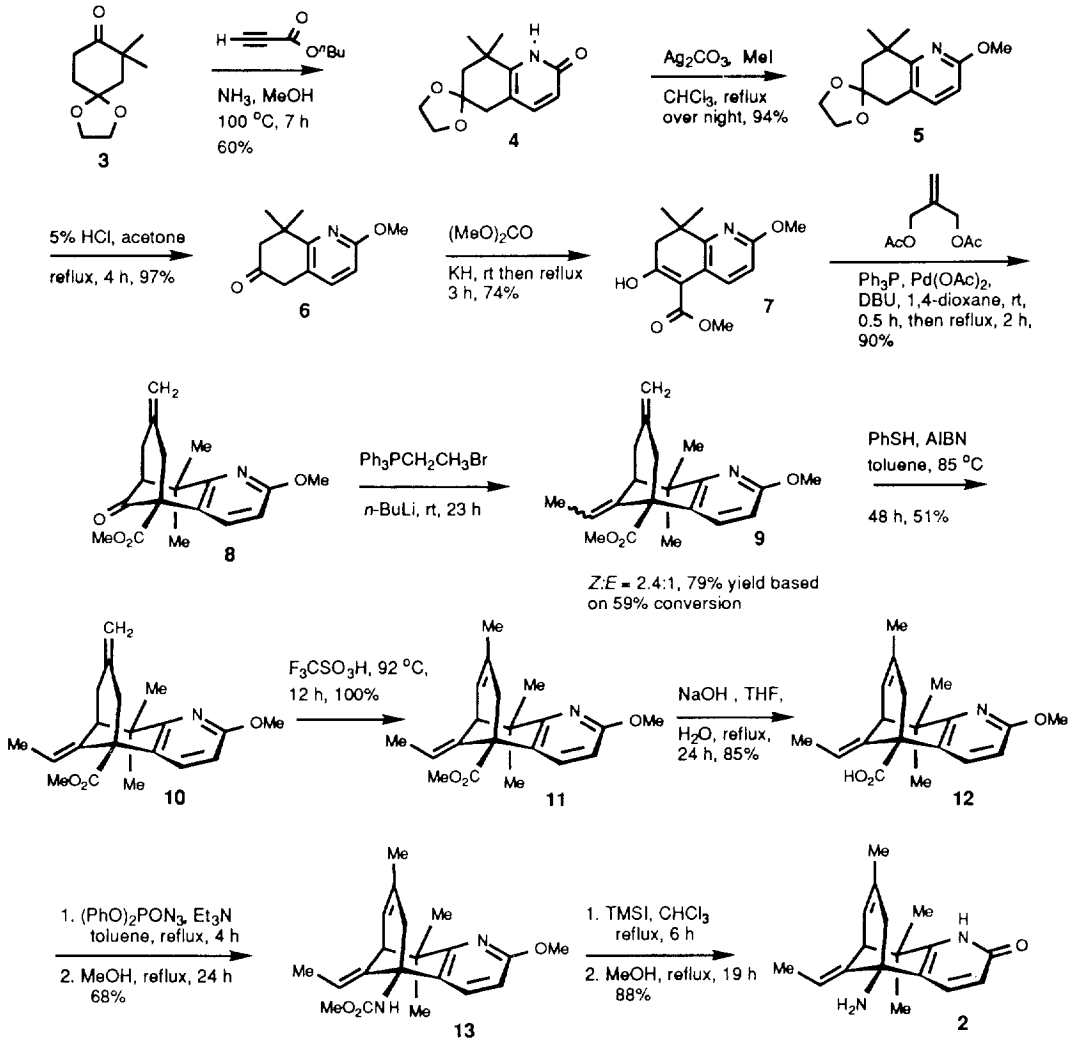
Huperzine A



C10-Dimethyl Analogue

Chemistry. 1,4-cyclohexanedione mono-ethylene ketal was dimethylated using a known procedure to yield **3**.[11] Treatment of ketone **3** in turn with *n*-butyl propiolate and ammonia in methanol at 100 °C in a pressure reactor for 7 hours resulted in the fused ring pyridone **4** in 60% yield. Next, the pyridone ring oxygen was *O*-methylated by reaction with silver carbonate and methyl iodide in refluxing chloroform. The methoxypyridine **5** was isolated in 94% yield after column chromatography using chloroform as eluent. Deprotection of ketal **5** with 5% HCl in acetone gave ketone **6** in 97% yield. Reaction of the ketone **6** with 3.5 equivalents of potassium hydride and excess dimethyl carbonate yielded the β-ketoester **7** in 74% yield. At this stage we chose to introduce the remaining three-carbon bridge by use of the previously described palladium catalyzed bicycloannulation protocol.[9] Accordingly, the β-ketoester **7** was reacted with 2-methylene-1,3-propanediol diacetate to provide the annulation product **8** in 90% yield. The standard Wittig olefination of tricycle **8** with ethylidenetriphenylphosphorane (prepared from the corresponding salt and *n*-butyllithium) proved to be sluggish. The reaction was incomplete after 23 hours, and appreciable decomposition of **8** was observed when the reaction time was prolonged further. The Wittig reaction was therefore stopped after 23 hours at room temperature; in this fashion 59% of the ketone **8** was consumed, and a 79% yield (calculated based upon unreacted **8**) of olefin **9** could be realized. Proton NMR revealed the olefin **9** to be a mixture of *Z* and *E*-isomers formed in a ratio of 2.4:1, respectively. Isomerization of the *Z*-isomer to the *E*-isomer was accomplished in turn by heating with thiophenol in toluene at 85 °C for 48 hours using AIBN as the radical initiator. The pure *E*-olefin **10** was isolated in 51% yield after column chromatography employing 10% ethyl acetate in hexanes as eluent. Next, a second isomerization step was conducted in order to effect migration of the exocyclic methylene double bond into the ring. This was accomplished by treating **10** with trifluoromethanesulfonic acid in 1,4-dioxane at 92 °C for 12 hours. A quantitative yield of **11** was obtained. Completion of the synthesis required that the ester group of **11** now be transformed into an amino group. Compound **11** was thus treated with sodium hydroxide in a mixture of THF, MeOH, and H_2O at reflux for 7 hours to provide carboxylic acid **12** in 85% yield. Next, the acid **12** was reacted with diphenyl azidophosphate and triethylamine in toluene to afford an isocyanate

Scheme 1. Synthesis of the 10,10-Dimethyl Analogue of Huperzine A.



which was trapped with methanol to give urethane **13** in 68% yield. Finally, double-deprotection of **13** with iodotrimethylsilane in refluxing chloroform gave the final, desired product, 10,10-dimethylhuperzine A (**2**) in 88% yield.[12]

Measurement of AChE Activity. These studies were carried out using AChE purified from fetal bovine serum (FBS).[13] AChE activity was measured in 50 mM sodium phosphate, pH 8.0, at 22 °C as described by Ellman *et al.*[14] using acetylthiocholine as the substrate. The kinetic and inhibition parameters for (±)-huperzine A, (-)-huperzine A, and its 10,10-dimethyl analogue are provided in Table 1.

Table 1. Kinetic and Inhibition Parameters for Huperzine A and its 10,10-Dimethyl Analogue.

Inhibitor	k_{on} ($M^{-1}min^{-1}$)	k_{off} (min^{-1})	K_I (nM)
(±)-Huperzine A*	$0.93 \pm 0.03 \times 10^6$	0.022 ± 0.003	23.6
(-)-Huperzine A	$3.4 \pm 0.8 \times 10^6$	0.020 ± 0.002	5.9
(±)-Dimethylhuperzine A	$0.78 \pm 0.10 \times 10^6$	0.013 ± 0.004	16.7

*Data for (±)-huperzine A taken from the following reference: Ashani, Y.; Peggins, J. O. III; Doctor, B. P. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 719.

Remarkably, as is apparent from an examination of the K_Is reported in Table 1, the 10,10-dimethyl analogue **2** is comparable in activity to (±)-huperzine A, but it is less active than enantiomerically pure (-)-huperzine A. Of particular interest is the fact that the off-rate of the dimethyl compound from AChE is slightly slower than huperzine A. The extra bulk provided by the two methyl groups must therefore impede release from the enzyme for steric reasons. While this result will be investigated further using computational methods, preparation of this new analogue in optically pure form will also be important to the refinement of these findings. Furthermore, the effect of the present structural change upon *in vivo* cognitive performance will be of interest for study.

Acknowledgment. We are indebted to Interneuron Pharmaceuticals for partial support of these studies.

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12. Spectral data for **2** follow: IR (film) 2964, 2913, 1654, 1593, 1448, 1202, 1070, 905, 731 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 10.15 (s, br., 1 H), 7.86 (d, 1 H, *J* = 9 Hz), 6.38 (d, 1 H, *J* = 9 Hz), 5.58 (q, 1 H, *J* = 6 Hz), 5.49 (d, 1 H, *J* = 3 Hz), 3.10 (d, 1 H, *J* = 3 Hz), 2.16 (d, 1 H, *J* = 18 Hz), 2.04 (d, 1 H, *J* = 18 Hz), 1.65 (d, 3 H, *J* = 6 Hz), 1.62 (s, 3 H), 1.46 (s, 3 H), 1.25 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 12.97, 22.58, 24.58, 28.33, 42.32, 45.37, 49.91, 54.93, 112.64, 117.72, 120.23, 122.57, 134.46, 140.34, 141.40, 149.22, 164.14.
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(*Received in USA* 4 *December* 1995; *accepted* 21 *December* 1995)