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Synthesis and Acetylcholinesterase Inhibition of 5-Desamino Huperzine A Derivatives

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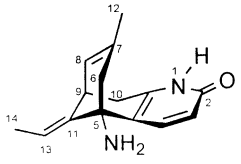
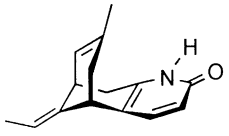
Abstract—(*E*)- and (*Z*)-5-Desamino huperzine A derivatives have been synthesized using a new synthetic strategy towards the huperzine A skeleton. These derivatives showed AChE inhibition constants in the low micromolar range and thus display better activity than all previously synthesized C5 derivatives.

One of the most promising symptomatic therapeutic approaches for the treatment of Alzheimer's disease (AD)[1] involves the reduction of the cholinergic dysfunction resulting from a deficiency in the neurotransmitter acetylcholine.[2] In this context, inhibition of acetylcholinesterase (AChE) has been extensively studied over the last two decades and resulted in the discovery and development of several AChE inhibitors.[3] Amongst these, the Lycopodium alkaloid huperzine A (**1**), initially isolated from the clubmoss *Huperzia serrata* (Thunb.), is one of the most potent agents.[4,5] This natural product was shown to have a superior profile compared to other AChE inhibitors due to its longer duration of action, higher selectivity (huperzine A has almost no effect on butyrylcholinesterase) and slow off-rate from the enzyme. In China, huperzine A has already been approved as a palliative drug for AD.[6]

The X-ray analysis of the complex of huperzine A and *Torpedo* AChE[7] led to the surprising result that only a few direct contacts between the inhibitor and the enzyme are responsible for the strong affinity. These include a strong hydrogen bond between the pyridone oxygen and a tyrosine residue and a more putative H-bond between the ethylidene methyl group and the main chain carbonyl oxygen of a histidine. Most interestingly, the bridgehead amino group of huperzine A is not part of a direct interaction with the protein, but hydrogen bonds mediated by at least two water molecules could be observed. The 5-dimethylamino as well as the 5-fluoro and 5-hydroxy huperzine A derivatives have been shown to have significantly less AChE activity than the parent molecule.[8,9] In the course of our project to develop a new synthetic strategy towards the huperzine A skeleton, we decided to prepare 5-desamino huperzine A (**2**) and investigate its biological activity to discover whether or not the amino functionality is necessary for biological activity.



1: huperzine A



2

Chemical Synthesis

The synthesis of **2** was achieved following our new synthetic strategy towards huperzine A derivatives (Scheme 1).[10] A double Michael addition of dimethyl 1,3-acetone dicarboxylate to benzoquinone monoketal **3**[11] furnished a diastereomeric mixture of diester **4**.[12] Selective monodecarboxylation with LiOH in DMF

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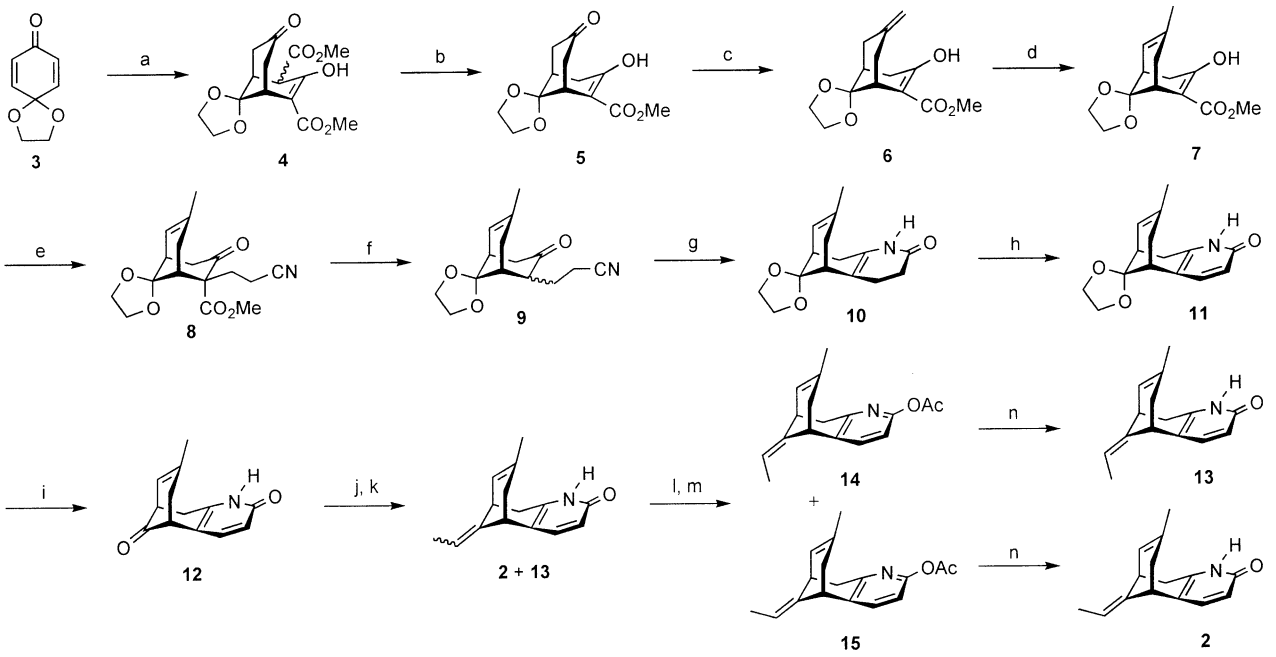

Scheme 1. Reagents and conditions: (a) 1.05 equiv $(MeO_2CCH_2)_2CO$, EtONa cat, EtOH, rt, 10 h, 85%; (b) 3 equiv $LiOH{\cdot}H_2O$, DMF, H_2O, 150 °C, 2 h, 91%; (c) 3 equiv Ph_3PCH_3Br, 3 equiv *n*-BuLi, THF, reflux, 22 h, 90%; (d) Pd–C cat, H_2, EtOH, rt, 14 h, 68%; (e) 10 equiv acrylonitrile, 1 equiv DBU, DMF, rt, 21 h, 48%; (f) 4 equiv LiBr, DMF, 150 °C, 15 h, 87% (g) satd NH_3 in MeOH, sealed tube, 120 °C, 66 h, 89%; (h) i. 1.05 equiv SO_2Cl_2, CH_2Cl_2, 0 °C, 10 min; ii. 120 °C (neat), 0.5 h, 95%; (i) 4 M H_2SO_4, acetone, 80 °C, 4 h, 84%; (j) 10 equiv Ph_3PEtBr, 9 equiv *n*-BuLi, THF, rt, 5 h; (k) 1.5 equiv PhSH, 1 equiv AIBN, PhMe, 85 °C, 18 h, 91% (from **12**), $E/Z = 1{:}1$; (l) 10 equiv Et_3N, 5 equiv Ac_2O, CH_2Cl_2, rt, 6 h, 64%; (m) HPLC separation (see text); (n) satd NH_3 in MeOH, CH_2Cl_2, rt, 6.5 h, 100%.

gave β-keto ester **5**. The non-enolized carbonyl group was then subjected to a Wittig olefination with an excess of phosphorus ylid, resulting in the formation of olefin **6**. The exo methylidene group could be isomerized to the thermodynamically favoured endocyclic position using Pd–C/H_2.[13] This transformation was completely regioselective and gave β-keto ester **7** in good yield. The three carbons needed to form the pyridone were then introduced by a Michael reaction of **7** with acrylonitrile to give **8**, which was successfully decarboxylated with LiBr in DMF and led to a diastereomeric mixture of keto nitriles **9**. Cyclization to the dihydropyridone **10** was achieved via a sealed tube reaction of **9** with a saturated solution of ammonia in methanol. Regioselective chlorination of the enamide double bond with sulfuryl chloride,[14] followed by dehydrochlorination led to pyridone **11**. The ketal was hydrolyzed with sulfuric acid and the resulting ketone **12** was subjected to a Wittig olefination to give a 2:3 *E*/*Z* mixture of desamino huperzine A (**2** and **13**, respectively). A thiophenyl radical addition/elimination sequence gave isomerization and isolation of a 1:1 mixture of the geometric isomers. The isomers turned out to be inseparable in a wide range of solvent systems and were therefore transformed into the less polar pyridone *O*-acetates **14** and **15**. At this stage, separation was successfully performed by semi-preparative HPLC using a Chiracel OD-H column eluting with ethyl acetate/hexane (1:98). Deprotection with a saturated solution of ammonia in methanol furnished the desired desamino huperzines **2** and **13**.[15]

Biological Activity

Materials and methods

AChE originated from the brain (striatum) of Sprague–Dawley rats, sacrificed by decapitation and dissected according to Glowinski and Iversen.[16] Striatal tissue was homogenized at 4 °C in the following buffer solution: Na_2HPO_4/KH_2PO_4, 0.1 M, pH 7.4 containing 1% of Triton-X-100 (1 vol tissue + 9 vol buffer). After centrifugation at 15,000*g* for 15 min (4 C), the clear supernatant was frozen at −70 °C in aliquots to serve as enzyme source.

Aliquots of the thawed enzyme solution (corresponding to 0.05 mg striatum) were placed (each in triplicate) in a 96-well flat-bottom micro-test plate. Enzymatic activity was determined by the method described by Ellman et al.[17] The reaction was started by adding the following substrate–reagent mixture: acetylthiocholine-iodide (ASCh) 0.08–0.5 mM and 0.25 mM 5,5′-dithiobis-(2-nitrobenzoic acid) in phosphate buffer pH 7.4 (0.1 M). The plate was then placed in the automatic Micro-Reader, which recorded the occurrence of the yellow reaction product at 405 nm. Reaction velocities were calculated as the change of absorbance per unit time. Liberation of thiocholine was quantified using an extinction coefficient for the thionitrobenzoate dianion of 13,300 $M^{-1}\,cm^{-1}$ (pH 7.4).

Enzymatic hydrolysis of acetylthiocholine iodide was measured as described above. The various inhibitors

Table 1. Rat brain AChE inhibition by desamino huperzine A derivatives

Compound	IC_{50} (μM)[a]	K_i (μM)[a]
1		0.024–0.040[b]
2	12.8±0.8	2.00
13	55.5±2.7	12.3
12	>500	>500

[a]IC_{50} and K_i values derived from three inhibition experiments (range of inhibitor concentrations 1–500 μM).
[b]See ref 18.

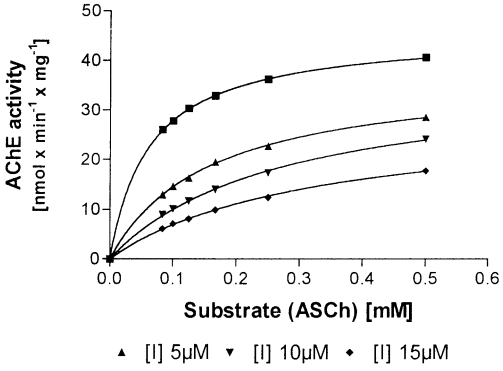

Figure 1. ASCh hydrolysis by rat striatal AChE and (*E*)-desamino huperzine A **2**.

were preincubated with the enzyme at 25 °C for 15 min. Kinetic data were calculated by fitting the rate of liberation of thiocholine versus substrate concentrations and applying double reciprocal plots followed by secondary plots of slopes and *y*-intercepts using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, California USA.

Results

The 5-desamino huperzine A derivatives **2** and **13** inhibited AChE in vitro with an IC_{50} in the micromolar range (Table 1), whereas ketone **12** had no influence on the enzyme up to a concentration of 0.5 mM. For comparison reasons, the K_i values of the investigated compounds are listed in Table 1 together with values for huperzine A taken from the literature.[19] The characteristics of the ASCh hydrolysis by AChE from rat striatum in presence or absence of compound **2** are shown in Figure 1. Substrate concentrations higher than 0.75 mM produced substrate inhibition, a phenomenon well known for AChE.[18]

Huperzine A and its desamino derivatives **2** and **13** displayed linear mixed inhibition of AChE using acetylthiocholine as substrate (Figs. 1–3 for compound **2**, similar figures were obtained for **13**). This behavior was indicated by intersection of the double-reciprocal lines in the upper left quadrant (Lineweaver–Burk plot) and the linear slope versus [I] replot (secondary plot L–B). From the *x*-intercept of the slope replot, the inhibition constant was determined to be in the lower micromolar range: $K_i = 2.0$ for **2** and $K_i = 12.3$ μM for **13**, respec-

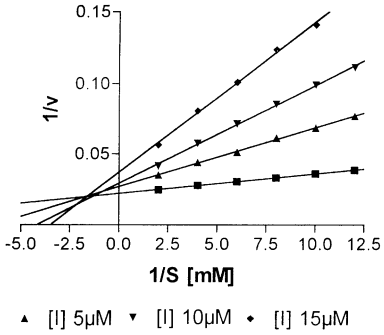

Figure 2. AChE inhibition by (*E*)-desamino huperzine A **2** as double reciprocal plot (Lineweaver–Burk): substrate versus velocity.

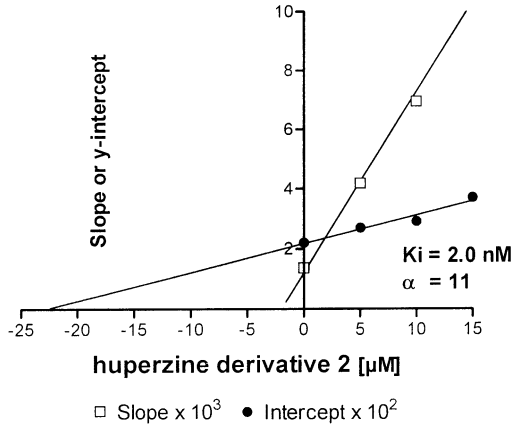

Figure 3. Mixed-linear AChE inhibition by (*E*)-desamino huperzine A **2**: secondary plot of slope and intercept of reciprocal plot (Lineweaver–Burk).

tively. The *y*-intercept replot was linear and afforded a value of $\alpha = 7.8$ for **2** and $\alpha = 11$ for **13**, respectively.

Discussion

Kinetic examination of the association of desamino huperzine A derivatives **2** and **13** with AChE affords inhibition constants in the micromolar range. For both compounds, the linear mixed inhibition of AChE reflects the presence of competitive and noncompetitive components. The value for $\alpha > 1$ indicates essentially reversible noncompetitive inhibition.[20] Such noncompetitive inhibition might arise through ligand association at a site remote from the active center. The catalytic subunit of AChE contains a peripheral anionic site that, while topographically distinct from the active center, exerts allosteric control over enzyme hydrolysis. Since we have no indications about an allosteric interaction with both inhibitors (Hill coefficient unchanged in presence of inhibitor, and the inhibition is linear) an interaction with the peripheral anionic site seems unlikely. The biological results revealed that the C5 heteroatom substitution of huperzine A is not prerequisite for the inhibition of AchE. Although 5-desamino huperzine A (**2**) showed about 100-fold less activity than the natural product, its IC_{50} is still 3 to 4 times better than the IC_{50} of the 5-fluoro and the 5-hydroxy derivative.[9] The conclusion from the X-ray structure that the

ethylidene methyl group is important for the binding[7] could be confirmed as the ketone precursor **12** was shown to have no effect on AChE up to a concentration of 0.5 mM. Interestingly, the geometry of the ethylidene double bond did only affect the binding constant by a factor of 5.

Acknowledgements

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