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SYNTHESIS AND ANTICHOLINESTERASE ACTIVITY OF HUPERZINE A ANALOGUES CONTAINING PHENOL AND CATECHOL REPLACEMENTS FOR THE PYRIDONE RING

Giuseppe Campiani,[a] Alan P. Kozikowski,[b*] Shaomeng Wang,[b] Liu Ming,[b] Vito Nacci,[a] Ashima Saxena,[c] and Bhupendra P. Doctor[c]

[a]*Dipartimento Farmaco Chimico Tecnologico, Siena University, Banchi di Sotto, 55, 53100 Siena, Italy,* [b]*Drug Discovery Program, Georgetown University Medical Center, Institute for Cognitive & Computational Sciences, 3970 Reservoir Road, N.W., Washington DC 20007, and* [c]*Division of Biochemistry, Walter Reed Army Institute of Research, Washington, DC 20307*

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Abstract: Based upon modeling results obtained using the crystal structure of huperzine A in complex with acetylcholinesterase (AChE), two novel analogues of this potent AChE inhibitor were designed with phenol or catechol rings replacing the pyridone ring. From the modeling studies, the catechol analogue appeared capable of replacing one of the crystallographic waters bridging huperzine with Tyr 130 and Glu 199 of AChE. The synthesis of these materials by use of a palladium catalyzed bicycloannulation strategy is detailed together with the results of AChE inhibition assays.

The role of acetylcholine (ACh) in the modulation of memory function in normal and pathological states has been extensively reviewed. Neurodegenerative conditions involving memory impairment, such as dementia of the Alzheimer's type, result in part from a loss of basal forebrain neurons and reduced cortical and hippocampal levels of the neurotransmitter ACh.[1] The observation of decreased ACh levels in AD patients prompted clinical investigations of a number of inhibitors of acetylcholinesterase (AChE), the enzyme responsible for the rapid degradation of ACh. At present, the use of reversible AChE inhibitors is considered to be a valuable therapeutic approach to the treatment of Alzheimer disease (AD), and recently, the AChE inhibitor Aricept appears to be well on its way to supplanting THA (tacrine). THA was the first AChE inhibitor to be approved for use in the US for the palliative treatment of AD patients. Additionally, the naturally occurring AChE inhibitor huperzine A (HA), isolated from the clubmoss *Huperzia serrata*, appears to be quite promising as a potent, selective, and safe AChE inhibitor.[2] Clinical studies of this drug in China have resulted in the approval of the compound for the treatment of AD in that country.

Previously, we have reported the synthesis of huperzine A and of a number of its analogues,[3] together with the X-ray structural analysis of HA in complex with *Torpedo* AChE.[4] To date we have been able to identify several analogues that have an activity comparable to or better than that of HA.[3] Herein we describe our efforts aimed at using the information available from the HA-AChE co-crystal structure analysis in the design of several new analogues. Specifically, we sought to examine the effect of replacing the pyridone ring of HA by a phenol or catechol moiety. From X-ray analysis, the pyridone ring forms one hydrogen bond with Tyr 130 (Figure 1) and another hydrogen bond with Glu 199 via a water bridge (H_2O 619).[4] Theoretically, if this water molecule can be incorporated into the inhibitor, the binding affinity may be improved through a gain in entropy. Based upon this concept and the X-ray structure information, the catechol and the phenol analogues **2a**,**b** were designed. Extensive docking studies using the MCDOCK

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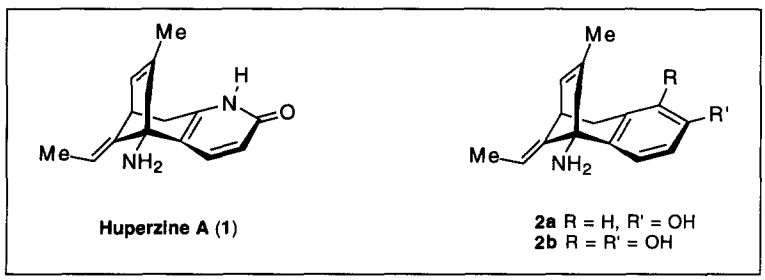
program[5] on huperzine A revealed that the X-ray determined binding mode is energetically the most stable binding mode starting from the X-ray determined conformation of AChE. Docking studies on the catechol analogue **2b** showed that the energetically most stable binding mode for this compound is almost identical to the X-ray determined huperzine A binding mode. Indeed, the catechol ring forms two hydrogen bonds with Tyr 130 and Glu 199, thus suggesting that the catechol ring can replace the pyridone ring and the water molecule 619 (Figure 1). Moreover, as a further point of comparison we chose to investigate simultaneously the activity of the phenol **2a**, as this compound should be capable of binding in a manner much like that of HA, in which Tyr 130 would engage in hydrogen bond formation with the phenolic hydroxyl group.



Chemistry

The synthesis of the phenolic analogue of HA, **2a**, was accomplished as shown in Scheme 1. The starting 6-methoxy-2-tetralone (**3**) was obtained in 70% yield from commercially available 6-methoxy-1-tetralone using a known procedure.[6] Methoxycarbonylation of **3** following Mander's method[7] gave intermediate **4** in 83% yield, which was subjected to a bicycloannulation reaction using DBU as base, 2-methylene-1,3-propanediol diacetate as the bis-electrophile, and tetrakis(triphenylphosphine)-palladium(0) as the catalyst to provide the exocyclic olefin **5** in 86% yield.[8] Next, a standard Wittig olefination reaction with ethylidenetriphenylphosphorane[9] followed by the triflic acid[10] promoted isomerization of the exocyclic double bond to the endocyclic olefin provided a mixture of *Z*- and *E*-olefins at C-11 in a ratio of 95:5. Isomerization of the *Z*-olefin to the *E*-olefin and saponification of the ester provided the *E*-acid **6** in good overall yield. Curtius reaction of the acid **6** by treatment with diphenyl azidophosphate and Et_3N followed by methanolysis of the resulting isocyanate provided the urethane **7** in 70% yield.[11] Reaction of urethane **7** with lithium *n*-propylmercaptide in HMPA at 120 °C gave the desired phenol **2a** in 78% yield (Scheme 1).[12]

The synthesis of the catechol analogue **2b** was accomplished as detailed in Scheme 2. The dimethoxycynnamic acid **3** served as a convenient starting material for the preparation of the required tetralone **11**. Accordingly, acid **8** was converted into the γ-phenylpropionic ester **9** by reaction with iodomethane in presence of DBU[13] followed by hydrogenation over palladium on charcoal.[14] Conversion of **9** to the intermediate β-keto sulfoxide **10** was accomplished in 95% yield upon reaction with the dimsyl anion. Cyclization with TFA[15] followed by desulfurization with Raney-nickel[16] provided the required 2-tetralone **11** in excellent yield. Steps identical to those delineated in Scheme 1 were then employed to convert **11** to the tricycle **14**. In the case of **14**, the ethylidene side chain at C-11 was introduced through a two step sequence involving first the construction of an intermediate β-lactone **15** by the addition of a thiol

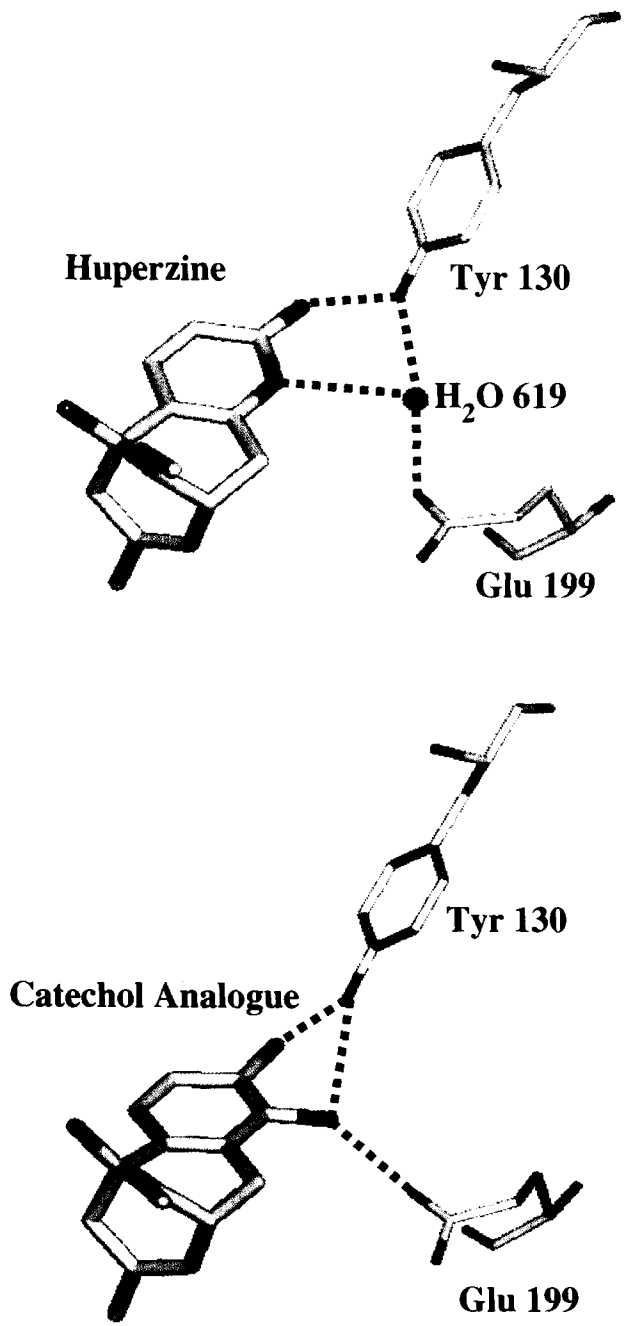
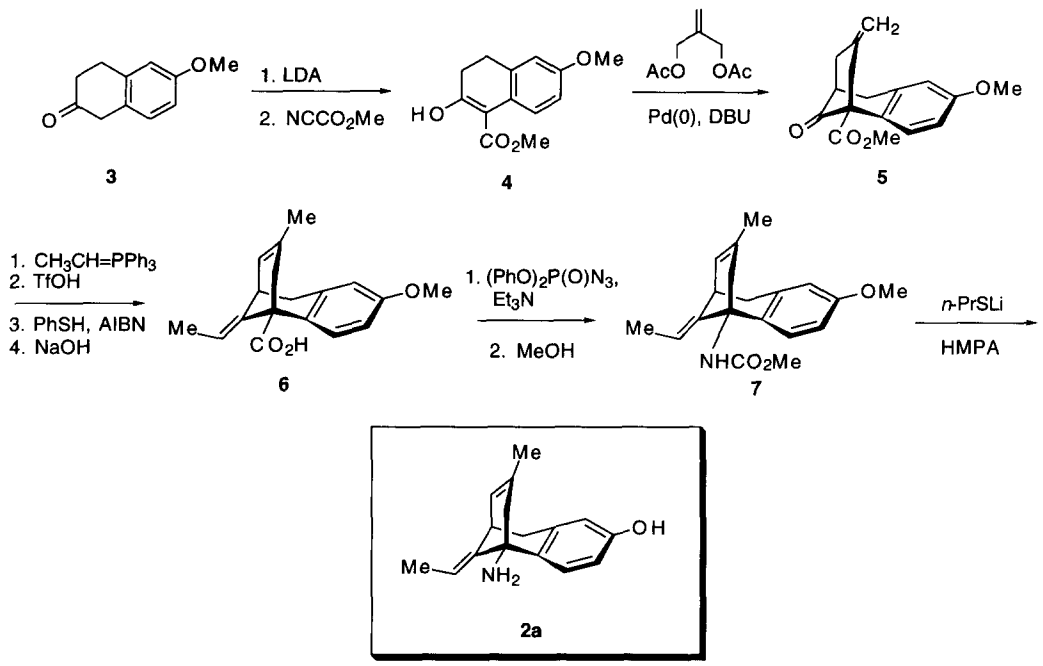


Figure 1. The hydrogen bond network formed between the pyridone ring of huperzine A and its surrounding amino acid residues in the X-ray determined structure of AChE (top) and the network of interactions found for the catechol analogue **2b** in the MCDOCK predicted structure (bottom).

ester enolate to the carbonyl group of **14**.[17] This reaction took place with complete stereospecificity to provide the sterically less encumbered diastereoisomer **15**. Upon heating of **15** in presence of silica gel in

Scheme 1. Synthesis of the Phenol Analogue **2a** of Huperzine A



dry toluene, a [2+2] cycloreversion reaction ensued with elimination of carbon dioxide to generate the required olefin **16**. The cycloreversion reaction is a stereospecific process, and solely the *E*-olefin results as evident from the NMR analysis. The urethane **17** was then generated in the standard way in 70% yield. Lastly, because the TMSI promoted deprotection of **17** in refluxing chloroform provided incomplete demethylation of the catechol system, we found it necessary to expose the urethane to lithium *n*-propylmercaptide in HMPA. In this fashion we were able to obtain **2b** in 64% yield.[18]

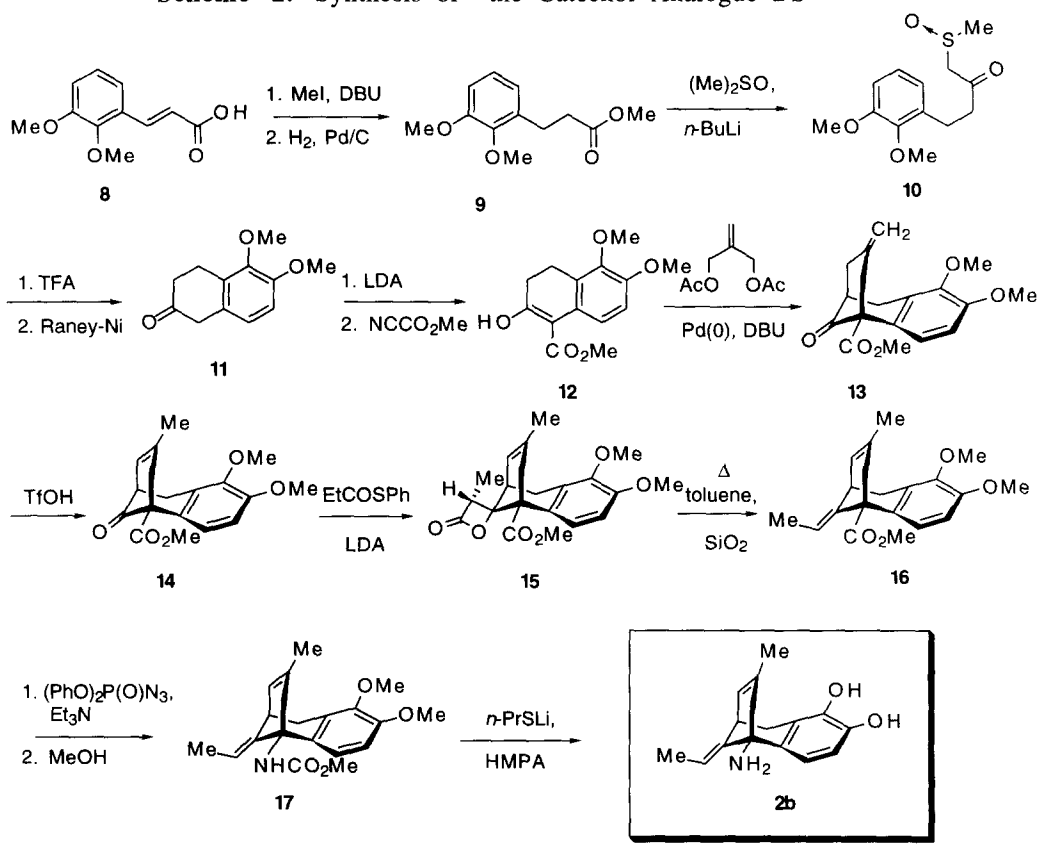
Measurement of AChE Activity

The biological activity of the analogues of huperzine A was evaluated using AChE purified from fetal bovine serum.[19] AChE activity was measured in 50 mM sodium phosphate, pH 8.0, at 22 °C as described previously using acetylthiocholine as the substrate.[20] The interaction of HA and its analogues with AChE can be described by the following:[21]

$$E + HA \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} E \cdot HA$$

The ratio k_{off}/k_{on} is the dissociation constant K_I. The K_I values for the inhibition of FBS AChE with analogues of HA were determined by equilibrating a known amount of enzyme (1–2 units/mL) with various concentrations of the analogue.

Scheme 2. Synthesis of the Catechol Analogue **2b**



Surprisingly we observed no inhibition of AChE when **2b** was tested at concentrations up to 68 μM, while **2a** was found to be inactive when tested at concentrations up to 54 μM!

We presently have no good explanation for these results. Since the modeling studies clearly indicate that both analogues are nicely accommodated by AChE, engaging in appropriate hydrogen bond interactions, steric factors are unlikely to play a role. Perhaps certain as yet poorly understood solvation effects are responsible. The ability to understand the specific factors responsible for the lack of activity of these analogues will be crucial to the de novo design of other HA analogues, and, in general, to improving upon the predictive power of modeling programs.

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18. Physical and spectroscopic data for analogues **2a** and **2b** follow: **2a**: mp: 224 °C (hexanes); IR ($CHCl_3$) 3330 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.12 (d, 1 H, *J* = 8.7 Hz), 6.20 (dd, 1 H, *J* = 2.6, 8.7 Hz), 6.00 (d, 1 H, *J* = 2.5 Hz), 5.05 (q, 1 H, *J* = 6.7 Hz), 4.94 (d, 1 H, *J* = 4.6 Hz), 3.17 (m, 1 H), 2.50 (dd, 1 H, *J* = 5.0, 16.1 Hz), 2.23 (m, 4 H), 1.74 (s, 2 H), 1.26 (d, 3 H, *J* = 6.8 Hz), 1.07 (s, 3 H); MS *m/z* 241 (100), 226, 212, 196, 186, 172, 159, 146, 129. Anal. Calcd. for $C_{16}H_{19}NO$: C, 79.63; H, 7.94; N, 5.80. Found: C, 79.55; H, 7.99; N, 5.93. **2b**: colorless prisms; mp 232–233 °C (from hexanes and EtOAc); *Rf* 0.18 (20% methanol in chloroform); IR ($CHCl_3$) 3470–3350 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.06 (d, 1 H, *J* = 8.2 Hz), 6.64 (d, 1 H, *J* = 8.1 Hz), 5.42 (q, 1 H, *J* = 6.7 Hz), 5.37 (m, 1 H), 3.70 (br s, 5 H), 2.88 (d, 1 H, *J* = 16.1 Hz), 2.68 (dd, 1 H, *J* = 16.5, 5.0 Hz), 2.25 (s, 2 H), 1.70 (d, 3 H, *J* = 6.3 Hz), 1.49 (s, 3 H); ^{13}C NMR ($CDCl_3$/DMSO-d_6 9/1) δ 12.3, 22.5, 32.1, 33.4, 50.7, 55.8, 109.8, 113.2, 116.1, 122.8, 124.6, 133.1, 137.6, 141.5, 142.0, 143.4; MS *m/z* 257 (100%, M^+), 242, 228, 202, 162. Anal. Calcd. for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.69; H, 7.41; N, 5.39.
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