Chemistry, Pharmacology, and Clinical Efficacy of the Chinese Nootropic Agent Huperzine A

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Introduction

Huperzine A (HA) (Figure 1) is an alkaloid from the clubmoss *Huperzia serrata* (Thunb.) Trev. = *Lycopodium serratum* Thunb.,[1] which is used in the treatment of Alzheimer's disease (AD).[2] *H. serrata* has been used in Chinese traditional medicine under the name Chien Tseng Ta to treat several illnesses.[3] In pharmacological studies of the late 1980s, HA proved to be a very potent inhibitor of the enzyme acetylcholinesterase (AChE). Recently, purified HA isolated from the clubmoss has undergone double-blind, placebo-controlled clinical trials in China in patients suffering from various memory disorders, including AD. Significant effects were noted in these patients in terms of both life quality and memory retrieval.[4]

Natural AChE inhibitors have been identified from other sources as well. Physostigmine (IC_{50} 0.01 μM), an alkaloid from *Physostigma venenosum* Balfour,[5] is presently used in topical applications for the treatment of glaucoma, and various analogues (e.g., heptylphysostigmine; IC_{50} 0.12 μM) have also been examined for use in Alzheimer's therapy with some success. The morphine alkaloid galanthamine (IC_{50} 0.36 μM) from *Galanthus woronowii* Vel. is currently in clinical trials for the treatment of AD with encouraging results.[6]

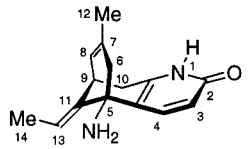

FIGURE 1. Structure and numbering of HA.

A Summary of Alzheimer's Disease and Its Possible Etiology

To understand the action of HA, a brief discussion of AD is useful. AD is a common neurodegenerative disorder that accompanies aging and afflicts an estimated 4 000 000 people in the U.S. alone,[7] leading to progressive loss in cognitive abilities, performance of routine tasks, time and space orientation, communication skills, abstract thinking, and personality. From the diagnosis of AD until death, an individual's median life expectancy is about eight years. Annual health care expenses associated with caring for afflicted individuals are estimated around $80 billion dollars.[8] Since life expectancy has been increasing, and since AD's incidence increases with age (approximately 40% of individuals over 85 have AD), more people will be at risk. One of the problems associated with AD is cholinergic dysfunction, resulting from the deficiency in the neurotransmitter acetylcholine,[9] which plays a fundamental role in memory. While deficiencies in other neurotransmitter systems have been found, the cholinergic connection seems to be one of the most pertinent ones. A proper interplay of communication among nerve cells is required to maintain their proper functioning; thus, deficiencies in cell-to-cell communication as well as in intracellular signaling pathways can lead to neuronal cell death.

If a cholinergic deficit exists, as in AD, inhibition of AChE should allow for the small amounts of acetylcholine that are still being synthesized and released to persist longer within the synaptic cleft and to interact with its postsynaptic targets, the muscarinic and nicotinic acetylcholine receptors. HA is a very potent (K_i = 20−40 nM) reversible inhibitor of AChE and shows superior inhibition characteristics relative to other cholinesterase inhibitors in that it possesses a very slow rate of dissociation from the enzyme ($t_{0.5}$ = 35 min in vitro)[10] and a long duration of action, as studies in rodents show that AChE remains inhibited by 33% after 6 h.[11]

Other deficiencies occur in the AD brain in addition to the cholinergic dysfunction.[12] Neurons must constantly be supplied with oxygen and glucose through tiny blood vessels, and the blood brain barrier (BBB) that allows these nutrients to enter the brain while blocking the admission of toxins and pathogens may be altered in AD. Insufficient nutrition of neurons can in turn result in their death, as occurs in the case of stroke or asphyxiation.[13] In any event, those neurons requiring particularly high levels of glucose, as needed, for example, in the synthesis of acetylcholine, may be placed in a particular state of metabolic vulnerability.

Alan P. Kozikowski was born in 1948 in Menominee, MI. He received his Ph.D. degree from the University of California, Berkeley, under the supervision of the late Professor W. G. Dauben. After postdoctoral work with Professor E. J. Corey at Harvard University, Dr. Kozikowski began his independent career at the University of Pittsburgh. After 14 years there, he spent 3 years at the Mayo Clinic and then moved on to the Georgetown University Medical Center, where he is the Director of the Drug Discovery Program. Dr. Kozikowski's interests are in the areas of drug abuse, medications development, second messenger based therapeutics for cancer, and novel chemical interventions for the treatment of Alzheimer's disease.

Werner Tückmantel was born in 1955 in Cologne, Germany. He received his Ph.D. in 1984 from the University of Cologne under the supervision of Professor Emanuel Vogel. After postdoctoral work with Professor Hitosi Nozaki (Kyoto University, Japan) and Professor Alan Kozikowski (then at the University of Pittsburgh), he spent 1.5 years at the University of Heidelberg, Germany, before returning in 1989 to Professor Kozikowski's team, with whom he has worked since.

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Additionally, excessive release of excitatory amino acids from presynaptic nerve terminals can also initiate events that result in neuronal death. Glutamate-activated neurons, just like the cholinergic system, play an important role in memory and learning. However, as in many biological systems, too much of a good thing can also be bad. Glucose-deprived neurons may be particularly susceptible to overstimulation by glutamate, which in turn leads to the influx of large amounts of extracellular calcium, and subsequent cell death through a cascade of biochemical events involving, among other things, the activation of certain enzymes known as kinases. Cell death may occur by necrosis or apoptosis (programmed cell death),[14] a process not involving subsequent inflammatory events.

In autopsies on the brains of AD patients, two types of lesions stand out in particular, namely, the so-called plaques and tangles.[15,16] The plaques consist of β-amyloid (Aβ) protein, which is in turn derived from the larger amyloid precursor protein (βAPP) whose own role in brain function is poorly understood.[15] It is clear at this point that Aβ contributes somehow to the disease process. All four known genetic alterations associated with familial AD lead to increased production or deposition of Aβ.[17] Suggestions have been made that Aβ may generate free radicals, very reactive chemical species capable of destroying neurons. Certainly, in some cases substantial numbers of amyloid plaques were observed in AD brains where no clear evidence of a dementia was apparent. Thus, it is likely that Aβ itself may not be the primary determinant of whether an individual will have AD or not. Additionally, other disease-modifying genes may play a role in determining an individual's susceptibility to Aβ toxicity.[18,19] Despite the still questionable role of Aβ in the disease process, much research is devoted to identifying inhibitors of the proteases involved in βAPP processing, so as to minimize Aβ production.[20] For example, activation of the intracellular kinase, protein kinase C (PKC), has been shown to increase the production of APPα (the nonamyloidogenic product derived from cleavage of βAPP by α-secretase) while diminishing Aβ production. In this respect, it is also of interest to note that alterations of potassium channel function have been identified in AD fibroblasts, and that a PKC activator based upon the indolactam V structure restores normal K^+ channel function in AD fibroblasts and enhances production of the soluble form of the amyloid protein.[21] Recent genetic evidence suggests that a common mutation in the gene encoding the protein α_2-macroglobulin (α2M) increases the likelihood for developing neurodegeneration. While more work is needed to identify the role of normal α2M, the suggestion has been made that it may remove neurotoxic proteins.[22]

A further contributor to the list of events that may conspire to lead to AD is oxidative stress. Aβ itself acquires radical character under certain conditions and possibly serves in the initiation of superoxide radical generation. Considerable credence has therefore recently been given to the study of antioxidant therapies in slowing down AD's progression. In particular, vitamin E has been studied in a multicenter clinical trial, and found when administered at sufficiently high doses to delay the time for the patient to enter a healthcare facility.[23] Further, amyloid is known to activate the classical complement system in the brain, thereby suggesting that the neurodegeneration associated with AD stems at least partially from inflammatory mechanisms. In fact, small-scale clinical trials have revealed that nonsteroidal antiinflammatory drugs such as indomethacin do slow AD's progression.[24]

Thus, there exist many possible points of intervention in the discovery of therapeutic agents for AD. As mentioned above, AChE inhibitors such as HA offer some benefit to AD patients. In this respect, HA works much like the currently marketed drug for AD treatment, Aricept or donepizil (E2020; IC_{50} 0.03 μM). In studies comparing HA with Aricept, HA is 1000-fold selective for AChE over butyrylcholinesterase, while Aricept shows a selectivity of only about 200-fold.[25] Clinical studies have demonstrated that inhibition of butyrylcholinesterase, which is abundant in human plasma, may be associated with potentiating side effects. Also, HA lacks any affinity for nicotinic and muscarinic receptors, while Aricept exhibits low micromolar activity at M1 and M2 muscarinic receptors.[25,26] Furthermore, enzyme inhibition by HA occurs without chemical modification of AChE, whereas two new drug candidates that have undergone extensive clinical testing, metrifonate and excelon, covalently modify the enzyme. As such, they interact chemically with the serine hydroxyl at the active site. Metrifonate is an organophosphate previously used as anthelmintic.[27] Excelon is a carbamate which, like pyridostigmine and physostigmine, carbamoylates the serine hydroxyl. The carbamoylation step can be reversed.[28]

Other interesting apects of HA's pharmacology relate to its neuroprotective properties.[29] For example, pretreatment of embryonic rat neuronal cultures with HA reduces glutamate-induced cell death. As HA pretreatment was found to decrease glutamate-induced calcium mobilization, it is likely that its neuroprotective properties may relate to its ability to block excitatory amino acid action at the NMDA type of glutamate receptors. On the basis of these findings, it would seem likely that HA may actually be able to slow the progression of AD, especially if some element of glutamate toxicity is actually involved in the disease process. Furthermore, preliminary studies of HA's action on βAPP processing reveal huperzine to be able to enhance the production of the soluble form of APP, perhaps thereby reducing the production of the toxic Aβ fragment. While repetitions of these studies are underway, findings such as these further enhance the therapeutic profile of this exciting molecule.[30]

Additionally, HA is a powerful pretreatment candidate for protection from nerve agent toxicity.[10] In mice, a protective ratio of approximately 2 was maintained for at leat 6 h after a single injection (0.5 mg/kg ip) of huperzine, without the necessity for an additonal postchallenge drug therapy. In contrast, pretreatment with physostigmine increased the LD_{50} of soman by 1.4−1.5-fold for only up

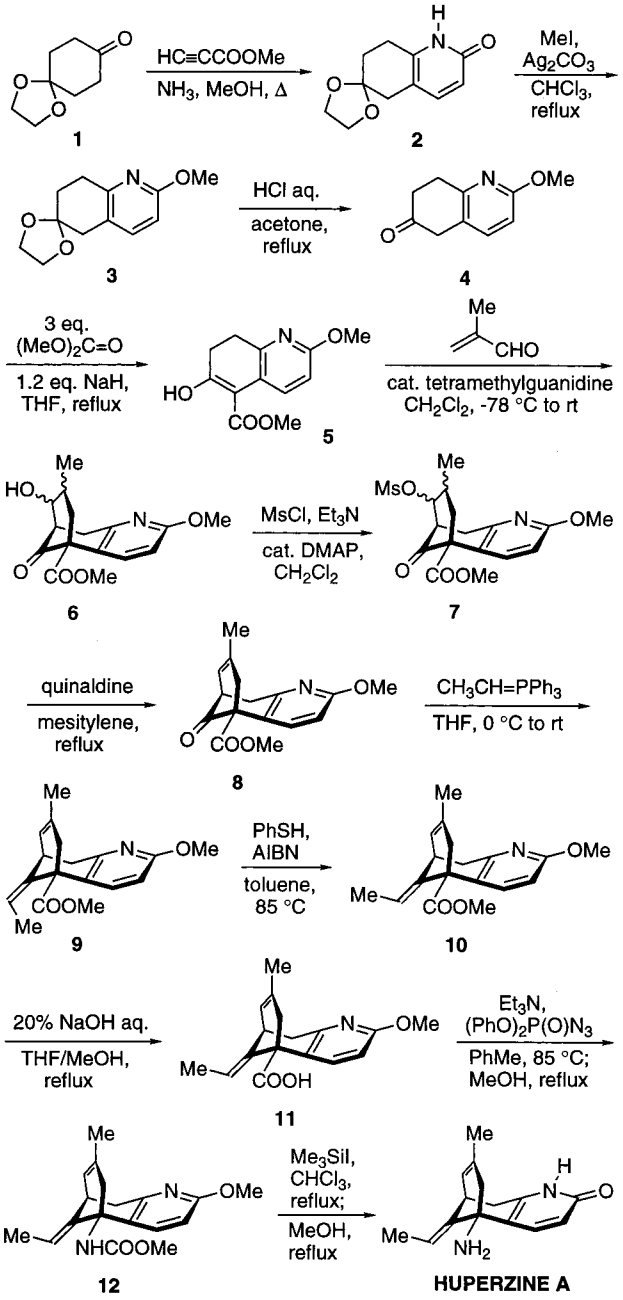
to 90 min. The long-lasting antidotal action of huperzine was correlated with the time course of its blood-AChE inhibition. Moreover, in guinea pigs challenged with soman, huperzine pretreatment at 0.5 mg/kg ip was found to totally prevent seizures and to ensure the survival of all animals for 24 h after intoxication. In comparison, all animals pretreated with pyridostigmine exhibited epileptic activity after soman poisoning, and five of the six animals died.[31] The hippocampal tissue of the huperzine-treated animals was free of any neuronal damage, whereas the sole surviving animal treated with pyridostigmine showed extensive neuropathology. In similar experiments, four Chinese rhesus monkeys administered intravenously with $1-1.3 \times LD_{50}$ soman at 65 min, 75 min, 5 h, and 14 h posttreatment with a sign-free dose of HA (50 μg/kg ip) survived without need for postexposure therapy. In marked contrast, physical deterioriation occurred with a nonprotected control monkey within 5 min of iv injection of $1 \times LD_{50}$ of soman.[32]

Synthesis of HA and Its Analogues. Discovery of a More Potent Analogue

HA's potent activity stimulated us[33,34] and others[35] to synthesize this compound. Scheme 1 shows our synthesis of racemic HA including recent improvements. The construction of the alicyclic part closely follows earlier work on *Lycopodium* alkaloids.[36] 1,4-Cyclohexanedione monoethylene ketal (**1**) was subjected to pyridone annulation (essentially a variation of Stork's method[37]) to form the tetrahydroquinolinone **2**.[38] The lactam was protected by *O*-methylation, and the ketal hydrolyzed. Methoxycarbonylation at C-5 provided β-ketoester **5**, which underwent with methacrolein and catalytic *N*,*N*,*N*′,*N*′-tetramethylguanidine a tandem Michael-aldol reaction, yielding the tricycle **6**. This material consists of four stereoisomers, resulting in relatively poor and erratic yields in the transformation of the derived mesylate **7** to olefin **8** by MsOH elimination. Although the conditions shown gave consistent yields around 50%, this step nevertheless remained unsatisfactory. Olefination of **8** produced predominantly (*Z*)-olefin **9** and little of the desired, more stable (*E*)-olefin **10**. *E*/*Z* equilibration was achieved by reversible addition of phenylthio radicals. Subsequent saponification left residual **9** unreacted for steric reasons. Curtius–Shioiri degradation of acid **11** gave urethane **12**, which on deprotection with iodotrimethylsilane yielded racemic HA. Compared with (−)-HA (IC_{50} for AChE inhibition 0.047 μM), the racemate exhibited an IC_{50} of 0.073 μM, which is, within error, as expected if the unnatural enantiomer is inactive.

The three-carbon bridge was more efficiently introduced by Pd-catalyzed alkylation[39] of **5** with 2-methyleneпropane-1,3-diyl diacetate on both sides of the ketone carbonyl (Scheme 2). The resulting exocyclic olefin **13**[40] was carried on to urethane **14** by steps analogous to those shown above. Deprotection with Me_3SiI caused partial double bond migration to yield HA besides its isomer **15**, doubtlessly due to acid traces, and indeed further acid

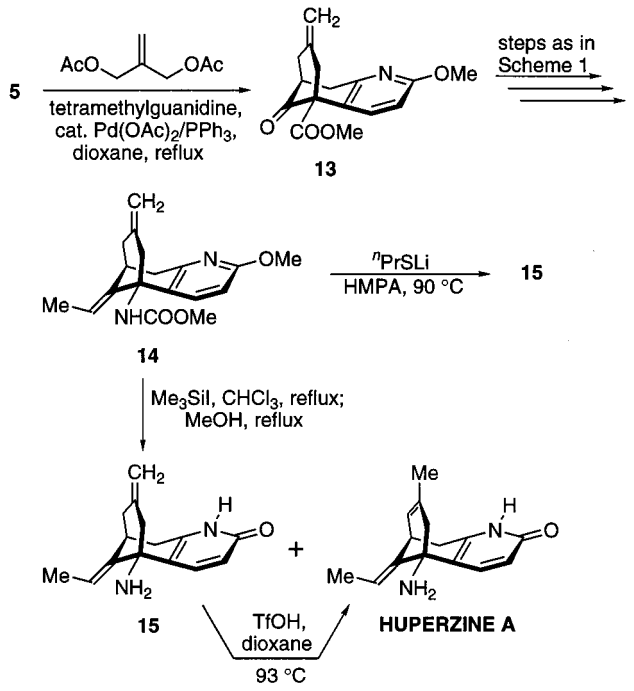
Scheme 1



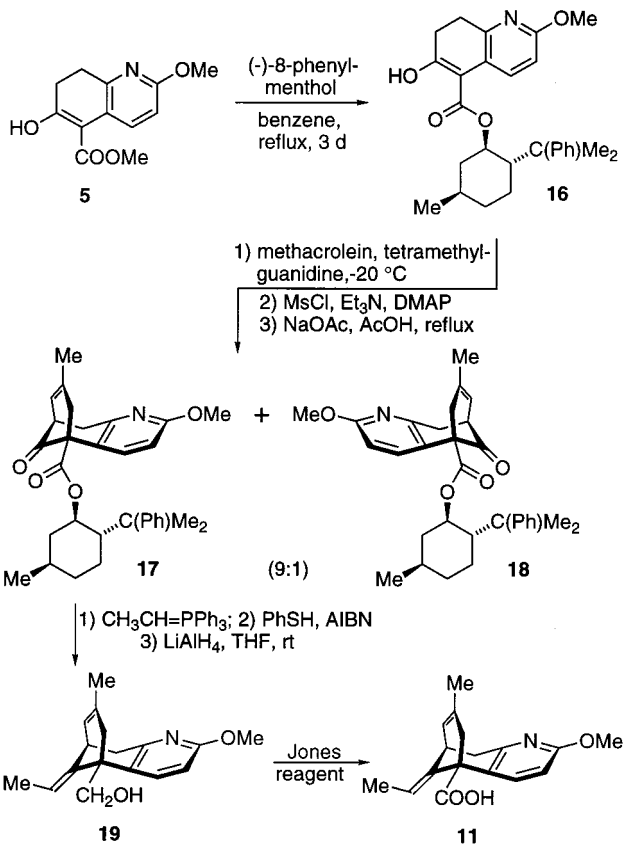
treatment converted **15** completely into HA. Pure **15** resulted from an alternative deprotection method.

Both approaches were amenable to enantioselective modifications (Scheme 3). Transesterification of **5** with (−)-8-phenylmenthol, Michael-aldol reaction at −20 °C, and dehydration yielded a separable 9:1 mixture of the diastereoisomers **17** and **18**. Since the bulky phenylmenthyl group prevented saponification, **17** was reduced to alcohol **19** with $LiAlH_4$ and **19** reoxidized to the enantiomerically pure acid **11**. The remaining steps proceeded as before. In an alternative approach better suited to the preparation of (+)-HA, racemic **19** was resolved by chromatographic separation of its diastereoisomeric Mosher acid esters, and individual enantiomers were recovered by $LiAlH_4$ treatment.[41] Others have used *Cinchona* alkaloids as Michael-aldol catalysts and optically active phos-

Scheme 2



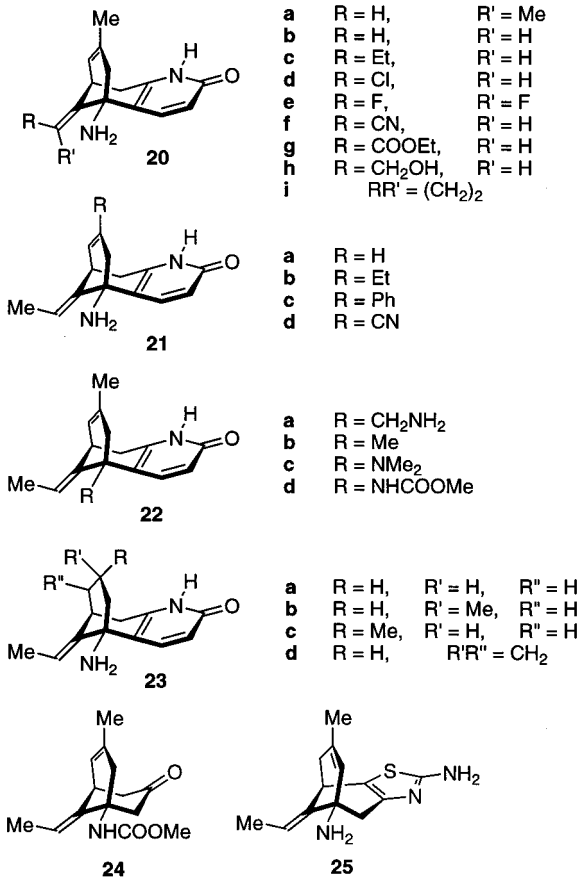
Scheme 3



phine ligands in the palladium-catalyzed annulation and obtained enantiomeric excesses ≤64%.[42] (+)-HA inhibits AChE with an IC_{50} of 1.44 μM and thus appears to be 30 times less active than its enantiomer. The actual difference may be significantly higher, as the sample of Mosher acid used had an enantiomeric excess around 99%, and the resulting (+)-HA therefore contained traces of (−)-HA. All compounds discussed below are racemic unless described otherwise.

From early on, we desired to understand which parts of the HA molecule are necessary to its activity, and whether a more active compound, or one that more readily crosses the BBB, could be generated by adding, omitting, or modifying substituents. Truncated versions of HA in which the three-carbon bridge had been replaced with a C-5 methyl, or even larger portions of the alicyclic moiety had been removed, lacked significant activity.[34,43] Several HA analogues were readily accessed by modifying the above schemes. Omission of the olefin isomerization (**9** to **10**) resulted in the *Z* isomer **20a** of HA (IC_{50} 6 μM). In the transformation of **8** to **9**, alternative olefination reagents gave intermediates from which HA analogues modified at the exocyclic double bond, **20b**−**i**, were obtained after following through the remaining steps.[34,40b,44] Removing or adding a single carbon atom (compounds **20b**,**c**) resulted in activity losses by factors of 70 and 270, respectively, and other compounds of this type performed similarly or worse, except for **20d** (IC_{50} 0.81 μM). Another obvious target for modifications was the C-7 methyl group. Replacement of methacrolein with other enals in the Michael-aldol step lead to HA analogues **21a**−**d**, which were again less active than HA, regardless of whether the substituent's size had been decreased or increased. One-carbon chain extension at C-5 or replacement of NH_2 by a methyl group of comparable size (compounds **22a**,**b**, accessible from *rac*-**19** via nucleophilic substitution or Barton deoxygenation) resulted in loss of activity as did *N*-methylation and *N*-methoxycarbonylation (compounds **22c**,**d**).[34,44] Yet another series of analogues **23a**−**d** was derived from HA by deleting the 7,8 double bond. The parent structure **23a** and the *anti* and *syn* isomers **23b**,**c** of the 7-methyl derivative were prepared by Barton deoxygenation of aldol **6** and the related acrolein-derived aldol.[43] Cyclopropane **23d** was synthesized via dibromocyclopropanation ($CHBr_3$, ^{t}BuOK) of the 12-nor analogue of **8**, followed by dehalogenation (Bu_3SnH, AIBN).[44] Into the same category of analogues falls the exocyclic olefin **15**. All of these compounds were significantly less active than HA. Bromination in position 3 also proved deleterious for biological activity[44] as did replacement of the pyridone with a benzene ring.[45] This result was, surprisingly, not improved upon by restoring hydrogen-bonding capabilities through introduction of a 2-hydroxyl group or of hydroxyl groups in both the 1- and 2-positions.[46]

It thus appeared that the structure of HA tolerates little modification. Neither could heteroatoms or π-bonds be omitted, nor could the olefinic methyl substituents be removed or modified without significantly reducing activity. There remained, however, several positions to be investigated. Specifically, we planned to replace C-3 and/or C-4 with heteroatoms and to introduce substituents at C-10. Initial approaches to heteroatom analogues via intermediate **24** failed as its enolate-based derivatization gave mostly products with the wrong regiochemistry.[47] Unexpectedly, compound **25**, obtained by deprotection

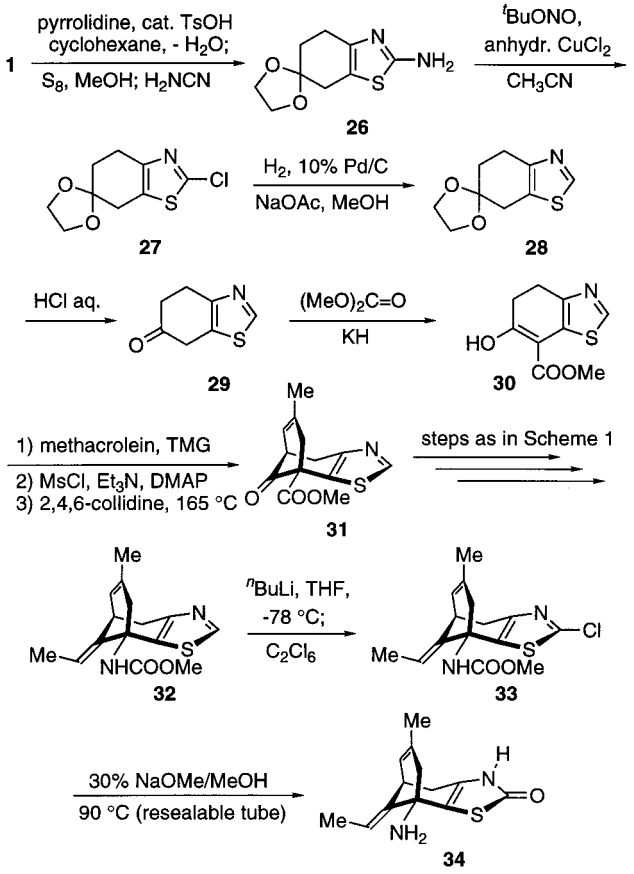


of one of those products, exhibited weak AChE-inhibitory activity (IC_{50} 37 μM).

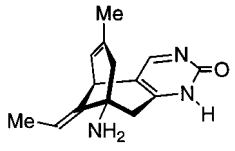
The thiazolone analogue **34** was therefore prepared in a fashion similar to our HA synthesis, beginning with a Gewald thiazole annulation to monoketal **1** (Scheme 4).[48] Masking of the future C-2 carbonyl as a methoxy group failed because of its excessive acid sensitivity, demethylation occurring in preference to ketal hydrolysis. Instead, the 2-substituent was removed, whereupon the following steps proceeded like those in the synthesis of HA. Arriving at the urethane **32**, the 2-substituent was reintroduced by deprotonation and chlorination. Finally, substitution of Cl by methoxide was under appropriate conditions accompanied by complete deprotection to furnish **34** in a single operation. This compound failed to inhibit AChE when tested at concentrations up to 14 μM.

The pyrimidinone analogue **42**, a compound capable of additional hydrogen bonding with AChE, was synthesized as shown in Scheme 5.[49] Again the heterocyclic ring was annulated to the starting material **1**; subsequently, the β-ketoester moiety was introduced, and the three-carbon bridge attached under Pd catalysis. Attempted Wittig or Takai olefination of the resulting tricycle **39** gave only ring cleavage or reduction. Conversely, the lithium enolate of phenyl thiolpropionate added to **39** to yield β-lactone **40** as a single stereoisomer. Its thermolysis in the presence of SiO_2 delivered the required intermediate **41** (*E* stereochemistry),[50] which gave after the usual steps the pyrimidinone analogue **42** (IC_{50} 31 μM). **42** was independently synthesized by others on the Michael-aldol

Scheme 4

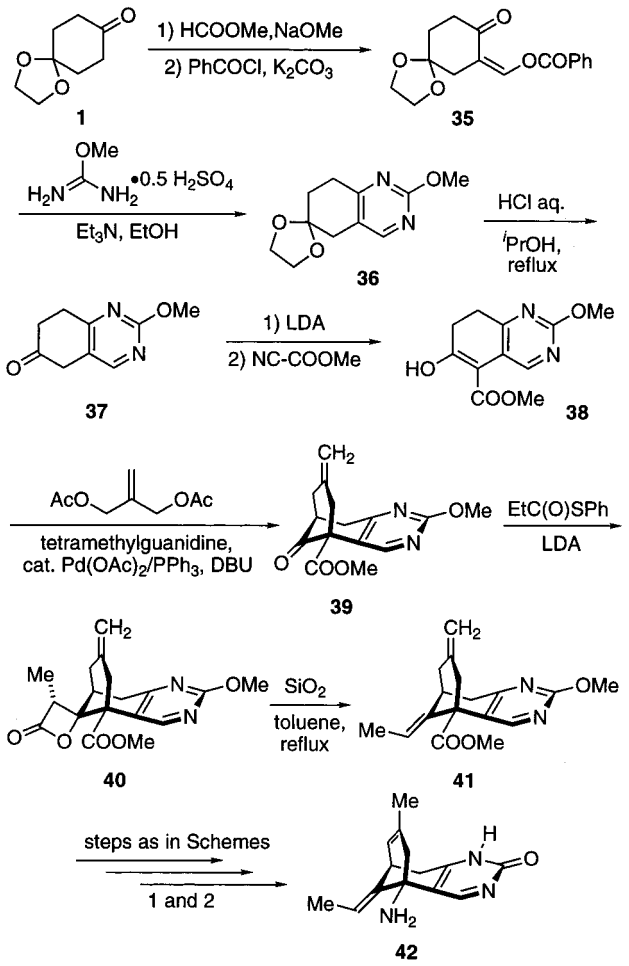


route.[51] Surprisingly, its regioisomer **43**, obtained via application of the pyrimidine annulation to ketone **24**, displays much better activity (IC_{50} 0.73 μM).[49]



To prepare racemic 10-substituted HA analogues (Scheme 6),[52−54] we employed the Pd-catalyzed approach but also obtained the 10-spirocyclopropane **53d** of natural chirality via the Michael-aldol route using the (−)-8-phenylmenthyl ester. The 10-substituents were introduced at the beginning by enolate alkylation or after pyridone annulation and *O*-methylation by modifying an allyl substituent. Stereoisomerism arises for 10-monosubstituted intermediates upon completion of the tricyclic skeleton (**49**, **50**). The stereoisomers are designated *exo* (axial substituent) and *endo* (equatorial substituent), depending on the substituent's orientation with regard to the three-carbon bridge. They were separated at the carboxylic acid or urethane stage (**51**, **52**). In the ethyl and propyl series, only the *exo* isomers were obtained pure. Some of the 10-substituted analogues showed impressive IC_{50} values: *exo*-**53b**, 0.003 μM; *endo*-**53b**, 0.035 μM; **53c**, 0.017 μM; **53d**, 0.012 μM; *exo*-**53e**, >200 μM; *exo*-**53f**, 2.0 μM (in this series of experiments, IC_{50}(HA) 0.024 μM).

Scheme 5



Space for a single carbon atom is therefore available in both the axial and equatorial regions at C-10, whereas a larger substituent at least in the axial region strongly inhibits binding. Only an axial substituent improves binding, whereas an equatorial one slightly reduces it, with geminally disubstituted derivatives approximately pulling even with or slightly surpassing HA. While *exo*-**53b** is remarkable as the only HA analogue reported to date that displays significantly higher AChE inhibition than HA itself, this compound and **53c** and **53d** are of additional interest with regard to their increased lipophilicity, which may render them better capable of crossing the BBB. Last, a kinetic investigation has revealed that **53c** dissociates more slowly from AChE than HA,[53] thereby suggesting a longer duration of action.

Molecular Modeling and X-ray Studies

We have carried out extensive modeling studies to define HA's binding site in *Torpedo californica* AChE for which the X-ray structure has been determined by Sussman and collaborators. This structure is remarkable by the fact that the catalytic site resides inside a narrow "gorge" lined with hydrophobic aromatic residues. We have refined our previously reported binding model[55] by including crystallographic waters, a refinement that was considered essential in light of the X-ray results reported for edrophonium (EDR), tacrine (THA), and decamethonium (DECA), all of which include crystallographic waters in binding to the enzyme.[56] The most likely binding mode of HA in AChE as derived from docking, energy minimization, and molecular dynamics studies is shown in Figure 2. The ammonium group of HA forms hydrogen bonds with Asp72, Trp84, and Asn85 through two bridging water molecules. These two water molecules are present in all three X-ray crystal structures of AChE in complex with EDR, THA, and DECA.[57] The ammonium group of HA may also interact with the aromatic ring of Trp84 through a cation−π interaction. The lactam NH forms H-bonds with the hydroxyl oxygen of Tyr130 and the carboxyl group of Glu199. The lactam carbonyl forms two H-bonds with the hydroxyl group of Tyr130 and the backbone amide group of Leu124. Interestingly, the site of HA's lactam carbonyl in the binding model was originally occupied by a water molecule in the structures of AChE in complex with both EDR and THA, suggesting that this position is indeed ideal for H-bonding. The hydrophobic interactions of HA with the enzyme occur primarily through five aromatic residues, Trp84, Tyr121, Phe290, Phe330, and Phe331, and two aliphatic residues, Ile439 and Ile444. Using the calculated binding site information, we can explain the different activities of the axial and equatorial isomers of **53b**: the C-10 axial methyl group points into a hydrophobic region of the enzyme, while the equatorial methyl is directed to a less favorable hydrophilic region. The predicted binding mode of *exo*-**53b** is shown with that of HA in Figure 2.

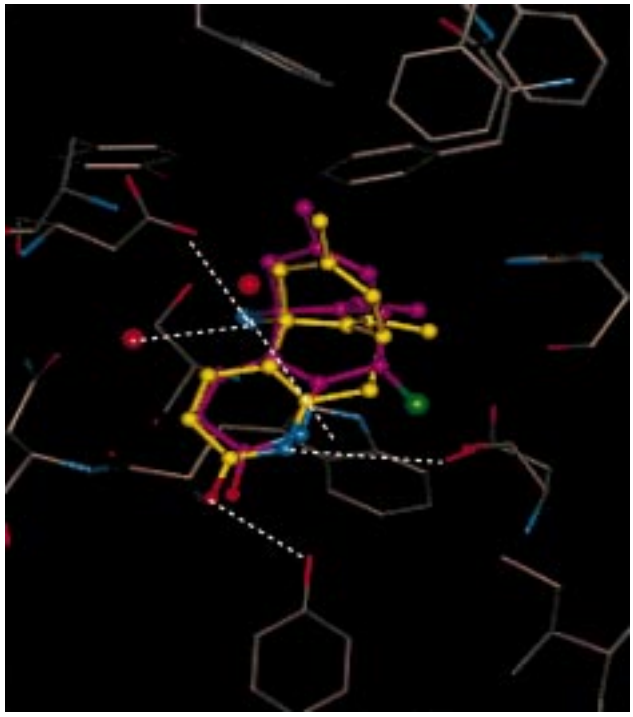
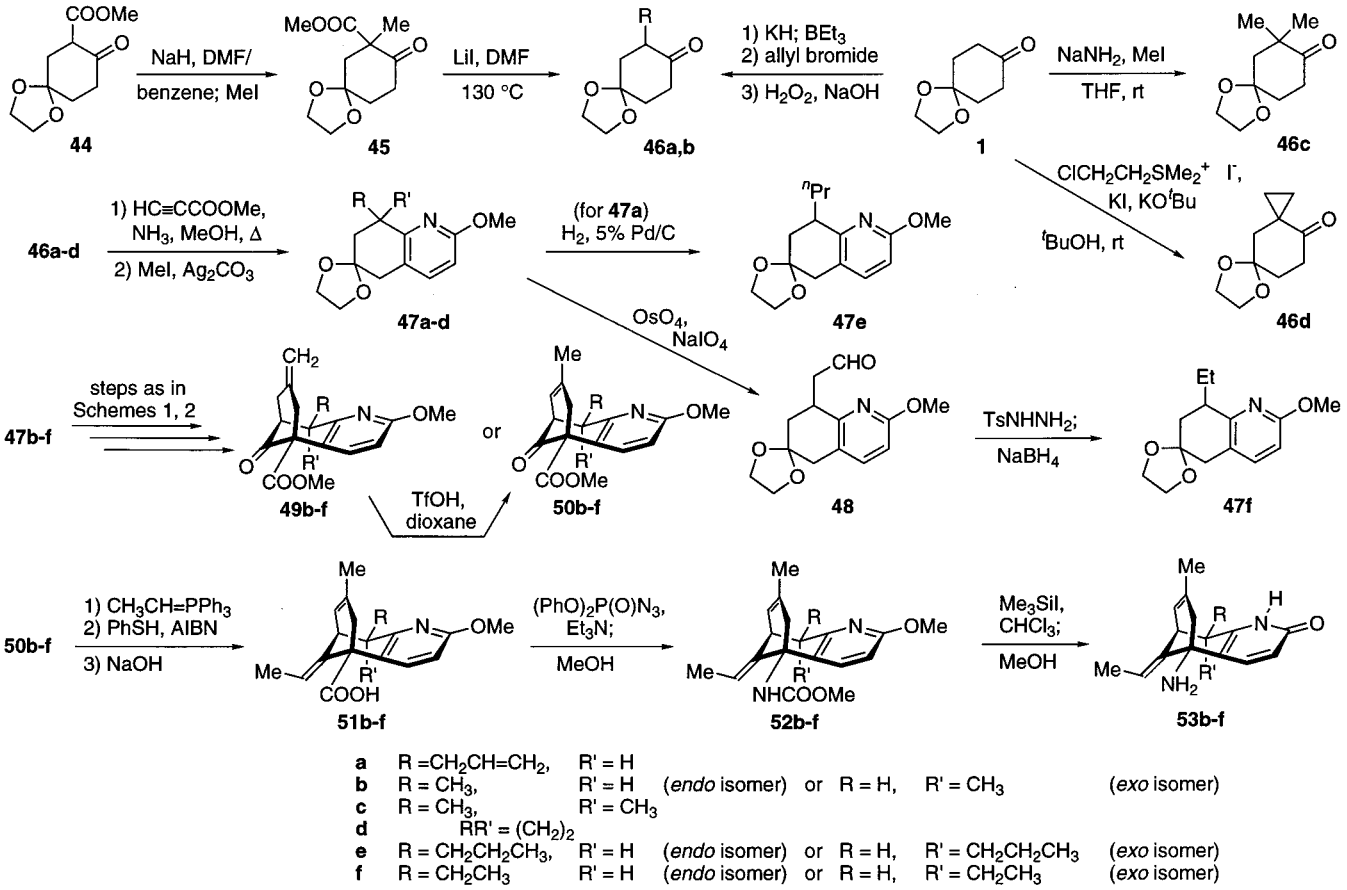


FIGURE 2. Predicted binding modes of HA (yellow) and its axial 10-methyl analogue *exo*-**53b** (fuchsia with the methyl group in green) in AChE (blue, nitrogen; red, oxygen). Dashed lines represent H-bonds or cation−π interactions.

In collaboration with Dr. Joel Sussman of the Weizmann Institute, we have completed a cocrystal X-ray structure of (−)-HA in complex with *Torpedo* AChE.[57] A

Scheme 6



a R = $CH_2CH=CH_2$, R' = H
b R = CH_3, R' = H (*endo* isomer) or R = H, R' = CH_3 (*exo* isomer)
c R = CH_3, R' = CH_3
d RR' = $(CH_2)_2$
e R = $CH_2CH_2CH_3$, R' = H (*endo* isomer) or R = H, R' = $CH_2CH_2CH_3$ (*exo* isomer)
f R = CH_2CH_3 R' = H (*endo* isomer) or R = H, R' = CH_2CH_3 (*exo* isomer)

schematic showing the main interactions between the protein and HA as determined from the X-ray data is provided in Figure 3. The principal protein−ligand interactions include (1) a strong hydrogen bond from the pyridone carbonyl of HA to Tyr130, (2) hydrogen bonds to water molecules within the active-site gorge which are themselves hydrogen-bonded to other waters or to side-chain and backbone atoms of the protein, notably to Glu199 and Tyr121, (3) interaction of the charged amino group of HA with the aromatic rings of Trp84 and Phe330, (4) an H-bond between the ethylidene methyl group and the main-chain carbonyl oxygen of His440, and (5) several hydrophobic contacts, notably with the side chains and main-chain atoms of Trp84 and His440 and with residues of the oxyanion hole, Gly118 through Ser122. Our calculated binding site is nearly identical to that determined from the X-ray work. Together, these studies provide a solid structural foundation for designing other HA analogues likely to be of therapeutic interest.

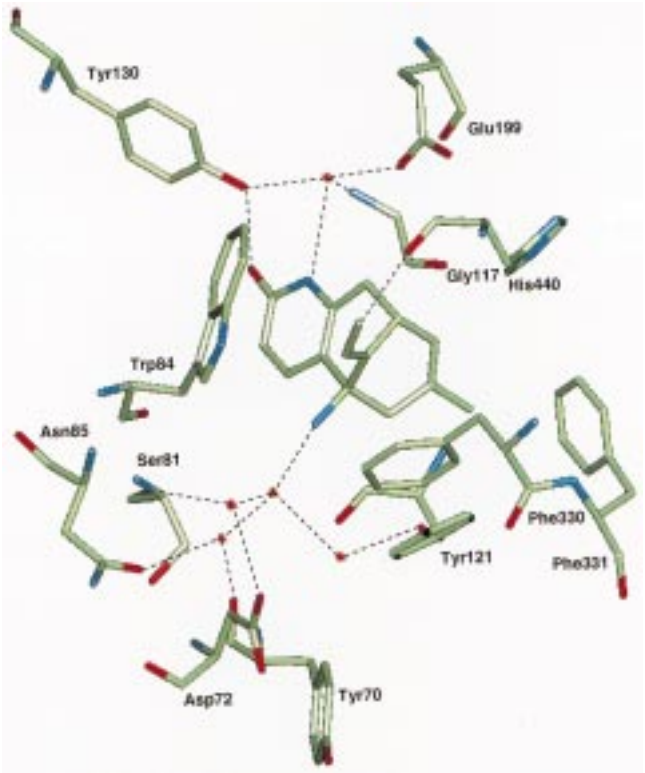


FIGURE 3. Main interactions between HA and AChE as determined by X-ray crystallography. Hydrogen bonds are indicated by dashed lines.

Future Prospects

HA has undergone double blind, placebo-controlled clinical trials in China in patients suffering from various memory disorders, including AD. Significant effects were noted in these patients in terms of their life quality; approximately 60% showed an improvement in their memory.[4] In view of the fact that *H. serrata* was used for many years in Chinese folklore medicine, and in accord

with the Dietary Supplement Health & Education Act of 1994 (DSHEA) in this country, it has been possible to introduce HA into the U.S. market as a dietary supplement. Accordingly, HA has become available to numerous individuals for the treatment of memory problems.

As we have discovered analogues of HA that are more potent than the parent structure, or that show slower off-rates from the enzyme, it will be of considerable interest to explore the cognitive-enhancing effects of such compounds in animal memory models. The manipulation of appropriate structural parameters may allow not only for further improvements in potency, but also for more ready penetration of the BBB. It will further be of value to elucidate the structural features of HA that are relevant to its neuroprotective properties and its effect on the processing of APP so that these particular properties may be improved upon.

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