Highly Potent, Selective, and Low Cost Bis-tetrahydroaminacrine Inhibitors of Acetylcholinesterase

STEPS TOWARD NOVEL DRUGS FOR TREATING ALZHEIMER’S DISEASE*

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Yuan-Ping Pang‡§, Polly Quiram¶, Tanya Jelacic¶, Feng Hong‡, and Stephen Brimijoin¶

From ‡Neurochemistry Research, Mayo Foundation for Medical Education and Research, Jacksonville, Florida 32224 and the ¶Department of Pharmacology, Mayo Foundation for Medical Education and Research, Rochester, Minnesota 55905

We report highly potent, selective, and low cost bi-functional acetylcholinesterase (AChE) inhibitors developed by our two-step prototype optimization strategy utilizing computer modeling of ligand docking with target proteins: 1) identify low affinity sites normally missed by x-ray crystallography; and 2) design bifunctional analogs capable of simultaneous binding at the computer-determined low affinity site and the x-ray-identified high affinity site. Applying this strategy to 9-amino-1,2,3,4-tetrahydroacridine (THA), a drug for Alzheimer’s disease, we obtained alkylene linked bis-THA analogs. These analogs were up to 10,000-fold more selective and 1,000-fold more potent than THA in inhibiting rat AChE and yet required one simple reaction to synthesize. Additionally, alkylene linked benzyl-THA analogs were developed to examine the specificity of the docking-derived low affinity AChE peripheral site and the x-ray-identified high affinity site. Applying this strategy to 9-amino-1,2,3,4-tetrahydroacridine (THA), a drug for Alzheimer’s disease, we obtained alkylene linked bis-THA analogs. These analogs were up to 10,000-fold more selective and 1,000-fold more potent than THA in inhibiting rat AChE and yet required one simple reaction to synthesize. Additionally, alkylene linked benzyl-THA analogs were developed to examine the specificity of the docking-derived low affinity AChE peripheral site in AChE. The present work and our previous computational studies strongly suggest that a low affinity AChE peripheral site exists in AChE. This peripheral site provides a structural basis for design of improved cholinesterase ligands for treating Alzheimer’s disease and for other health-related purposes.

The agent THA1 is approved by the United States Food and Drug Administration for palliative treatment of mild and moderate AD (1). Despite evidence of clinical utility, the mechanism of the therapeutic effect of THA is uncertain, since this drug has many actions in the central nervous system and interacts with multiple receptors and ligand-binding sites. However, one well characterized effect of THA is inhibition of acetylcholinesterase (AChE), the enzyme that regulates synaptic availability of the neurotransmitter acetylcholine (2, 3).

Unfortunately, THA is not without serious toxicity, so it would be logical to develop additional potent and selective inhibitors of AChE as potential therapeutic agents. We have aimed at compounds that are more potent and selective for AChE inhibition but otherwise similar to THA. Such inhibitors may or may not produce all of the clinical effects of THA, but these THA analogs can serve as indispensable pharmacological tools for evaluating the role of AChE inhibition in AD therapy. If AChE inhibition is an important component of the therapeutic response, and if structural modification introduces no additional toxicity, increased potency might provide clinical advantage. With these aims, we previously devised an automated computer docking program, SYSDOC, to guide the directed synthesis of THA analogs with superior therapeutic potential. This program systematically translates and rotates a guest (such as THA) in the putative binding pocket of a host (such as AChE) to evaluate energetically favorable binding sites for eachavailable conformation of the two partners. The affinity of binding in a docking study is estimated from the potential energy of the complex relative to the potential energies of the guest and host in their free state, assuming that the differences in entropy and solvation energies between two binding sites can be neglected (4).

Work with SYSDOC identified putative binding sites for THA and other inhibitors in the three-dimensional structure of AChE (4, 5). One interesting prediction of the SYSDOC analysis was THA docking at two different loci on the Torpedo AChE, not only at the catalytic site per se but also near residues Trp297, Tyr315, and Phe286. The second region constitutes AChE’s so-called peripheral site (4, 6–8). It is located at the opening of the enzymatic binding pocket (gorge) and is far from the catalytic site at the bottom of the gorge (Fig. 1). The catalytic site has been shown by x-ray crystallography to bind THA; however, a crystal complex of THA-AChE contained no ligand at the peripheral site (5). Pharmacological evidence for interaction of THA with AChE’s peripheral site is also conflicting (9–11). Nonetheless, we propose that the SYSDOC-determined peripheral AChE site does exist and is functionally relevant in the Torpedo AChE but not in the related enzyme mammalian butyrylcholinesterase (BChE) (12). We further propose that this peripheral site also exists in mammalian AChE, whose primary structure is similar to the Torpedo enzyme, especially in the conserved 14 aromatic residues lining the active site gorge (13).

The peripheral site in AChE probably evolved to increase the concentration of acetylcholine at the opening of the active site gorge, facilitating passage through the narrower portion of the gorge toward the catalytic site. This function is best accomplished with interactions of relatively low affinity (4, 6, 13), for tight binding at the peripheral site would actually lower sub-
strate concentrations at the catalytic site. A peripheral site that serves to concentrate acetylcholine would not be an advantage for BChE, however, since the active site gorge of this enzyme is wider throughout (12). It seems likely that low affinity explains why crystallography revealed no THA binding at the peripheral site. This low affinity peripheral site can also be responsible for nonspecific, low affinity binding of inhibitors such as 9-aminoacridine (11).

The peripheral site predicted by SYSDOC suggested a strategy for improving on the potency and selectivity of AChE inhibition by THA. This strategy was to connect two THA molecules with an alkylene chain spaced to permit simultaneous binding at the catalytic and peripheral sites (Fig. 1). This design takes advantage of Koshland’s “proximity and orientation” effect (14), facilitating productive interactions between ligand and protein by decreasing the entropy loss of the ligand in binding. At the same time, since BChE lacks a peripheral site for THA, selectivity for AChE should be increased. Additional reasons for producing bifunctional THA analogs were 1) to minimize efforts in synthesis of THA analogs and toxicity studies of the respective metabolites and synthetic intermediates; 2) to develop economical insecticides and parasiticides; 3) to confirm the existence of AChE’s peripheral THA site; and 4) to evaluate the role of AChE inhibition by THA in treating AD. Bifunctional THA analogs are well suited to the last aim, since varying the linking chain length may vary AChE inhibition while preserving other pharmacologic properties of the THA molecule.

Effective alkylene-bridged analogs require chains of the correct length (number of methylene units) coupled to the parent structure at the appropriate point. Inhibitory potency toward AChE is reportedly preserved or raised by attaching an n-pentyl or phenethyl group to the amino group of THA (15). Therefore, guided also by simulations of AChE complexed with two bound THA molecules we chose to link the amino nitrogen atoms. This choice simplified the synthesis of analogs because the nitrogen atom served as a nucleophilic center. Approximate chain length was determined by the growth method, in which sp^3 carbon atoms were added sequentially starting at one connecting point and ending near the other. At each step, the new atom’s energetically favored location in AChE’s active-site gorge was determined. Energy evaluation with the CHARMM force field involved rotating the torsional angle of the newly introduced atom at increments of 30° over a 360° range of arc (16).

Once these calculations had established the approximate chain length (9 methylene units), the specified compound 1c was readily synthesized according to Scheme I as illustrated in Fig. 1. Given the ease of synthesis, it was efficient to make homologs without performing full scale docking studies and the dual topology (17) free energy perturbations for accurate chain length. Therefore, three homologs with chains one and two atoms shorter and one atom longer than in 1c were produced at the same time for empirical evaluation.

Newly synthesized compounds were tested in vitro for selectivity and potency as enzyme inhibitors. Rat serum and extracts from rat brain were used, respectively, as sources of BChE and AChE for this screening work. As compared with THA, compound 1a proved 1,000 times more potent and 10,000 times more selective in inhibiting rat brain AChE (Table I). Inhibitory potency within the series of compounds was related to the length of the alkylene chain (Table I). The best chain
length determined by experiment was reached in compound 1a, with 7 methylene groups between THA residues. Based on a conformational search of compound 1a, the heptylene chain allows the ring nitrogen atoms of the two THA moieties to lie up to 18 Å apart. This spacing amply bridges the distance between the two SYSDOC-determined THA-binding sites (approximately 16 Å between the ring nitrogen atoms).

The modest discrepancy by 2 methylene units between the experimental result and the highly simplified computational result is hardly surprising. The growth method ignores solvent and entropy effects as well as the molecular flexibility of both enzyme and inhibitor. These factors all contribute to the experimentally observed binding affinity. Nonetheless, the predictive power of the growth method is evident in the fact that compound 1c, although not optimal in the series, still demonstrates dramatic improvement in potency and selectivity relative to THA (Table I).

For a rigorous test of the anticholinesterase properties of our compounds, the substrate kinetics of enzyme inhibition were examined in a highly purified preparation of human brain AChE. According to the reciprocal slope-replot procedure of Segel (18), class 1 analogs, like THA itself, produced a linear line as substrate and 10 M thiocholine as substrate in the presence of 10 M BW284C51 as AChE inhibitor. IC50 values were computed by a nonlinear least squares regression program that also provided an estimate of statistical precision (standard error of the mean). NA, not available.

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inhibitory potency conferred by the additional THA residue in compound 1a but not by the toluene residue in compound 2e implies a secondary site that is specific to THA. The relative potency of compound 2e is not as great as that of compound 1a. A likely explanation is that the small amount of favorable interaction energy gained from the nonspecific binding of toluene is largely offset by the loss of entropy energy when the alkylene chain binds and by the steric hindrance of the chain. In our view, this secondary site probably corresponds to the recognized "peripheral anionic site" of AChE. Additional enzymological studies of our THA analogs might be pursued for new insights into the highly evolved catalytic machinery inherent in the protein structure of AChE.

Inestrosa et al. (28) recently reported that AChE accelerates amyloid formation from amyloid-β peptide, which alone produces few amyloid-like fibrils, and that the formation of amyloid induced by the association of AChE with amyloid-β peptide can be inhibited by peripheral anionic site ligands such as propidium and decamethonium (28). Compounds 1 and 2 are novel peripheral anionic site ligands with different functional groups occupying the peripheral anionic site. It will be interesting to investigate whether these compounds can effectively inhibit the AChE-induced amyloid formation and therefore offer additional mechanistic advantage of treating AD relative to the current drug THA.

In conclusion, we have produced a series of highly potent, selective, and low cost bis-THA AChE inhibitors. These compounds are more hydrophobic than THA because of the introduced alkylene chain and interact simultaneously with the catalytic site and the peripheral anionic site. They might therefore serve as improved drugs for treating AD and as potential insecticides and parasiticides. The present work and the results of our previous computational studies (4, 6) strongly suggest that a low affinity THA peripheral site exists in AChE. This unique secondary site in AChE therefore provides a structural basis for designing novel antidotes for chemical warfare agents (29) and developing novel BChE-selective compounds that do not fit AChE’s peripheral site. Such analogs can serve as tools to evaluate permissive or causative roles of neural AChE and glial BChE in the neuropathology of AD (30).

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