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Lead identification of acetylcholinesterase inhibitors–histamine H_3 receptor antagonists from molecular modeling

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Abstract—Currently, the only clinically effective treatment for Alzheimer's disease (AD) is the use of acetylcholinesterase (AChE) inhibitors. These inhibitors have limited efficacy in that they only treat the symptoms and not the disease itself. Additionally, they often have unpleasant side effects. Here we consider the viability of a single molecule having the actions of both an AChE inhibitor and histamine H_3 receptor antagonist. Both histamine H_3 receptor antagonists and AChE inhibitors improve and augment cholinergic neurotransmission in the cortex. However, whereas an AChE inhibitor will impart its effect everywhere, a histamine H_3 antagonist will raise acetylcholine levels mostly in the brain as its mode of action will primarily be on the central nervous system. Therefore, the combination of both activities in a single molecule could be advantageous. Indeed, studies suggest an appropriate dual-acting compound may offer the desired therapeutic effect with fewer unpleasant side effects [*CNS Drugs* **2004**, *18*, 827]. Further, recent studies[2] indicate the peripheral anionic site (PAS) of AChE interacts with the β-amyloid (βA) peptide. Consequently, a molecule capable of disrupting this interaction may have a significant impact on the production of or the aggregation of βA. This may result in slowing down the progression of the disease rather than only treating the symptoms as current therapies do. Here, we detail how the use of the available crystal structure information, pharmacophore modeling and docking (automated, manual, classical, and QM/MM) lead to the identification of an AChE inhibitor–histamine H_3 receptor antagonist. Further, based on our models we speculate that this dual-acting compound may interact with the PAS. Such a dual-acting compound may be able to affect the pathology of AD in addition to providing symptomatic relief.
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1. Introduction

Alzheimer's disease is the most common form of neurodegenerative dementia.[3] It accounts for approximately 50–60% of the overall cases of dementia among individuals over the age of 65 years.[4] Unfortunately, the therapeutic options for Alzheimer's disease are limited. No true disease-modifying therapies are known and at best physicians can only hope to alleviate the cognitive deficits that characterize this disease with symptomatic treatments. The most frequently prescribed anti-Alzheimer's drugs are the acetylcholinesterase (AChE) inhibitors, which promote memory function and delay the cognitive decline without altering the underlying pathology.[5] These compounds, however, leave much to be desired, both in efficacy and tolerability. Their efficacy is limited; the preferred target patient population suffers a mild to moderate cognitive impairment.[5] Additionally, they are associated with unpleasant mechanism-based side effects such as gastrointestinal discomfort and risk of bradycardia, especially the nonselective AChE inhibitors, which inhibit AChE as well as butyrylcholinesterase.[6]

Clinical studies suggest combination therapies can provide synergistic benefits in some instances.[1] An alternative approach taken by several pharmaceutical companies is to combine[7] AChE inhibition with other mechanisms targeted at symptoms of Alzheimer's disease using a single molecular entity. For instance, inhibition of the serotonin reuptake transporter is expected to

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bestow antidepressant efficacy, which may alleviate some of the psychiatric symptoms observed in Alzheimer's populations. Dual-acting compounds of AChE and the serotonin transporter have been synthesized.[8] The argument in favor of the combination of an AChE inhibitor and histamine H_3 receptor antagonist can be separated into a neurochemical and a procognitive argument. Neurochemically it is believed the decline in cognitive function in normal aging, mild cognitive impairment, Alzheimer's disease, and related conditions is caused by a decrease in cholinergic neurotransmission in the cortex. Both AChE inhibitors and histamine H_3 receptor antagonists improve and augment cholinergic neurotransmission in the cortex, albeit by different mechanisms. Therefore, the combination of both activities in a single molecule may lead to a potentiation of cholinergic neurotransmission. Procognitive benefits are expected from both AChE inhibitors and histamine H_3 receptor antagonists as both exhibit memory-enhancing effects. The combination of both activities in a single molecule might be expected to have significant synergistic or additive effects on cognitive function and memory.

Inhibition of AChE increases the synaptic levels of acetylcholine and thereby promotes cholinergic neurotransmission. The effect of AChE inhibitors is thus to stabilize and prolong the biological half-life of acetylcholine. In contrast, histamine H_3 receptor antagonists increase cholinergic neurotransmission via a distinct mechanism. The presynaptic inhibitor–histamine H_3 receptor, when activated, decreases the release of acetylcholine from cholinergic neurons. This has been shown both in the gastrointestinal[9] and central nervous system.[10] Consequently, when a histamine H_3 receptor antagonist is present, an increased release of acetylcholine is observed. Thus, both mechanisms contribute to the same neurochemical end result (increased synaptic levels of acetylcholine) in different ways. The histamine H_3 receptor antagonists increase the amount of acetylcholine molecules entering the synaptic space, and AChE inhibitors prolong their survival time and hence the likelihood of their interacting with a postsynaptic cholinergic neurotransmitter and eliciting a biological effect.

In order to achieve a specific, cognition-enhancing level of cholinergic neurotransmission, a lower dose of an AChE inhibitor–histamine H_3 receptor antagonist compound will likely be required than of either an AChE inhibitor or histamine H_3 receptor antagonist alone; a combination molecule will have a higher potency for increasing acetylcholine levels than either an AChE inhibitor alone or histamine H_3 receptor antagonist alone. It is likely reduced compound requirements for efficacy will then improve the side effect profile. At present, not much is known about the side effects of H_3 antagonists. For AChE inhibitors, on the other hand, the major side effects are nausea and gastrointestinal discomfort.[6] By decreasing the amount of acetylcholine released in the periphery through augmentation of its release centrally via histamine H_3 receptor antagonism, fewer side effects may result.

Further, there is evidence for some AChE inhibitors exhibiting therapeutic benefits beyond influencing cholinergic transmission. AChE inhibitors able to interact with the peripheral anionic site (PAS) are of particular relevance. In addition to their ability to stimulate the cholinergic system, these inhibitors may be able to disrupt the interactions between AChE and the β-amyloid (βA) peptide and thereby be capable of slowing the progression of the disease by inhibiting the production or aggregation of the βA promoted by AChE.[3]

We describe herein the molecular modeling efforts that lead to the identification of an AChE inhibitor–histamine H_3 receptor antagonist. The use of available crystal structure information, pharmacophore modeling, classical, and quantum calculations lead us to the discovery. Our models also predict this dual-acting compound can interact with the PAS thus possibly having the potential to directly impact the disease itself unlike current therapies.

2. The acetylcholin esterase enzyme

The ability to study AChE by molecular modeling methods[11] (in particular by structure-based design) is benefited by the numerous crystal structures available both of the apo and complexed forms. The three-dimensional structure of *Torpedo californica* AChE was initially solved at a resolution of 2.8 Å[12] and later refined to 2.5 Å.[13] For our modeling purposes we used the structure of *T. californica* (PDB ID: 1EVE) complexed with the AChE inhibitor Aricept® (donepezil).[14] Reference to residue numbers will follow from this structure. The enzyme monomer is an α/β serine hydrolase consisting of 537 residues with a 12-stranded mixed β sheet surrounded by 14 α helices.[12] The active site is striking in that it is a deep, narrow tunnel approximately 20 Å long. This tunnel penetrates halfway into the enzyme and widens out at the end. Fourteen highly conserved residues cover a substantial portion of the tunnel. Crystallographic and theoretical analyses suggest approximately 20 water molecules occupy the tunnel[15,16] and indeed, the presence of water is necessary for the deacylation of acetylcholine. Approximately 4 Å above the bottom of the tunnel is the catalytic machinery: a catalytic triad consisting of Ser 200, His 440, and Glu 327. AChE is the first serine protease discovered to contain a Glu rather than an Asp as a catalytic member. It is in this area of the active site that deacylation of acetylcholine occurs. The electrophilic carbon on the ester group of acetylcholine undergoes nucleophilic attack from Ser 200 to form a tetrahedral intermediate followed by acylation of the enzyme and displacement of choline. Subsequent hydrolysis restores the enzyme with the generation of acetic acid. Proximal to the catalytic Ser 200 are the residues Gly 118, Gly 119, and Ala 201, which comprise the oxyanion hole. The amido protons of these residues aid in the deacylation process by forming hydrogen bonds to the carbonyl oxygen of acetylcholine. This orients the electrophilic carbon for nucleophilic attack by Ser 200. Directly across from the catalytic triad is Trp 84, which binds the quaternary

amine of acetylcholine through a cation-π interaction. (This region is known by the unfortunate name of 'anionic' site, AS, since it was initially believed that binding in this region occurred due to a collection of negative charges.) This finding was rather unexpected but is further substantiated by theoretical calculations showing the large stability of cation-π interactions.[17,18] There is also the peripheral 'anionic' site (PAS)[19] corresponding to Trp 279. The role of this site (with regard to the substrate) appears to be to attract, via cation-π and/or hydrophobic interactions with the quaternary amine moiety, acetylcholine into the active site. One can imagine Trp 279 attracting acetylcholine into the active site and assisting in aligning it into the correct orientation for subsequent deacylation. The PAS has gained much interest in the design of newer drugs for AD. Recent data suggest inhibitors interacting with the PAS could prevent the βA peptide from interacting with AChE. This could result in the elimination of AChE's role in the fibril formation process.[20] Consequently, it is believed AChE inhibitors interacting with the PAS could have an impact on the pathology of AD, rather than just treating the symptoms as current therapies do.

3. Molecular modeling

Our goal was to design an AChE inhibitor–histamine H_3 receptor antagonist capable of interacting with the PAS of AChE. Our initial efforts focused on virtual screening our in-house library. We sought to use the abundance of crystal structure data on AChE, our knowledge of the three-dimensional pharmacophore features of the histamine H_3 receptor[21] and modeling to optimize this effort. Our review of the crystal structure data focused on Aricept® (donepezil),[14] tacrine,[22] galantamine,[23] rivastigmine,[24] and decamethonium.[22] Decamethonium was of particular interest to us.

Shown in Figure 1 is the crystal structure of decamethonium[22] in the active site of AChE. The crystal structure

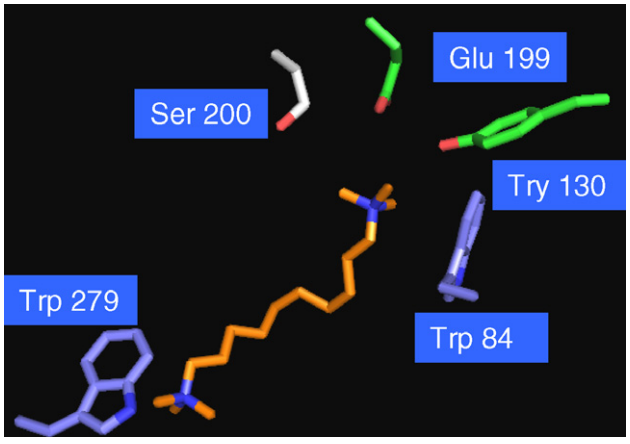


Figure 1. The crystal structure binding mode of decamethonium in the active site of AChE. The most notable interactions are those of the quaternary amines of decamethonium with the protein. One quaternary amine interacts with Trp 84 (the AS) and the other with Trp 279 (the PAS).

reveals two sets of key interactions between the protein and the ligand. One set of interactions is between the Trp 84 (the AS), located at the base of the gorge, and one of the quaternary amines of decamethonium. The other important interaction occurs between the Trp 279 (the PAS), located at the opening of the tunnel, and the other quaternary amine on the ligand. Both of these are cation-π interactions.[17,18] In Figure 2 is the proposed three-dimensional pharmacophore for histamine H_3 based on our previous work[21] along with a compound (**1** in Table 1) known to have good activity for the histamine H_3 receptor. The pharmacophore modeling was carried out with Catalyst[25] using the Hip-Hop option. This procedure takes three-dimensional conformations for each training molecule, identifies common features, and overlays them in space.[26] We used the automatic conformer generation feature employing the flexible option. Three active compounds we felt captured all of the features required for activity, while providing significant structural variation as well, were used to define the three-dimensional pharmacophore model. The three-dimensional pharmacophore model consists of three features: two positive ionic centers and an aromatic ring forming the center of the molecule. The model well explains known experimental data.[27]

For the most part, decamethonium fits this H_3 pharmacophore rather well with its two quaternary amines and hydrophobic alkyl chain acting as a replacement for the aromatic center. However, we expected an aromatic center would indeed be favored over a general hydrophobic for two reasons: first, we concluded from our previous work[21] the central aromatic ring can make π-stacking interactions with the residues Tyr 115 and Trp 371 in the histamine H_3 receptor; AChE contains several aromatic resides in its active site (the gating residues)[28] capable of π-stacking interactions. We hypothesized this pharmacophore model may well describe inhibitors of AChE (capable of interactions with the PAS) and histamine H_3 receptor antagonists. Starting from this premise we performed similarity and substructure searches around **1**. In conjunction with this effort we also performed manual docking[29] of **1** in the active site of AChE using Insight II version 2005[25] and the consistent valence force field (CVFF),[30] which is expected to be sufficient for our system. All modeling requiring a protein structure used *T. californica* (PDB ID: 1EVE) complexed with Aricept® (donepezil).[14] While this effort provided useful information, the force field is not parameterized to handle the important cation-π interactions between the basic amines and the key tryptophans (Trp 84 and Trp 279). To build off this original effort and reconcile the issue with the cation-π interactions we further performed automated docking with Glide[31] followed by QM/MM[32–34] calculations using QSite.[31] All Glide dockings were done in extra precision mode. The docking grid was generated with the default size using the aforementioned structure. All QM/MM were performed with the protein fixed using B3LYP/6-31G**. The QM region contained the following residues: Ser 200, Glu 327, His 440 (the catalytic triad); Trp 84 (the AS); Trp 279 (the PAS); Tyr 121, Phe 290, Phe

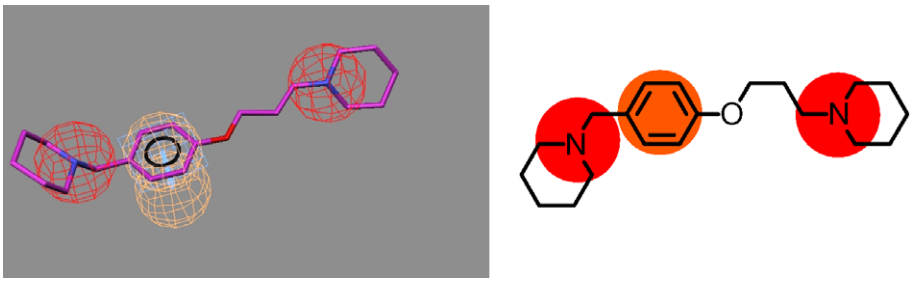
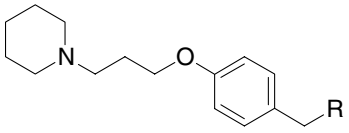
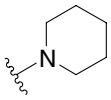
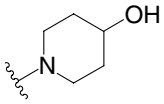
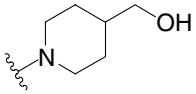
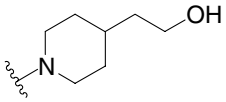


Figure 2. The proposed three-dimensional pharmacophore for histamine H_3 with **1**. The main features are two positive ionic centers and an aromatic ring forming the center of the molecule.

Table 1. Data for hydroxyl linkers of various lengths in the four position of the piperidine ring



Compound	R	AChE IC_{50} (nM)	H_3 (nM)
1		3200 ± 400	0.53 ± 0.49
7		2300 ± 100	0.72 ± 0.19
2		1100	1.30 ± 0.60
6		350 ± 10	0.98 ± 0.21
	Decamethonium	5900 ± 1400	820 ± 170
	Donepezil	160 ± 30	350 ± 70

330, Phe 331, Tyr 334 (the gating residues) and Gly 117, Glu 199, Tyr 130 (other residues showing the potential for interaction). For these residues only the side chains were chosen except for Gly 117 where we chose the whole residue since the important interaction was with the carbonyl backbone. All other settings for the QM/MM were the defaults chosen by QSite. The automated docking with Glide allowed us to get a more robust sampling of the possible binding modes for **1** in AChE. Clearly, it is not feasible to perform QM/MM on all the binding modes returned by Glide. However, one mode stood out among the rest and was followed up with QM/MM. This mode scored high in Glide, was similar to the initial binding mode from the manual docking with Insight II and, upon overlaying the Glide binding mode and the binding mode of decamethonium from the crystal structure, showed excellent overlap of their pharmacophore features. This was especially true for the positively charged nitrogens.

4. Results and discussion

Figure 3 shows the QM/MM binding mode for **1** overlaid with the crystal structure binding mode of decamethonium (the residues shown are of the crystal structure of decamethonium[22]). There is excellent overlap of the pharmacophore features with the most notable being between the basic amines of **1** and the quaternary nitrogens of decamethonium. The central aryl ring of **1** is also coincident with the alkyl chain of decamethonium. From the results of the QM/MM binding mode and from the shown overlay we suggest **1** is making cation-π interactions between the basic amines and the key tryptophans (Trp 84 and Trp 279). Also notable in Figure 3 are the residues Tyr 130 (green), Glu 199 (green), and the catalytic serine Ser 200 (gray). It was speculated these nearby residues could interact favorably with pendant substituents on the proximal piperidine ring of **1** and thus improve the affinity of **1** for AChE. The QM/MM binding mode for **6** is shown in Figure 4 (the residues shown are from the crystal structure of *T. californica* (PDB ID: 1EVE) complexed with donepezil).[14] As with **1**, **6** also makes cation-π interactions with the key tryptophans. Additionally the hydroxyl group is well aligned to make hydrogen bond interactions with Glu 199 and Ser 200 and possibly Tyr 130. Table 1 shows the data for hydroxyl linkers of various lengths in the

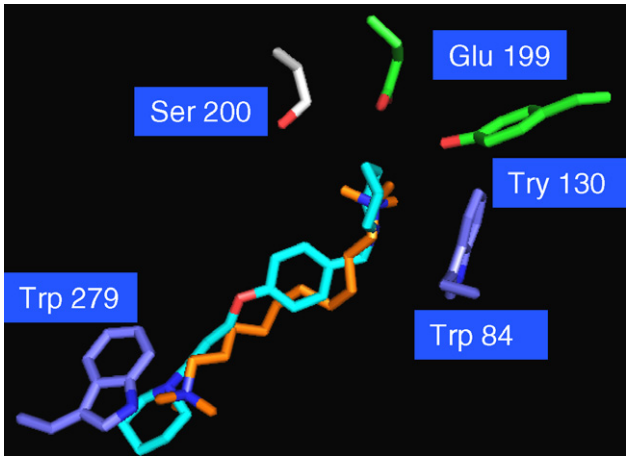


Figure 3. The QM/MM binding mode for **1** overlaid with the crystal structure binding mode of decamethonium in the active site of AChE. The residues shown are from the crystal structure of decamethonium. Note the near perfect overlay of the basic amines with the quaternary nitrogens of decamethonium. Further, the central aryl ring of **1** is coincident with the alkyl chain of decamethonium.

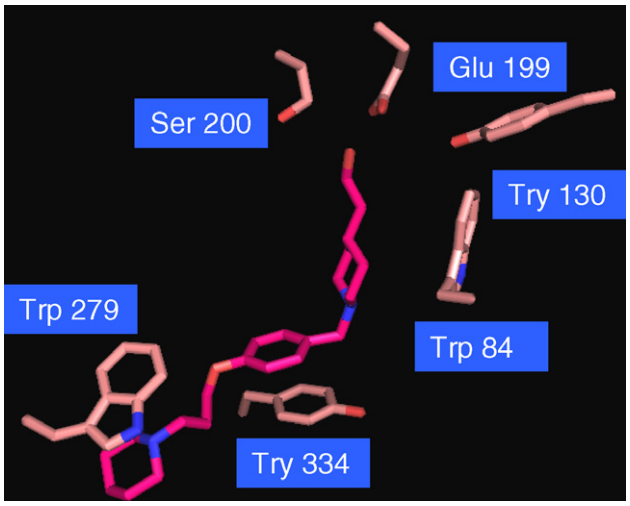


Figure 4. The QM/MM binding mode of compound **6**. The hydroxyl group is positioned to make hydrogen bond interactions with Ser 200 and Glu 199 and possibly Tyr 130. Further, the central aromatic ring appears well situated to make a π-stacking interaction with Tyr 334. The residues shown are from the protein of *Torpedo californica* (available from the PDB) complexed with donepezil.

four position of the piperidine ring. Docking calculations suggest the hydroxyethyl moiety provides the minimum linker length needed to have the opportunity to make interactions with Glu 199, Ser 200, and Tyr 130 and indeed, **6** is the most potent compound of the three analogs shown in Table 1. Finally, the central aromatic ring of **6** appears to be well positioned for π-stacking interactions with Tyr 334. We believe the central aromatic ring of **6** makes π-stacking interactions in the active sites of both AChE and histamine H_3 receptor (in histamine H_3 receptor the residues Tyr 115 and Trp 371 are most likely providing this interaction).

5. Conclusions

Currently, AChE inhibitors represent the most common therapeutic option for AD. Their procognitive and memory-enhancing effects are well known in the literature.[35] In addition, there is a considerable body of experimental work suggesting antagonism of the histamine H_3 receptor translates into increased cognitive performance.[36–38]

Herein we demonstrated how the use of available crystal structure information, pharmacophore modeling, docking (automated, manual, classical, and QM/MM) lead us to a compound (**6**) (and potential series) with excellent activity at both AChE and the histamine H_3 receptor. In addition, our models suggest such a dual-acting compound may interact with the PAS and, based on current thinking, could potentially slow the progression of AD.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.12.048.

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