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***J. Comput. Method. Mol. Design*, 2011, 1 (1): 44-51**
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Identification of Potential Inhibitors against Acetylcholinesterase Associated With Alzheimer's Diseases: A Molecular Docking Approach

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ABSTRACT

Cholinesterase inhibitors (ChE-Is) are the standard of therapy for treatment of patients with Alzheimer's disease (AD) and are the only class of drugs approved by the Food and Drug Administration (FDA) for treatment of this condition. In this paper we used the new approach utilizing cheminformatics tools such as CORINA, Yet Another Scientific Artificial Reality Application (YASARA), and molecular docking program to identify binding affinity and mechanism of interaction between the ChE-Is with the target proteins. This approach should be helpful to understand the selectivity of the given drug molecule in the treatment of Alzheimer's disease.

Keywords*:* Alzheimer's disease; Acetylcholinesterase; Cholinesterase inhibitors; Molecular Docking.

INTRODUCTION

Alzheimer's disease (AD) is the most common single cause of dementia in our ageing society. AD is estimated to account for between 50 and 60% of dementia cases in persons over 65 years of age [1-3] and is progressive, neurodegenerative disease that primarily affects the elderly population. The symptoms associated with AD involve decline in cognitive dysfunction, primarily memory loss [4, 5] and in the later stages of the disease language deficits, depression, agitation, mood disturbances and psychosis are often seen [6]. AD is associated with substantial reductions in the activity of the enzyme responsible for acetylcholine (ACh) synthesis, choline acetyltransferase (ChAT) and a subsequent decline in levels of ACh in the brain [7].

Early attempts to treat AD using precursors of ACh met with little success [8]. More recent efforts have focused on augmenting cholinergic transmission by blocking the activity of cholinesterases that degrade ACh at the synaptic junction [9-11]. Several cholinesterase inhibitors (ChEIs) are available and have been shown, with varying degrees of efficacy, to slow the AD-associated decline in behavior, cognition, and the ability to perform activities of daily living (ADL). Four ChE-Is have been approved by the United States Food and Drug Administration (U.S. FDA) are marketed for the treatment of Alzheimer's disease are donepezil (Aricept), rivastigmine (Exelon), galantamine (Reminyl) and tacrine (Cognex). These four agents represent different classes of ChE-Is and have different pharmacologic properties beyond inhibition of Acetylcholinestrase (AChE) [12].

Previous clinical experience of the drug indicated only mild cholinergic side effects with high levels of AChE inhibition (>80%) and short treatment periods [13]. The safety of the ChE-Is in long-term treatment is currently under review, with recent trials highlighting a possible link with muscle weakness. The side effects of the AChE inhibitors generally attributable to peripheral cholinergic effects. Nausea, vomiting and diarrhoea were the most frequently reported [14].

Despite recommendations for their use, ChE-Is remains a relatively unfamiliar class of agents for many practitioners. The means of initiating therapy, assessing benefit, surveying side effects, and determining the appropriate length of therapy are critical to their successful implementation but have received limited discussion. The present paper describes the work undertaken to study the effectiveness of ChE-Is and the mechanism of interactions by computational analysis. The results should be highly useful and may provide a convenient platform for the development of a more analog of ChE-Is towards the treatment of Alzheimer's disease.

MATERIALS AND METHODS

Data set

The three dimensional structure of AChE was obtained from the Protein Data Bank (PDB ID-1B41) [15]. We selected 4 small molecule/inhibitor, donepezil (Aricept), rivastigmine (Exelon), galantamine (Reminyl) and tacrine (Cognex) for our investigation. Structural formulas for all the selected drug molecules were given in Fig. 1. The SMILES strings were collected from PubChem, a database maintained National Center for Biotechnology Information (NCBI) [16] and submitted to CORINA (www.molecularnetworks.com/online_demos/corina_demo.html) for constructing the 3D structure of small molecule.

Determination of binding site

Binding and active sites of proteins are often associated with structural pockets and cavities. The catalytic site of AChE obtained from the information available from the literature. The catalytic residue further examined with the help of Q-SiteFinder [17] and Computed Atlas of Surface Topography of proteins (CASTp) server [18]. Q-SiteFinder uses the interaction energy between the protein and a simple van der Waals probe to locate energetically favorable binding sites.

CASTp server uses the weighted Delaunay triangulation and the alpha complex for shape measurements. It provides identification and measurements of surface accessible pockets as well

as interior inaccessible cavities, for proteins and other molecules. It measures analytically the area and volume of each pocket and cavity, both in solvent accessible surface (SA, Richards' surface) and molecular surface (MS, Connolly's surface).

Target Structure Minimization

Energy minimization for 3D structures was performed by using YASARA [19]. YASARA, which runs molecular dynamics simulations of models in explicit solvent, using a new partly knowledge-based all atom force field derived from Amber, whose parameters have been optimized to minimize the damage done to protein crystal structures. The LEE-SERVER, which makes extensive use of conformational space annealing to create alignments, to help Modeller build physically realistic models while satisfying input restraints from templates and Chemistry at HARvard Molecular Mechanics (CHARMM) stereochemistry, and to remodel the side-chains. ROSETTA, whose high resolution refinement protocol combines a physically realistic all atom force field with Monte Carlo minimization to allow the large conformational space to be sampled quickly. Finally UNDERTAKER, which creates a pool of candidate models from various templates and then optimizes them with an adaptive genetic algorithm, using a primarily empirical cost function that does not include bond angle, bond length, or other physics-like terms.

Computation of docking score between the inhibitor and acetylcholineesterase

We used the program molecular docking server [20] to compute the free energy of binding (ΔG) of docked complexes. 3D coordinates of the AChE and the inhibitor was submitted in PDB format with default parameters. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged and rotatable bonds were defined. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [21]. The grid points and spacing were generated using the Autogrid program [21]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [22]. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

RESULTS AND DISCUSSION

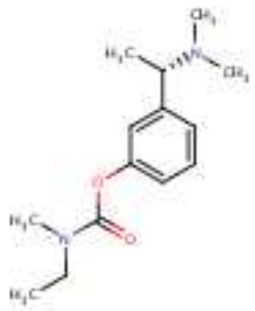
Binding site analysis

The experimental analysis shows that Ser-203, His-447, and Glu-334 could be the catalytic site residues present in the structure of AChE [23]. We have evaluated the catalytic residues by means of various computational tools such as Q-SiteFinder and CASTp. From the view of Q-SiteFinder, we observed that catalytic site residues such as Ser-203, His-447, and Glu-334 were present in the first predicted site of volume 635.1 $Å^3$. The evidences available suggest that catalytic residues of more than 90% of the protein were present at least in one of the top three predicted sites when tested using Q-SiteFinder [17]. We have also observed the same kind of results in our analysis. The program CASTp also supports the results of Q-SiteFinder. The

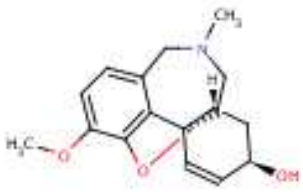
catalytic site residues in the structure of AChE were shown in Fig. 2. These computational analysis along with experimental fact support that Ser-203, His-447 and Glu-334 act as catalytic residues in the three dimensional structure of AChE [23].

Energy minimization

The energy minimization was performed by YASARA program. Higher the total energy, less stable the protein structure will be. The total energy of the structure showed that original structure is little higher in energy there by means that structure is unstable. To depict the *in vivo* interaction, we have minimized the energy of the target protein before performed the docking operations. The total energy for the given structure before and after minimization was found to be 165391.1kJ/mol and −32, 5320.5 kJ/mol, respectively (Fig. 3.). It shows that the minimized structure is more stable than the original one. Thus we hope that our results may exactly correlate with *in vivo* situations.

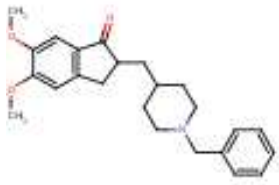


Rivastigmine



Galantamine

Tacrine



Donepezil

Fig 1. Two dimensional structures of selected drug molecule

Docking studies of AChE with inhibitor

Our investigation showed the behavior of protein–ligand complex of AChE with ChE-Is. The PyMOL view of docked complexes shown in Fig. 4. The estimated free energy of binding (ΔG) for the target molecule, AChE with donepezil (Aricept), rivastigmine (Exelon), galantamine (Reminyl) and tacrine (Cognex) were found to be 3.58, -5.61, -7.86 and -6.95 kcal/mol respectively (Table 1). The donepezil shows positive ΔG value there by means that binding was not appropriate. It is also observed that galantamine have the better binding affinity with AChE than the other drug molecules. The gradual decrease in ΔG from galantamine to donepezil may be attributed to the intermolecular interaction energy between the AChE and drug molecule. The number of intermolecular interactions in the docked complexes shown in Table 2. It shows that

number of intermolecular interaction is higher in the case of galantamine compared with other drug molecule (Table 2). This may leads to the efficient binding of galantamine with AChE. Since the binding affinity is higher, the value of inhibition constant was very less for galantamine than the other drug molecule. From this observation, we understand that galantamine have better binding affinity with the target molecule, AChE, leads to the lesser requirement for the inhibition. The result reported by our work in this study is well supported by an experimental study carried out earlier [24].

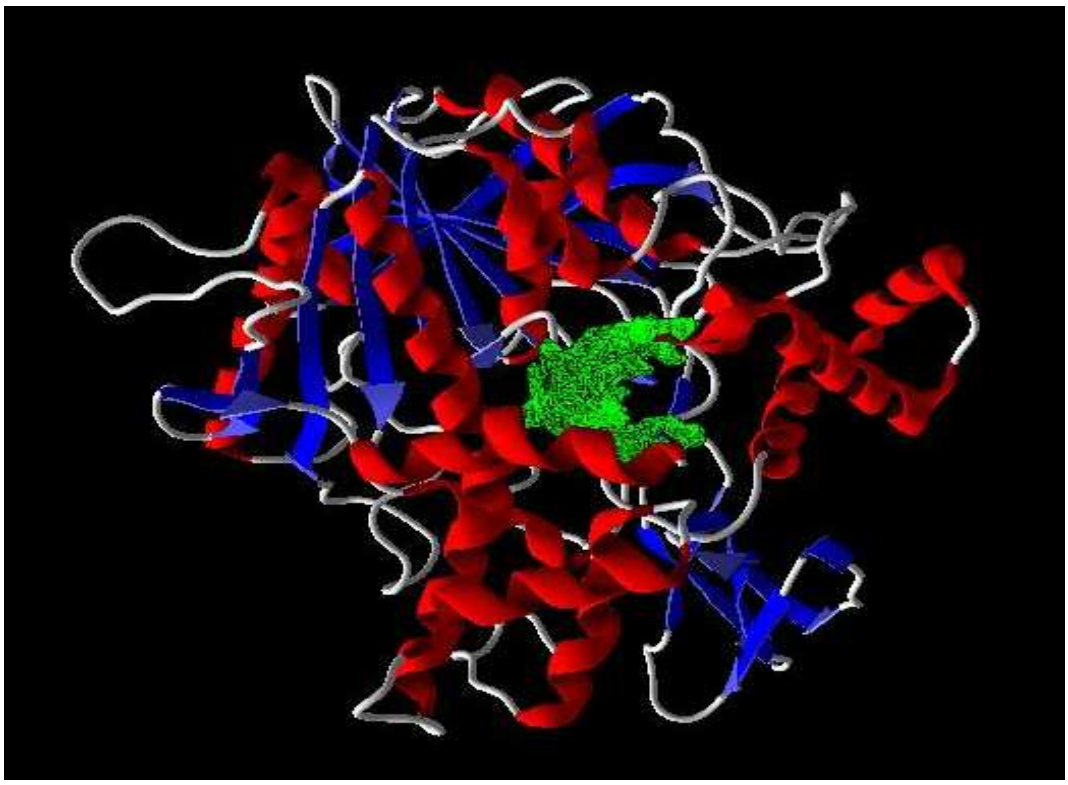


Fig. 2. Schematic view of Binding site in the structure of AChE

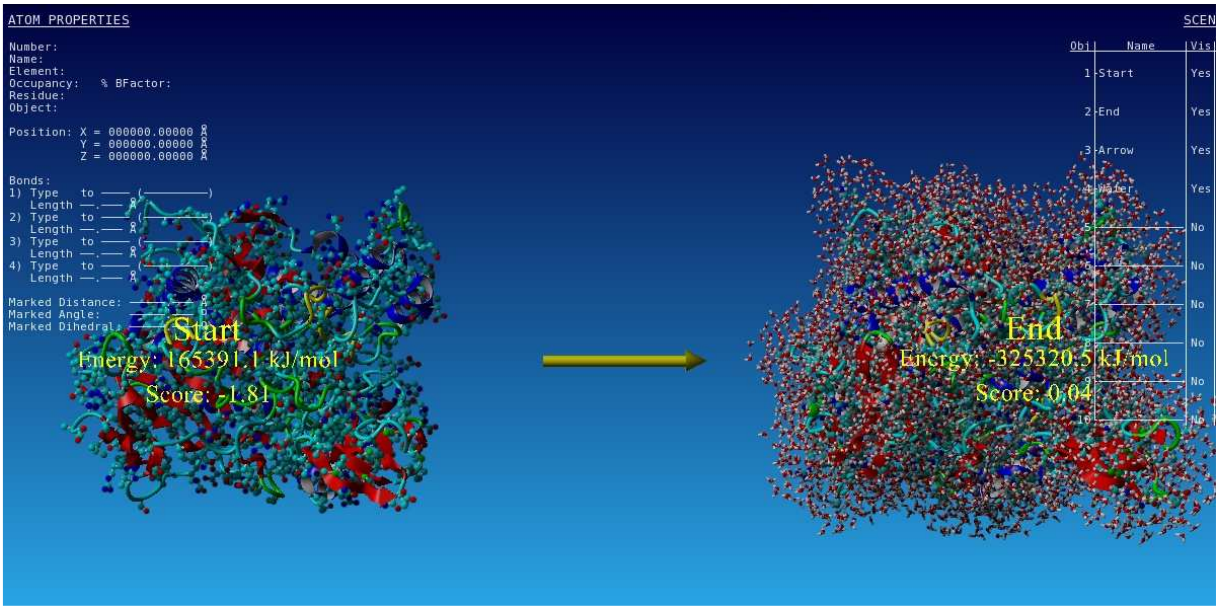
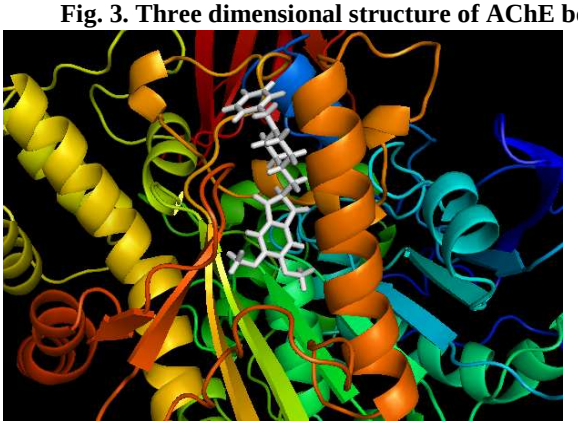
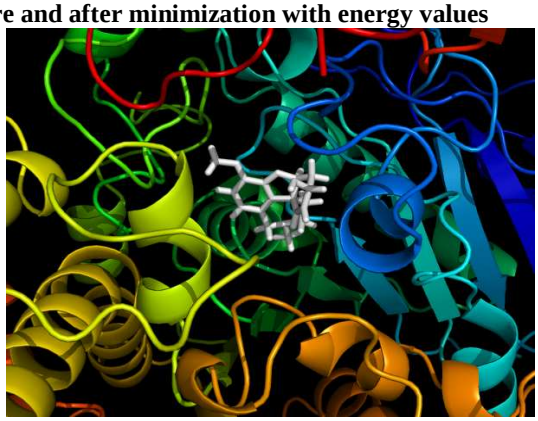


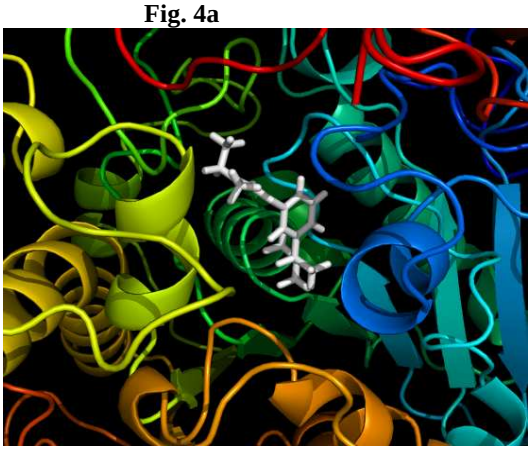
Fig. 3. Three dimensional structure of AChE before and after minimization with energy values



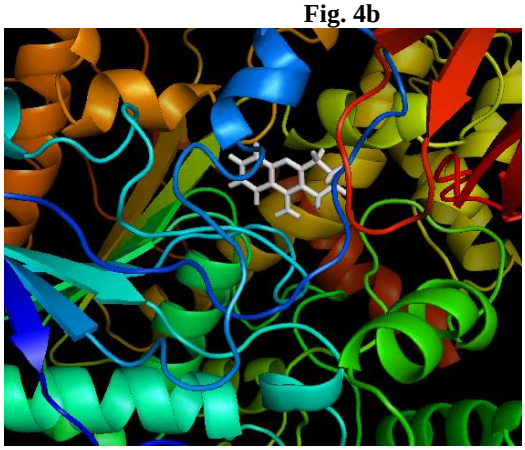
AChE - Donepzil complex
Fig. 4a



AChE - Galantamine complex
Fig. 4b



AChE - Rivastigmine complex
Fig. 4c



AChE - Tacrin complex
Fig. 4d

Fig. 4. PyMOL view of docked complexes

Table 1 Docking analysis of AChE with selected drug molecules

Drug (Ligand) molecule	Receptor molecule	Estimated free energy of binding (ΔG), kcal/mol	Estimated inhibition constant, μM	Total intermolecular interaction energy, kcal/mol
Tacrine	AChE	-6.95	8.03	-7.25
Rivastigmine	AChE	-5.61	77.72	-6.53
Galantamine	AChE	-7.86	1.73	-7.91
Donepezil	AChE	3.58	-	-1.82

Table 2 Details of intermolecular interactions in the binding site of docked complexes

Complex name	Number of H-bond	Number of polar interactions	Number of hydrophobic interactions	Number of π-π interactions	Number of Cation-π interactions	Other weak forces	Total number of interactions
AChE-Tacrin	2	2	1	8	2	16	31
AChE-Rivastigmine	1	2	6	6	1	13	29
AChE-Galantamine	3	7	11	3	2	12	36
AChE-Donepezil	1	4	7	5	1	10	28

CONCLUSION

With the current deluge of data, computational methods have become indispensable to biological investigations. Here we have used computational approach to understand the mechanism of interactions and binding affinity between AChE with drug molecules. The present analysis allows us to draw the number of conclusions. The computational methods such as Q-SiteFinder and CASTp are the potential tool for the analysis of catalytic site of the given AChE. The molecular docking programs helpful in understanding the interaction between the AChE with various drug/lead molecules. Our analysis also shows that galantamine could be the potential lead molecule for the inhibition of AChE. Hence galantamine could be used as the template for designing therapeutic lead molecule. We strongly hope that the ingenuity and success of the computational efforts discussed above bode well for the future prospects of finding new inhibitors which could results into massive reductions in therapeutics development time.

Acknowledgments

The authors thank the management of Vellore Institute of Technology, for providing the facilities to carry out this work.

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