



## Docking Study of Benzothiazole–Piperazines: AChE Inhibitors for Alzheimer's Disease

Kaliappan Ganesan \*

Department of Biotechnology, School of Bioengineering, SRM University, SRM Nagar, Kattankulathur, Chennai, India.

\*Corresponding author's E-mail: [kganeshnew@gmail.com](mailto:kganeshnew@gmail.com)

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### ABSTRACT

Acetylcholinesterase (AChE) is an essential enzyme that terminates neurotransmission by hydrolyzing the neurotransmitter acetylcholine in the synapse. Defects in cholinergic neurotransmission are significant for the progression of Alzheimer's disease. Hence, the most efficacious treatment approach for Alzheimer's disease is considered to increase cholinergic neurotransmission in the brain. Currently four AChE inhibitors including tacrine, donepezil, rivastigmine and galantamine had been clinically approved by the FDA for the treatment of Alzheimer's disease. In our study, we have performed molecular docking using Surflex dock module on the series of newly identified benzothiazole–piperazines which is a potent AChE inhibitors. Here we have used two known drugs (donepezil and galantamine) and two protein crystal structures (4EY6 and 4EY7) during docking. To verify whether the benzothiazole–piperazines could act similarly as known drugs the active site of known drugs were employed during protomol generation. The docking scores and interaction of the few inhibitors were comparable with the known drug donepezil.

**Keywords:** AChE; Alzheimer's disease, Benzothiazole–piperazines; Docking.

### INTRODUCTION

Acetyl cholinesterase (AChE) is one of the most efficient enzymes of nervous system and plays an essential role in acetylcholine-mediated neurotransmission. AChE is concentrated at the cholinergic synapses and at neuromuscular synapses where it rapidly hydrolyses the neurotransmitter acetylcholine (ACh) in to choline and acetate thus playing an essential role in cholinergic neurotransmission. Acetyl cholinesterase is a very fast enzyme, functioning at a rate approaching that of a diffusion-controlled reaction. The enzyme inactivation, induced by various inhibitors, leads to acetylcholine accumulation, hyper stimulation of nicotinic and muscarinic receptors, and disrupted neurotransmission. Hence, acetyl cholinesterase inhibitors, interacting with the enzyme as their primary target, are applied as relevant drugs and toxins. Acetyl cholinesterase (acetylcholine acetylhydrolase, E.C. 3.1.1.7) is found in many types of conducting tissue: nerve and muscle, central and peripheral tissues, motor and sensory fibers, and cholinergic and noncholinergic fibers. The activity of AChE is higher in motor neurons than in sensory neurons. AChE is also found in the red blood cell membranes, where it constitutes the Yt blood group antigen. The enzyme exists in multiple molecular forms, which possess similar catalytic properties, but differ in their oligomeric assembly and mode of attachment to the cell surface. AChE is a serine hydrolase mainly found at neuromuscular junctions and cholinergic brain synapses. Its principal biological role is termination of impulse transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter ACh to acetate and choline. AChE has a remarkably high specific catalytic activity, especially for a serine hydrolase.<sup>1-10</sup>

ACh can be degraded by acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Compared with BuChE, AChE draws more attention from pharmaceutical chemists since it accounts for nearly 80% ACh hydrolysis in the brain. Defects in cholinergic neurotransmission are significant for the progression of Alzheimer's disease (AD). Alzheimer's disease (AD), the most common form of dementia, is a chronic neurodegenerative disorder, which is clinically characterized by impairment in memory, complex cognition, language, emotion, and behavioural disturbances. Although the pathogenesis of AD is not fully understood, currently the most efficacious treatment approach for AD is considered to increase cholinergic neurotransmission in the brain by lowering ACh hydrolysis. Therapeutically, controlled application of AChE inhibitors is used to increase synaptic levels of acetylcholine in diseases that impair acetylcholine neurotransmission, such as Alzheimer's disease and myasthenia gravis. AChE inhibitors are used in treatment of various neuromuscular disorders, and have provided the first generation of drugs for the treatment of Alzheimer's disease. Cholinesterase inhibitors such as donepezil, galantamine, rivastigmine, and huperzine A, have been extensively studied as symptomatic treatments for AD. AChE inhibitors are preferred in the treatment of AD to keep ACh levels normal. Donepezil is the most favourable AChE inhibitor since it gives a relatively positive response in AD treatment. Furthermore, compared to other AChE inhibitors, donepezil has some advantages, such as blood–brain barrier permeability, non-hepatotoxicity, the least side efficacy, and consumption once daily.<sup>11-22</sup>

In this study, the series of benzothiazole–piperazines which is a potent AChE inhibitors were used for molecular



docking. The crystal structure of Recombinant Human Acetylcholinesterase in Complex with Donepezil (4EY7) and Galantamine (4EY6) were used to perform docking. The molecular docking was performed using the Surflex dock module of SYBYL software. The protocol for docking was generated based on the co-crystal ligand molecules. This was done to analyze whether the inhibitors binds and interacts in the active site of the protein similar to the known drugs donepezil and galantamine. The docking scores and interaction of the few inhibitors were comparable with the known drugs in 4EY7.

## MATERIALS AND METHODS

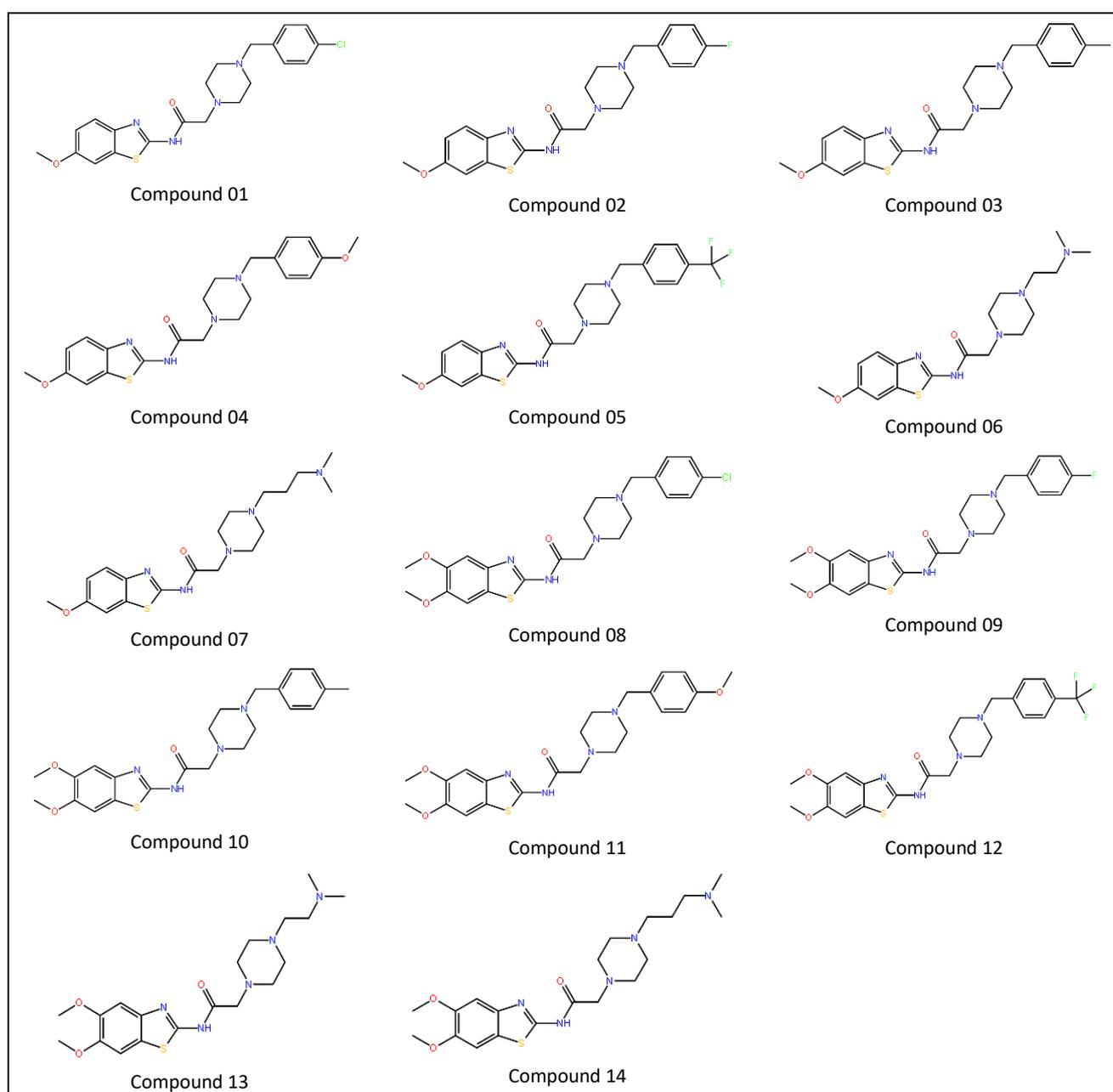
### Preparation of Protein structure

The three dimensional structures of Acetylcholinesterase (4EY6 and 4EY7) used for docking were prepared using

protein preparation tool in biopolymer module of SYBYL. During preparation, hydrogen molecules and Gasteiger Huckel charge were added and water molecules were removed from the crystal structures. It was followed by energy minimization utilizing Tripos force field, Gasteiger Huckel charge and Powell method for 100 steps.<sup>23</sup>

### Preparation of ligand molecules

The chemical structure of fourteen benzothiazole–piperazines as AChE inhibitors were taken from the literature. The antagonists were sketched using sketch molecule function in SYBYL software. The energy minimization of all the molecules was performed using Tripos force field and atomic charges were assigned using Gasteiger Huckel method. The structures of all molecules are shown in Figure 1.<sup>24</sup>



**Figure 1:** The structure of benzothiazole–piperazines derivatives.

## Molecular Docking

Molecular docking was performed utilizing Surflex dock module of SYBYL. Fourteen inhibitors of AChE and two known drugs were docked into AChE receptor. The docking algorithm in surflex dock uses an idealized active site called protomol. The protomol is the representation of intended binding site to which the ligand molecules were docked. Two parameters, such as threshold and bloat, determine the extent of a protomol. The protomol was generated using ligand mode. Surflex dock uses an empirical scoring function to score the docked ligand conformation which takes into account several terms, including hydrophobic, polar, repulsive, entropic and solvation. To evaluate the docking results, the docking scores are expressed in terms of  $-\log_{10}K_d$  units, where  $K_d$  represents a dissociation constant of a ligand.<sup>25-26</sup>

## RESULTS AND DISCUSSION

### Validation of Surflex dock

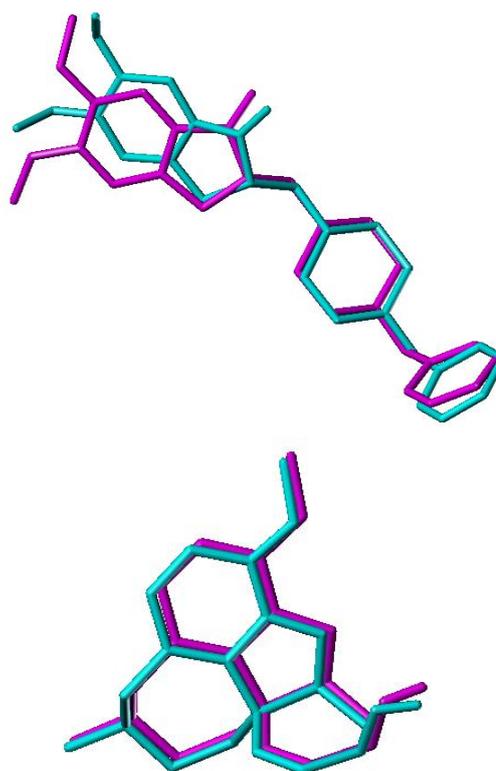
To validate the Surflex dock software, we have performed re-docking on the crystal structure of human Acetyl cholinesterase utilizing the co-crystallized ligand molecule Donepezil (4EY7) and Galantamine (4EY6) into the binding pocket of AChE. The Surflex score of 8.46 was obtained for donepezil molecule and it forms H-bond with the residue PHE295 and 7.89 was obtained for galantamine and it forms H-bond with GLU202, SER203 and GLY122. It was reported that if the RMSD of the best conformation is  $<2.0\text{\AA}$  from the bound ligand in the experimental crystal structure then the used scoring function is successful. Therefore, the docked mode of donepezil and galantamine was compared to the crystal structure of bound ligand-protein complex. The RMSD of the docked pose of donepezil and galantamine with the co-crystal donepezil and galantamine was found to be  $<2.0\text{\AA}$  which authenticates the accuracy of the software. The superimposition of docked pose of donepezil and galantamine with the co-crystal conformation is shown in Figure 2a and 2b.<sup>27</sup>

### Molecular Docking

Molecular docking was performed for recently reported Acetylcholinesterase inhibitors (benzothiazole-piperazines) and the known drugs (donepezil and galantamine).

Before performing docking the protomol was generated in the active sites of the protein utilizing the co-crystal ligands in 4EY6 and 4EY7 to verify whether these molecules binds similar to known drugs. All the ligand molecules were docked into 4EY6 and 4EY7 to compare the interaction of benzothiazole-piperazines with donepezil and galantamine actives sites. When performing docking, 20 different conformations were generated for each molecule and the best conformation

was chosen based on surflex score and interaction with the residues. The docking score and H-bond forming residues for all the molecules are tabulated in Table 1.



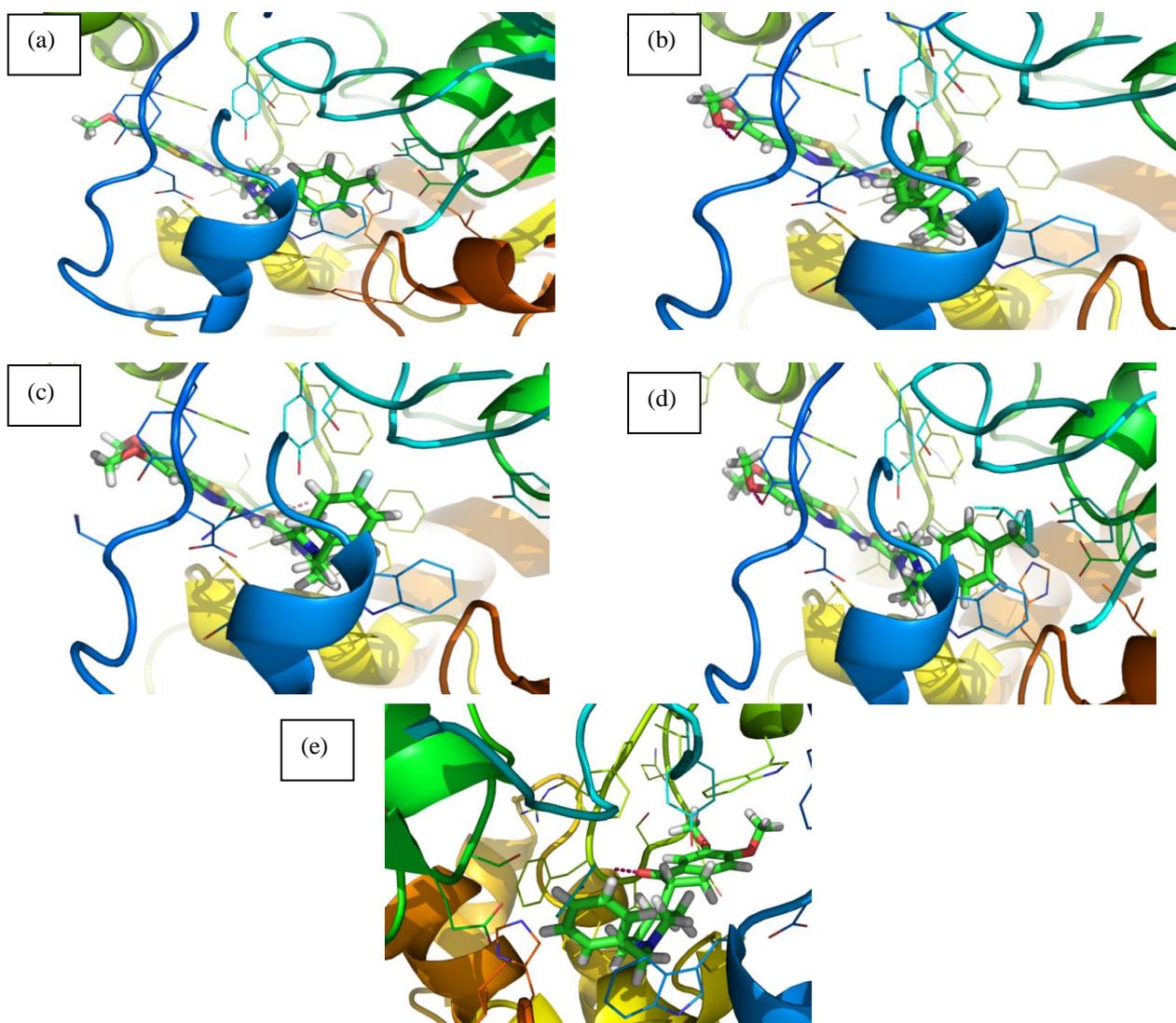
**Figure 2:** Superimposition of the docked poses of co-crystal ligand dopamine (a) and galantamine (b) (shown in cyan) with co-crystal conformation (shown in pink).

We observed that the docking score of benzothiazole-piperazines derivatives were comparable with donepezil whereas galantamine scored more than the new inhibitors in its active site.

The H-bond forming residues of donepezil in 4EY7 were comparable to benzothiazole-piperazines. Also, we noticed, PHE295 play a major role in interaction of many of the benzothiazole-piperazines inhibitors with the AChE protein. The same was with donepezil drug molecules. This validates that these inhibitors could bind efficiently with the protein similar to known drugs. Figure 3 shows the docking interaction of known drug donepezil and benzothiazole-piperazines inhibitors. These results authenticates that benzothiazole-piperazines derivatives have same binding interaction as known drugs donepezil which validates these compounds can be used for Alzheimer disease

**Table 1:** Docking scores and H-bond interactions formed between human AChE and its inhibitors

Compound	4EY7		4EY6	
	Surflex Score	H-bond formation	Surflex Score	H-bond formation
1	7.82	-	4.75	TYR124
2	7.38	-	4.88	GLY120, TYR337
3	8.09	PHE295	4.94	-
4	7.47	SER203, GLY122	5.24	-
5	7.98	TYR133	4.54	PHE295, TYR124
6	8.61	-	5.95	-
7	7.65	-	5.91	-
8	8.35	PHE295	5.10	SER125
9	7.76	PHE295	7.18	-
10	8.51	-	3.37	TYR124
11	8.15	-	5.04	TYR124
12	7.75	TYR133, PHE295	4.33	SER125, TYR124
13	8.13	-	6.12	TYR124
14	8.97	-	6.88	-
Donepezil/ Galantamine	8.46	PHE295	7.89	GLU202, SER203, GLY122

**Figure 3:** Interaction between the AChE and the benzothiazole–piperazines derivatives (a-d) and donepezil (e).

## CONCLUSION

In the present study, molecular docking has been performed on newly synthesized 14 benzothiazole piperazines derivatives and two known drugs donepezil and galantamine. The docking results demonstrate that these inhibitors docked well within the active site of AChE protein. The interactions of benzothiazole–piperazines were comparable with known drug donepezil. H-bond formation of piperazine with AChE occurred mostly at PHE295 residue. Thus we validated that these molecules were capable of binding inside the AChE active site. Hence, benzothiazole–piperazines could be used for the treatment of Alzheimer's disease.

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