








Computational Investigation of the Interaction Mechanism of Some anti-Alzheimer Drugs with the Acetylcholinesterase Enzyme

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Abstract - The molecular structures of the lowest-energy conformers of donepezil (C₂₄H₂₉NO₃), rivastigmine (C₁₄H₂₂N₂O₂), and galantamine (C₁₇H₂₁NO₃), which are extensively used in Alzheimer's disease and other memory disorders, were identified using the Spartan06 program and the MMFF method. The optimized geometries, obtained with the same method, were used as initial data in molecular docking investigations with the Acetylcholinesterase enzyme. The binding modes, binding affinities, and interactions were comparatively determined as consequence of the calculations.

Keywords: Acetylcholinesterase Enzyme, Alzheimer, Donepezil, Rivastigmine, Galantamine, Molecular docking.

1. Introduction

Alzheimer's disease is a common cause of cognitive impairment acquired in middle age and old age. However, age alone is not a major risk factor for this disease. Presence of one or more apolipoprotein gene E4 alleles (APOE4), severe traumatic brain injuries and cardiovascular factors are also important risk factors for Alzheimer's disease [1]. Apathy, anxiety and irritability are the most prominent neuropsychiatric symptoms in this disease. Symptoms that occur after dementia are appetite and sleep disturbances, disinhibition, hallucinations, or thought changes [2]. Brain tomography allows the detection of moderate and severe amyloid deposition in the brain [3].

Alzheimer's disease is also biologically characterized by the presence of plaques containing beta-amyloid and neurofibrillary tangles containing the tau gene or tau protein. Neurodegenerative diseases are characterized by the degeneration of some nerve cells by developing filamentous inclusions [4, 5]. The filamentous inclusions are found in diseases such as Alzheimer's disease, corticobasal degeneration, types of dementia head syndrome, Down syndrome, Parkinsonism, and Pick's disease. Normal aging contains hyperphosphorylated microtubule-associated tau protein, these diseases are known as taupathies [6, 7].

The name of a neurodegenerative disease, that occurs with abnormal tau protein accumulation in the brain, is taupathies [8,9]. The pathophysiologically important proteins in Alzheimer's disease are tau proteins [10]. Pick's disease, advanced paralysis, and corticobasal degeneration are tau pathologies [11]. Amyloid deposition in the brain is thought to begin 10-20 years before clinical symptoms appear [12]. In the dementia disease practice guide of the American Academy of Neurology, it is stated that it is possible to treat mild and moderate Alzheimer's patients with acetylcholinesterase inhibitors [13]. The entorhinal cortex is the primary source of information flow to the hippocampus. Granule cells are involved in this cortex [14]. There is an exchange of signals between the entorhinal cortex and the hippocampus [15]. The decrease in entorhinal cortex volume is

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associated with Alzheimer's disease. The entorhinal cortex is associated with the cognitive disorder schizophrenia [16]. Memory loss occurs due to memory and spatial learning function. The hippocampus is a region in the medial temporal lobe of the brain that plays an important role in memory and navigation and influences particularly short-term memory [17-18].

The most used cholinergic neuronal markers are choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) and their loss is one of the most consistent neurotransmitter changes found in the brain of Alzheimer's patients [19-23]. Alzheimer's disease is expressed as the loss of learning and memory abilities in the elderly population [24]. The enzyme acetylcholinesterase (AChE) plays an important role in the hydrolysis of the neurotransmitter acetylcholine, and because of this feature, it is the therapeutic target of most drugs used in the treatment of Alzheimer's disease [25]. Since the exact mechanism of this disease is unknown, its treatment is still not possible. AChE is an enzyme that catalyzes the hydrolysis of a chemical bond. This enzyme enables the development of mechanism-based inhibitors [26, 27]. It inhibits β -amyloid-induced damage and cytokine release from cell free radical toxicity [28].

Increasing the level of acetylcholine in the brain using acetylcholinesterase inhibitors is an important approach to treat this disease [29]. Various AChE inhibitors are under investigation for the treatment of Alzheimer's disease. AChE inhibitors such as tacrine, donepezil, rivastigmine, and galantamine have been approved by the Food and Drug Administration in the United States [30]. Among other strategies not explored, monoamine oxidase inhibitors have also been proposed for the treatment of AD [29].

Acetylcholinesterase (AChE) is a carboxylesterase enzyme secreted from muscle and primarily found at postsynaptic neuromuscular junctions, especially in muscles and nerves. Its altered activity is caused to some congenital and acquired diseases [31]. Sudden blockade of AChE is lethal, and its gradual loss is associated with progressive deterioration of cognitive, autonomic, and neuromuscular functions, as in Alzheimer's disease, multiple system atrophy, and other conditions [32]. It immediately hydrolyzes a neurotransmitter acetylcholine (ACh) and breaks it down into acetic acid and choline [32]. The primary role of AChE is to prevent ACh dispersal and activation of nearby receptors, in order to terminate neuronal transmission and signaling between synapses. It also plays a role in neural development [32]. Organophosphorus compounds such as pesticides and nerve agents are AChE inhibitors. Since the 1990s, AChE inhibitors have been known to provide some benefits for Alzheimer's disease [33]. Inhibition of AChE reduces the breakdown of ACh and as a result ACh accumulates. This accumulated ACh leads to increased stimulation of muscarinic and nicotinic receptors, resulting in some therapeutic relief for memory deficits in Alzheimer's disease [34]. Thus Acetylcholinesterase (AChE) is an important target for symptomatic improvement in Alzheimer's disease (AD) [35].

The microtubule-associated protein tau is one of the major pathophysiologically relevant proteins in Alzheimer's disease [36]. Tau pathology is the underlying cause of a variety of sporadic and genetic illnesses. These are referred to as tauopathies. The spectrum of tau pathologies includes Pick's disease, progressive supranuclear palsy, and corticobasal degeneration [37, 38]. Emerging pathologies include primary age-related tauopathies, including globular glial tauopathies, chronic traumatic encephalopathy, and aging-related tau astroglial pathology [39]. Clinical symptoms include frontotemporal dementia, corticobasal syndrome, Richardson syndrome, parkinsonism, and rarely motor neuron symptoms [40, 41].

Today, some special molecules are used in treatment of this disease. These molecules are donepezil ($C_{24}H_{29}NO_3$), rivastigmine ($C_{14}H_{22}N_2O_2$) and galantamine ($C_{17}H_{21}NO_3$). Since Alzheimer's disease is not a reversible disease, treatment is provided only by using acetylcholinesterase inhibitor drugs such as donepezil, rivastigmine and galantamine to stop or reduce the progression of the disease [42, 43].

The development of donepezil began in 1983 at the Japanese pharmaceutical company Eisai [44]. The active ingredient was first approved in the USA in 1996. Generics with the active ingredient donepezil have been on the market since 2010 [45, 46]. In the treatment of Alzheimer's disease in which cholinergic neurons are deficient, it works with the principle of operating the decreased acetylcholine amount more effectively by inhibiting the acetylcholinesterase enzyme, which is the enzyme that breaks down acetylcholine [47]. At the same time Donepezil is a medicine that most commonly prescribed for the treatment of Alzheimer's disease. Functional studies have shown that donepezil conditions slow the progression of mild-to-moderate Alzheimer's disease [48, 49].

Rivastigmine is used to treat dementia in persons with Alzheimer's disease (a brain ailment that affects the ability to remember, think effectively, communicate, and perform daily activities, as well as mood and personality changes) [50]. Rivastigmine belongs to the cholinesterase inhibitors group of drugs and it improves mental function (such as memory and reasoning) in the brain by increasing the amount of a natural substance [51]. Furthermore, rivastigmine is utilized to treat Lewy body dementia on occasion [52].

Galantamine is a reversible and selective competitive AchE inhibitor obtained from several botanical sources. AchE inhibitors have historically been the first line of treatment for Alzheimer's Disease. Galantamine also has a lower rate of adverse drug responses than other AchE inhibitors [53,54]. In addition Galantamine is it is also used in the treatment of Alzheimer's, polio, narrow-angle glaucoma and as an antidote after poisoning [55]. It is currently under investigation for use in the treatment of alcohol and nicotine addiction, schizophrenia, and cognitive memory disorders [56].

In this study, due to close relation between structure and function of the bioactive molecules, firstly the conformation analyses were performed on donepezil, rivastigmine, and galantamine molecules, and their lowest energy conformations were determined. Afterwards, the interaction of the donepezil, rivastigmine, and galantamine with Acetylcholinesterase enzyme were investigated by in silico docking simulations, and binding affinities and binding modes were determined.

2. Methods and Calculations

The most stable molecular structures (Figure 1) of donepezil, rivastigmine, and galantamine molecules were determined using the MMFF method [57] and the Spartan06 [58] program, by conformational analysis, following by geometric optimization. The optimized molecular structures were then used as the starting data for docking with the acetylcholinesterase enzyme. The docking studies were performed by using AutoDock-Vina program [59].

3. Results and Discussions

3.1. Structure

The most stable conformers of donepezil ($C_{24}H_{29}NO_3$), rivastigmine ($C_{14}H_{22}N_2O_2$) and galantamine ($C_{17}H_{21}NO_3$) molecules are shown in **Figure 1**.

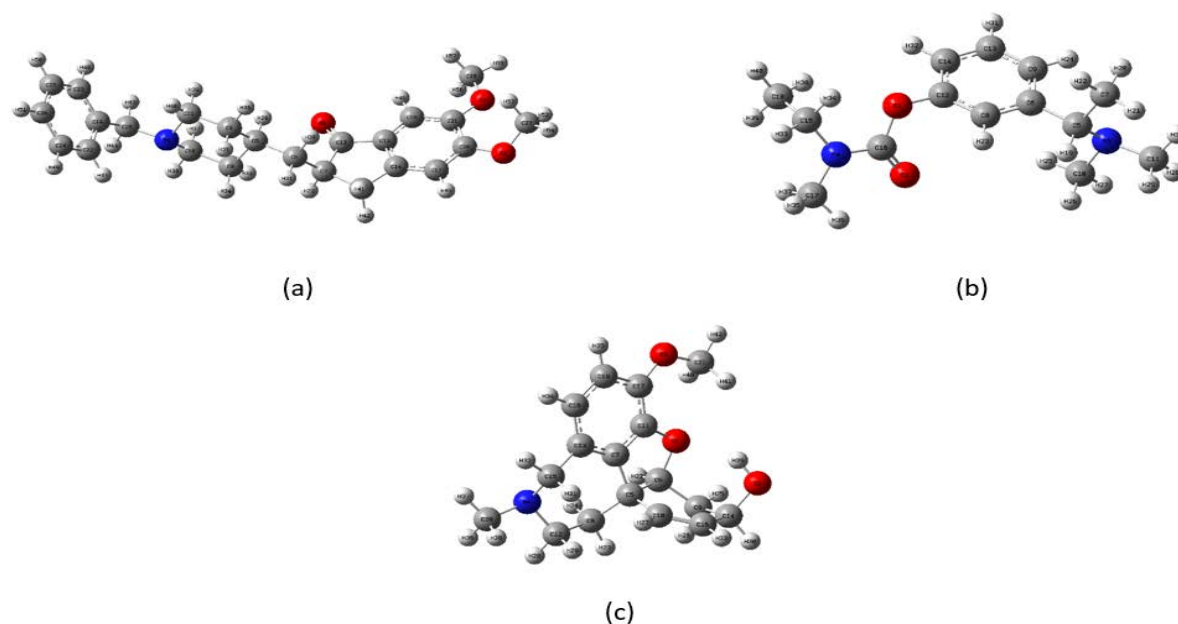


Figure 1. The most stable conformer of donepezil (a), rivastigmine (b) and galantamine (c).

3.2. Molecular Docking

The crystal structures of Acetylcholinesterase enzyme (PDB ID:1DX4, 4EY4, 4EY7) was obtained from the protein database [60, 61]. The acetylcholinesterase enzyme was prepared for docking by removing water molecules and replacing them with polar hydrogens. Its Kollman charges were computed before the docking study. The partial charges of the donepezil, rivastigmine and galantamine molecules were assigned by Geisterger method and the active region of Acetylcholinesterase enzyme was labeled as $40\text{\AA} \times 40\text{\AA} \times 40\text{\AA}$ grid.

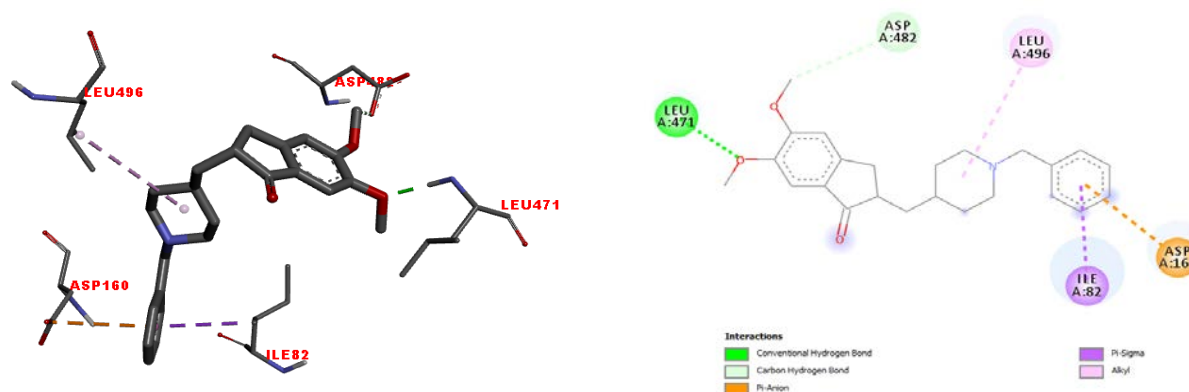


Figure 2. The 3D docked views of the most stable conformer of donepezil in active site of acetylcholinesterase (PDB ID:1DX4, -7.4 kcal/mol).

The 3D docked views of donepezil, rivastigmine and galantamine were shown in Figures 2-4.

As seen in **Figure 2**, the donepezil molecule interacted with:

Ile82 through pi-sigma interaction with 3.86\AA length;

Asp160 through pi-anion interaction with 4.14\AA length;

Leu471 through 1.83\AA long hydrogen bond interaction;

With Asp482 through 3.39\AA long carbon hydrogen bond interaction;

and with Leu496 through an alkyl pi-sigma interaction with a length of 5.47\AA

The binding affinity was determined to be -7.4 kcal/mol .

In the molecular docking studies of triterpenoid (azadirachtin A) into acetylcholinesterase enzyme (PDB ID:1DX4) performed by Rodrigues et al., it was found that triterpenoid (azadirachtin A)

interacted with Tyr71, Gly79, Glu80, Ile82, Trp83, Asn84, Tyr148, Gly149, Gly150, Gly151, Phe152, Met153, Thr154, Gly155, Leu159, Tyr162, Trp321, Tyr324, Phe330, Tyr370, Phe371, Leu479, His480, Asp482 and Glu485 amino acids of AChE receptor [62].

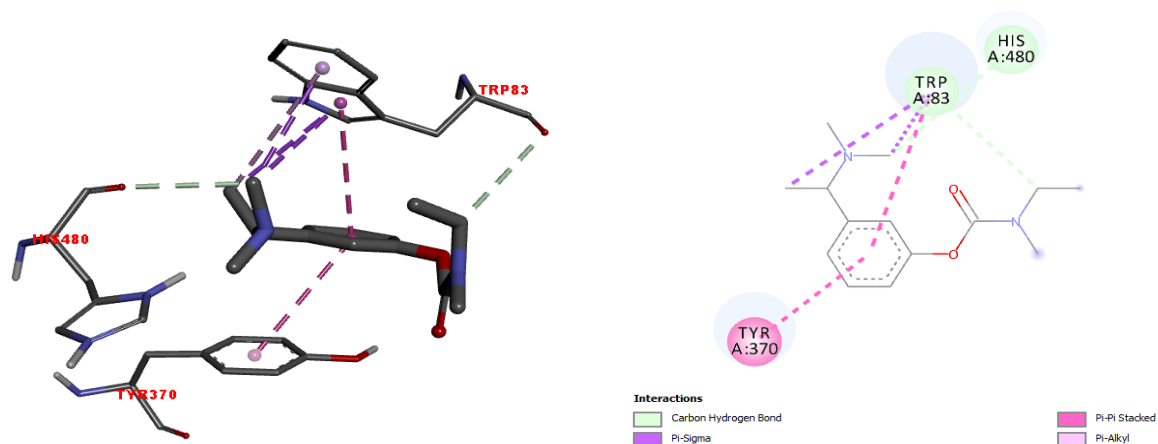


Figure 3. The 3D docked views of the most stable conformer of rivastigmine in active site of acetylcholinesterase (PDB ID:1DX4, -7.0 kcal/mol).

As seen in **Figure 3**, rivastigmine was interacted with;

Trp83 through carbon hydrogen bond interaction with 3.77 Å length, pi-pi stacked interaction with 4.28 Å length, pi-sigma interactions with 3.72, 3.97 and 4 Å length, pi-alkyl interaction with 4.81 Å length;

Tyr370 through pi-pi stacked interaction with 4.45 Å length;

and with His480 through a carbon hydrogen bond interaction with a length of 3.32 Å (Figure 3). The binding affinity was calculated as -7.0 kcal/mol.

In the molecular docking studies of Tacrine and Hesperetin molecules into AChE receptor (PDB ID:1DX4) performed by Kondapalli et al., it was found that Tacrine molecule interacted with Trp83, Tyr370, His480 amino acids and Hesperetin molecule interacted with Trp83, Thr154, His480 amino acids of AChE receptor. Our results are in accord with the previous findings [63].

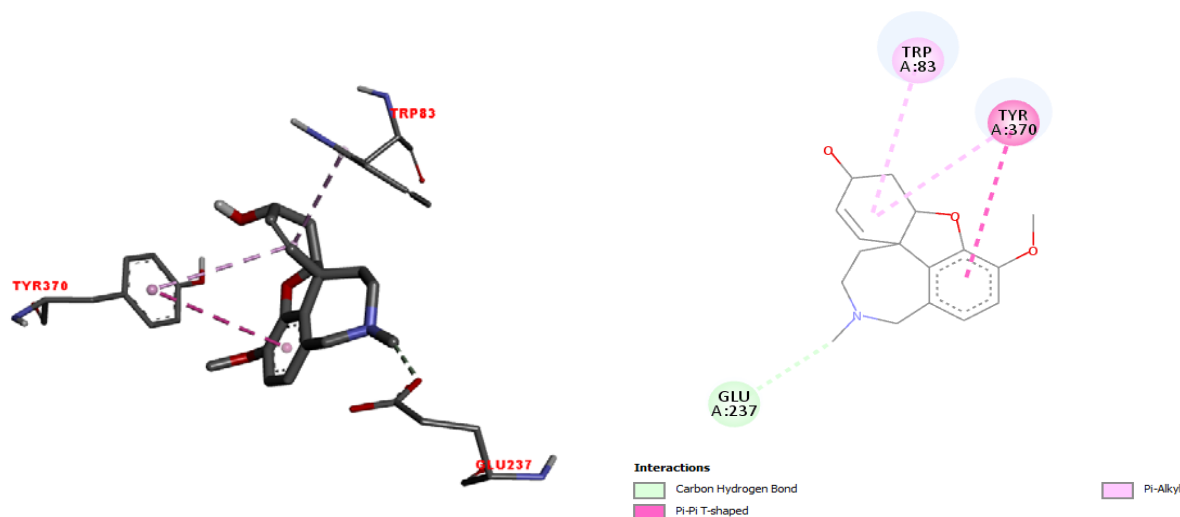


Figure 4. The 3D docked views of the most stable conformer of galantamine in active site of acetylcholinesterase (PDB ID:1DX4, -9.7 kcal/mol).

As seen in **Figure 4**, galantamine molecule was interacted with:
Trp83 through pi-alkyl interaction with 4.74Å length;

Glu237 through carbon hydrogen bond interaction with 3.53Å length; and with Tyr370 through a pi-alkyl interaction with a length of 5.09Å and a pi-pi t-shaped interaction with a length of 5.17Å. The binding affinity was determined to be -9.7 kcal/mol.

In the molecular docking study of Methomyl and 9-(3-phenylmethylamine)-1,2,3,4-tetrahydroacridine molecules into AChE receptor (PDB ID:1DX4), Methomyl molecule was found to interact with Trp83, Tyr370, Trp472, Leu479, His480 amino acids and 9-(3-phenylmethylamine)-1,2,3,4-tetrahydroacridine found to interact with Trp83, Gly150, Gly151 ve His480 residues of the AChE receptor [64]. Our findings are consistent with those of Rodrigues et al. [64].

The donepezil, rivastigmine and galantamine drugs, used in the study are known as AChE inhibitors, and their half maximal inhibitory concentrations (IC_{50}) which are measure of their potency in inhibiting AChE are available in the literature [65]. The results of the docking of these molecules to AChE were compared with the pIC_{50} results of the molecules and were given in **Table 1**.

Table 1. The calculated binding affinities of donepezil, rivastigmine and galantamine molecules to acetylcholinesterase enzyme, in comparison with their experimental half-maximal inhibitory concentration (pIC_{50}).

	PDB ID: 1DX4 Binding affinity (kcal/mol)	PDB ID: 4EY4 Binding affinity (kcal/mol)	PDB ID: 4EY7 Binding affinity (kcal/mol)	pIC_{50}^a
	This study			[65]
Donepezil	-7.4	-8.6	-11.4	8.699
Rivastigmine	-7.0	-6.0	-7.9	7.268
Galantamine	-9.7	-7.5	-10.1	6.523

^a: $pIC_{50} = -\log IC_{50}$ (IC_{50} values were taken from ref [65]).

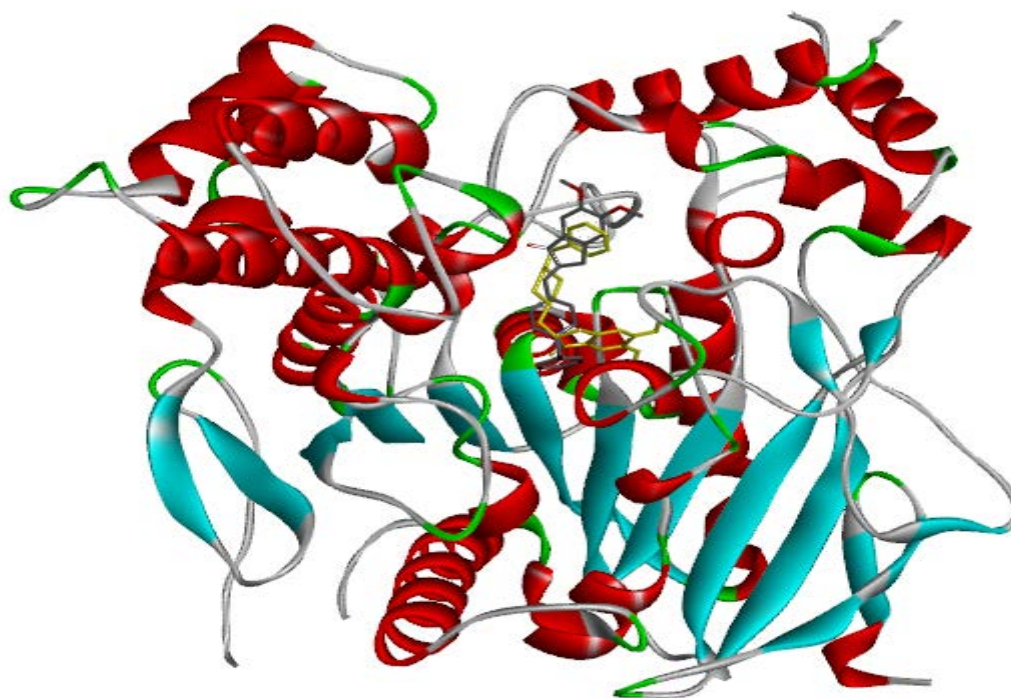


Figure 5. The orientations of donepezil docked into AChE, obtained in this study (yellow) and donepezil in the crystal structure of AChE-Donepezil complex taken from (PDB ID: 4EY7) (grey).

The 3D orientation of Donepezil in AChE target, obtained the study, was compared with the existing ligand orientation in the AChE-donepezil complex crystal structure (see **Figure 5**) and were found to be in agreement.

Recently, Baskaran et al [66] were performed molecular docking calculations of Donepezil and Rivastigmine molecules into 4EY7 target receptor, using AutodockVina program and found the binding affinities as -9.8 and 7.1 kcal/mol, respectively. In this study the binding affinities of

donepezil and risvatigmine molecules to the 4EY7 receptor (AChE) were found to be -11.4 and -7.9 kcal/mol, respectively. The results indicated that the active site found in this study was more stable. Moreover, as seen in Fig 5 that the location donepezil binding site in the AChE target is highly compatible with the location of the donepezil molecule in donepezil-AChE complex.

4. Conclusions

Because the function-activity relationship is so important for bioactive molecules, the most stable conformer of donepezil, rivastigmine, and galantamine molecules was determined. To shed light into their biological activities and to examine the inhibitory effects on Acetylcholinesterase enzyme, the binding mechanisms of donepezil, risvastigmine and galantamine with Acetylcholinesterase enzyme were elucidated by performing molecular docking studies with AChE targets (PDB IDs: 1DX4, 4EY4 and 4EY7). Donepezil, rivastigmine, and galantamine were found to bind to Acetylcholinesterase enzyme (PDB ID:1DX4) with -7.4, -7.0, and -9.7 kcal/mol binding affinities, respectively. In the molecular docking studies of triterpenoid (azadirachtin A) into acetylcholinesterase enzyme (PDB ID:1DX4) [62], It was found that triterpenoid was interacted also with Ile82, Trp83, Tyr370, His480, Asp482 residues of the target receptor. Our results are highly consistent with previous findings [62]. The docking studies revealed that donepezil, rivastigmine, and galantamine binds to 4EY4 with -8.6, -6.0 and -7.5 kcal/mol and to 4EY7 with -11.4, -7.9 and -10.1 kcal/mol binding affinities, respectively. The results indicated that the investigated molecules have good inhibitory impact on the Acetylcholinesterase enzyme.

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References

1. Soreq, H., Seidman, S. Acetylcholinesterase — new roles for an old actor. *Nature Reviews Neuroscience* 2, 294–302 (2001). <https://doi.org/10.1038/35067589>.
2. Villemagne, V. L., Burnham, S., Bourgeat, P., Brown, B., Ellis, K. A., Salvado, O., ... & Masters, C. L. (2013). Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *The Lancet Neurology*, 12(4), 357-367.
3. Jack, C. R., Knopman, D. S., Jagust, W. J., Shaw, L. M., Aisen, P. S., & Weiner, M. W. & Trojanowski JQ (2010). *Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade*. *The Lancet Neurology*, 9(1), 119-128.
4. Huang, H. C., & Jiang, Z. F. (2009). Accumulated amyloid- β peptide and hyperphosphorylated tau protein: relationship and links in Alzheimer's disease. *Journal of Alzheimer's disease*, 16(1), 15-27.
5. Iqbal, K., & Grundke-Iqbal, I. (2010). Alzheimer's disease, a multifactorial disorder seeking multitherapies. *Alzheimer's & Dementia*, 6(5), 420-424.
6. Shoghi-Jadid, K., Small, G. W., Agdeppa, E. D., Kepe, V., Ercoli, L. M., Siddarth, P., ... & Barrio, J. R. (2002). Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease. *The American Journal of Geriatric Psychiatry*, 10(1), 24-35.

7. De Felice, F. G., Wu, D., Lambert, M. P., Fernandez, S. J., Velasco, P. T., Lacor, P. N., ... & Klein, W. L. (2008). Alzheimer's disease-type neuronal tau hyperphosphorylation induced by A β oligomers. *Neurobiology of aging*, 29(9), 1334-1347.
8. Norstrom, E. (2017). Metabolic processing of the amyloid precursor protein—new pieces of the Alzheimer's puzzle. *Discovery Medicine*, 23(127), 269-276.
9. Edwards III, G., Zhao, J., Dash, P. K., Soto, C., & Moreno-Gonzalez, I. (2020). Traumatic brain injury induces tau aggregation and spreading. *Journal of neurotrauma*, 37(1), 80-92.
10. Kovacs, G. G. (2018). Tauopathies. *Handbook of clinical neurology*, 145, 355-368.
11. Iqbal, K., Liu, F., Gong, C. X., & Grundke-Iqbal, I. (2010). Tau in Alzheimer disease and related tauopathies. *Current Alzheimer Research*, 7(8), 656-664.
12. Panza, F., Lozupone, M., Logroscino, G., & Imbimbo, B. P. (2019). A critical appraisal of amyloid- β -targeting therapies for Alzheimer disease. *Nature Reviews Neurology*, 15(2), 73-88.
13. Doody, R. S., Stevens, J. C., Beck, C., Dubinsky, R. M., Kaye, J. A., Gwyther, L. M. S. W., ... & Cummings, J. L. (2001). Practice parameter: Management of dementia (an evidence-based review): Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*, 56(9), 1154-1166.
14. Frank, L. M., Brown, E. N., & Wilson, M. (2000). Trajectory encoding in the hippocampus and entorhinal cortex. *Neuron*, 27(1), 169-178.
15. Pennanen, C., Kivipelto, M., Tuomainen, S., Hartikainen, P., Hänninen, T., Laakso, M. P., ... & Soininen, H. (2004). Hippocampus and entorhinal cortex in mild cognitive impairment and early AD. *Neurobiology of aging*, 25(3), 303-310.
16. Gómez-Isla, T., Price, J. L., McKeel Jr, D. W., Morris, J. C., Growdon, J. H., & Hyman, B. T. (1996). Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *Journal of Neuroscience*, 16(14), 4491-4500.
17. Eichenbaum, H., & Lipton, P. A. (2008). Towards a functional organization of the medial temporal lobe memory system: role of the parahippocampal and medial entorhinal cortical areas. *Hippocampus*, 18(12), 1314-1324.
18. Goldman-Rakic, P. S. (1996). The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 351(1346), 1445-1453.
19. Perry, J. (1977). Frege on demonstratives. *The philosophical review*, 86(4), 474-497.
20. PERRY, E. K., Perry, R. H., Blessed, G., & Tomlinson, B. E. (1978). Changes in brain cholinesterases in senile dementia of Alzheimer type. *Neuropathology and applied neurobiology*, 4(4), 273-277.
21. Atack, J. R., Perry, E. K., Bonham, J. R., Perry, R. H., Tomlinson, B. E., Blessed, G., & Fairbairn, A. (1983). Molecular forms of acetylcholinesterase in senile dementia of Alzheimer type: selective loss of the intermediate (10S) form. *Neuroscience letters*, 40(2), 199-204.
22. Fishman, E. B., Siek, G. C., MacCallum, R. D., Bird, E. D., Volicer, L., & Marquis, J. K. (1986). Distribution of the molecular forms of acetylcholinesterase in human brain: alterations in dementia of the Alzheimer type. *Annals of neurology*, 19(3), 246-252.
23. Palmert, M. R., Podlisny, M. B., Witker, D. S., Oltersdorf, T., Younkin, L. H., Selkoe, D. J., & Younkin, S. G. (1989). The beta-amyloid protein precursor of Alzheimer disease has soluble derivatives found in human brain and cerebrospinal fluid. *Proceedings of the National Academy of Sciences*, 86(16), 6338-6342.

24. Zhang, L., Tang, W., Chao, F. L., Zhou, C. N., Jiang, L., Zhang, Y., ... & Tang, Y. (2020). Four-month treadmill exercise prevents the decline in spatial learning and memory abilities and the loss of spinophilin-immunoreactive puncta in the hippocampus of APP/PS1 transgenic mice. *Neurobiology of Disease*, 136, 104723.
25. Çokuğraş, A. N. (2003). Butyrylcholinesterase: structure and physiological importance. *Turk J Biochem*, 28(2), 54-61.
26. Dasarathy, S., & Merli, M. (2016). Sarcopenia from mechanism to diagnosis and treatment in liver disease. *Journal of hepatology*, 65(6), 1232-1244.
27. Walsh, C. T. (1984). Suicide substrates, mechanism-based enzyme inactivators: recent developments. *Annual review of biochemistry*, 53(1), 493-535.
28. Chao, C. C., Hu, S. X., Ehrlich, L., & Peterson, P. K. (1995). Interleukin-1 and tumor necrosis factor- α synergistically mediate neurotoxicity: involvement of nitric oxide and of N-methyl-D-aspartate receptors. *Brain, behavior, and immunity*, 9(4), 355-365.
29. Enz, A., Amstutz, R., Boddeke, H., Gmelin, G., & Malanowski, J. (1993). Brain selective inhibition of acetylcholinesterase: a novel approach to therapy for Alzheimer's disease. *Progress in brain research*, 98, 431-438.
30. Zarotsky, V., Sramek, J. J., & Cutler, N. R. (2003). Galantamine hydrobromide: an agent for Alzheimer's disease. *American journal of health-system pharmacy*, 60(5), 446-452.
31. McHardy, S. F., Wang, H. Y. L., McCowen, S. V., & Valdez, M. C. (2017). Recent advances in acetylcholinesterase inhibitors and reactivators: an update on the patent literature (2012-2015). *Expert opinion on therapeutic patents*, 27(4), 455-476.
32. Trang, A., & Khandhar, P. B. (2021). Physiology, acetylcholinesterase. In *StatPearls [Internet]*. StatPearls Publishing.
33. McGleenon, B. M., Dynan, K. B., & Passmore, A. P. (1999). Acetylcholinesterase inhibitors in Alzheimer's disease. *British journal of clinical pharmacology*, 48(4), 471.
34. Lazarevic-Pasti, T., Leskovac, A., Momic, T., Petrovic, S., & Vasic, V. (2017). Modulators of acetylcholinesterase activity: From Alzheimer's disease to anti-cancer drugs. *Current medicinal chemistry*, 24(30), 3283-3309.
35. Mehta, M., Adem, A., & Sabbagh, M. (2012). New acetylcholinesterase inhibitors for Alzheimer's disease. *International Journal of Alzheimer's disease*, 2012.
36. Gong, C. X., Liu, F., Grundke-Iqbal, I., & Iqbal, K. (2005). Post-translational modifications of tau protein in Alzheimer's disease. *Journal of neural transmission*, 112(6), 813-838.
37. Komori, T. (1999). Tau-positive dial Inclusions in Progressive Supranuclear Palsy, Corticobasal Degeneration and Pick's Disease. *Brain pathology*, 9(4), 663-679.
38. Kovacs, G. G. (2019). Molecular pathology of neurodegenerative diseases: principles and practice. *Journal of clinical pathology*, 72(11), 725-735.
39. Iida, M. A., Farrell, K., Walker, J. M., Richardson, T. E., Marx, G. A., Bryce, C. H., ... & Crary, J. F. (2021). Predictors of cognitive impairment in primary age-related tauopathy: an autopsy study. *Acta Neuropathologica Communications*, 9(1), 1-12.
40. Respondek, G., & Höglinger, G. U. (2016). The phenotypic spectrum of progressive supranuclear palsy. *Parkinsonism & related disorders*, 22, S34-S36.
41. Levin, J., Kurz, A., Arzberger, T., Giese, A., & Höglinger, G. U. (2016). The differential diagnosis and treatment of atypical parkinsonism. *Deutsches Ärzteblatt International*, 113(5), 61.
42. Faujan, N. H., Zakaria, N., & Mohammad, N. N. (2019). Molecular docking studies on the interaction of anti-Alzheimer compounds with amyloid beta peptides. *J. Multidiscip. Eeng. Sci. Technol.*, 6, 132-136.

43. Zarini-Gakiye, E., Amini, J., Sanadgol, N., Vaezi, G., & Parivar, K. (2020). Recent updates in the Alzheimer's disease etiopathology and possible treatment approaches: a narrative review of current clinical trials. *Current Molecular Pharmacology*, 13(4), 273-294.
44. Sugimoto, H., Ogura, H., Arai, Y., Imura, Y., & Yamanishi, Y. (2002). Research and development of donepezil hydrochloride, a new type of acetylcholinesterase inhibitor. *The Japanese journal of pharmacology*, 89(1), 7-20.
45. Heravi, M. M., & Zadsirjan, V. (2020). Prescribed drugs containing nitrogen heterocycles: An overview. *RSC Advances*, 10(72), 44247-44311.
46. Fowler, A. C. (2020). Pharmaceutical line extensions in the United States.
47. Yan, Z., & Feng, J. (2004). Alzheimer's disease: interactions between cholinergic functions and β -amyloid. *Current Alzheimer Research*, 1(4), 241-248.
48. Howard, R. J., Juszczak, E., Ballard, C. G., Bentham, P., Brown, R. G., Bullock, R., ... & Rodger, M. (2007). Donepezil for the treatment of agitation in Alzheimer's disease. *New England Journal of Medicine*, 357(14), 1382-1392.
49. Hashimoto, M., Kazui, H., Matsumoto, K., Nakano, Y., Yasuda, M., & Mori, E. (2005). Does donepezil treatment slow the progression of hippocampal atrophy in patients with Alzheimer's disease?. *American Journal of Psychiatry*, 162(4), 676-682.
50. Emre, M., Aarsland, D., Albanese, A., Byrne, E. J., Deuschl, G., De Deyn, P. P., ... & Lane, R. (2004). Rivastigmine for dementia associated with Parkinson's disease. *New England Journal of Medicine*, 351(24), 2509-2518.
51. Ravisankar, P., Parvathi, Y. S., Sri, K. C., Ameen, S. A., Kiranmai, D., Ram, R. S., ... & Babu, P. S. (2017). A Comprehensive Analysis on Different Types of Hypothesis, Diagnosis and Treatment of Alzheimer's Disease. *IOSR-JDMS*, 16(4), 97-108.
52. Hampel, H., Mesulam, M. M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T., ... & Khachaturian, Z. S. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*, 141(7), 1917-1933.
53. Scott, L. J., & Goa, K. L. (2000). Galantamine. *Drugs*, 60(5), 1095-1122.
54. Rodda, J., Morgan, S., & Walker, Z. (2009). Are cholinesterase inhibitors effective in the management of the behavioral and psychological symptoms of dementia in Alzheimer's disease? A systematic review of randomized, placebo-controlled trials of donepezil, rivastigmine and galantamine. *International psychogeriatrics*, 21(5), 813-824.
55. Shanbhag, T., & Shenoy, S. (2015). *Pharmacology: Prep Manual for Undergraduates E-book*. Elsevier Health Sciences.
56. Sugarman, D. E., De Aquino, J. P., Poling, J., & Sofuoglu, M. (2019). Feasibility and effects of galantamine on cognition in humans with cannabis use disorder. *Pharmacology Biochemistry and Behavior*, 181, 86-92.
57. Halgren, T. A. (1996). Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *Journal of computational chemistry*, 17(5-6), 490-519.
58. Shao, Y., Molnar, L. F., Jung, Y., Kussmann, J., Ochsenfeld, C., Brown, S. T., ... & DiStasio Jr, R. A. (2006). Advances in methods and algorithms in a modern quantum chemistry program package. *Physical Chemistry Chemical Physics*, 8(27), 3172-3191.
59. Trott, O.; Olson, A.J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010,31, 455-461.
60. Harel, M., Kryger, G., Rosenberry, T. L., Mallender, W. D., Lewis, T., Fletcher, R. J., ... & Sussman, J. L. (2000). Three-dimensional structures of *Drosophila melanogaster*

- acetylcholinesterase and of its complexes with two potent inhibitors. *Protein Science*, 9(6), 1063-1072.
61. Cheung, J., Rudolph, M. J., Burshteyn, F., Cassidy, M. S., Gary, E. N., Love, J., ... & Height, J. J. (2012). Structures of human acetylcholinesterase in complex with pharmacologically important ligands. *Journal of medicinal chemistry*, 55(22), 10282-10286.
 62. Rodrigues, G. C., Scotti, L., & Scotti, M. (2017). Molecular docking study of triterpenoid azadirachtin A on acetylcholinesterase of *Drosophila melanogaster* (Diptera: Drosophilidae), *MOL2NET*, 3, 10.3390/mol2net-03-05054.
 63. Kondapalli, N., Sruthi, K. (2020). Novel Tacrine and Hesperetin analogues: Design, Molecular docking and in silico ADME studies to identify potential Acetyl choline esterase inhibitors for Alzheimer's disease. *Journal of Faculty of Pharmacy of Ankara University*, 44(1), 18-32.
 64. Rodrigues, G. C. S., dos Santos Maia, M., Silva Cavalcanti, A. B., de Sousa, N. F., Scotti, M. T., & Scotti, L. (2021). In silico studies of lamiaceae diterpenes with bioinsecticide potential against *Aphis gossypii* and *Drosophila melanogaster*. *Molecules*, 26(3), 766.
 65. NIH, National Library of Medicine, <https://pubchem.ncbi.nlm.nih.gov>.
 66. Baskaran, K. P., Arumugam, A., Kandasamy, R., & Alagarsamy, S. (2020). Insilico method for prediction of maximum binding affinity and ligand-protein interaction studies on Alzheimer's disease. *Int J Res Granthaalayah*, 8(11), 362-370.