



Comparative molecular interaction study of Alzheimer's Disease target with existing drugs

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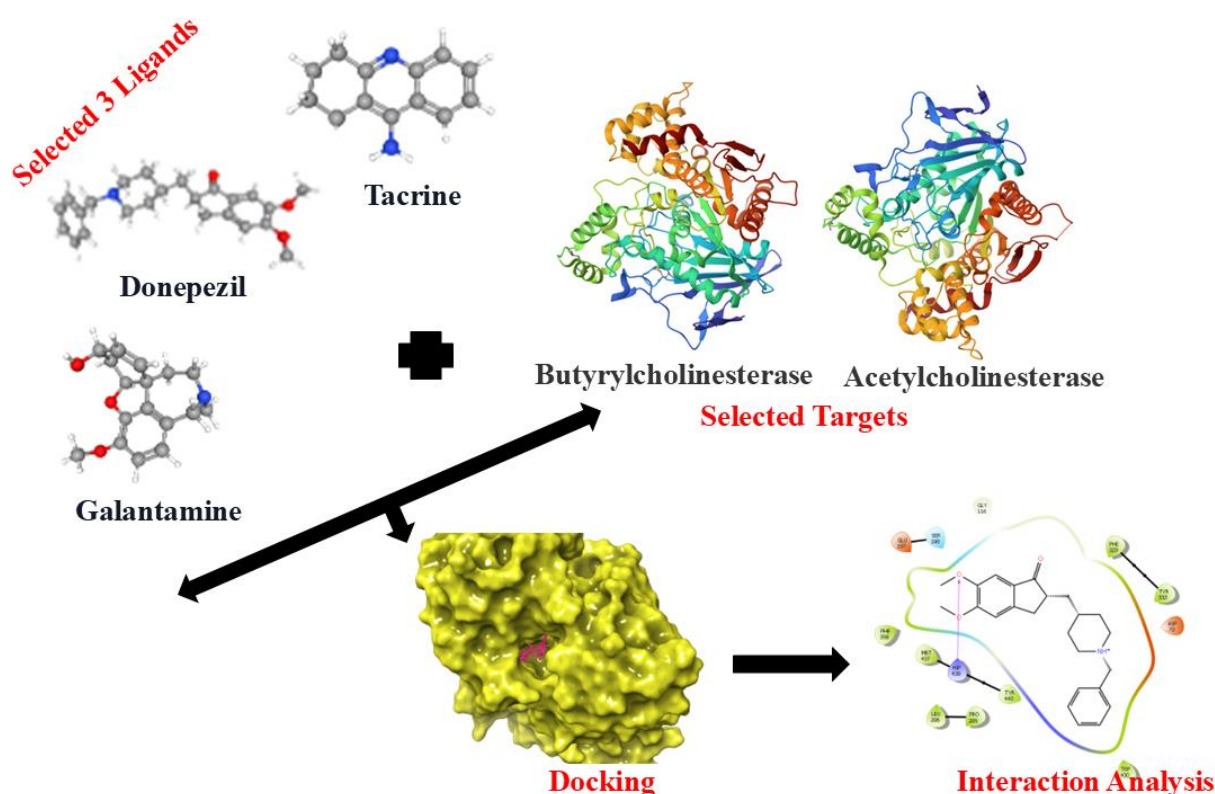
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Abstract : With few effective disease-modifying therapies, Alzheimer's disease continues to be an unsolvable problem in the study of neurodegenerative diseases. Due to inadequate pharmacokinetic characteristics and safety concerns, there aren't many new compounds hitting the market. In our study, we have taken 3 existing drugs and performed docking studies against the 2 targets, like Butyrylcholinesterase, Acetylcholinesterase. The molecular docking results are compared, and the best ligand interactions are aimed at Butyrylcholinesterase, Acetylcholinesterase in the pathobiology of Alzheimer's disease. Potential therapeutic advantages can be investigated by blocking these targets. Drug discovery for Alzheimer's disease is aided by insights into these important biochemical processes provided by molecular docking simulations and a careful selection of ligands and proteins. The current investigation has revealed the possibility of key medications Tacrine, Donepezil and Galantamine for Alzheimer's disease with a binding free energy of -6.692, -8.07 and -6.053 kcal/mol against the target Butyrylcholinesterase whereas the binding free energy of -8.515, -15.305 and -11.871 kcal/mol against the target Acetylcholinesterase demonstrating strong binding affinities. These compounds exhibited promising druggability profiles in both molecular docking and interaction studies, indicating their potential as novel Butyrylcholinesterase and Acetylcholinesterase for AD treatment. However, further experimental validation is necessary to verify their effectiveness and safety.

Keywords: Alzheimer's disease, Butyrylcholinesterase, Acetylcholinesterase, Molecular docking, Glide and Interaction studies.

Graphical Abstract



Introduction

Alzheimer's disease (AD) is a complex and heterogeneous condition characterized by distinct pathobiological features. Contemporary evidence indicates that late-onset Alzheimer's disease is a complex polygenic condition involving disrupted interactions among various molecular pathways [1]. The hallmark neuropathological criteria for diagnosing AD include the presence of extracellular amyloid- β deposits in the form of neuritic plaques and the intracellular accumulation of hyperphosphorylated tau, which forms neurofibrillary tangles. β -secretase (BACE1) is a key target in AD research because it plays a critical role in the production of toxic A β 42 peptides. Inhibiting BACE1 could potentially reduce the formation of these plaques and mitigate their harmful effects. However, clinical trials targeting BACE1 have encountered challenges, including issues related to efficacy and side effects. This underscores the complexity of Alzheimer's pathology and the need for a multifaceted approach in developing effective therapies. Ongoing research continues to explore alternative targets and treatment strategies [2]. Recent research, however, has uncovered additional critical cellular and molecular processes that play significant roles in the disease's pathology. Despite these advances, effective disease-modifying treatments remain elusive, and many phase 3 clinical trials have not shown meaningful benefits.

Several cholinesterase (ChE) inhibitors have been developed for the treatment of Alzheimer's disease (AD), with donepezil, galantamine, rivastigmine, and memantine currently available on the market [3]. However, the efficacy of these drugs is limited, and they can cause various dose-related side effects, especially at higher doses. Galantamine and donepezil specifically inhibit acetylcholinesterase (AChE), while rivastigmine is a reversible inhibitor of both AChE and butyrylcholinesterase (BChE). Notably, donepezil shows a high selectivity for AChE over BChE [4]. Memantine is safe and effective for the symptomatic treatment of Alzheimer's disease (AD). Although its neurobiological mechanisms are not fully understood, memantine differs from current AD therapies as it is not a cholinesterase inhibitor. It interacts with various ligand-gated ion channels, with NMDA receptors identified as a primary therapeutic target at effective concentrations [5]. Memantine acts as an uncompetitive NMDA receptor antagonist, blocking the channel. At high concentrations, memantine can inhibit synaptic plasticity mechanisms that are crucial for learning and memory. However, at lower, clinically relevant doses, it may promote synaptic plasticity and enhance memory in animal models of AD. Additionally, memantine offers protection against the excitotoxic damage to cholinergic neurons [6]. By blocking NMDA receptors, memantine may have disease-modifying potential in AD by mitigating NMDA receptor-dependent excitotoxicity, which is thought to contribute to progressive neuronal loss. Recent in vitro studies also suggest that memantine may counteract β -amyloid (A β) toxicity and potentially inhibit A β production. There has been considerable interest in exploring theories that explain memantine's better tolerability compared to other NMDA receptor antagonists [7].

The aetiology of AD is not completely understood, yet it is complex and multifactorial. Although several treatment strategies have been proposed [3,4], most current therapeutic options involve restoring acetylcholine levels in the brain. hAChE [human AChE (acetylcholinesterase)] inhibitors donepezil (Aricept®), rivastigmine (Exelon®) and galanthamine (Reminyl®) are currently approved anti-AD drugs [5]. In previous studies, BChE (butyrylcholinesterase) received attention owing to its role in modulating acetylcholine levels in cholinergic neurons under normal conditions [6] and when AChE activity decreases [7,8]. Consequently, both enzymes are important targets in AD treatment [9–11]. The complex aetiology of AD has prompted the development of MTDLs (multitarget-directed ligands) that act simultaneously on different components of AD pathology, e.g. hAChE and amyloid. Inhibitors of hAChE have been used as scaffolds to synthesize such MTDLs [12–15].

Rational design of more potent hAChE and hBChE (human BChE) inhibitors would benefit from a better understanding of how the current inhibitors bind to the active sites of these enzymes. Structural data on hAChE in complex with anti-AD drugs have been available only recently and have shown how important it is to analyse complexes formed with the human enzyme rather than with AChE from other species [16].

In the present paper, we describe the crystal structures of Butyrylcholinesterase and Acetylcholinesterase with the existing aforesaid ligands tacrine, donepezil, and galantamine, against AD treatment. This study aims to identify new compounds with promising inhibitory activity using in silico methods, targeting Alzheimer's disease treatment. Subsequently, virtual screening of the phytochemical library was conducted using rigid receptor-molecular docking to identify compounds capable of effectively inhibiting.

Materials and methods

Preparation of proteins

The complex formed by Acetylcholinesterase and Butyrylcholinesterase plays a pivotal role in AD treatment. We retrieved the protein structures of human Acetylcholinesterase in complex with Donepezil from the Protein Data Bank (PDB ID: 4EY7) and HUMAN Butyrylcholinesterase in complex with tacrine from the Protein Data Bank (PDB ID: 4BDS). The initial protein structures obtained from the PDB database required optimization. To achieve this, we used Schrödinger's Protein Preparation Wizards. During this optimization process, we addressed various issues, including ensuring proper bond orders, adding hydrogen atoms, filling in missing residues, correcting formal charges for hetero groups, and generating ionization states suitable for physiological pH 7.0. Finally, we performed a minimization step using the OPLS_2005 force field. This comprehensive preparation procedure ensured that the protein structures were suitably configured for subsequent molecular docking studies [15].

Ligand preparation

The LigPrep module of Schrödinger suite was employed for the pre-processing of the selected existing ligands (tacrine, rivastigmine, donepezil, galantamine and memantine). In this preprocessing step, the formation of tautomers and ionization states at pH 7.0 ± 2.0 using Epik, hydrogen atoms were added, charged groups were neutralised, and geometry of the ligands was optimised [16]. The determination of ligand chirality was based on their three-dimensional structures.

Target protein grid generation

To prepare for prospective molecular docking studies, it is essential to establish a well-defined grid box around the protein's active site. In this regard, we defined the active site by creating an enclosing box with dimensions of $36 \times 36 \times 36$ Å. Predicted active site residues guided the generation of this grid. For configuring the grid, we maintained default settings, where the van der Waals radii were scaled with a factor of 1.0 Å, and a cutoff of less than 0.25 was applied to partial atomic charges [18]. During this process, we did not designate any rotatable groups, and constraints were left undefined. This approach ensured a suitable grid for subsequent molecular docking investigations.

GLIDE (Grid-based Ligand Docking with Energetics)

Molecular docking is a crucial technique used to predict the binding orientation of small molecules to their respective protein targets. This process helps estimate the affinity and activity of these small molecules, making it a vital component of structure-based drug design [19]. Glide serves as a comprehensive solution for ligand-receptor docking. It offers a range of options balancing speed and accuracy. These options include the HTVS (high-throughput virtual screening) mode, which efficiently handles large compound libraries, the SP (standard precision) mode for accurately docking tens to hundreds of thousands of ligands, and the XP (extra precision) mode. The XP mode is particularly valuable as it enhances accuracy by conducting more extensive sampling and employing advanced scoring methods to eliminate false positives, thereby achieving higher enrichment.

In this study, we utilized Glide v11.9 to assess the binding interactions between the target protein and ligands. The molecular docking was carried out in the extra precision (XP) mode, ensuring a high level of accuracy in our binding interaction predictions. The interactions between the receptor and phytochemical were calculated as Glide scores, i.e., G-scores, based on the following equation:

$$\text{Glide score} = 0.065 \times \text{vdW} + 0.130 \times \text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}$$

where,

vdW = van der Waals energy,

Coul = Coulomb energy,

Lipo = lipophilic term derived from hydrophobic grid potential,

Hbond = hydrogen-bonding term,

Metal = metal-binding term,

BuryP = penalty for buried polar groups,

RotB = penalty for freezing rotatable bonds, and

Site = polar interactions present in the active site.

Results

Molecular Docking

In our molecular docking investigation, we obtained three-dimensional SDF structures of the selected compounds from the PubChem database. These structures were subjected to molecular docking analysis using the Glide docking panel within the Maestro 11.9 Schrodinger suite. The results of the docking experiments, including the docking score, the number of hydrogen bonds formed, and the interacting residues, were compiled and presented in Table 1 and Figures 1 to 6.

In our study, all three ligands were subjected to molecular docking analysis in complex with human Acetylcholinesterase and Butyrylcholinesterase. Among the docked compounds, it demonstrated the most favourable binding affinity, as evidenced by its significantly lower glide score of -8.07 kcal/mol for Donepezil against Butyrylcholinesterase and -15.305 kcal/mol for Donepezil against Acetylcholinesterase. This negative value indicates a strong binding interaction between Acetylcholinesterase and the ligands, along with the Butyrylcholinesterase as another target. In the context of our investigation, it can be confidently identified as the "hit molecule Donepezil" due to its outstanding binding affinity when compared to the other 2 ligands for the target.

Table 1. Docking score of the screened compound.

S. No.	Compound name	PubChem ID	Target	Docking Score	Number of H-bonds	Interacting Residue
1	Tacrine	1935	Butyrylcholinesterase	-6.692	1	Hip 438
2	Donepezil	3152		-8.07	1	Hip 438
3	Galantamine	9651		-6.053	1	Glu 197
4	Tacrine	1935	Acetylcholinesterase	-8.515	-	No Interaction
5	Donepezil	3152		-15.305	1	Phe 295
6	Galantamine	9651		-11.871	1	Hip 447

This finding implies that Donepezil hold promise as a potential lead compound for further exploration and development as a therapeutic agent targeting human Acetylcholinesterase and Butyrylcholinesterase. Its strong binding affinity suggests that it may have the desired biological activity required for inhibiting Acetylcholinesterase and Butyrylcholinesterase, making it a valuable candidate for drug development efforts in the context of oncology research or other relevant fields.

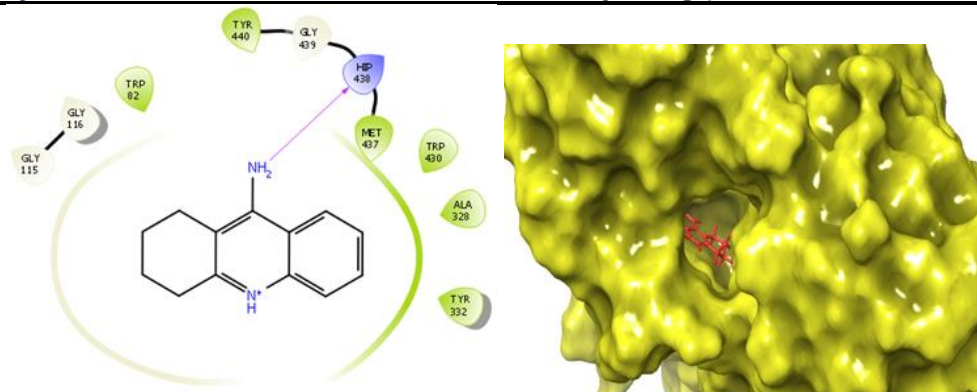


Figure 1. 2D interaction and surface view of 4BDS in complex with Tacrine.

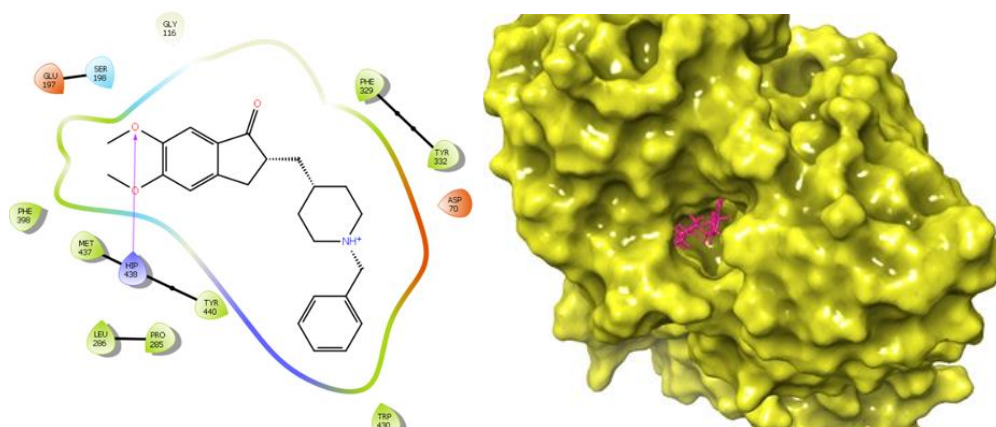


Figure 2. 2D interaction and surface view of 4BDS in complex with Donepezil.

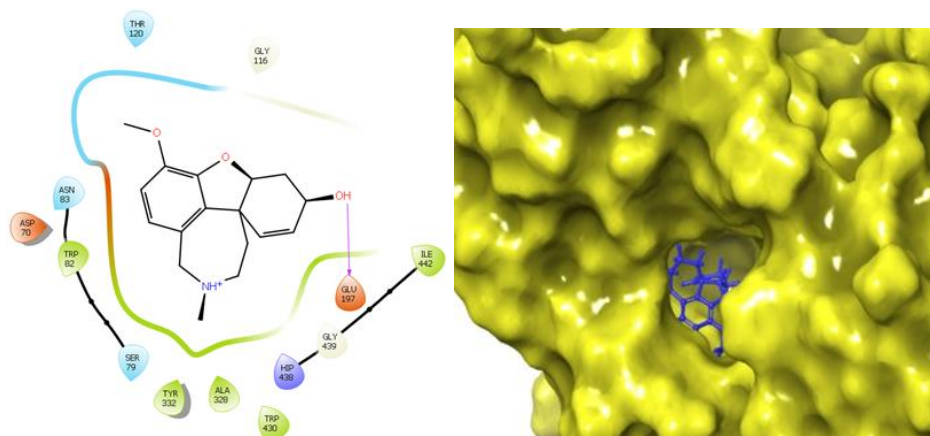


Figure 3. 2D interaction and surface view of 4BDS in complex with Galantamine.

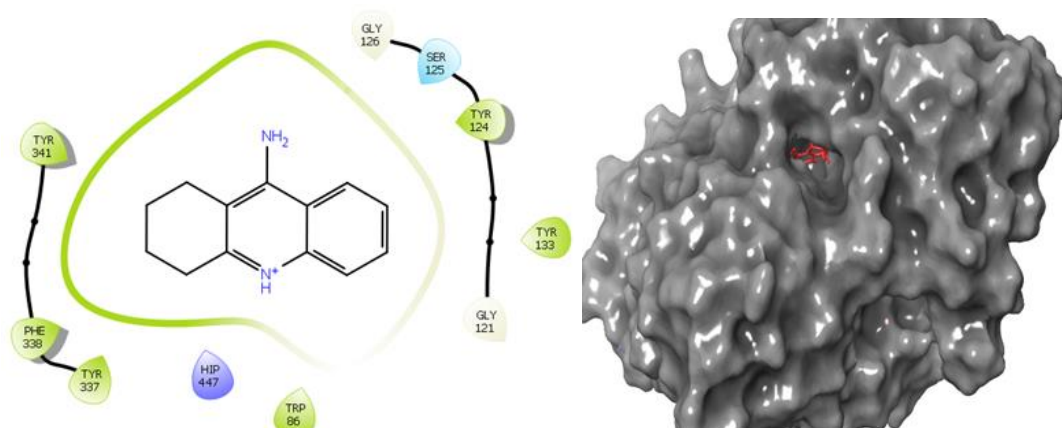


Figure 4. 2D interaction and surface view of 4EY7 in complex with Tacrine.

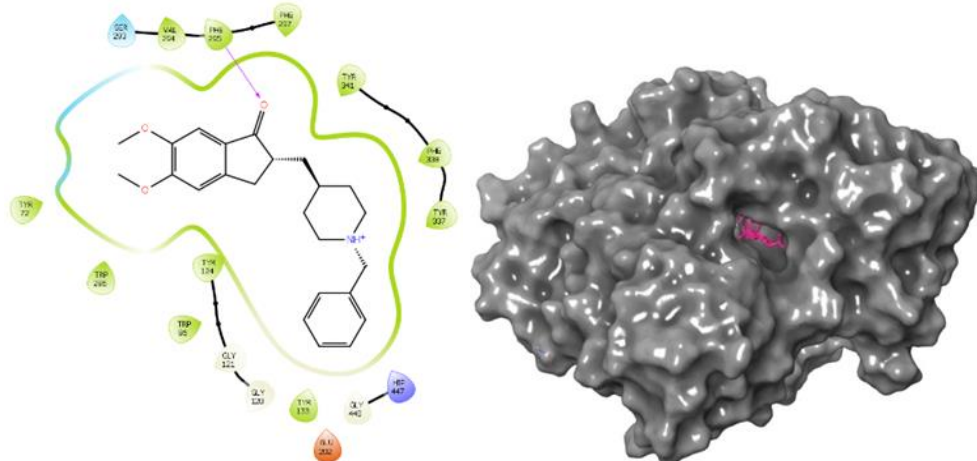


Figure 5. 2D interaction and surface view of 4EY7 in complex with Donepezil.

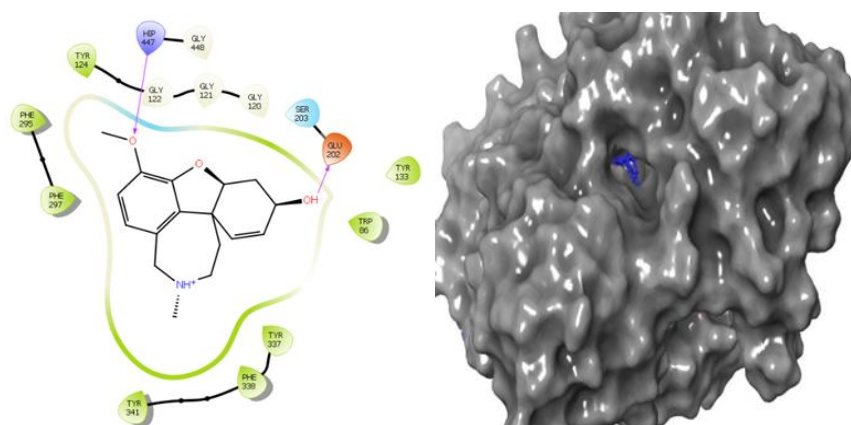


Figure 6. 2D interaction and superimposed surface view 4EY7 in complex with Galantamine.

Conclusion

This study highlights the importance of molecular docking in drug discovery for AD, demonstrating its potential to screen ligands that target Acetylcholinesterase and Butyrylcholinesterase. As the search for effective AD treatments continues, molecular docking remains a valuable tool to elucidate ligand interactions, guiding future therapeutic developments. Despite the laborious efforts, presently, very few drugs are in the pipeline due to the limitations associated with molecules to satisfy the ADME profile. Due to these limitations, the present study explored the identification of new drugs with better binding energy, favorable pharmacokinetics, and a toxicology profile. From our study, we revealed that Donepezil was found to be the best candidate for the inhibition of Acetylcholinesterase and Butyrylcholinesterase protein. Further studies are need to evident this. Hence, in silico-based drug screening has been able to identify promising results which might be useful therapeutically in AD.

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