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Article

Rational drug repurposing for alzheimer's treatment using in-silico ligand and structure-based approaches

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Alzheimer's disease is a devastating neurodegenerative disorder characterized by memory loss and cognitive decline. New AD treatments are essential, and drug repositioning is a promising approach. In this study, we combined ligand-based and structure-based approaches to identify potential candidates among FDA-approved drugs for AD treatment. We used the human acetylcholinesterase receptor structure (PDB ID: 4EY7) and applied Rapid Overlay of Chemical Structures and Swiss Similarity for ligand-based screening.Computational shape-based screening revealed 20 out of 760 FDA approved drugs with promising structural similarity to Donepezil, an AD treatment AChE inhibitor and query molecule. The screened hits were further analyzed using docking analysis with Autodock Vina and Schrodinger glide. Predicted binding affinities of hits to AChE receptor guided prioritization of potential drug candidates. Doxazosin, Oxypertine, Cyclopenthiazide, Mestranol, and Terazosin exhibited favorable properties in shape similarity, docking energy, and molecular dynamics stability.Molecular dynamics simulations confirmed the stability of the complexes over 100 ns. Binding free energy analysis using MM-GBSA indicated favourable binding energies for the selected drugs. ADME, formulation studies offered insights into therapeutic applications and predicted toxicity.This comprehensive computational approach identified potential FDA-approved drugs (especially Doxazosin) as candidates for repurposing in AD treatment, warranting further investigation and clinical assessment.

Keywords: Alzheimer's disease.structure-based screening. ligand-based screening.FDA approved drugs. Docking. Swiss similarity.

INTRODUCTION

The cerebral cortex and the hippocampus, two of the most sensitive regions of the brain, are affected in Alzheimer's disease (AD) leads to multifaceted and diverse illness. The most common symptoms of AD, a progressive age-related neurodegenerative disease, are memory loss and declining cognition. The accumulation of amyloid (Aβ) peptides, along with tau protein malfunction that affects the cholinergic system, mitochondrial biogenesis, and microtubular structure, considered as pathophysiological hallmarks of AD (Parvez, 2022).The development of new treatment options for AD typically involves symptomatic treatment that can treat, slow the progression of the illness, postpone its onset, or even prevent it. A variety of computational tools are used today for rational selection of drug for repositioning (Hassan *et al.*, 2019). Drug repositioning or repurposing as a supplement to conventional medication that offer benefits including rapid discovery and regulatory approval(Parvez, 2022). The definition of drug repositioning, often referred to as drug rediscovery, redisposition, or drug rescue, is "the application of established pharmacological molecules

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to new therapeutic indications (Kore *et al.*, 2012). Comparatively less expensive and more promising than de novo drug development strategies (Kore *et al.*, 2012). The recent success stories of CADD application in drug discovery have shown potential value in the field of drug repurposing. For target identification, validation, lead selection, small-molecule screening, and optimization, CADD techniques play important role (Macalino*et al.,*2015). Acetyl-Cholinesterase (AChE) is one of the potential targets for the symptomatic treatment of AD and related dementias(Abbasi *et al.*, 2018; Cygler *et al.*, 1993; Tougu, 2001)AChE, the most important cholinesterase that hydrolyzes Acetylcholine into acetic acid and choline and terminates its neuronal transmission and signalling between synapses and nearby receptors. Thus, AChE is employed as the target molecule to show the inhibitory potential of newly developed chemical structures in the treatment of AD (Abbasi *et al.*, 2018). Another study discovered that AChE is validated drug target for symptomatic improvement because cholinergic insufficiency is a constant finding in AD (Tougu, 2001). The centrally acting reversible AChE inhibitor donepezil (Aricept), used as a potential treatment option for AD, that increases cortical acetylcholine levels (Mehta, Adem, Sabbagh, 2012). The therapeutic effects of donepezil are attained by inhibiting AChE that reduces the degradation of acetylcholine. As a result, acetylcholine levels at cholinergic synapses increase. Donepezil has also been studied for treatment of dementia and vascular dementia (Lee *et al.*, 2015; Rojas-Fernandez, 2001; Malouf, Birks, 2004). In our study, we proposed a strategy for drug repurposing in Alzheimer's disease (AD) that combines ligand-based and structure-based approaches. We aimed to identify potential candidates among FDA-approved drugs as shown in Figure1 by utilizing the human acetylcholinesterase (AChE) receptor structure, specifically the PDB ID: 4EY7 (available at https://www.rcsb.org/structure/4ey7).For the ligand-based screening, we employed the Swiss Similarity method, which involved using ROCS to evaluate shape, atom similarity, and electrostatic properties. We utilized donepezil as the query molecule to search for compounds with similar 2D, 3D shape, and electrostatic characteristics. The screened hits, along with their 2D, 3D shape, and electrostatic similarity scores, were further analyzed using Schrödinger software.To perform docking analysis, we employed Autodock Vina, which allowed us to predict the binding affinities of the screened hits to the AChE receptor. This step enabled us to prioritize potential drug candidates for further investigation.Overall, our study employed a comprehensive approach that integrated ligand-based and structure-based methods to identify FDA-approved drugs with potential therapeutic benefits for AD (Hassan *et al.*, 2019). as shown in Figure2

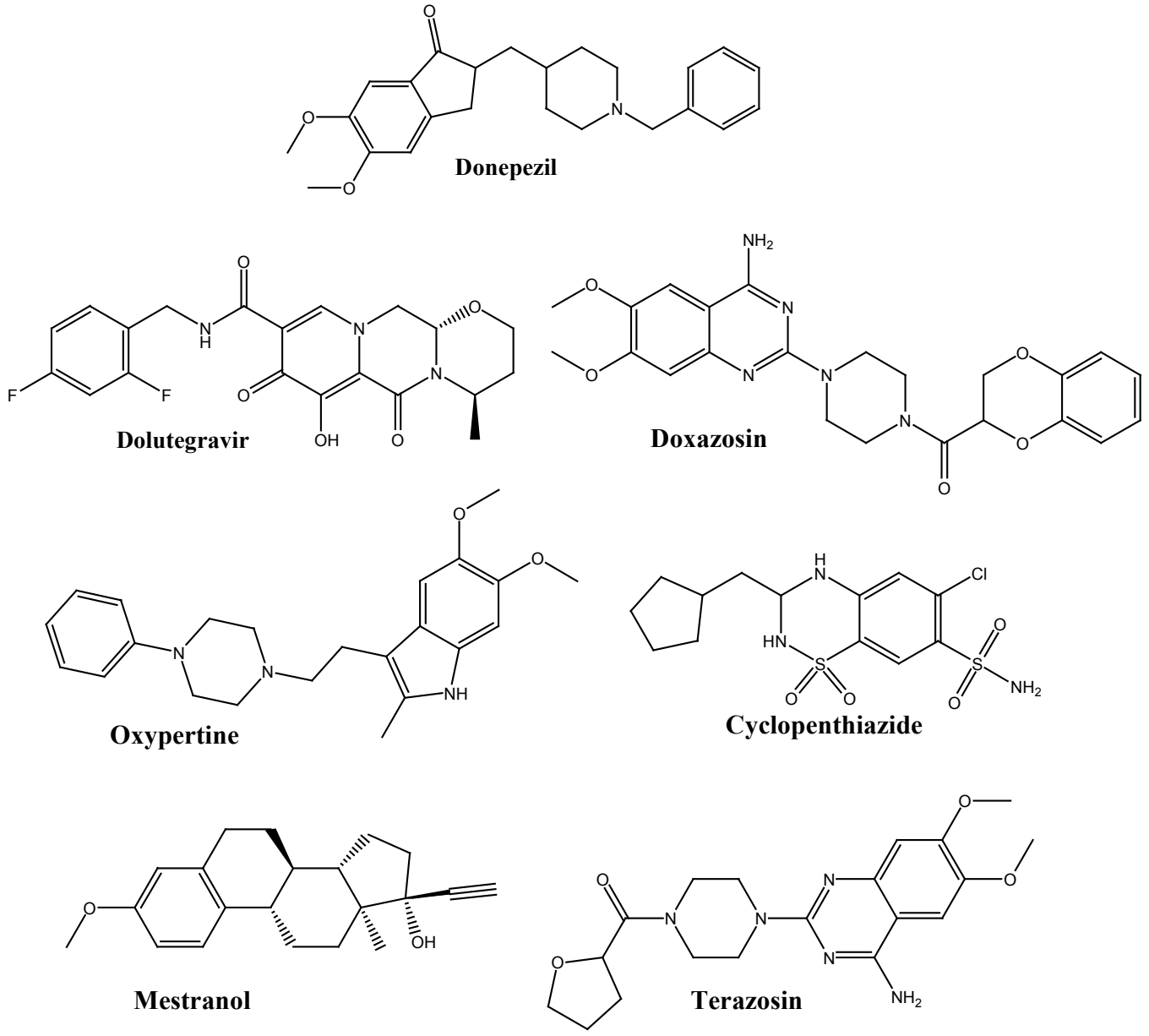


FIGURE 1 - Structures of proposed molecules for repurposing for the treatment of AD.

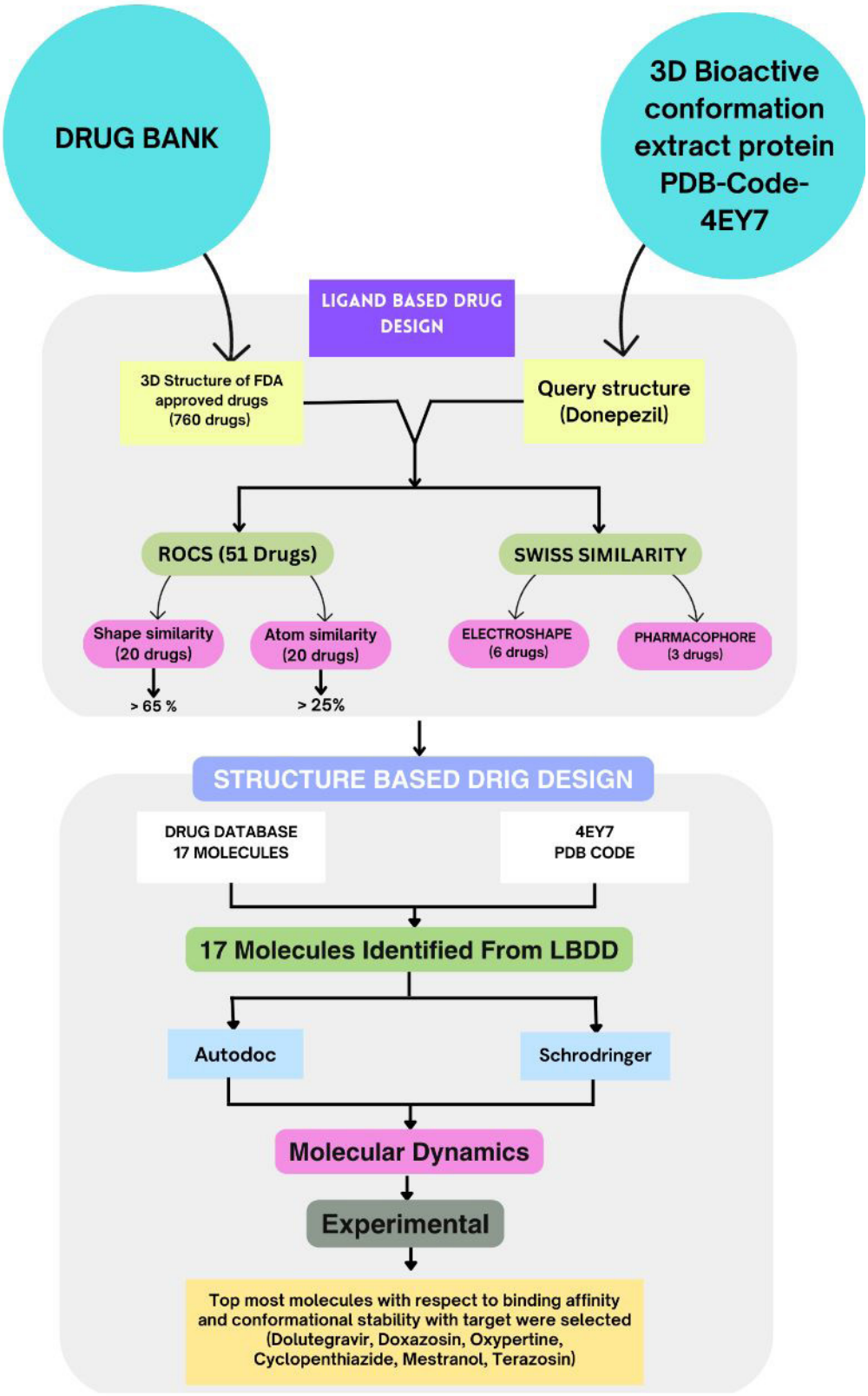


FIGURE 2 - Work flow.

MATERIAL AND METHODS

Selection of target protein, Query molecule and FDA approved drug database

We have selected Donepezil as a query structure for shape and electrostatic similarity studies used for treatment of AD by increasing the level of cortical acetylcholine (Dallakyan, Olson, 2015) Donepezil exerts their therapeutic action by inhibiting AChE that as a result the acetylcholine level is increased at cholinergic synapses. Donepezil is an orally bio available drug with lower toxicities. Donepezil also reported for its application (therapeutic) in other cognitive disorders including Lewy body dementia and vascular dementia(Sastry *et al*., 2013; Cheung*et al*., 2012) Here in, Donepezil was used as standard template to screen the similar ligand structures from FDA approved drug data bank using Swiss Similarity and Rapid overlay of chemical structures (ROCS)further potential candidates were processed for docking studies. AChE enzyme with PDB code 4EY7 was selected for docking studies as it is co-crystalised with donepezil and having required features for computational studies (Seltzer, 2007; Cacabelos, 2007).

3D shape, atom based, pharmacophore based similarity studies using ROCS (Open Eye Scientific Software) and swiss software

The 3D shape and atom-based similarity analysis was conducted using the ROCS (Rapid Overlay of Chemical Structures) tool (OpenEye Scientific Software version:). The procedure involved preparing the input files (3D structure of Donepezil, Dolutegravir, Pantoprazole, Pretomanid, Terazosin from drug data bank was obtained), setting up the ROCS configuration, running the similarity search, and analyzing the results (Hawkins, Skillman, Nicholls, 2007; Grant, Gallardo, Pickup, 1996; Hartshorn, 2002) The 3D molecular structures of the query molecule Donepezil in its bioactive confirmation obtained from co-crystalised structure of protein (PDB code- 4EY7) and the molecules for virtual screening were obtained from drug bank. All these molecules were carefully checked and prepared considering their protonation states, appropriate ionization, and tautomeric forms. All the parameters and options for the ROCS calculation were specified. This includes defining the desired scoring function, search options, and other relevant parameters for shape and electrostatic similarity studies (O'Boyle *et al*., 2011; Pruitt, 2009; Singh *et al*., 2020). The ROCS similarity search was initiated by executing the ROCS command including the paths to the query and target molecule files, setting of all required parameters. The ROCS tool performed the similarity search by aligning the query molecule to the target molecules and generating a similarity score for each alignment. The obtained results, including the similarity scores and alignment information, were carefully analyzed. The top-ranked hits and alignment poses were extracted for further evaluation and interpretation (Humphrey, Dalke, Schulten, 1996). To gain insights into the similarities and differences, the aligned structures and their atom-based features were visualized using molecular visualization software. The described procedure followed the guidelines provided by the official ROCS documentation and user manual, ensuring accurate and reliable results. The usage of ROCS in this study allowed for efficient 3D shape and atom-based similarity analysis, facilitating the identification of molecules with similar structural features (Grant *et al*., 2007; Open Eye Software).

The shape and pharmacophore-based similarity analysis was performed using the Swiss Similarity tool (www.swisssimilarity.ch). The molecular structures of the query molecule (donepezil) for screening were obtained from drug bank, ensuring proper protonation states, ionization, and tautomeric forms. The Swiss Similarity web interface was accessed, and the 'electro shape and pharmacophore' section was utilized to study shape and pharmacophore similarity (Gobbi, Lee, 2003). In this study, we utilized a query molecule file containing the structure of Donepezil and a drug database molecule file comprising the structures of FDA-approved drugs for comparative analysis. To provide flexibility to the user, we implemented multiple options for inputting the query molecule, either by drawing it in an embedded molecular editor or by pasting its SMILES representation in the dedicated text box. Furthermore, users were given the opportunity to select the desired class of compounds,

such as drugs, bioactive compounds, commercial compounds, or synthesizable compounds. Subsequently, they could choose the compound library and screening method for analysis, such as pharmacophore or electro shape analysis. Finally, upon submitting the molecule, the system generated results displaying molecules that exhibited similarity to the query molecule (Willett, Barnard, Downs, 1998; Sheridan *et al*., 2010; Leelananda, Lindert, 2016; Rueda-Zubiaurre, Tietze, Medina, 2020). In this similarity search process Swiss Similarity performed the electro shape similarity and pharmacophore-based similarity between the query molecule (Donepezil) and the FDA approved drugs (Willett, 2006). The obtained results, including the similarity scores or rankings, were carefully analyzed. Molecules with high similarity scores or favourable rankings were identified as potential hits exhibiting similar electro shape and pharmacophore features to the query molecule-(Donepezil) (Koes, Camacho, 2011; Schneider, Fechner, 2005). The similarity score ranges from 0 for totally different molecules to 1 for identical compounds. It corresponds to a Tanimoto score for FP2 fingerprints, Align-IT and Shape-IT and to a Manhattan-based score for Electroshape-5D and Spectrophores.

Molecular docking using Auto dock vina and Schrodinger (Glide)

Molecular docking studies were performed using the Auto dock vina 1.5.7 software, an established tool for predicting the binding interactions between ligands and protein targets. The procedure involved preparing the input files, configuring the docking parameters, running the docking simulations, and analyzing the results (Schrödinger Release, 2020; Schneidman *et al*., 2008).The three-dimensional structures of the target protein 4EY7 was obtained from a PDB Data Bank. The protein structure was prepared by removing water molecules, hydrogen, adding missing atoms or residues if necessary, and optimizing the protein's geometry. The ligand molecules, representing potential small molecule inhibitors or drug candidates, were prepared by generating their three-dimensional structures and assigning appropriate partial charges. This involved defining the search space around the protein's active site, specifying the search algorithm setting the number of dockings runs and generations, and selecting the scoring function (e.g., Auto dock Vina scoring).The docking simulations were initiated by running Autodock with the prepared protein and ligand files and the specified parameters. The software explored the conformational space of the ligands and predicted their binding modes and affinities within the protein's active site.The obtained docking results were carefully analyzed. The docked poses were examined to identify potential binding interactions, such as hydrogen bonds, hydrophobic interactions, and electrostatic interactions. The binding affinities or scores were recorded to assess the relative binding strength of the ligands .

The molecular docking procedure was performed using the Schrödinger software suite, by utilizing the Glide module. The three-dimensional structure of the receptor protein was obtained from the Protein Data Bank (PDB)- 4EY7 and prepared using Schrödinger's protein preparation tools. The ligand molecule(s) were obtained from a drug bank and subjected to ligand preparation, including the optimization of protonation states and stereochemistry. A receptor grid was generated using Schrödinger's Grid Generation panel in Glide, defining the binding site of interest. The grid size and resolution were adjusted to cover the relevant region adequately. Docking settings were configured within the Glide module, including the choice of docking algorithm (Glide SP) search options (such as sampling method and number of poses). The docking job was submitted, and the progress was monitored until completion. Upon completion, the docking results, including predicted binding poses and docking scores, were obtained. The docking results were further analyzed using Schrödinger's analysis tools, such as the Ligand Interaction Diagram and GlideScore, and visually inspected using Maestro, a visualization tool within the Schrödinger suite .Comparison of docking scores was made between auto dock vina and Schrodinger. The docking results were interpreted in the context of the research objectives, focusing on the binding modes, key protein-ligand interactions, and potential ligand-protein interactions.(Daina, Michielin, Zoete, 2017; Jain, 2003; Hawkins *et al*., 2010; Morris *et al*., 2009)

Molecular dynamics simulation (MD) and Binding free energy analysis

The MD simulations studies were carried on the dock complexes for complex 1 and complex 3using the Desmond 2020.1from Schrödinger, LLC. The OPLS-2005 force field , and explicit solvent model with the SPC water molecules were used in this system. Na^+ ions were added to neutralize the charge. 0.15 M, NaCl solutions added to the system to simulate the physiological environment. Initially, the system was equilibrated using NVT ensemble for 100 ps to retrainover the protein-quercetin complex. Followed by this a short run equilibration and minimization using NPT ensemble for 12 ps. The NPT ensemble was set up using the Nose-Hoover chain coupling scheme with temperature 27 ºC, the relaxation time of 1.0 psand pressure 1 bar maintained in all the simulations. A time step of 2 fs was used. The Martyna-Tuckerman–Klein chain coupling scheme barostat method was used for pressure control with a relaxation time of 2 ps. The particle mesh Ewald method was used for calculating long-range electrostatic interactions, and the radius for the coulomb interactions were fixed at 9Å. RESPA integrator was used for a time step of 2 fs for each trajectory to calculate the bonded forces. The root mean square deviation (RMSD), radius of gyration (Rg), root mean square fluctuation (RMSF) and solvent accessible surface area (SAS Area) were calculated to monitor the stability of the MD simulations (Trott, Olson, 2010).

The molecular mechanics combined with generalized Born surface area (MM-GBSA) approach was used to compute the binding free energies of the complex 1 and complex 3. MM-GBSA binding free energy was calculated using the Python script thermal_mmgbsa.py in the simulation trajectory and the OPLS_2005 force field over last 50 frames with a 100-step sampling size. The binding free energy of Prime MM-GBSA (kcal/mol) was estimated using the principle of additivity, in which individual energy modules such as columbic, covalent, hydrogen bond, van der Waals, self-contact, lipophilic, solvation, and - stacking's of ligand and protein were collectively added. The equation used to calculate ΔG_{bind} is the following:

Where

- $\mathbf{\Delta G}_{bind}$ designates the binding free energy,
- $\mathbf{\Delta G}_{MM}$ designates difference between the free energies of ligand-protein complexes and the total energies of protein and ligand in isolated form,
- $\mathbf{\Delta G}_{Solv}$ designates difference in the GSA solvation energies of the ligand-receptor complex and the sum of the solvation energies of the receptor and the ligand in the unbound state,
- $\mathbf{\Delta G}_{SA}$ designates the difference in the surface area energies for the protein and the ligand.

RESULTS

Selection of target protein, Query molecule, structure database

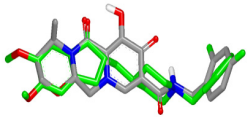
PDB-4EY7 was selected as the target protein based on its significant involvement in the disease pathway under investigation. Extensive literature review and preliminary studies indicated that PDB-4EY7 plays a critical role in the progression of the target disease, making it an attractive candidate for drug development. Additionally, the three-dimensional structure of PDB-4EY7 was readily accessible in the Protein Data Bank (PDB), ensuring the availability of reliable structural information for subsequent computational and experimental analyses.Donepezil, an FDA-approved drug primarily used for the treatment of Alzheimer's disease, was chosen as the query molecule due to its well-characterized pharmacological properties and structural features. By utilizing Donepezil as a template, we aim to leverage its established activity and build upon its scaffold to design new molecules with enhanced potency, selectivity, and improved pharmacokinetic properties.

Electroshape, pharmacophore similarity studies

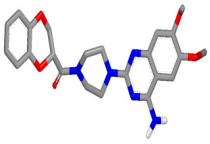
Derivatives of the identified cores were sought such that the 3D shape and volume of the new molecules were like the shape and volume of Donepezil. We surmised that by means of this strategy, the new derivatives would retain the potency inherent to Donepezil (MIC 0.7–1.5 µg/mL). The Tanimoto shape similarity coefficient (TSSC)

was used to quantify the shape similarity with Donepezil, and is expressed quantitatively in the range 0–1.0. A value of 1.0 indicates complete similarity, while 0 indicates no similarity as shown in Figure 3. A database curated from literature was virtually screened against the five lowest energy conformations of Donepezil. Terazosin sahpe tanimoto score is 0.615, Colour Tanimoto score is 0.338, and combo tanimoto score is 0.953 (e.g. compound 6) is closest in shape to Donepezil; next is the Dolutegravir sahpetanimoto score is 0.687, Colour Tanimoto score is 0.256, and combo tanimoto score is 0.943 (e.g. compound 1), followed by Doxazosin sahpe tanimoto score is 0.636, Colour Tanimoto score is 0.223, and combo tanimoto score is 0.86, Oxypertine sahpe tanimoto score is 0.545, Colour Tanimoto score is 0.229, and combo tanimoto score is 0.775 (e.g. compound 3) Cyclopenthiazide sahpetanimoto score is 0.579, Colour Tanimoto score is 0.17, and combo tanimoto score is 0.751 (e.g. compound 3). The compound Mestranol was not selected from the list as its steriodal derivative and because of its adverse effects. Terazosin showed best effect in electroshape and pharmacophore similarities, while it also showed best score from Autodock and Molecular dynamics studies as shown in Table I

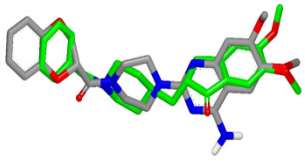
Dolutegravir_Only



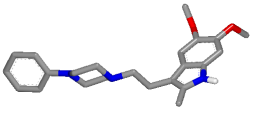
Dolutegravir _Overlay



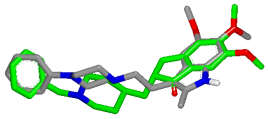
Doxazosin_Only



Doxazosin_Overlay



Oxypertine_Only



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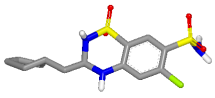
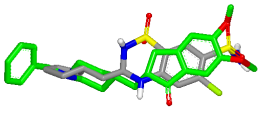


Cyclopenthiazide_Only



Cyclopenthiazide_Overlay

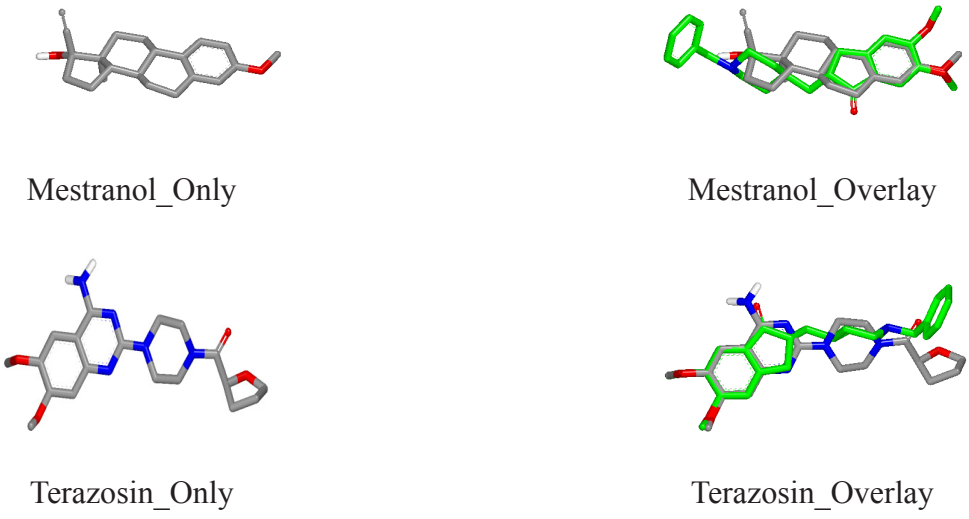


FIGURE 3 - Shape, electrostatic similarity studies.

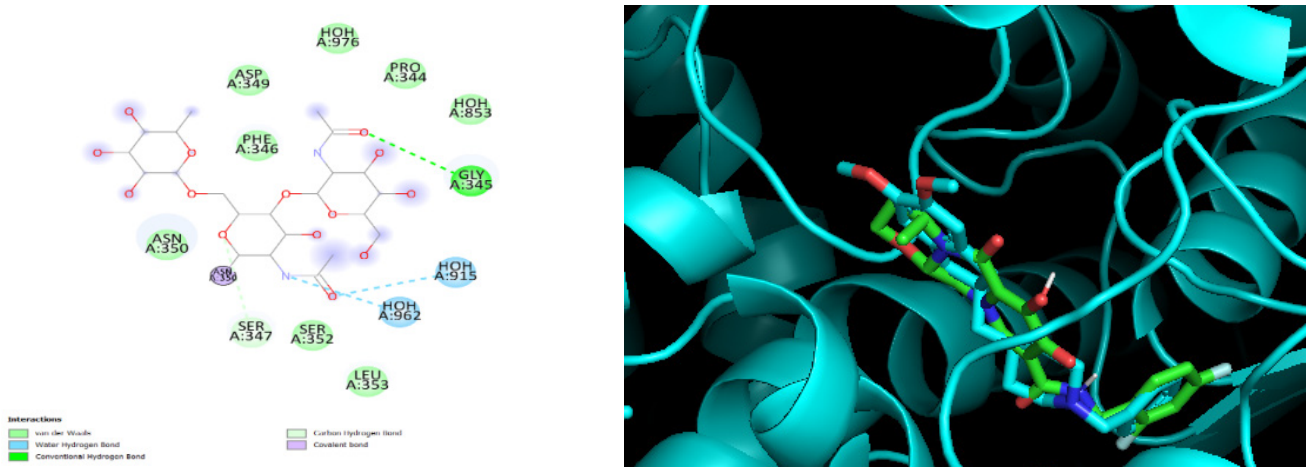
TABLE I - ROCS & Swiss Similiarity scoring values of screened drugs

Sr. No	Drugs Name	Shape Tanimoto	Colour Tanimoto	Combo Tanimoto	Swiss similarity (Electroshape score)	Swiss similarity (Pharmacophore score)
1.	Donepezil	1	1	1	1.000	1.000
2.	Doxazosin	0.636	0.223	0.86	--	--
3.	Oxypertine	0.545	0.229	0.775	0.869	--
4.	Mestranol	0.536	0.28	0.816	--	--
5.	Terazosin	0.615	0.338	0.953	--	--
6.	Cyclopenthiazide	0.579	0.17	0.751	--	--
7.	Dolutegravir	0.687	0.256	0.943	--	--
8.	Cinitapride	--	--	--	0.848	--
9.	Niaprazine	--	--	--	0.842	--
10.	Fenoverine	--	--	--	0.837	--
11.	Clebopride	--	--	--	0.834	--
12.	1-Benzeyl-4	--	--	--	--	1.000
13.	Zanapezil	--	--	--	--	0.382

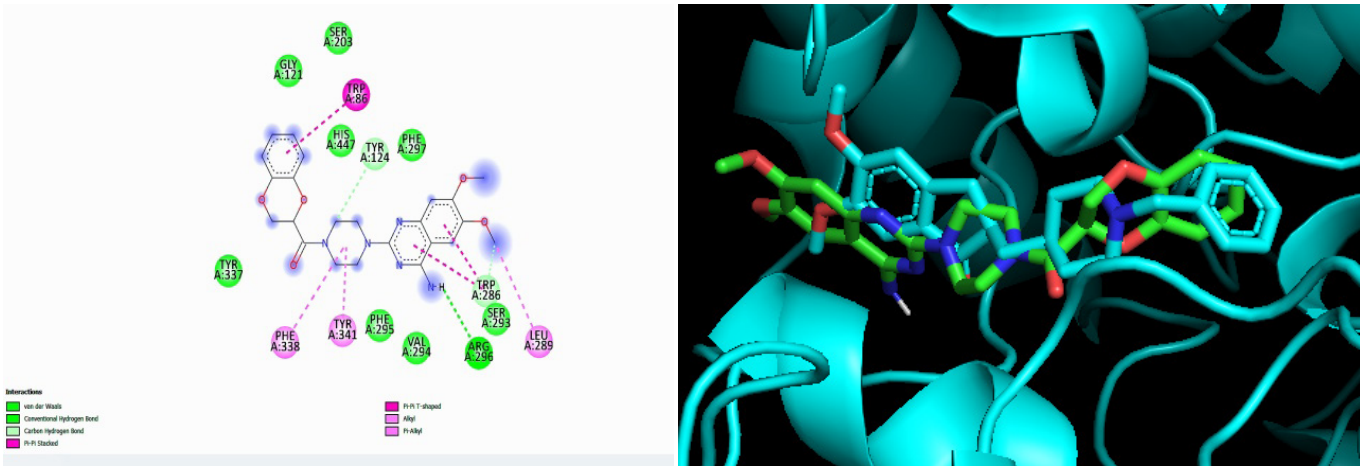
Molecular Docking

Molecular docking serves as a powerful tool for evaluating the binding affinities of drugs against target proteins. In our study, we employed this technique to investigate the binding conformation of various drugs within the active site of AChE. By analyzing the obtained binding energy values, we aimed to identify the most promising drug candidate. The results, as shown in Table II, indicated that the majority of screened drugs exhibited energy values comparable to that of donepezil, standard inhibitor of AChE. Notably, by autodock software dolutegravir displayed the highest binding affinity value (-11.5 kcal/mol) among all the screened drugs, suggesting its potential as a strong candidate. Furthermore, doxazosin, oxypertine, cyclopenthiazide, mestranol, and terazosin also demonstrated favorable docking energy values (-10.9, -10.3, -10.0, -10.0, -9.9 kcal/mol, respectively). To ensure a fair comparison, we conducted docking of donepezil against AChE using identical parameters, revealing a binding energy of -11.7 kcal/mol. It showed proposed drugs could be potential AChE inhibitors as ashown in Figure4.

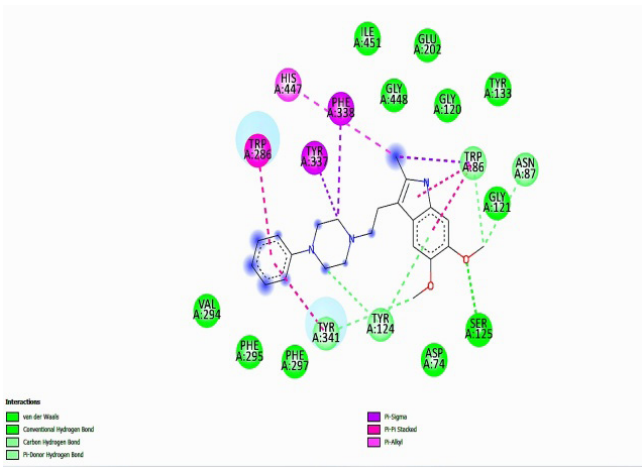
Dolutegravir

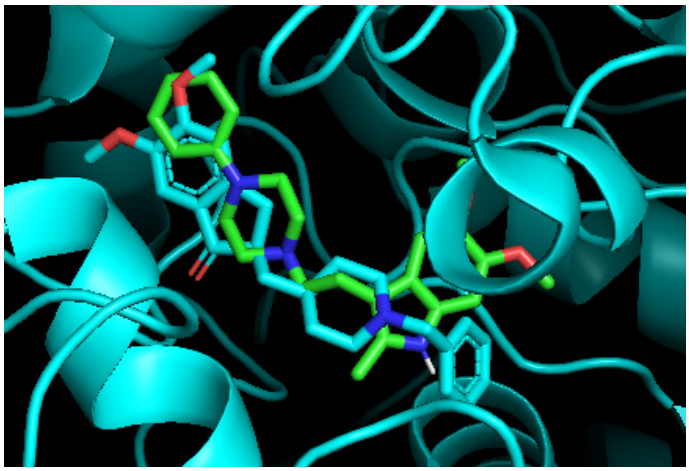


Doxazosin

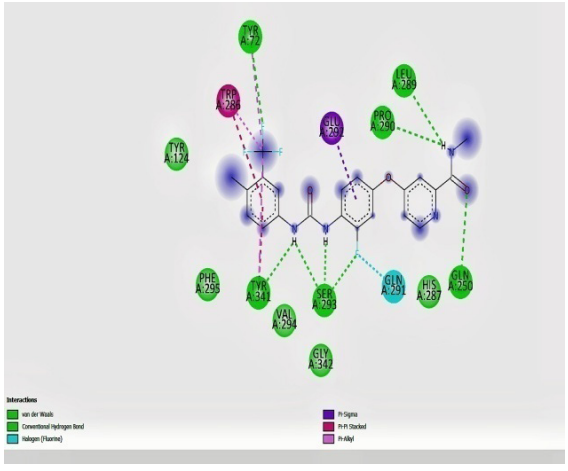


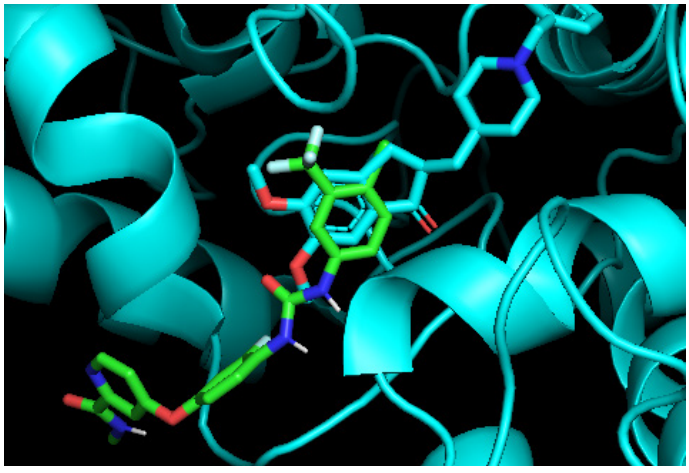
Oxypertine



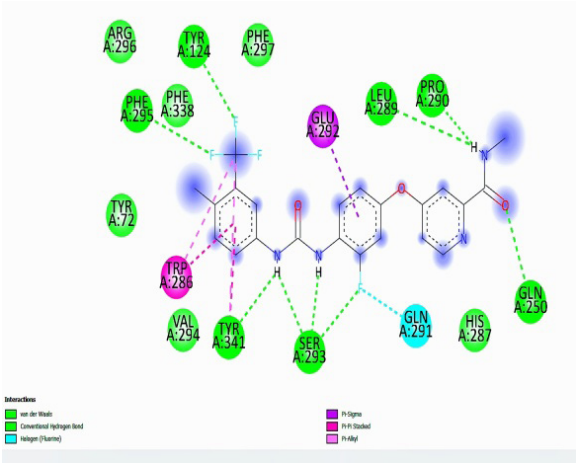


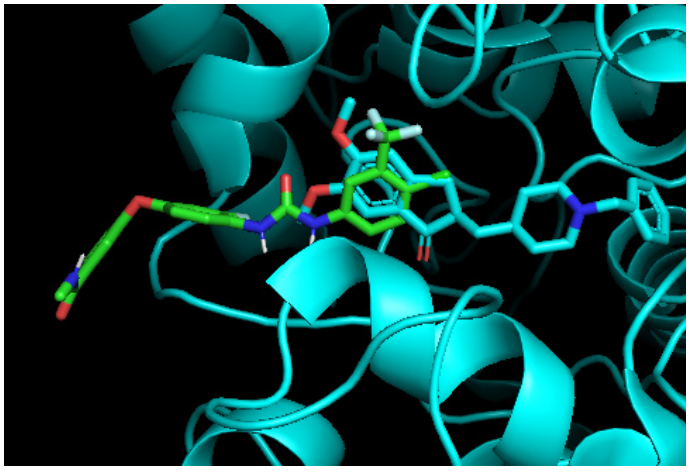
Cyclopenthiazide



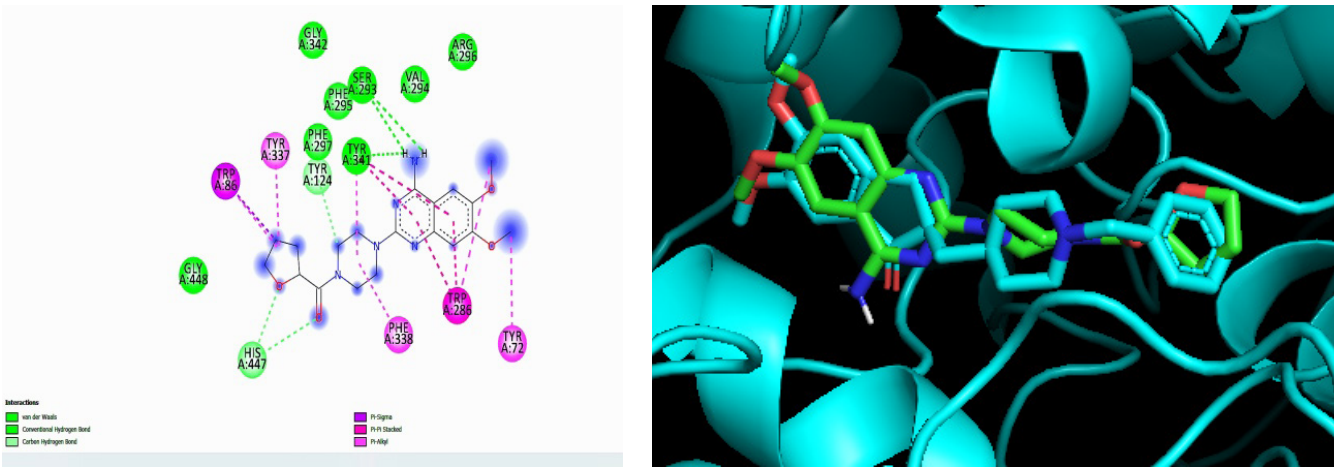


Mestranol





Terazosin



Donepezil

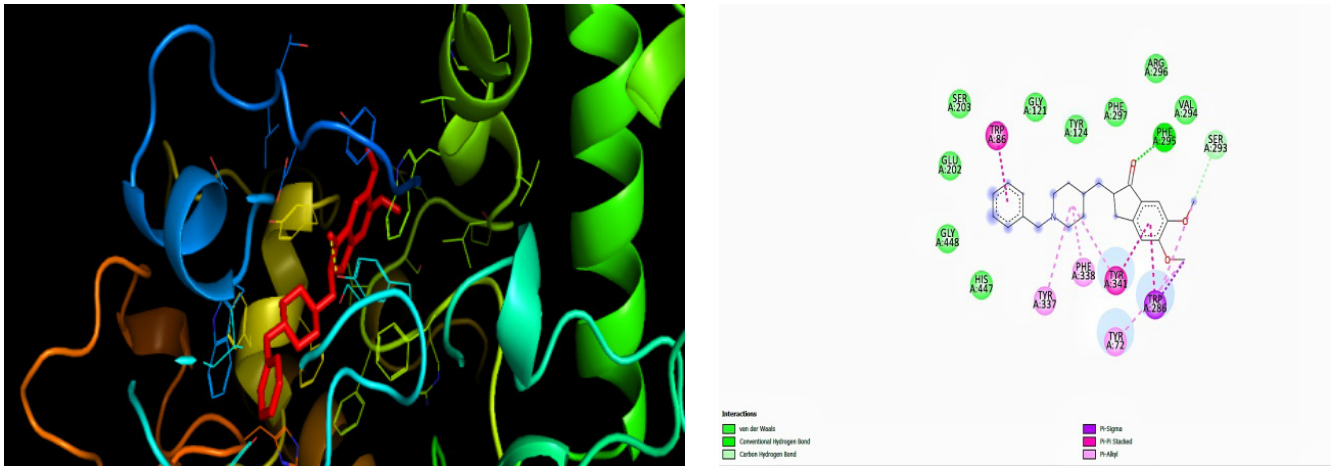


FIGURE 4 - Docking.

Based on docking energy values six drugs (Doxazosin, Oxypertine, Cyclopenthiazide, Mestranol and Terazosin) showed promising results compared with donepezil docking energy value and selected forfurther analyses. The binding interactions similar to donepezil are serine, tyrosine, tryptophan and the common binding residues are 293, 72, 86 respectively as shown in Table II. This represents the binding interactions of potential molecule with AChE.

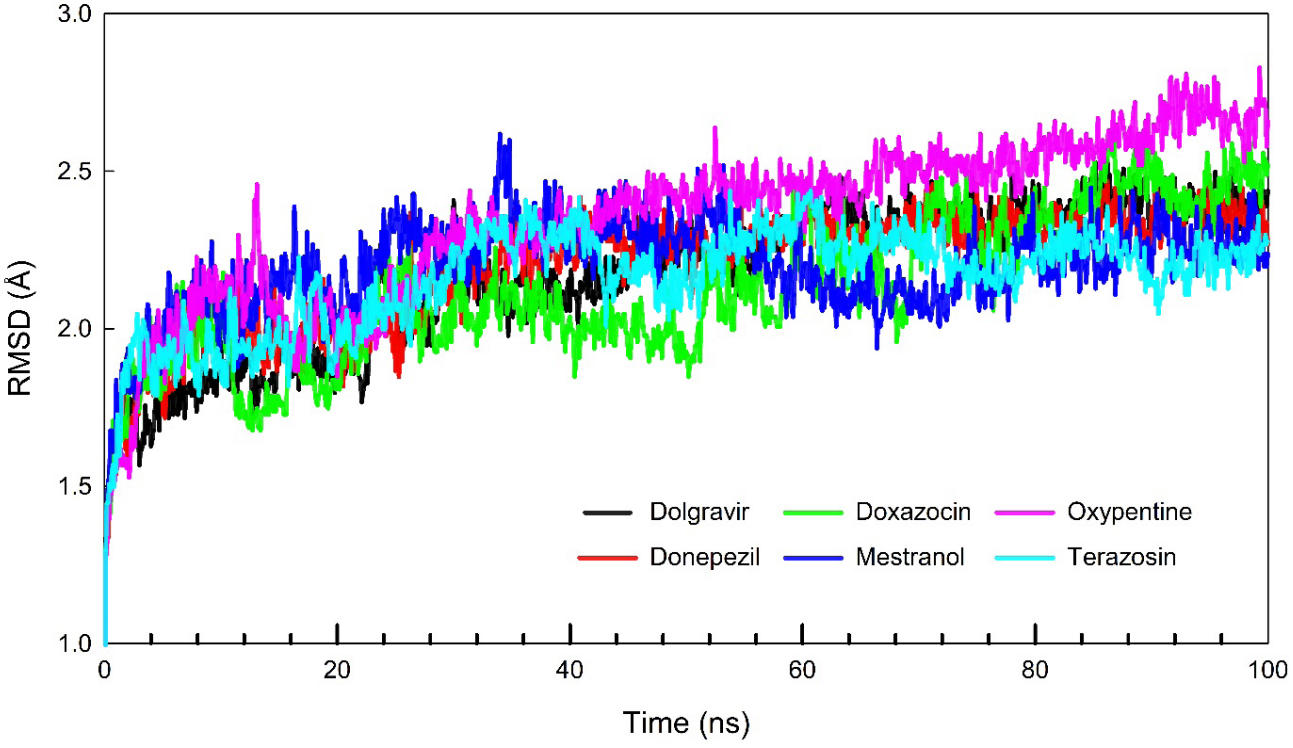
TABLE II - Docking energy values of donepezil against AChE

Sr. no	Drugs	Serine		Phenylalanine		Tyrosine					Tryptophan		Histidine	Glycine		Glutamic acid		Valine	Arginine	Dock Score
		203	293	295	297	338	72	124	337	341	86	286	447	448	121	202		294	296	
	Donepezil																			
1.	Auto Doc Vina																			-11.7
	Schrodinger																			-8.672
	Doxazosin																			
2.	Auto Doc Vina																			-10.9
	Schrodinger																			-9.164
	Oxypertine																			
3.	Auto Doc Vina	125																		-10.3
	Schrodinger																			-8.621
	Mestranol																			
4.	Auto Doc Vina															292				-10
	Schrodinger																			-8.168
	Terazosin																			
5.	Auto Doc Vina																			-9.9
	Schrodinger																			-14.64
	Cyclopenthiazide																			
6.	Auto Doc Vina													250						-10
	Schrodinger																			--

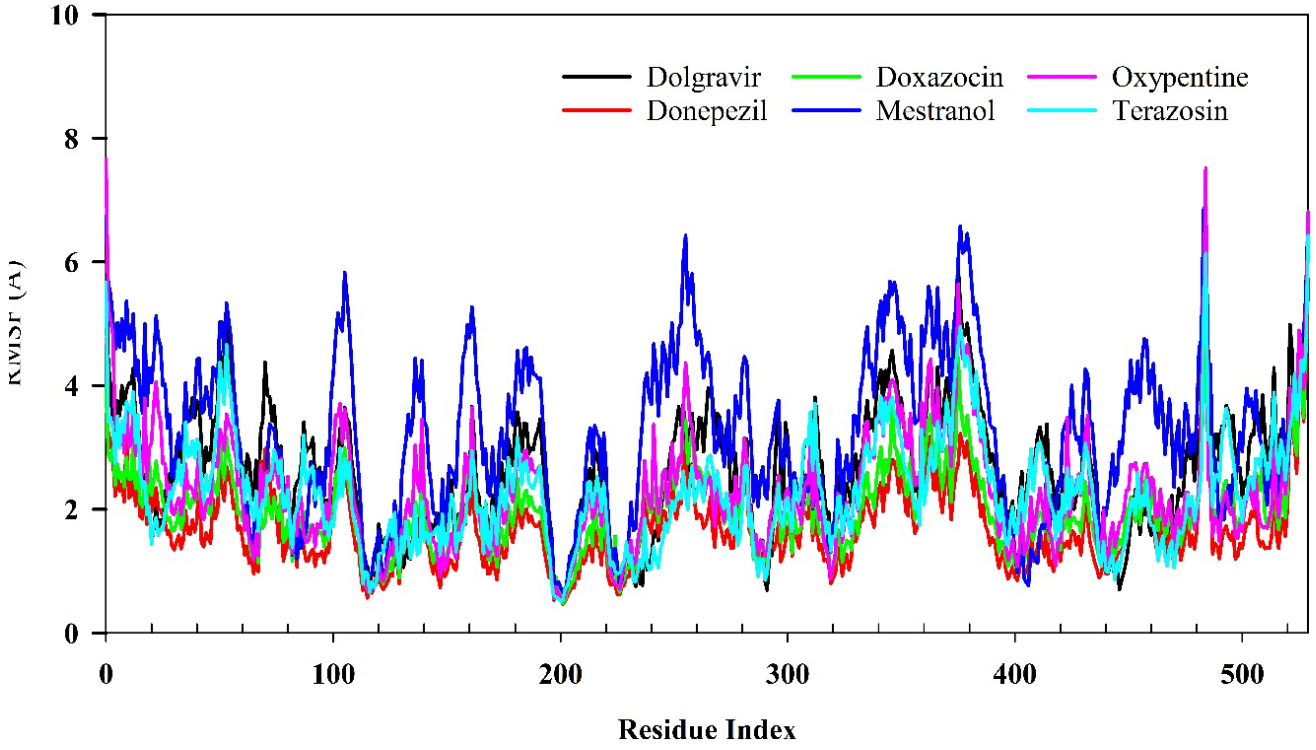
Molecular Dynamics and Post-simulation binding free energy analysis

Molecular dynamics and simulation (MD) studies were carried out in order to determine the stability and convergence of Dolutegravir, Donepezil, Doxazocin, Mestranol, Oxypentine&Terazosin with human acetylcholinesterase. Each simulation of 100 ns displayed stable conformation while comparing the root mean square deviation (RMSD) values.The Cα-backbone of Dolutegravir, Donepezil, Doxazocin, Mestranol, Oxypentine& Terazosin exhibited a deviation of 2.15 Å, 2.20 Å, 2.13 Å, 2.20 Å, 2.35 Å & 2.16 Å respectively (Figure 5A). RMSD plots are within the acceptable range signifying the stability of the ligand-bound state before and after simulation and it can also be suggested that the complexes are quite stable due to the higher affinity of the ligand. The plots for root mean square fluctuations (RMSF) displayed a significant spike of fluctuation at amino acid residuesLeucine (L) 275, Aspartic Acid (D) 385, and Glutamine (Q) 494 in Mestranol (M) and Oxypentine (O)bound proteins while the rest of the residues less fluctuating during the entire100 ns simulation (Figure 5B). The higher fluctuating residues are due to loop and turn conformation. Therefore, for RMSF plots it can be suggested that the protein structures were stable during simulation andhave flexible regions for acquiring the best conformations. The radius of gyration is the measure of the compactness of the protein.Here in this study, protein forming complex with Donepezil, Doxazocin, and Mestranol displayed less fluctuating radius of gyration (Rg) and became stable (Figure 5C).

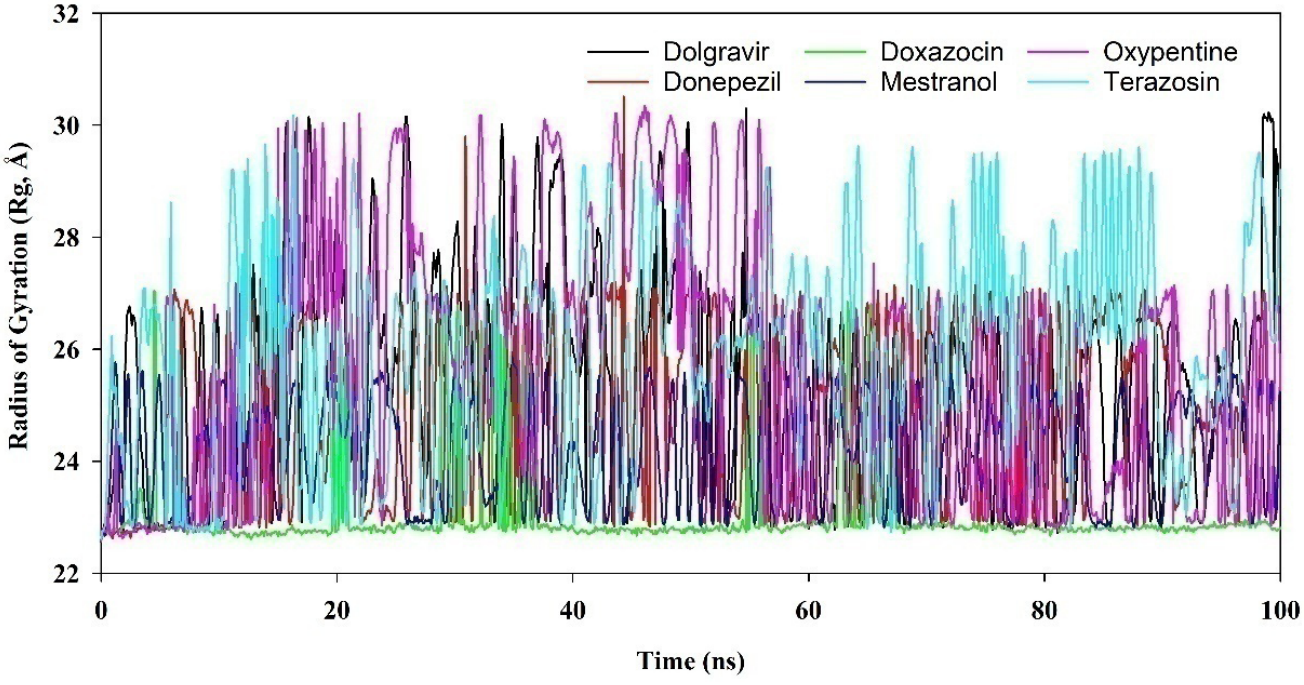
On the other hand, stable Rgwas observed in the case of protein complexes with Oxypentine, Terazosin& Dolutegravir having a little uprise of the peak conforming into less compact as compared to other complexes(Figure 5C).From the overall quality analysis from RMSD and Rg it can be suggested that Oxypentine bound to the protein targets posthumously in the binding cavities and played a significant role in the stability of the proteins.The number of hydrogen bonds formed between protein and ligand is an important factor to analyze for a stable complex throughout the simulation time. Here in this case, the number of H-bonds formed in all complexes displayed constant interactions end of the 100 ns simulation (Figure 5D). The stabilization of the ligand must be maintained via strong H-bonded interactions apart from other intermolecular interactions.



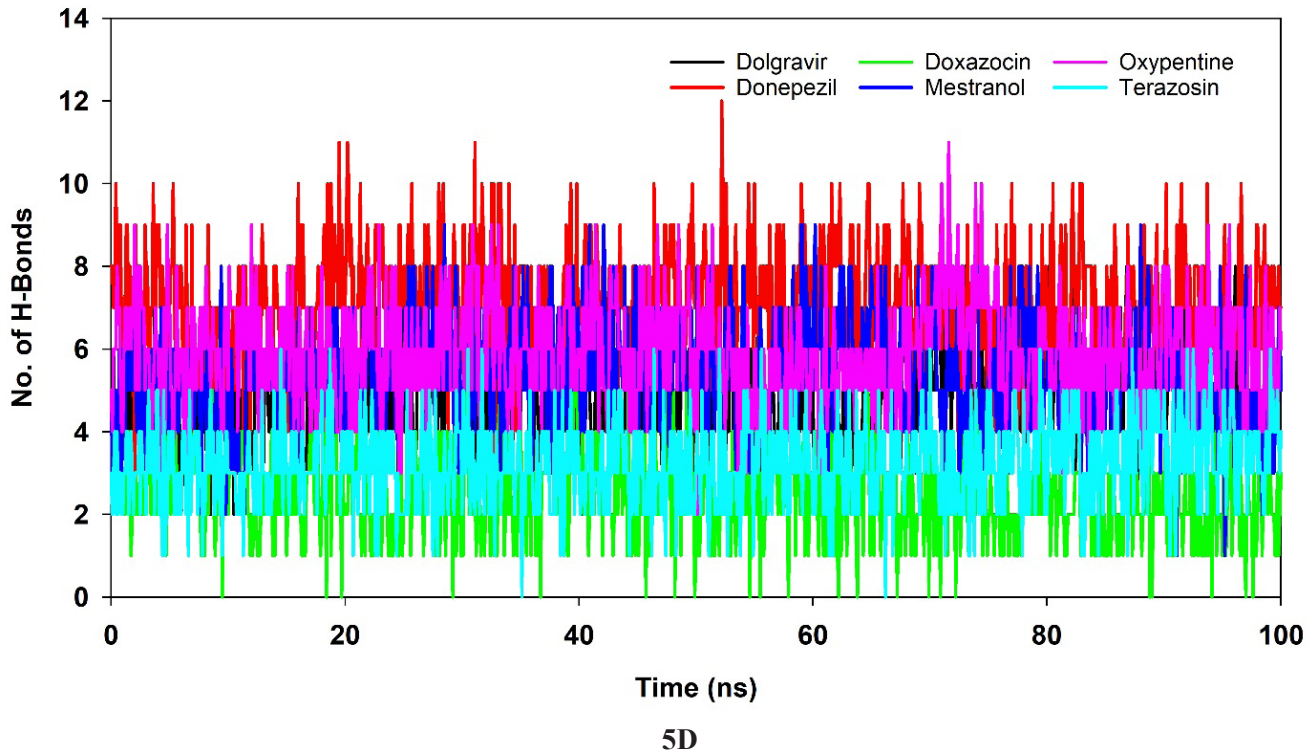
5A


Dolgravir
Donepezil
Doxazocin
Mestranol
Oxypentine
Terazosin
RMSF (Å)
Residue Index

5B


Dolgravir
Donepezil
Doxazocin
Mestranol
Oxypentine
Terazosin
Radius of Gyration (Rg, Å)
Time (ns)

5C



5D

FIGURE 5 - MD simulation analysis of 100 ns trajectories (A) RMSD of Cα backbone of protein-ligand complexes. (C) RMSF of Cα backbone of protein withDolgravir (black), Donepezil (red), Doxazocin (green), Mestranol (blue), Oxypentine(pink) &Terazosin(aqua) (C) Radius of gyration (Rg) of Cα backbone of protein Complexes (D) Formation of hydrogen bonds in Cα backbone of Protein Complexes.

MMGBSA is a popular method for calculating the binding energy of ligands to protein molecules. The estimation of the binding free energy of each of the complexes, as well as the role of other non-bonded interaction energies, were estimated. It is evidenced from Table III, the binding free energy (ΔG_{bind}) of complexes with revealed by the dynamics studies.The average binding energies of all the protein ligand complexes displayed in table (Table III). The ΔG_{bind} is influenced by of various types of non-bonded interactions, including $\Delta G_{bindCoulomb}$, $\Delta G_{bindCovalent}$, $\Delta GbindH\text{-}bond$, $\Delta G_{bindLipo}$, $\Delta G_{bindSolvGB}$ and $\Delta G_{bindvdW}$ interactions. Among all the types of interactions $\Delta G_{bindvdW}$, $\Delta G_{bindLipo}$and $\Delta G_{bind\ Coulomb}$ energies contributed most to achieve the average binding energy (Table III). In contrast, $\Delta G_{bindSolvGB}$ and $\Delta G_{bind\ Covalent}$ energies contributed the lowest to attain the final average binding energies. In addition, the values of $\Delta G_{bind\ H\text{-}bond}$ interaction of the ligands to protein complexes showed stable hydrogen bonds with the amino acid residues. In all the complexes $\Delta G_{bindSolvGB}$ and $\Delta G_{bind\ Covalent}$ showed unfavourable energy contributions and thus opposed binding (Table III). Generally, a more negative value shows stronger binding, which is clearly shown in table.

TABLE III - Binding energy calculation of complexes and non-bonded interaction energies from MMGBSA trajectories

Energies (kcal/mol)*	Dolutegravir	Donepezil	Doxazocin	Mestranol	Oxypentine	Terazosin
ΔG_{bind}	-56.84±4.09	-78.39±2.47	-57.41±3.31	-62.06±2.58	-57.98±4.62	-76.01±2.45
$\Delta G_{bindLipo}$	-24.67±1.19	-29.01±0.92	-29.49±1.25	-15.99±0.83	-18.75±0.91	-36.84±1.21
$\Delta G_{bindvdW}$	-47.10±1.61	-58.14±1.96	-55.48±2.10	-53.67±1.53	-46.00±3.32	-55.24±1.56
$\Delta G_{bindCoulomb}$	-16.43±3.54	-17.44±1.92	-18.44±3.96	22.35±7.98	-10.62±5.88	-25.64±6.17
$\Delta G_{bindHbond}$	-0.53±0.28	-1.04±0.10	-0.16±0.21	-0.29±0.18	-2.59±0.66	-0.53±0.06
$\Delta G_{bindSolvGB}$	35.25±2.28	35.17±2.63	51.54±3.89	-12.78±7.40	21.67±4.21	45.52±6.66
$\Delta G_{bindCovalent}$	2.57±1.25	3.52±0.42	0.80±0.51	3.20±2.30	2.11±1.41	1.80±0.49

ADME & formulation studies of shortlisted molecules

Donepezil, an acetylcholinesterase inhibitor used in Alzheimer's disease treatment, exhibited a log P value of 4.3 and a molecular weight of 379.5. It was administered orally in tablet or solution form, with doses ranging from 5-10mg. Doxazosin, an alpha adrenergic blocker for benign prostatic hyperplasia and high blood pressure, had a log P value of 2.5 and a molecular weight of 451.5, taken orally in tablet form at doses from 1-16mg. Oxypertine, an antipsychotic used in schizophrenia treatment, had a log P value of 4.3 and a molecular weight of 379.5, with an oral tablet dose of 20mg/day. Mestranol, an estrogen medication, exhibited a log P value of 4 and a molecular weight of 310.4, taken orally in tablet form at doses of 300-600µg. Terazosin, an alpha adrenergic receptor antagonist for high blood pressure, had a log P value of 1.4 and a molecular weight of 387.4grams per mole (g/mol), available in oral tablet and capsule forms at doses ranging from 1-10mg. Cyclopenthiazide, a thiazide diuretic used in heart failure and hypertension treatment, showed a log P value of 1.3 and a molecular weight of 379.9, administered orally in tablet or eye drop form, with doses from 0.004 to 0.014mg/kg. These drugs were assessed for their therapeutic uses, dosage forms, blood-brain barrier permeability, and predicted and experimental toxicity (as shown in Supplementary Table I&II)

Supplementary TABLE I - Drug Like properties

Sr. No	Name of Compound	Log P Value	Mol. wt	Hydrogen Bond Donar	Hydrogen Bond Accepter	R.O. A	Therapeutic Uses	Doage Form	Dose	BBB permability		Toxicity	
										Predicted	Experimental	Predicted	Experimental
1.	**Donepezil**	4.3	379.5	0	4	Oral, Transdermal	Acetylcholinesterase Inhibitor (Used to treat AD)	Tablet, orally disintegrating, Tablet, film coated, Solution	5-10mg orally, 23mg	Yes	Yes	Class 4	Nausea, Diarrhoea, Vomitting, difficulty sleeping, muscle cramps.

Supplementary TABLE I - Drug Like properties

Sr. No	Name of Compound	Log P Value	Mol. wt	Hydrogen Bond Donar	Hydrogen Bond Accepter	R.O. A	Therapeutic Uses	Doage Form	Dose	BBB permability		Toxicity	
										Predicted	Experimental	Predicted	Experimental
2.	**Doxazosin**	2.5	451.5	1	9	Oral	Alpha adrenergic blocker (Used to treat Benign Prstatic Hyperplasia, High B. P)	Oral tablet	1-16mg	No	Yes	Class 5	Hypotension, changes in heart rate, and drowsiness
3.	**Oxypertine**	4.3	379.5	1	4	Oral	Antipsychotic (Used in schizophrenia)	Oral tablet	20mg/day	Yes	Yes	Class 4	Mouse : Convulsions, effect on seizures.
4.	**Mestranol**	4	310.4	1	2	Oral	Estrogen medecation (used in birth control pills, menopausal harmone therapy, menstraul disorders)	Oral tablet	300–600 µg	Yes	Yes	Class 6	Nausea, breast tension, edema, and breakthrough bleeding ,Estrogens increase the hepatic synthesis of sex hormone binding globulin (SHBG), thyroid-binding globulin (TBG), and other serum proteins and suppress follicle-stimulating hormone (FSH) from the anterior pituitary
5.	**Terazosin**	1.4	387.4	1	8	Oral	Adrenergic Receptor (alpha) antagonist (Used to treat high BP)	Oral tablet, Capsule	1-10mg	No	Yes	Class 6	Priapism and low blood pressure, Renal Function
6.	**Cyclopenthiazide**	1.3	379.9	3	7	Oral	Thiazide Diuretic (Used in heart failure & hypertension)	Oral tablet, Eye drops	0.004 to 0.014 mg/kg	No	No	Class 4	Damage to the bladder (haemorrhagic cystitis), immunosuppression (when not desired) and alopecia

Supplementary TABLE II - Comparative study of structure-based and ligand-based drug design study

Sr. No	Drug Name	Dock score		Shape Tanimoto	Colour Tanimoto	Combo Tanimoto
		Auto Doc Vina	Schrodinger			
1.	Dolutegravir	-11.5	-8.155	0.687	0.256	0.943
2.	Doxazosin	-10.9	-9.164	0.636	0.223	0.86
3.	Oxypertine	-10.3	-8.621	0.545	0.229	0.775
4.	Cyclopenthiazide	-10	---	0.579	0.17	0.751
5.	Mestranol	-10	-8.168	0.536	0.28	0.816
6.	Terazosin	-9.9	-14.642	0.615	0.338	0.953
7.	Donepezil	-11.7	-8.672	1	1	1

DISCUSSION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that poses significant challenges in terms of treatment options. In this study, we employed a comprehensive computational approach to identify potential candidates for AD treatment among FDA-approved drugs. By combining ligand-based and structure-based methods, we screened a database of FDA-approved drugs using the human acetylcholinesterase (AChE) receptor structure as a target.

Through ligand-based screening using Swiss Similarity and Rapid Overlay of Chemical Structures (ROCS), we identified 20 drugs with promising structural similarity to Donepezil, an AChE inhibitor used in AD treatment. Further analysis using docking simulations with Autodock Vina revealed that five drugs (Doxazosin, Oxypertine, Cyclopenthiazide, Mestranol, and Terazosin) exhibited favourable binding affinities and docking energy, comparable to Donepezil.

To assess the stability of the drug-protein complexes, we conducted molecular dynamics simulations over a 100 ns timescale. The simulations demonstrated that the complexes remained stable, with minimal deviations in the root mean square deviation (RMSD) and radius of gyration (Rg). Additionally, binding free energy analysis using MM-GBSA indicated favorable binding energies for the selected drugs, further supporting their potential as candidates for AD treatment.

ADME and formulation studies provided insights into the therapeutic uses, dosage forms, blood-brain barrier permeability, and predicted toxicity of the shortlisted drugs. Among the selected candidates, Doxazosin showed promising results in terms of shape similarity, docking energy, molecular dynamics stability, and binding free energy analysis.

This study highlights the potential of drug repurposing as a strategy for AD treatment. The integration of ligand-based and structure-based computational approaches proved effective in identifying potential candidates among FDA-approved drugs. The identified drugs, especially Doxazosin, warrant further investigation and clinical assessment to validate their efficacy and safety for AD treatment. These findings contribute to the growing body of research on drug repurposing and offer potential alternatives for AD therapy, accelerating the drug discovery process and providing new avenues for addressing this debilitating disease.

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