RESEARCH ARTICLE

REVISED Molecular docking studies and molecular dynamic simulation analysis: To identify novel ATP-competitive inhibition of Glycogen synthase kinase-3β for Alzheimer's disease

[version 2; peer review: 2 approved, 1 approved with reservations, 1 not approved]

Suggala Ramya Shri, Yogendra Nayak, Sreedhara Ranganath Pai

Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India

v2 **First published:** 08 Jul 2024, **13**:773
https://doi.org/10.12688/f1000research.145391.1
Second version: 27 Aug 2024, **13**:773
https://doi.org/10.12688/f1000research.145391.2
Latest published: 27 May 2025, **13**:773
https://doi.org/10.12688/f1000research.145391.3

Abstract

Background

The discovery of an ideal and effective therapy is urgently required for the treatment of Alzheimer's disease. The main pathological hallmarks of Alzheimer's disease that appear before the clinical symptoms are neurofibrillary tangles, amyloid plaques, brain inflammation, and neuronal atrophy throughout the cerebral cortex and hippocampus. GSK-3β (Glycogen Synthase Kinase-3β) is regarded as the most important and promising target for therapeutic use because GSK-3β expression levels increase with age and are the most abundant and hyperactive in the brains of patients with Alzheimer's disease.

Methods

We used Maestro, which is Schrodinger, for our computational simulation studies. In the present work, we have used different modules that were used in previous studies with a little modification, the modules such as Protein Preparation with the help of Protein Preparation Wizard, Ligand Preparation with the help of LigPrep, for ADME (Absorption, Distribution, Metabolism and Excretion) prediction we used Qikprop, Docking studies we used Glide module, Binding energy prediction we used Prime and Molecular dynamic simulation

Open Peer Review

Approval Status ✗ ✓ ? ✓

	1	2	3	4
version 3 (revision) 27 May 2025				
version 2 (revision) 27 Aug 2024			? view	
version 1 08 Jul 2024	✗ view	✓ view		✓ view

1. **Qingchun Zhao**, Shenyang Pharmaceutical University, Shenyang, China
2. **Jigna Samir Shah**, Nirma University, Ahmedabad, India
3. **Sachchida Rai**, Banaras Hindu University, Varanasi, India
   Payal Singh, Banaras Hindu University, Varanasi, India
4. **Shvetank Bhatt**, Dr. Vishwanath Karad MIT World Peace University, Pune, India

studies by Desmond

Results

Our focus is mainly on an *in-silico* approach, focusing on library generation; we first drew an imidazo [1,5-a]pyridine-3-carboxamide (IMID 2) scaffold structure at Enamine and subjected it to a substructure search to target the receptor grid region (ATP-competitive site) of 6Y9R. They were then subjected to various screening processes. Finally, we selected nine compounds and subjected them to molecular dynamic simulation studies.

Conclusions

Nine compounds showed good results with the most stable interactions. Further experiments and studies are required to confirm these results.

Keywords
Alzheimer's disease, GSK-3β, ATP-competitive study, Protein preparation, Ligand Preparation, Qikprop, Glide module, Prime MM-GBSA, Molecular dynamic simulation.

This article is included in the Manipal Academy of Higher Education gateway.

This article is included in the Bioinformatics gateway.

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Sreedhara Ranganath Pai (ksr.pai@manipal.edu)

Author roles: Shri SR: Conceptualization, Data Curation, Formal Analysis, Methodology, Software, Writing – Original Draft Preparation; **Nayak Y**: Formal Analysis, Resources, Supervision, Writing – Review & Editing; **Ranganath Pai S**: Conceptualization, Formal Analysis, Methodology, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: Indian Council of Medical Research (ICMR), New Delhi, India, for ICMR-SRF (#2021-11506_F1) to Suggala Ramya Shri.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Shri SR, Nayak Y and Ranganath Pai S. **Molecular docking studies and molecular dynamic simulation analysis: To identify novel ATP-competitive inhibition of Glycogen synthase kinase-3β for Alzheimer's disease [version 2; peer review: 2 approved, 1 approved with reservations, 1 not approved]** F1000Research 2024, **13**:773
https://doi.org/10.12688/f1000research.145391.2

First published: 08 Jul 2024, **13**:773 https://doi.org/10.12688/f1000research.145391.1

REVISED **Amendments from Version 1**

In the revised manuscript, we have updated the abstract by incorporating details about the various modules utilised in the study, and we have added a new paragraph in the introduction discussing the selection of the core imidazole scaffold.

Any further responses from the reviewers can be found at the end of the article

Introduction

One of the greatest threats to public health is neurodegenerative diseases because there is no exact therapy. Therefore, the discovery of an ideal and effective therapy is urgently needed for the treatment of Alzheimer's disease.[1] Only five to seven cases are due to genetic mutations, and the remaining cases are due to environmental factors and sporadic mutations. The main pathological hallmarks of Alzheimer's disease that appear before the clinical symptoms are neurofibrillary tangles, amyloid plaques, brain inflammation, and neuronal atrophy throughout the cerebral cortex and hippocampus.[2] Memory loss can be elicited in Alzheimer's patients, such as episodic short-term memory impairment followed by a lack of motivation, disorganization, and impairment in solving problems, judgment, and executive functioning. In early stage impairments, such as visuospatial skills, neuropsychiatric symptoms are most common in mild and late-stage Alzheimer's disease.[3] The heritability of AD is estimated to be between 60% and 80%, and these components allow for the identification and determination of pathophysiological processes, diagnostic markers, biological targets, and new treatment targets through genomics translational studies.[4] Glycogen synthase kinase-3 (GSK-3) is a member of the protein kinase family and is widely expressed in tissues. GSK-3 is a Serine/Threonine kinase that transfers a phosphate group to either the serine or threonine residue of its substrate target.[5] GSK-3β is regarded as the most important and promising therapeutic target. The etiology and pathogenesis of AD are not completely understood. However, available treatments have failed to show novel approaches and effectiveness, and the efficacy of drugs varies from one person to another.[6] GSK-3β expression levels increase with age and are most abundant and hyperactive in the brains of patients with AD. Hence, dysregulation of GSK-3β automatically affects amyloid beta plaques, which have been previously shown in in vitro and in vivo Alzheimer's disease models.[7] GSK-3 plays a very important key role in the metabolic process and regulating structural processes in adult neurons as well as in developing neurons.[8] In the present study, we used a molecular modeling approach. For better BBB permeation, the structure was finalized based on a wet lab. So, based on this literature search, we have selected core imidazole scaffold for our study, and from that core imidazole scaffold, we have drawn sub-structures in the enamine database. Then docking studies were done on the compounds and subjected to ADME (Qikprop). A molecular dynamic simulation study was conducted on nine compounds.

Methods

Software used for this computational study

We used Maestro for our computational simulation studies; the graphical interface was Schrödinger. In the present work, we used different modules that were used in previous studies.: Protein Preparation Wizard, Ligand Preparation with the help of LigPrep, for ADME (Absorption, Distribution, Metabolism and Excretion) prediction, we used Qikprop, Docking studies we used the Glide module, and binding energy prediction we used Prime and Molecular dynamic simulation studies by Desmond.[9–11] Alternatively freely available software are AutoDock 4, ArgusLab, and Gromacs.

Selection and preparation of protein

We used PDB I.D: 6Y9R (http://www.rcsb.org/)[12] of GSK-3β, which is bound to a co-crystallized ligand with a Resolution of 2.08 Å consisting of only chain A, downloaded from the protein databank site,[10,11] and imported this PDB I. D to the Maestro interface. This is followed by preparing the protein molecule with the help of the protein preparation wizard panel of the Schrödinger suite. Alternatively, the AutoDock 4 which is freely available software can be used. The first step for protein preparation is preprocessing in the workspace the protein structure is selected then Assigned bond orders, using the CCD database, explicit hydrogen to the structure, zero-order bonds to metals, creating disulfide bonds, and optimizing missing side chain atoms by running a Prime job, fill in missing loops by running the Prime job was selected and delete waters that are further than specified distance beyond 5.00 Å from any of the het groups including ions. This is recommended for Glide and virtual screening, but Molecular Dynamics applications should keep these water as they will help the equilibrate the solvent box faster and generate het states using Epik pH: 7.5±0.0, and clicked for preprocessing. The second step is to review and modify here when a new chain, water, or het is selected to zoom to fit the selection to the workspace, select waters and hets within 5.0 Å of selected chains, and keep the remaining residues and chains as the default setting and generate a state pH of 7.5±0.0. The third step is to refine we have selected sample water orientations in addition to other groups, and the protonation states of residues and ligands were set by using PROPKA pH: 7.5 then it simulated the exact experimental conditions and clicked to automatically optimize hydroxyl, Asn, Gln, His states using ProtAssign, removing water that is further than beyond hets 3.0 Å, including ions. This is recommended for Glide and virtual screening, but Molecular Dynamics applications should keep this water as it will help

equilibrate the solvent box faster. We used the OPLS3e force field for the restrained minimization of proteins. After minimization, the glycerol and acetate ions were removed from the minimized protein.

Ligand selection and preparation

Imidazo [1,5-a]pyridine-3-carboxamide (IMID 2) scaffolds were used for this study. This scaffold possesses fewer hydrogen bond donors and improved Central Nervous System (CNS) penetration at micromolar concentrations (μM), based on studies by Buonfiglio et al.[13] To generate the focus library, we first drew an imidazo[1,5-a]pyridine-3-carboxamide (IMID 2) scaffold structure at Enamine and subjected it to a substructure search. Then, we obtained enamine realdb_IMID2_Scaffold_molecules (1400 molecules), followed by ligand preparation using LigPrep in the Schrödinger suite software (Schrödinger 2021-3).[14] Alternatively, the AutoDock 4 which is freely available software can be used. The 3D coordinates for the compounds were generated using the LigPrep tool, and the Epik module predicted the most probable charge form for the ionization state of compounds at pH: 7.5±0.0, generated tautomers and stereoisomers, determined chiralities from the 3D structure, generated at most 32 per ligand, and finally, the minimization of the drug molecule process was performed using the force field OPLS3e.[15,16]

ADME prediction

After ligand preparation, all ligands were subjected to Qikprop in the Schrödinger suite software to predict the ADME profile of the compounds. QikProp predicts the drug-like properties of all selected compounds, such as molecular weight, Hydrogen Bond Donors, Hydrogen Bond Acceptor, QPlogS (predicted aqueous solubility), QPlogPo/w (predicted octanol/water partition coefficient), QPlogBB (predicted brain/blood partition coefficient), QPlogPw (predicted water/gas partition coefficient), QPlogPC16 (predicted hexadecane/gas partition coefficient), QPlogPoct (predicted octanol/gas partition coefficient), PSA (Van der Waals surface area of polar nitrogen and oxygen atoms), QPPCaco (predicted apparent Caco-2 cell permeability), and QPPMDCK (predicted apparent MDCK cell permeability).[17]

Molecular docking studies

Here, we used the Schrödinger suite Glide module to better understand the prediction of the protein-ligand involved in assessing the fitting of all conformations of compounds at the binding site followed by ranking and modes Schrödinger Release 2021-3. The alternative freely accessible softwares are AutoDock and Gromacs. The molecular docking study process was used for assessment before molecular docking studies, and the default settings were used for the Receptor Grid generation module. The binding site was recognized using the Receptor Grid generation module, and this binding site was specified as a box of $10 \times 10 \times 10$ Å^3 centered on the centroid of the co-crystallized ligand of GSK-3β, which was taken into consideration for grid generation.[18] We subjected all prepared compounds to the standard precision (SP) scoring function mode.[19] Glide SP docking ligand sampling is in flexible mode and selected sample nitrogen inversions, sample ring conformations, and amides only penalize non-planar conformations, adding Epik state penalties to the docking score. In the output file, we selected the pose viewer file that includes the receptor, written out at most one pose per ligand, performed post-docking minimization, the number of poses per ligand to include was five, and the RMSD was computed to input ligand geometries and run the job. Finally, the results were analyzed, separated, and selected based on the VAL135 residue interaction and physicochemical properties reported in the literature. These compounds were then subjected to the prime-MMGBSA module.

MM-GBSA binding energy

MM-GBSA (molecular mechanics-generalized born energies & surface area/accessibility continuum solvation method) was used to calculate the binding free energy and binding analysis of 523 SP docked protein-ligand complexes. MM-GBSA exhibits many energy properties for proteins, ligands, and complex structures. Prime MM-GBSA analysis is based on the solvent model of VSGB 2.0, and the OPLS3e force field is used to calculate the binding affinity of the respective protein-ligand complex.[20]

Molecular dynamics simulation with desmond

This model was used to precisely predict the interaction of the ligand with 6Y9R (protein), the stability of their binding under physiological conditions, and to analyze their motion at the molecular and atomic levels. The protein-ligand complex was subjected to a system built here, and different solvent models were selected; an orthorhombic box with dimensions of 10 Å × 10 Å × 10 Å was used to determine the structure geometry to have the minimum box volume, and the checked boundary box is shown in the workspace. The next step is to load the previous system builder file and enter 100ns in simulation time, 1000frames were captured, ensemble class is NPT (Normal Pressure Temperature), 300°K temperature, pressure bar is 1, and relax model system before simulation was used for molecular dynamic simulation studies.[21] After Molecular dynamic simulation, the analysis was done with the help of the RMSD (Root Mean Square Deviation), the RMSD of ligand, RMSD of protein. RMSD is mainly used to determine the stability of the protein-ligand complex, RMSF (Root Mean Square Fluctuations), ligand-protein contacts, and ligand interaction diagram. Alternatively, NAMD molecular simulation along with VMD software can be used which is freely available.

Results and Discussion

When we searched for PDB in UniProt, we identified 89 GSK-3β human PDBs. out of 89 PDB entries, 8 PDB literature are yet to be published, and some PDBs with position 3-12 amino acids residual chains C and D are removed from my PDB selection list. PDBs that have chain A/B are taken into consideration, and we studied all the PDBs individually. Finally, we decided on PDB: 6Y9R.[25]

The co-crystal structure of 2 was in complex with PDB I. D 6Y9R, which is a GSK-3β enzyme. While INDZ was located at the hinge region, the piperidine chain was exposed to the solvent towards ARG141, Pyridine formed a hydrogen bond with LYS85, nearer to the ligand several hydration sites with residues such as THR138, ASN186, GLN185, LEU132, ASP200, and IIE62 were found to be fully explained in a previous study. The INDZ core scaffold was located in the ATP-competitive binding site of GSK-3β between the N-terminal lobe and the C-terminal lobe region of the protein, and INDZ was previously developed as a novel GSK-3β inhibitor using computational tools. The N-2 of the core and VAL135 N-H group, hydrogen at position N-1 of the core with the ASP133 carbonyl group, hydrogen of the carboxamide group, and

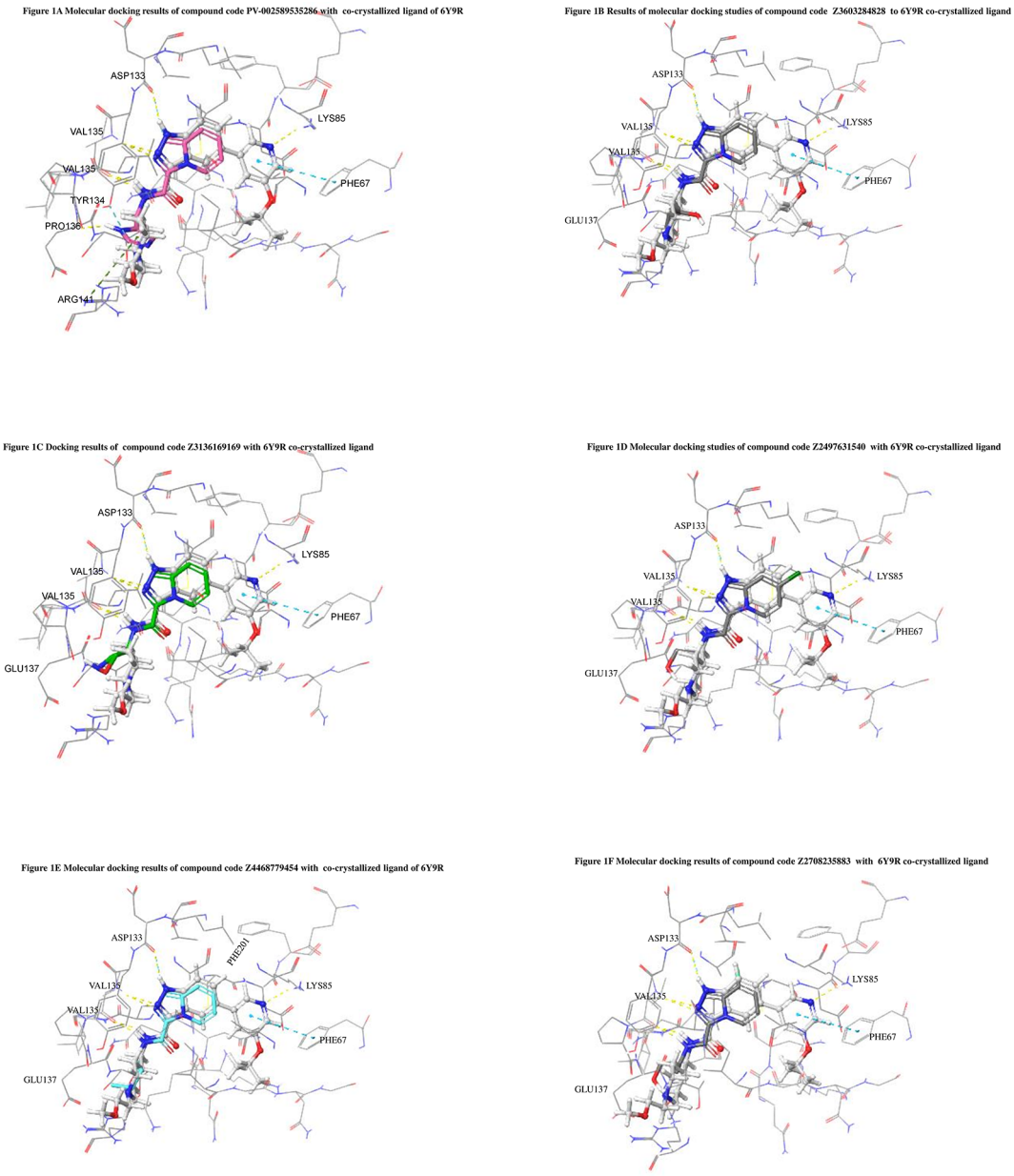


Figure 1. Overlap of co-crystal structure of 6Y9R and IMID2 substructures.

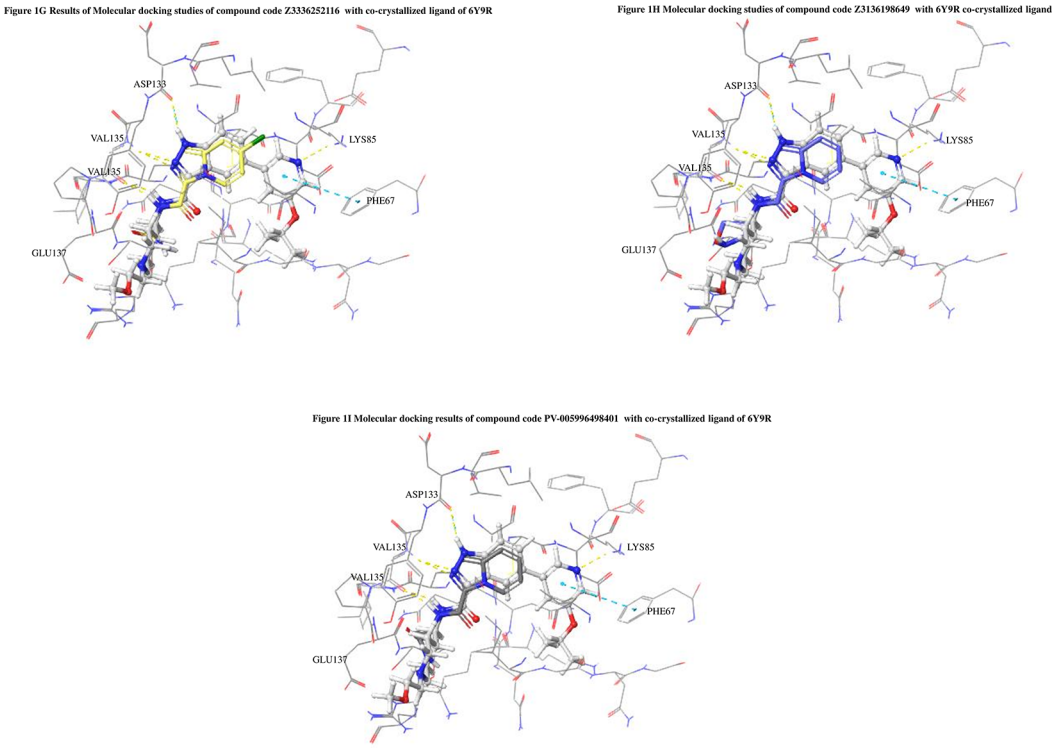


Figure 1. (continued)

VAL135 carbonyl group are important interactions. The R1 substituent appended to the core is oriented towards ARG141 in the external solvent-accessible part of the solvent, and the R2 substituent appended to the LYS85 inner cavity proximity. The indazole N-Ha was then replaced with the C-Ha group, and the ring nitrogen atoms were shuffled, resulting in the IMID2 core.[13]

The docked ligands accommodated in the ATP-binding site are similar to the co-crystal structure of the 6Y9R protein. The overlap of the co-crystal structures of the 6Y9R and IMID2 substructures is shown in Figure 1 (Figure 1A to 1I).

The docking of ligands to a particular receptor grid generated a region of the protein 6Y9R. In this study, SP docking was used. After docking, the compounds were separated and selected based on the VAL135 residue interaction in the literature. Therefore, based on the VAL135 N-H group {the H group of the VAL135 protein should show direct interaction with the nitrogen group of the ligand}. Through this process, we finalized 523 molecules of the IMID2 scaffold. Compounds with good SP-scores are listed in Table 1.

ADME prediction

The Absorption, Distribution, Metabolism, and Excretion (ADME) or drug-likeness parameters are the most important and helpful parameters for screening compounds after SP docking studies during the drug discovery process.[22] Generally, for CNS drugs, the physicochemical properties range from molecular weight 100 Da to 450 Da, hydrogen bond acceptors range from 0 to 5, hydrogen bond donor values range from 0 to 3, and the topological polar surface area value is $\leq$76 Å2.[23] The results of this study are listed in Table 2. Whereas the previous ADME properties of 13 trial compounds with an IMID2 core scaffold molecular weight of 393.48, the hydrogen bond donor was 1. In the14 trial compound of the IMID2 core scaffold, the molecular weight was 451.56, and the hydrogen bond donors were 1. In the 15 trial compounds of the IMID2 core scaffold, the molecular weight was 336.39, and the hydrogen bond donors were 1. In 16 trials of the IMID2 core scaffold, the molecular weight was 394.47, and the number of hydrogen bond donors was 1.[13]

Molecular dynamic simulation analysis

Compounds were selected based on the screening process described above. Only short-listed compounds were subjected to desmonds for molecular dynamic simulations. These studies provide information related to the stability and flexibility of the molecular docking complexes. We performed this study using the MM-GBSA. This study provides information

Table 1. Intermolecular interactions with amino acids and docking score of the top 9 selected compounds.

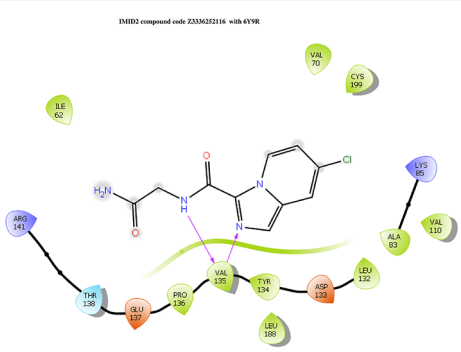
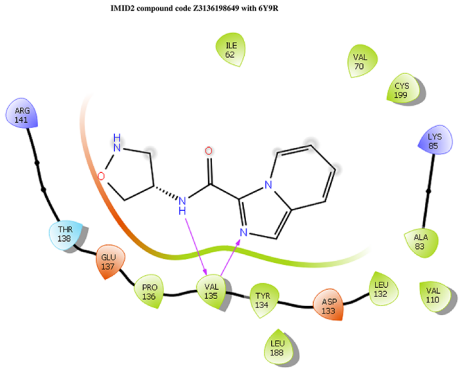
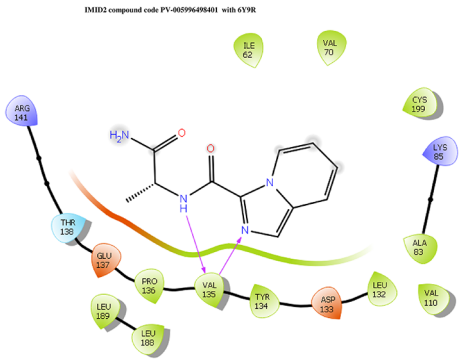
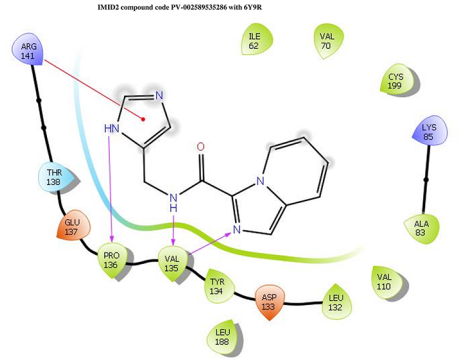
Name	Docking score	Structure	Bonded interaction	Non-bonded interaction
Z3336252116	-8.535		H-bond: VAL135,	ILE62, ARG141, THR138, GLU137, PRO136, TYR134, LEU188, ASP133, LEU132, ALA83, VAL110, LYS85, CYS199, VAL70
Z3136198649	-8.285		H-bond: VAL135	ILE62, ARG141, THR138, GLU137, PRO136, TYR134, LEU188, ASP133, LEU132, ALA83, VAL110, LYS85, CYS199, VAL70
PV-005996498401	-8.132		H-bond: VAL135	ARG141, THR138, GLU137, PRO136, LEU189, LEU188, TYR134, ASP133, LEU132, VAL110, ALA83, LYS85, CYS199, VAL70, ILE62
PV-0025895352867	-7.541		H-bond: VAL135, PRO136 PI-cation: ARG141	VAL110, ALA83, LYS85, CYS199, VAL70, ILE62, LEU132, ASP133, TYR134, LEU188, GLU137, THR138

Table 1. *Continued*

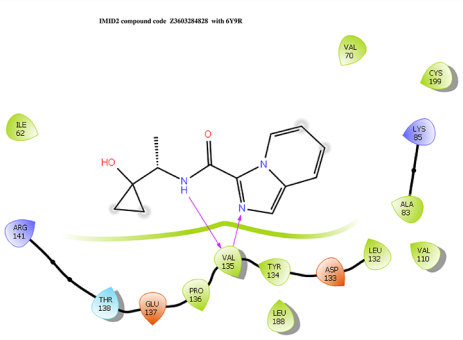
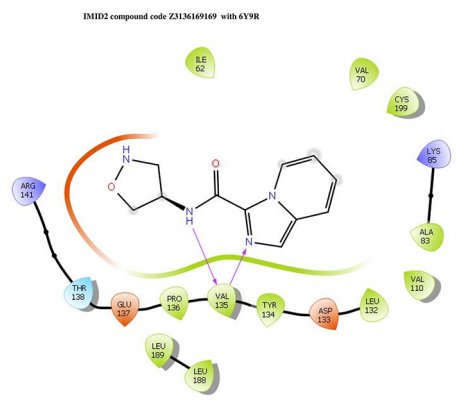
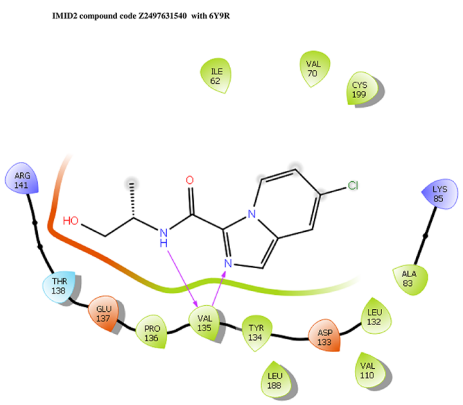
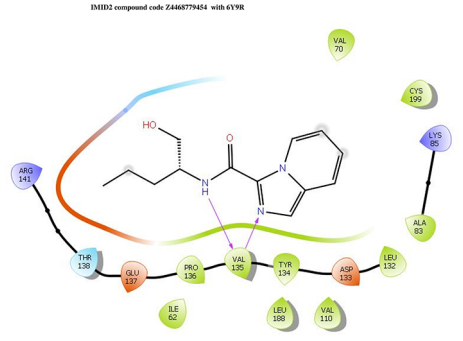
Name	Docking score	Structure	Bonded interaction	Non-bonded interaction
Z3603284828	-7.765		H-bond: VAL135	ILE62, ARG141, THR138, GLU137, PRO136, TYR134, LEU188, ASP133, LEU132, ALA83, VAL110, LYS85, CYS199, VAL70
Z3136169169	-8.268		H-bond: VAL135	ARG141, THR138, GLU137, PRO136, LEU189, LEU188, TYR134, ASP133, LEU132, VAL110, ALA83, LYS85, CYS199, VAL70, ILE62
Z2497631540	-7.967		H-bond: VAL135	ARG141, THR138, GLU137, PRO136, TYR134, LEU188, ASP133, LEU132, VAL110, CYS199, ALA83, LYS85, VAL70, ILE62
Z4468779454	-7.306		H-bond: VAL135	ARG141, THR138, GLU137, PRO136, TYR134, LEU188, ASP133, LEU132, VAL110, CYS199, ALA83, LYS85, VAL70, ILE62

Table 1. *Continued*

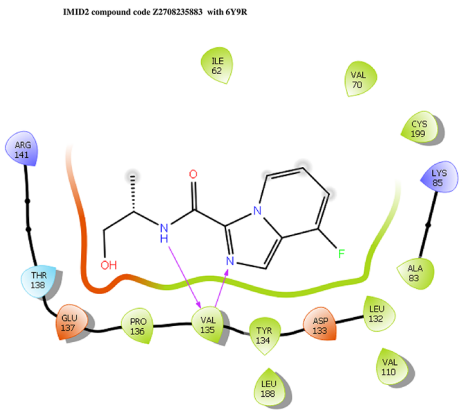
Name	Docking score	Structure	Bonded interaction	Non-bonded interaction
Z2708235883	-7.961		H-bond: VAL135	ARG141, THR138, GLU137, PRO136, TYR134, LEU188, ASP133, LEU132, VAL110, CYS199, ALA83, LYS85, VAL70, ILE62

Table 2. MM-GBSA score and Qikprop ADME prediction results of the top-9 selected compounds.

Name	MM-GBSA score	Molecular weight	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Polar Surface Area
Z3336252116	-36.94	252.660	2	3	105.021
Z3136198649	-40.67	232.243	2	4	81.022
PV-005996498401	-33.07	232.243	2	3	101.470
PV-002589535286	-37.94	241.252	2	5.5	78.034
Z3603284828	-40.91	245.282	2	3	72.320
Z3136169169	-40.53	232.243	2	4	81.001
Z2497631540	-42.12	253.688	2	5.7	73.124
Z4468779454	-46.04	247.296	2	5.7	70.083
Z2708235883	-39.16	237.233	2	5.7	73.152

related to protein-ligand interactions, RMSD variation can be assessed, and RMSF fluctuations can be assessed for protein-ligand complexes. The complex was regarded as stable if it fell within the 3 Å range. These are the compounds

IMID2 PV-002589535286: During MD simulations, the ligand-protein complex showed hydrogen-bonded interactions. The protein backbone and ligand structure exhibited higher RMSD fluctuations over the first 0-20nsec. The protein backbone and ligand fluctuations stayed within the range of 0.6 Å and 1.3 Å over the last 80nsec. The amino acid residue VAL135 formed a 95% direct hydrogen bonding interaction with the amide carbonyl, the amino acid residue VAL135 formed 42% direct hydrogen bonding interaction with imidazole in the ring structure, the amino acid residue TYR134 had 33% direct hydrogen bonding interaction, and ARG141 had a 72% PI-cation interaction with 1H-imidazole. The amide carbonyl was exposed to an H_2O molecule through which it interacted with residue ILE62 (49%), as shown in Figure 2; however, it was reported in **13** triads (previous literature) that the amide carbonyl was exposed to water-mediated contacts with residues such as ILE62 and GLN185 for the IMID2 core scaffold. The 16-triad isopropoxyl group confirmed the probable water molecule interaction with the residue ASP200. The **17** triads showed an indirect interaction between the ortho substituent and residue ASN186. In the 18 triad, the di-F phenyl group was orientated towards LYS85, thus promoting an electrostatic interaction. However, VAL135 interaction formed direct hydrogen bonding interactions with amide carbonyl in all 13, 16, 17, and 18 trial compounds of previous IMID2 scaffolds.[13]

Interaction diagram of 6Y9R with IMID2 compound code PV-002589535286 was observed during RMSD, Ligand interaction with amino acid residues of Protein Contact during Molecular dynamics simulation, protein–ligand contacts (cont.) (On the x-axis trajectory frame, the number is present, and amino acid residues are seen on the y-axis. The amino acid residues were in greater contact with ligands in the trajectory frame that appeared as a dark color shade). The

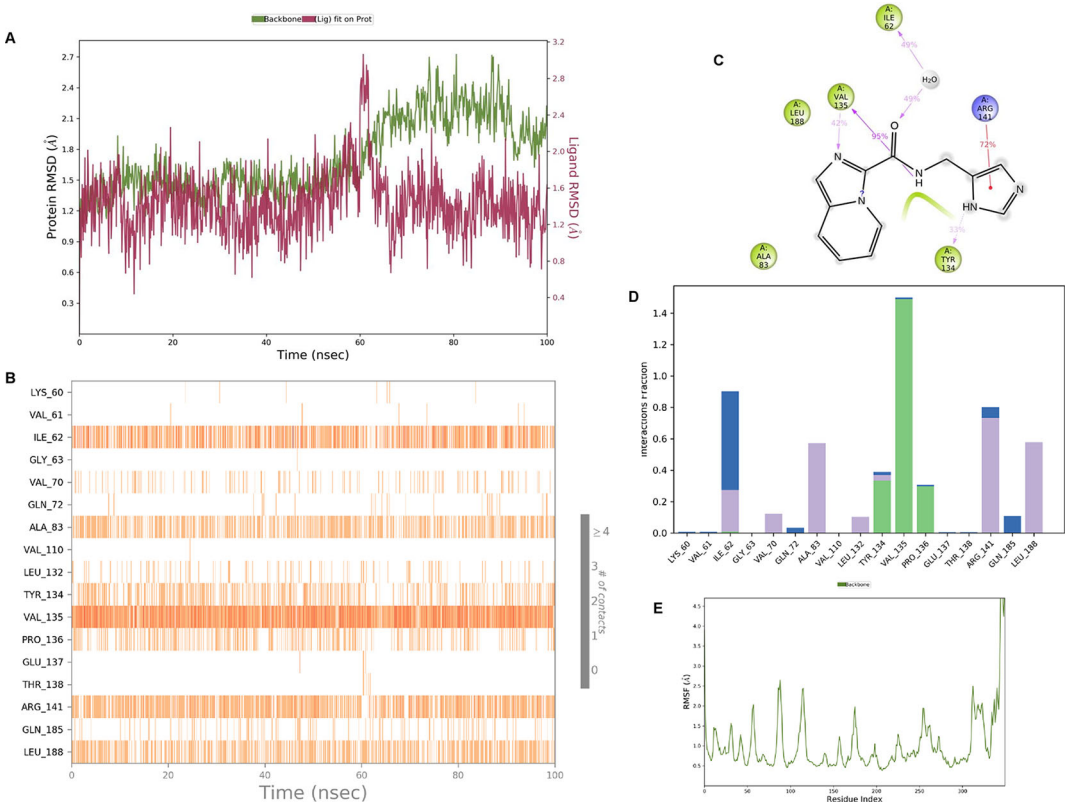


Figure 2. Pictorial representation of 6Y9R interactions with the IMID2 compound code PV-002589535286 monitored during the course of MD Simulation trajectory (A) Protein-Ligands RMSD; (B) Protein-Ligand Contacts; (C) Ligand-Protein Contacts; (D) Protein-Ligand Contacts described as histogram; (E) Protein RMSF.

VAL135 interaction fraction approached 1.4, whereas the ASP133 interaction fraction was missing in comparison to the core IMID2 scaffold from the previous literature. Protein root mean square fluctuation (RMSF) is mostly helpful for predicting changes that occur locally along the enzyme chain. These peaks provide information on how much the protein fluctuated during the molecular dynamic simulation study.

IMID2 compound code Z3603284828: During the MD simulations, the ligand-protein complex exhibited hydrogen-bonded interactions. The protein backbone and ligand structure exhibited higher RMSD fluctuations during the first 20 ns. The protein backbone and ligand fluctuations stayed within the range of 0.3 Å and 0.6 Å over the last 70 nsec shown in Figure 3. The amino acid residue VAL135 formed 93% direct hydrogen bonding interaction with the amide carbonyl, the amino acid residue VAL135 formed 55% direct hydrogen bonding interaction with imidazole, and the amino acid residue ILE62 formed 35% water-mediated direct hydrogen bonding interaction with 1-hydroxycyclopropyl.

Interaction diagram of 6Y9R with IMID2 compound code Z3603284828 observed during RMSD, Ligand interaction with amino acid residues of Protein Contact during Molecular dynamics simulation, protein–ligand contacts (cont.) (On the x-axis, a trajectory frame with a number is present, and amino acid residues are seen on the y-axis. The amino acid residues that were in greater contact with ligands in the trajectory frame appeared as a dark color shade). The VAL135 interaction fraction approached 1.4, whereas the ASP133 interaction fraction was missing in comparison to the core IMID2 scaffold from previous literature, and protein RMSF is mostly helpful for predicting the changes that occur locally along the enzyme chain. These peaks provide information on how much the protein fluctuated during the molecular dynamic simulation study.

IMID2 compound code Z3136169169: During the MD simulations, the ligand-protein complex exhibited hydrogen-bonded interactions. The protein backbone and ligand structure exhibited higher RMSD fluctuations during the first 20 ns. The protein backbone and ligand fluctuations stayed within the range of 0.8 Å and 0.3 Å over the last 70 nsec shown in Figure 4. The amide carbonyl is exposed to hydrogen bonding and interacts with residue VAL135, and the amino acid residue VAL135 forms 76% direct hydrogen bonding interactions with imidazole.

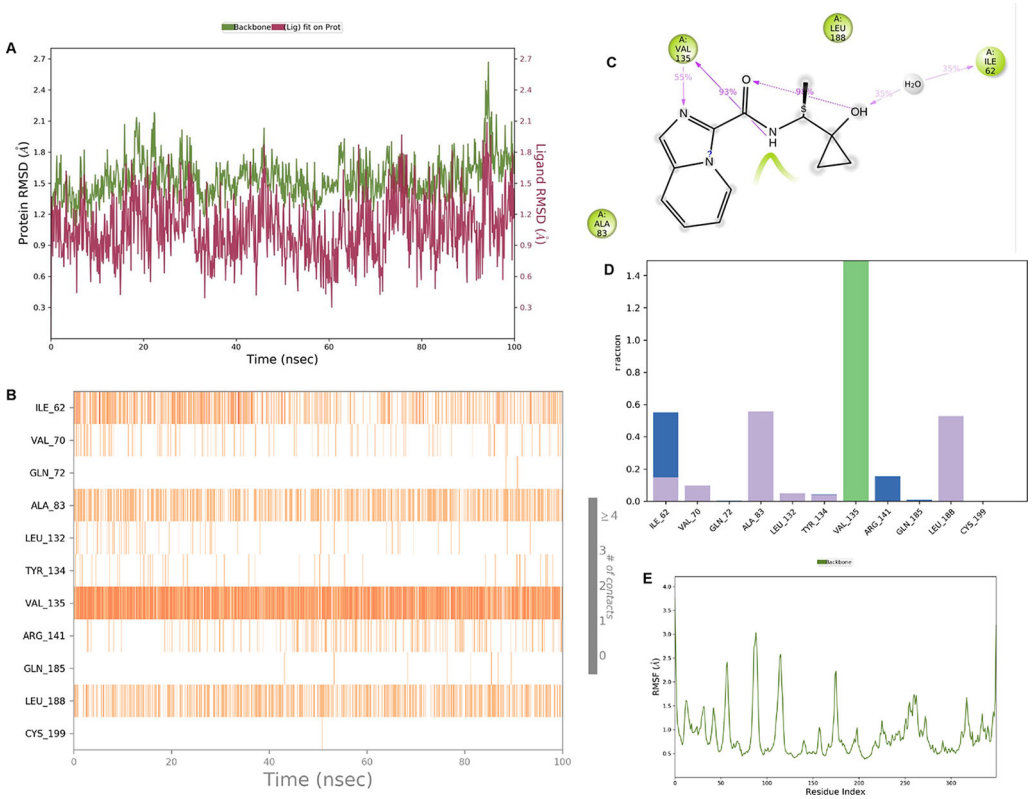


Figure 3. Pictorial representation of 6Y9R interaction with the IMID2 compound code Z3603284828 monitored during the course of MD Simulation trajectory (A) Protein-Ligands RMSD; (B) Protein-Ligand Contacts; (C) Ligand-Protein Contacts; (D) Protein-Ligand Contacts described as histogram; (E) Protein RMSF.

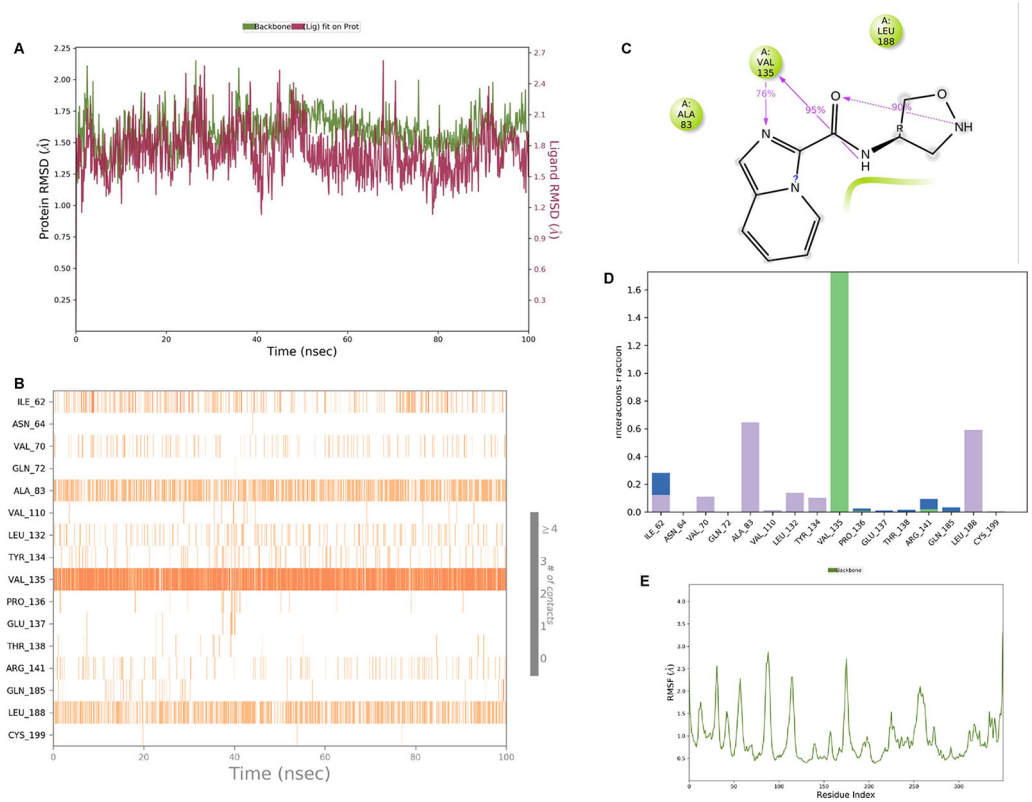


Figure 4. Pictorial representation of 6Y9R interaction with the IMID2 compound code Z3136169169 monitored during the course of Molecular Dynamic Simulation trajectory (A) Protein-Ligand RMSD; (B) Protein-Ligands Contacts; (C) Ligand-Protein Contacts; (D) Protein-Ligand Contacts described as histogram; (E) Protein RMSF.

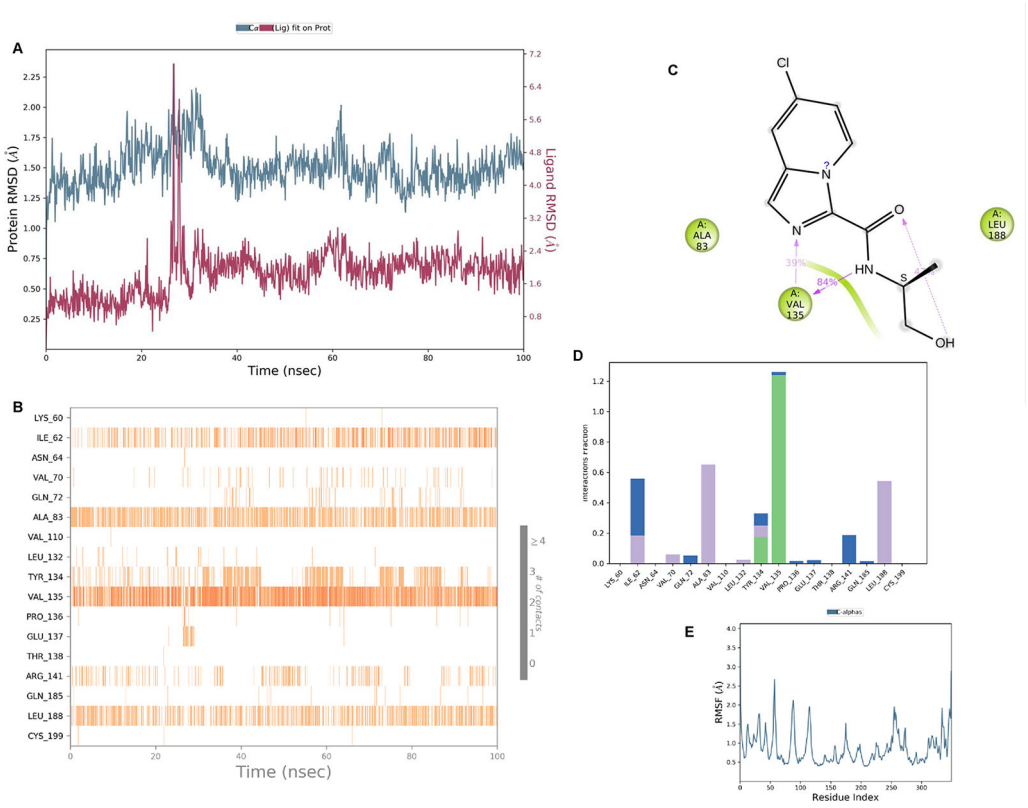


Figure 5. Pictorial representation of 6Y9R interaction with the IMID2 compound code Z2497631540 monitored during the course of MD Simulation trajectory (A) Protein-Ligand RMSD; (B) Protein-Ligand Contacts; (C) Ligand-Protein contacts; (D) Protein-Ligand Contacts described as histogram; (E) Protein RMSF.

Interaction diagram of 6Y9R with IMID2 compound code Z3136169169 observed during RMSD, Ligand interaction with amino acid residues of Protein Contact during Molecular dynamics simulation, protein–ligand contacts (cont.) (On the x-axis trajectory frame, the number is present, and amino acid residues are seen on the y-axis. The amino acid residues that were in greater contact with ligands in the trajectory frame appeared as a dark color shade). The VAL135 interaction fraction approached 1.6, whereas the ASP133 interaction fraction was missing in comparison to the core IMID2 scaffold from previous literature and protein RMSF is mostly helpful for predicting the changes that occur locally along with the enzyme chain. These peaks provide information on how much the protein fluctuated during the molecular dynamic simulation study.

IMID2 compound code Z2497631540: During the MD simulations, the ligand-protein complex exhibited hydrogen-bonded interactions. The protein Cα and ligand structures exhibited higher RMSD fluctuations over the first 20 ns. The protein backbone and ligand fluctuations stayed within the range of 0.4 Å and 3.7 Å over the last 70 nsec shown in Figure 5. The amino acid residue VAL135 formed 84% direct hydrogen bonding interactions with the amide carbonyl, and the amino acid residue VAL135 formed 39% direct hydrogen bonding interactions with imidazole in the ring structure.

Interaction diagram of 6Y9R with IMID2 compound code Z2497631540 observed during RMSD, Ligand interaction with amino acid residues of Protein Contact during Molecular dynamics simulation, protein–ligand contacts (cont.) (On the x-axis, a trajectory frame with a number is present, and amino acid residues are seen on the y-axis. The amino acid residues that were in greater contact with ligands in the trajectory frame appeared as a dark color shade). The VAL135 interaction fraction approached 1.2, whereas the ASP133 interaction fraction was missing in comparison to the core IMID2 scaffold from previous literature, and protein RMSF is mostly helpful for predicting the changes that occur locally along the enzyme chain. These peaks provide information on how much the protein fluctuated during the molecular dynamic simulation study.

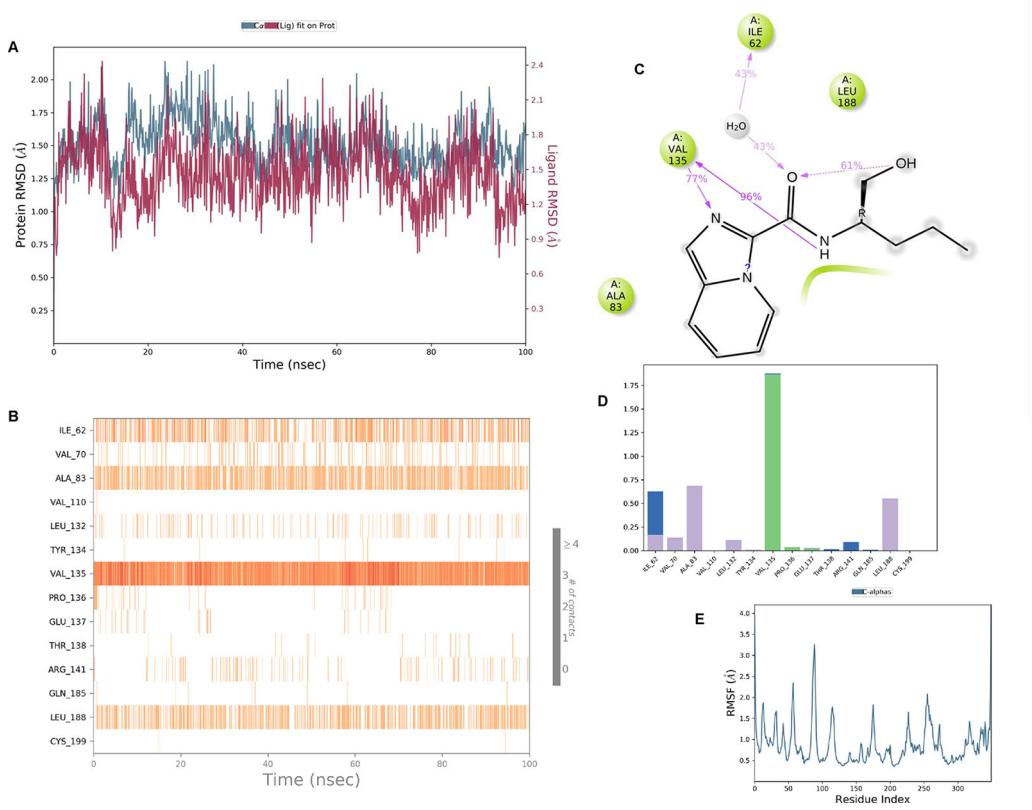


Figure 6. Pictorial representation of 6Y9R interaction with the IMID2 compound code Z4468779454 monitored during the course of Molecular Dynamic Simulation trajectory (A) Protein-Ligand RMSD; (B) Protein-Ligand contacts; (C) Ligand-Protein contacts; (D) Protein-Ligand contacts described as histogram; (E) Protein RMSF.

IMID2 compound code Z4468779454: During the MD simulations, the ligand-protein complex exhibited hydrogen-bonded interactions. The protein Cα and ligand structures exhibited higher RMSD fluctuations over the first 20 ns. The protein backbone and ligand fluctuations stayed within the range of 0.5 Å and 0.4 Å over the last 70 nsec shown in Figure 6. The amino acid residue VAL135 formed a 96% direct hydrogen bonding interaction with the amide carbonyl, the amino acid residue VAL135 formed a 77% direct hydrogen bonding interaction with imidazole, and the amide carbonyl was exposed to the H_2O molecule through which it interacted with residue ILE62, with 43% and 61% interaction with 1-hydroxypentan-2-yl.

Interaction diagram of 6Y9R with IMID2 compound code Z4468779454 observed during RMSD, Ligand interaction with amino acid residues of Protein Contact during Molecular dynamics simulation, protein–ligand contacts (cont.) (On the x-axis trajectory frame, the number is present, and amino acid residues are seen on the y-axis. The amino acid residues were in greater contact with ligands in the trajectory frame that appeared as a dark color shade). The VAL135 interaction fraction approached 1.75, whereas the ASP133 interaction fraction was missing in comparison to the core IMID2 scaffold from previous literature, and protein RMSF is mostly helpful for predicting the changes that occur locally along the enzyme chain. These peaks provide information on how much the protein fluctuated during the molecular dynamic simulation study.

IMID2 compound code Z2708235883: During the MD simulations, the ligand-protein complex exhibited hydrogen-bonded interactions. The protein backbone and ligand structure exhibited higher RMSD fluctuations during the first 20 ns. The protein Cα and ligand fluctuations stayed within the range of 0.5 Å and 0.2 Å over the last 70 nsec. The amino acid residue VAL135 formed 93% direct hydrogen bonding interactions with the amide carbonyl, and the amino acid residue VAL135 formed 53% direct hydrogen bonding interactions with imidazole in the ring structure, as shown in Figure 7.

Interaction diagram of 6Y9R with IMID2 compound code Z2708235883 observed during RMSD, Ligand interaction with amino acid residues of Protein Contact during Molecular dynamics simulation, protein–ligand contacts (cont.)

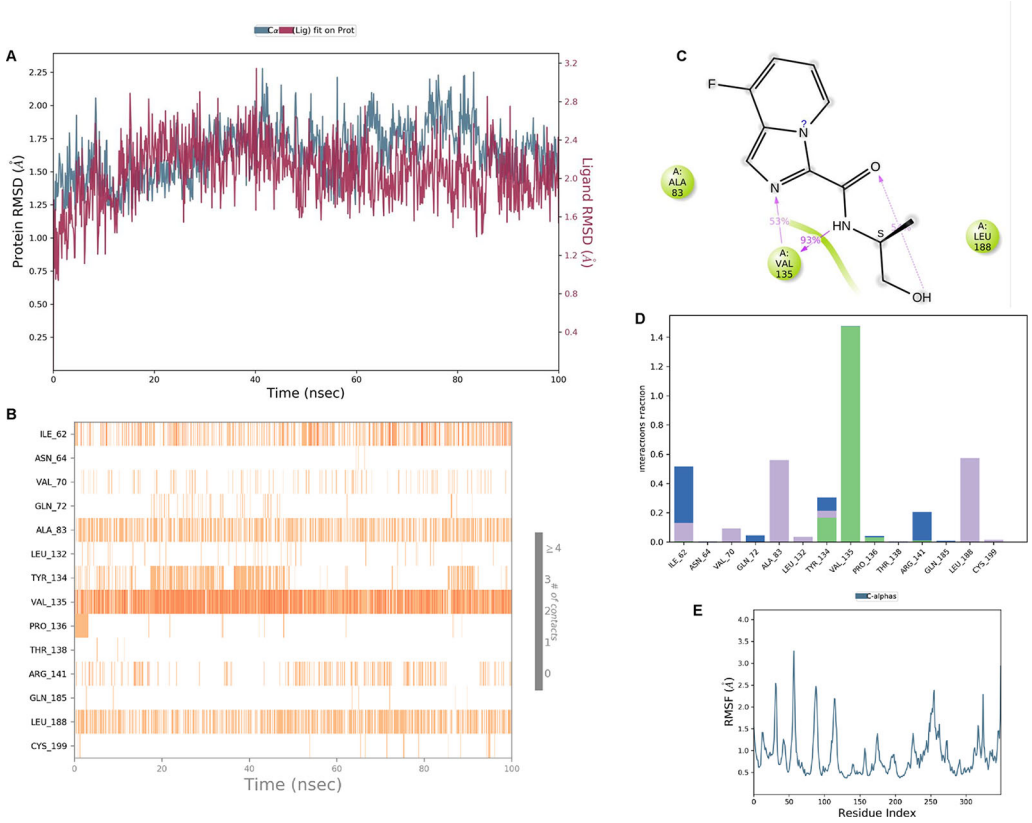


Figure 7. Pictorial representation of 6Y9R interaction with the IMID2 compound code Z2708235883 monitored during the course of MD Simulation trajectory (A) Protein-Ligand RMSD; (B) Protein-Ligand Contacts; (C) Ligand-Protein Contacts; (D) Protein-Ligand Contacts described as histogram; (E) Protein RMSF.

(On the x-axis trajectory frame, the number is present, and amino acid residues are seen on the y-axis. The amino acid residues that have been in more contact with ligands in the trajectory frame appear to have a dark color shade). The VAL135 interaction fraction approached 1.4, whereas the ASP133 interaction fraction was missing in comparison to the core IMID2 scaffold from previous literature, and protein RMSF is mostly helpful for predicting the changes that occur locally along the enzyme chain. These peaks provide information on how much the protein fluctuated during the molecular dynamic simulation study.

IMID2_compound code Z3336252116: During MD simulations, the ligand-protein complex showed hydrogen-bonded interactions. The protein backbone and ligand structure exhibited higher RMSD fluctuations over the first 0-20nsec. The protein backbone and ligand fluctuations stayed within the range of 0.6 Å and 2.2 Å over the last 80nsec. The amino acid residue VAL135 formed 93% direct hydrogen bonding interactions with the amide carbonyl, and the amino acid residue VAL135 formed 48% direct hydrogen bonding interactions with imidazole in the ring structure, which was exposed to the H_2O molecule through which it interacted with residue ILE62 (43%), as shown in Figure 8.

Interaction diagram of 6Y9R with IMID2_compound code Z3336252116 observed during RMSD, Ligand interaction with amino acid residues of Protein Contact during Molecular dynamics simulation, protein–ligand contacts (cont.) (On the x-axis trajectory frame, the number is present, and amino acid residues are seen on the y-axis. The amino acid residues that have been in more contact with ligands in the trajectory frame appear to have a dark color shade). The VAL135 interaction fraction approached 1.5, whereas the ASP133 interaction fraction was missing in comparison to the core IMID2 scaffold from the previous literature. Protein RMSF is mostly helpful for predicting changes that occur locally along the enzyme chain. These peaks provide information on how much the protein fluctuated during the molecular dynamic simulation study.

IMID2 compound code Z3136198649: During MD simulations, the ligand-protein complex showed hydrogen-bonded interactions. The protein backbone and ligand structure exhibited higher RMSD fluctuations over the first 0-20nsec.

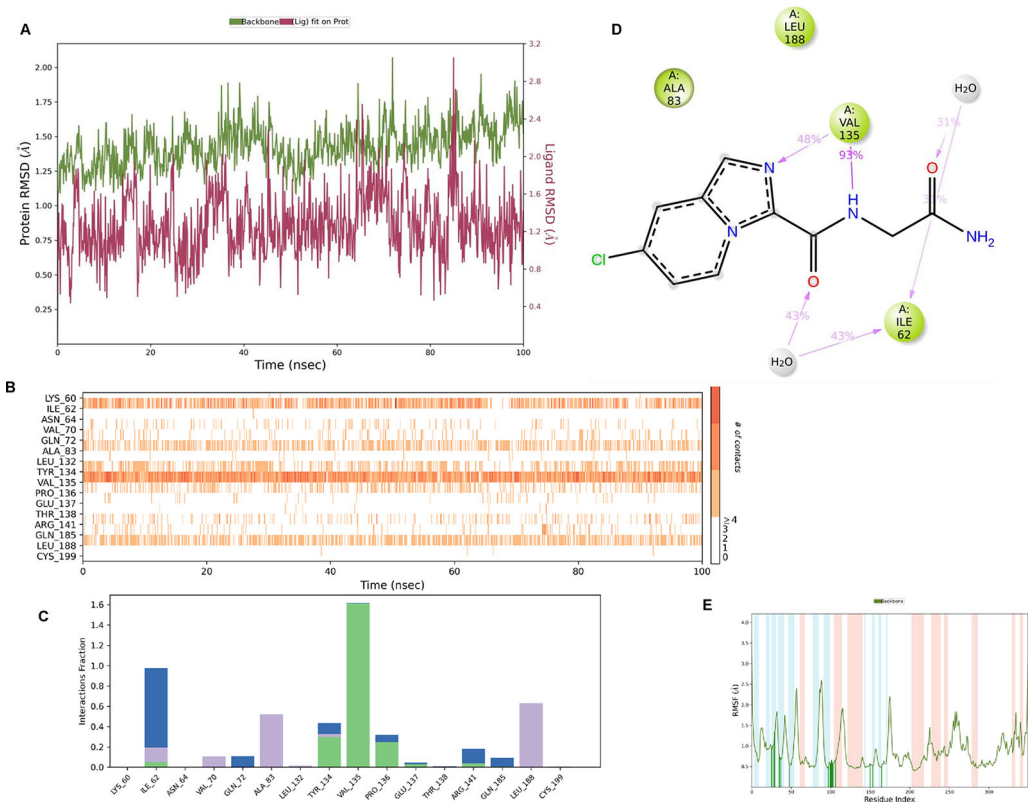


Figure 8. **Pictorial representation of 6Y9R interaction with the IMID2 compound code Z3336252116 monitored during the course of MD Simulation trajectory (A) Protein-Ligand RMSD; (B) Protein-Ligand Contacts; (C) Protein-Ligand Contacts described as histogram; (D) Ligand-Protein Contacts; (E) Protein RMSF.**

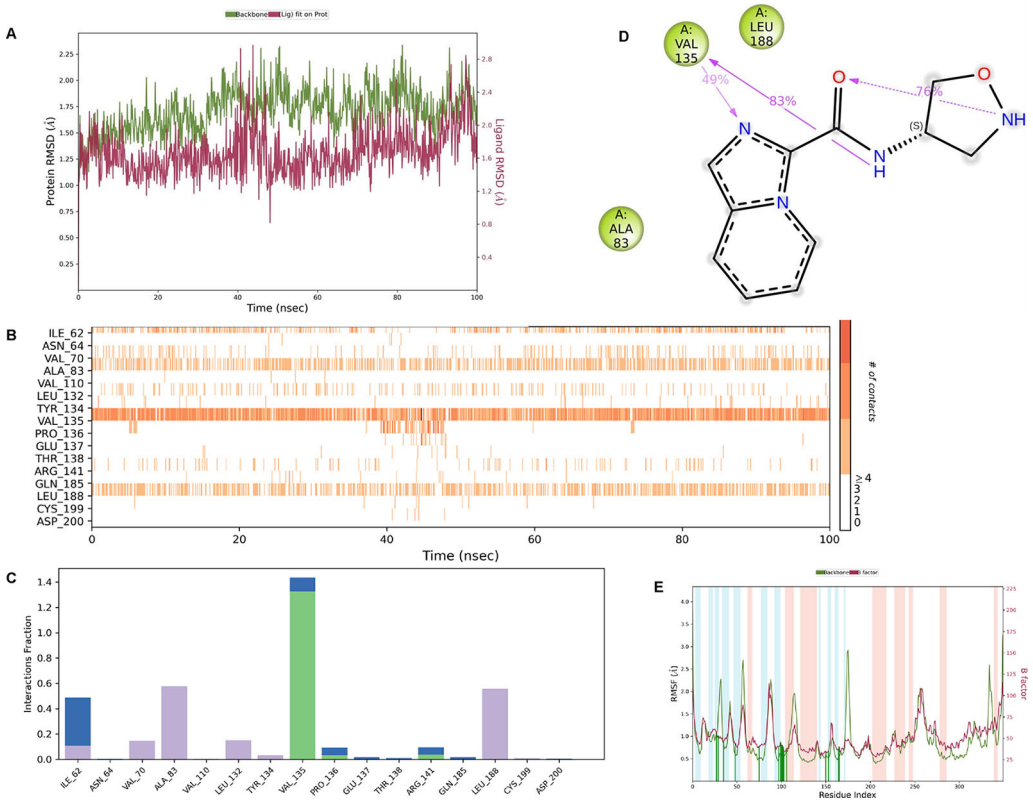


Figure 9. **Pictorial representation of 6Y9R interactions with the IMID2 compound code Z3136198649 monitored during the course of Molecular Dynamic Simulation trajectory (A) Protein-Ligands RMSD; (B) Protein-Ligand Contacts; (C) Protein-Ligand Contacts described as histogram; (D) Ligand-Protein Contacts; (E) Protein RMSF.**

The protein backbone and ligand fluctuations stayed within the range of 0.7 Å and 0.5 Å over the last 80nsec. The amino acid residue VAL135 formed 83% direct hydrogen bonding interaction with amide carbonyl, and the amino acid residue VAL135 formed 49% direct hydrogen bonding interaction with imidazole in the ring structure given in Figure 9.

Interaction diagram of 6Y9R with IMID2 compound code Z3136198649 observed during RMSD, Ligand interaction with amino acid residues of Protein Contact during Molecular dynamics simulation, protein–ligand contacts (cont.) (On the x-axis trajectory frame, the number is present, and amino acid residues are seen on the y-axis. The amino acid residues that have been in more contact with ligands in the trajectory frame appear to have a dark color shade). The VAL135 interaction fraction approached 1.4, whereas the ASP133 interaction fraction was missing in comparison to the core IMID2 scaffold from the previous literature. Protein RMSF is mostly helpful for predicting changes that occur locally along the enzyme chain. These peaks provide information on how much the protein fluctuated during the molecular dynamic simulation study.

IMID2 compound code PV-005996498401: During MD simulations, the ligand-protein complex showed hydrogen-bonded interactions. The protein backbone and ligand structure exhibited higher RMSD fluctuations over the first 0-20nsec. The protein backbone and ligand fluctuations stayed within the range of 1.0 Å and 0.6 Å over the last 80 nsec. The amino acid residue VAL135 formed 90% direct hydrogen bonding interactions with the amide carbonyl, and the amino acid residue VAL135 formed 47% direct hydrogen bonding interactions with imidazole in the ring structure, as shown in Figure 10.

Interaction diagram of 6Y9R withIMID2 compound code PV-005996498401 observed during RMSD, Ligand interaction with amino acid residues of Protein Contact during Molecular dynamics simulation, protein–ligand contacts (cont.) (On the x-axis trajectory frame, the number is present, and amino acid residues are seen on the y-axis. The amino acid residues that have been in more contact with ligands in the trajectory frame appear to have a dark color shade). The VAL135 interaction fraction approached 1.85, whereas the ASP133 interaction fraction was missing in comparison to the

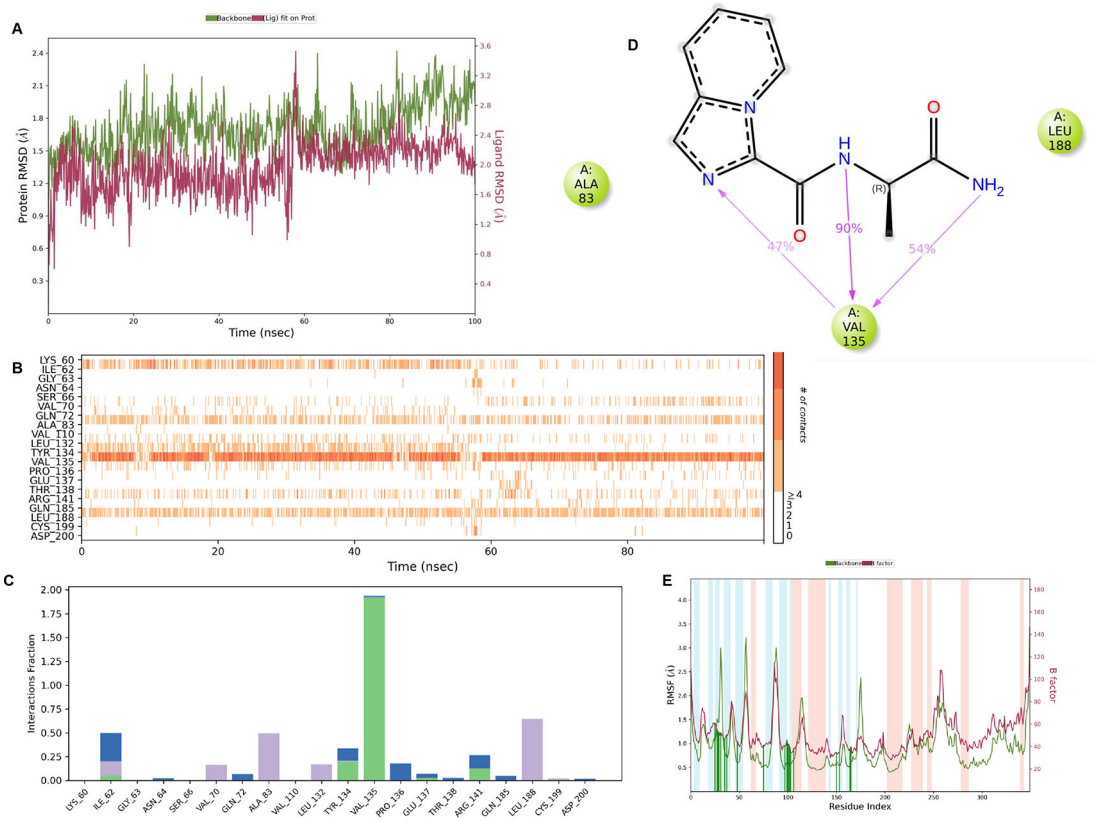


Figure 10. Pictorial representation of 6Y9R interaction with the IMID2 compound code PV-005996498401 monitored during the course of Molecular Dynamic Simulation trajectory (A) Protein-Ligand RMSD; (B) Protein-Ligand contacts; (C) Protein-Ligand contacts described as histogram; (D) Ligand-Protein contacts; (E) Protein RMSF.

core IMID2 scaffold from the previous literature. Protein RMSF is mostly helpful for predicting changes that occur locally along the enzyme chain. These peaks provide information on how much the protein fluctuated during the molecular dynamic simulation study.

Conclusion

In this study, we used focused library generation to target the receptor grid region (ATP-competitive site) of 6Y9R. After docking, the compounds were separated and selected based on the VAL135 residue interaction. Further prediction was performed using Qikprop and Prime MM-GBSA assays and Molecular dynamic simulation studies. Further experimental studies are required to confirm these findings.

Ethical considerations

Ethics and written consent were not applicable.

Data availability

Figshare: Molecular docking studies and molecular dynamic simulation to identify GSK-3β inhibitors for Alzheimer's disease, https://doi.org/10.6084/m9.figshare.24592716.v1.[24]

The underlying data for this project are:

- Enamine_IMID2_Scaffold.csv
- Enamine_IMID2_Scaffold_with nitrogen_realdb_molecules 17_35_52.sdf
- Ligprep_enamine_IMID2_Scaffold_withnitrogen_realdb_out.csv
- Ligprep_enamine_IMID2_SCAFFOLD_WITHNITROGEN_REALDB-out.mae
- Prime_mmgbsa_3_out.csv
- prime_mmgbsa_3-out.maegz
- Prime_mmgbsa_VAL135_INTERACTION_IMID2_ENAMINE_WITHNITROGEN-out.csv
- prime_mmgbsa_VAL135_INTERACTION_IMID2_ENAMINE_WITHNITROGEN-out.maegz
- QIKPROP_LIGPRE_IMID2_ENAMINE_WITHNITROGEN-out.csv
- QIKPROP_LIGPREP_ENAMINE_IMID2_SCAFFOLD_WITHNITROGEN_REALDB-out.mae

Protein Data Bank: 6Y9R, https://doi.org/10.2210/pdb6Y9R/pdb.[25]

Acknowledgements

The authors would like to thank the Indian Council of Medical Research (ICMR), New Delhi, India, for the ICMR-SRF (#2021-11506_F1) to Suggala Ramya Shri.

References

1. Rippin I, Eldar-Finkelman H: **Novel Modality of GSK-3 Inhibition For Treating Neurodegeneration.** *J Neurol Neuromedicine.* 2018; **3**(6): 5–7.
Publisher Full Text

2. Sheppard O, Coleman M: **Enfermedad de Alzheimer: etiología, neuropatología y patogenia.** *Enferm Alzheimer Descub fármacos.* 2020; 1–22.

3. Morris JC, McDade EM: **Alzheimer Disease.** *Contin Lifelong Learn Neurol.* 2022; **28**(3): 648–675.
Publisher Full Text

4. Bellenguez C, Küçükali F, Jansen IE, *et al.*: **New insights into the genetic etiology of Alzheimer's disease and related dementias.** *Nat. Genet.* 2022; **54**(4): 412–436.
PubMed Abstract | Publisher Full Text | Free Full Text

5. Pandey MK, DeGrado TR: *Glycogen synthase kinase-3 (GSK-3)-targeted therapy and imaging. Vol. 6, Theranostics.* Ivyspring International Publisher; 2016 [cited 2021 Apr 14]; pp. 571–593.
Free Full Text

6. Shri SR, Manandhar S, Nayak Y, *et al.*: **Role of GSK-3β Inhibitors: New Promises and Opportunities for Alzheimer's Disease.** *Adv Pharm Bull.* 2023; **13**: 688–700.
PubMed Abstract | Publisher Full Text | Free Full Text

7. Lauretti E, Dincer O, Praticò D: **Glycogen synthase kinase-3 signaling in Alzheimer's disease.** *Biochim Biophys Acta Mol Cell Res.* 2020; **1867**(5): 118664.
PubMed Abstract | Publisher Full Text | Free Full Text

8. Rippin I, Eldar-Finkelman H: **Mechanisms and therapeutic implications of gsk-3 in treating neurodegeneration.** *Cells.* 2021; **10**(2): 1–22.
Publisher Full Text

9. Baby K, Maity S, Mehta CH, *et al.*: **Computational drug repurposing of Akt - 1 allosteric inhibitors for non - small cell lung cancer.** *Sci. Rep.* 2023; **13**: 1–25.
PubMed Abstract | Publisher Full Text | Free Full Text

10. Baby K, Maity S, Mehta CH, *et al.*: **Targeting SARS-CoV-2 RNA-dependent RNA polymerase: An in silico drug repurposing for COVID-19.** *F1000Res.* 2020; **9**(September): 1166.
PubMed Abstract | Publisher Full Text | Free Full Text

11. Priya K, Manandhar S, Sankhe R, *et al.*: **Structure Based Virtual Docking and Molecular Dynamics Guided Identification of Potential Phytoconstituents from Traditionally Used Female Antifertility Plant.** *Tabriz Univ Med Sci.* 2022; **28**(2): 285–294.
Publisher Full Text

12. Altunkaya A, Bi C, Bradley AR, *et al.*: **OUP accepted manuscript.** *Nucleic Acids Res.* 2016; **45**(October 2016): 271–281.

13. Buonfiglio R, Prati F, Bischetti M, *et al.*: **Discovery of novel imidazopyridine GSK-3β inhibitors supported by computational approaches.** *Molecules.* 2020; **25**(9).
PubMed Abstract | Publisher Full Text | Free Full Text

14. Madhavi Sastry G, Adzhigirey M, Day T, *et al.*: **Protein and ligand preparation: Parameters, protocols, and influence on virtual screening enrichments.** *J Comput Aided Mol Des.* 2013; **27**(3): 221–234.
PubMed Abstract | Publisher Full Text

15. Roos K, Wu C, Damm W, *et al.*: **OPLS3e: Extending Force Field Coverage for Drug-Like Small Molecules.** *J Chem Theory Comput.* 2019; **15**(3): 1863–1874.
PubMed Abstract | Publisher Full Text

16. Chen IJ, Foloppe N: **Drug-like bioactive structures and conformational coverage with the ligprep/confgen suite: Comparison to programs MOE and catalyst.** *J Chem Inf Model.* 2010; **50**(5): 822–839.
PubMed Abstract | Publisher Full Text

17. Manual U: **Schrödinger. Schrödinger.** 1987.

18. Kumar A, Rathi E, Kini SG: **E-pharmacophore modelling, virtual screening, molecular dynamics simulations and in-silico ADME analysis for identification of potential E6 inhibitors against cervical cancer.** *J Mol Struct.* 2019; **1189**: 299–306.
Publisher Full Text

19. Kumar A, Rathi E, Kini SG: **Drug repurposing approach for the identification and designing of potential E6 inhibitors against cervical cancer: an in silico investigation.** *Struct Chem.* 2020; **31**(1): 141–153.
Publisher Full Text

20. Li J, Abel R, Zhu K, *et al.*: **The VSGB 2.0 model: A next generation energy model for high resolution protein structure modeling.** *Proteins Struct Funct Bioinforma.* 2011; **79**(10): 2794–2812.
PubMed Abstract | Publisher Full Text | Free Full Text

21. Bowers KJ, Chow E, Xu H, *et al.*: **Scalable algorithms for molecular dynamics simulations on commodity clusters.** *Proc 2006 ACM/IEEE Conf Supercomput SC'06.* 2006;(November).

22. Attique SA, Hassan M, Usman M, *et al.*: **A molecular docking approach to evaluate the pharmacological properties of natural and synthetic treatment candidates for use against hypertension.** *Int J Environ Res Public Health.* 2019; **16**(6): 1–17.

23. Kharkar PS: **Drugs acting on central nervous system (CNS) targets as leads for non-CNS targets.** *F1000Res.* 2014; **3**: 1–7.
Publisher Full Text

24. Shri SR, Nayak Y, Pai KSR: Molecular docking studies and molecular dynamic simulation to identify GSK-3β inhibitors for Alzheimer's disease. Dataset. *figshare.* 2023.
Publisher Full Text

25. Krapp S, Griessner A, Blaesse M, *et al.*: **Crystal structure of GSK-3b in complex with the 1H-indazole-3-carboxamide inhibitor 2.** *Protein Data Bank.* 2020.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ✗ ✓ ? ✓

Version 2

Reviewer Report 28 September 2024

https://doi.org/10.5256/f1000research.170347.r318530

?

Sachchida Rai
Centre of Experimental Medicine and Surgery (CEMS), Institute of Medical Sciences (IMS), Banaras Hindu University, Varanasi, Uttar Pradesh, 221005, India

Payal Singh
Department of Zoology, Banaras Hindu University, Varanasi, Uttar Pradesh, India

I have some questions on the manuscript which are as follows.
How do the identified GSK-3β inhibitors compare to existing therapeutics in terms of binding affinity and specificity?
What specific modifications were made to the previously used computational methods, and how did they enhance the prediction accuracy?
Can you provide more details on the ligand candidates identified? Were any novel chemical structures or scaffolds discovered?
How do the ADME predictions of the identified ligands suggest their potential success in in vivo studies?
Were there any challenges encountered in using the computational simulation tools, and how were they addressed in this study?
Have you considered testing the identified GSK-3β inhibitors in relevant Alzheimer's disease animal models?
Did the molecular dynamics simulations provide insights into the stability and conformational changes of the protein-ligand complexes over time?
How do you plan to validate the computational results experimentally, and what are the next steps in your drug development pipeline?
Was there any off-target effects observed for the identified inhibitors in the computational analyses?
Include some relevant bibliographic studies like Ramakrishna K, et al., 2024 (Ref 1), Tripathi PN, et al., 2024 (Ref 2), Singh M, et al., 2024 (Ref 3), Tripathi PN, et al., 2019 (Ref 4), Srivastava P, et al., 2019 (Ref 5), & Rai SN, et al., 2020 (Ref 6) in your manuscript.
Could targeting GSK-3β alone be sufficient to address the complex pathology of Alzheimer's disease, or do you propose a combination therapy approach?
How did the focused library generation improve the targeting accuracy of the ATP-competitive site

in 6Y9R?
What criteria were used to select compounds based on the VAL135 residue interaction, and how critical is this interaction for inhibitory activity?
How reliable are the Qikprop and Prime MM-GBSA assays in predicting the efficacy of the compounds before experimental validation?
Can you elaborate on the molecular dynamics simulation results, specifically regarding the stability of the protein-ligand complexes?
Were any alternative residues besides VAL135 considered for interaction, and how might they impact the binding efficiency?
How were the docking scores correlated with the Qikprop predictions and MM-GBSA binding energy calculations?
What experimental techniques do you propose to validate the computational findings, and what challenges do you anticipate in this process?
How do the identified compounds compare to existing inhibitors of 6Y9R in terms of predicted binding affinity and selectivity?
Did the molecular dynamics simulations reveal any significant conformational changes in the receptor that might affect ligand binding?
How do you plan to prioritize the compounds for further experimental testing, given the computational predictions?

References

1. Ramakrishna K, Karuturi P, Siakabinga Q, T A G, et al.: Indole-3 Carbinol and Diindolylmethane Mitigated β-Amyloid-Induced Neurotoxicity and Acetylcholinesterase Enzyme Activity: In Silico, In Vitro, and Network Pharmacology Study.*Diseases*. 2024; **12** (8). PubMed Abstract | Publisher Full Text
2. Tripathi PN, Lodhi A, Rai SN, Nandi NK, et al.: Review of Pharmacotherapeutic Targets in Alzheimer's Disease and Its Management Using Traditional Medicinal Plants.*Degener Neurol Neuromuscul Dis*. 2024; **14**: 47-74 PubMed Abstract | Publisher Full Text
3. Singh M, Agarwal V, Pancham P, Jindal D, et al.: A Comprehensive Review and Androgen Deprivation Therapy and Its Impact on Alzheimer's Disease Risk in Older Men with Prostate Cancer.*Degener Neurol Neuromuscul Dis*. 2024; **14**: 33-46 PubMed Abstract | Publisher Full Text
4. Tripathi PN, Srivastava P, Sharma P, Tripathi MK, et al.: Biphenyl-3-oxo-1,2,4-triazine linked piperazine derivatives as potential cholinesterase inhibitors with anti-oxidant property to improve the learning and memory.*Bioorg Chem*. 2019; **85**: 82-96 PubMed Abstract | Publisher Full Text
5. Srivastava P, Tripathi PN, Sharma P, Rai SN, et al.: Design and development of some phenyl benzoxazole derivatives as a potent acetylcholinesterase inhibitor with antioxidant property to enhance learning and memory.*Eur J Med Chem*. 2019; **163**: 116-135 PubMed Abstract | Publisher Full Text
6. Rai SN, Singh C, Singh A, Singh MP, et al.: Mitochondrial Dysfunction: a Potential Therapeutic Target to Treat Alzheimer's Disease.*Mol Neurobiol*. 2020; **57** (7): 3075-3088 PubMed Abstract | Publisher Full Text

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My research focuses on exploring the molecular mechanisms and therapeutic interventions for neurodegenerative diseases, particularly Alzheimer's and Parkinson's disease. I investigate the roles of biomarkers, signaling pathways, and epigenetic regulators in disease progression. My work integrates computational biology, drug discovery, and experimental models to identify novel therapeutic targets, including GSK-3β and other key enzymes. I also explore natural compounds, such as those derived from nutraceuticals and endophytic fungi, for their neuroprotective potential. Additionally, I study autophagy, oxidative stress, and inflammation as critical factors in neurodegeneration, aiming to develop targeted strategies for chronic disease mitigation and brain health.

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 16 May 2025

Sreedhara Ranganath Pai

Comment 1. How do the identified GSK-3β inhibitors compare to existing therapeutics in terms of binding affinity and specificity?
Answer to the comment: The binding affinity and specificity are generally related in that high binding affinity usually leads to high specificity for a particular target. The binding affinity and specificity of GSK-3β inhibitors vary depending on the type of inhibitors/ lignads used.

Comment 2. What specific modifications were made to the previously used computational methods, and how did they enhance the prediction accuracy?
Answer to the comment: Instead of a similarity structure in this study, substructure search was used for IMID2 scaffold at Enamine and the compounds were subjected to Qikprop to predict the accuracy of the compounds to cross the BBB (QPlogBB). In a previous study, QPlogBB was not used, whereas other physicochemical parameters were used, such as molecular weight, hydrogen bond donor and hydrogen bond acceptor.

Comment 3. Can you provide more details on the ligand candidates identified? Were any novel chemical structures or scaffolds discovered?
Answer to the comment: In this study, the substructure compound search was used for ligand identification. All the compounds used in this study were novel, but scaffold was similar to previous literature.

Comment 4. How do the ADME predictions of the identified ligands suggest their potential success in in vivo studies?
Answer to the comment: Absorption is how the drugs go through the organs of the body to reach the systemic circulation. Distribution of the drug/compound transported from one tissue to another tissue or from one organ to another organ. The transportation or distribution of compounds/drugs into the brain/CNS is the main focus in drug discovery. The metabolism is also referred to as the biotransformation of exogenous compounds/drugs to increase their water solubility and hydrophilicity. Finally, the water solubility facilitates their excretion process. Using the information to evaluate the compound's drug safety and risk outcomes.

Comment 5. Were there any challenges encountered in using the computational simulation tools, and how were they addressed in this study?
Answer to the comment: As the computation methods are regularly and routinely used, there are no challenges encountered in using the computational simulation tools.

Comment 6. Have you considered testing the identified GSK-3β inhibitors in relevant Alzheimer's disease animal models?
Answer to the comment: No, yet to consider testing the identified GSK-3β inhibitors in relevant Alzheimer's disease animal models.

Comment 7. Did the molecular dynamics simulations provide insights into the stability and conformational changes of the protein-ligand complexes over time?
Answer to the comment: Yes, the molecular dynamics (MD) simulations can provide insights into the stability and conformational changes of protein-ligand complexes over time. MD simulation studies predict how protein-ligand interactions occur, analyse dynamic changes and conformational changes in the proteins.

Comment 8. How do you plan to validate the computational results experimentally, and what are the next steps in your drug development pipeline?
Answer to the comment: The finalized compounds will be subjected to in vitro and in vivo studies. If the molecules are showing good results, they can be translated into clinical studies.

Comment 9. Was there any off-target effects observed for the identified inhibitors in the computational analyses?
Answer to the comment: No such off-target effects were observed for inhibitors in computational analyses using the Schrodinger Maestro tool.

Comment 10. Include some relevant bibliographic studies like Ramakrishna K, et al., 2024

(Ref 1), Tripathi PN, et al., 2024 (Ref 2), Singh M, et al., 2024 (Ref 3), Tripathi PN, et al., 2019 (Ref 4), Srivastava P, et al., 2019 (Ref 5), & Rai SN, et al., 2020 (Ref 6) in your manuscript.
Answer to the comment: These references are not related to our objectives, hence these are not included as relevant references.

Comment 11. Could targeting GSK-3β alone be sufficient to address the complex pathology of Alzheimer's disease, or do you propose a combination therapy approach?
Answer to the comment: As most of the current study focuses on targeting GSK-3β, and the preclinical data to a greater extent prevent Alzheimer's disease, however, the question of whether GSK-3β alone is sufficient to address the complex pathology of Alzheimer's disease remains to be answered through clinical investigations.

Comment 12. How did the focused library generation improve the targeting accuracy of the ATP-competitive site in 6Y9R?
Answer to the comment: The identified GSK-3β inhibitors particularly bind to the specific ATP-competitive site in 6Y9R protein region and reduce the off-target effects.

Comment 13. What criteria were used to select compounds based on the VAL135 residue interaction, and how critical is this interaction for inhibitory activity?
Answer to the comment: This was based on previous literature, in this study N-region of the core and VAL135 at N-H group of the ligand.

Comment 14. How reliable are the Qikprop and Prime MM-GBSA assays in predicting the efficacy of the compounds before experimental validation?
Answer to the comment: Qikprop: Predicts pharmaceutically relevant properties of the compounds and physically significant descriptors of an individual compound. Prime MM-GBSA: Predicts the protein-ligand binding free energy of an individual compound. This was accurate to estimate the relative binding free energy of the compound/ligand.

Comment 15. Can you elaborate on the molecular dynamics simulation results, specifically regarding the stability of the protein-ligand complexes?
Answer to the comment: We have elaborated this part in the revised manuscript.

Comment 16. Were any alternative residues besides VAL135 considered for interaction, and how might they impact the binding efficiency?
Answer to the comment: The alternative residue, PRO 136, forms a hydrogen bond interaction with the core of IMID2.

Comment 17. How were the docking scores correlated with the Qikprop predictions and MM-GBSA binding energy calculations?
Answer to the comment: The docking score gives an idea about the score of a compound and is used to predict the binding affinity of protein and ligand when it is subjected to a molecular docking study. Qikprop gives an idea about the compound's ability to cross the BBB or not. The MM-GBSA gives an idea about the sum of intermolecular interactions present between the protein and ligand.

Comment 18. What experimental techniques do you propose to validate the computational

findings, and what challenges do you anticipate in this process?
Answer to the comment: In the present study, a molecular docking study was used to subject these compounds to Qikprop, then the compounds which cross the standard range of physicochemical properties. Those compounds were segregated based on VAL135 at N-H group followed by subjecting those compounds to Prime MM-GBSA and Molecular dynamic simulation studies.

Comment 19. How do the identified compounds compare to existing inhibitors of 6Y9R in terms of predicted binding affinity and selectivity?
Answer to the comment: The identified compounds are better compared to the existing inhibitors of 6Y9R.

Comment 20. Did the molecular dynamics simulations reveal any significant conformational changes in the receptor that might affect ligand binding?
Answer to the comment: The interactions during the MD simulation were stable for a larger period, hence, the shortlisted molecules can be better GSK-3β inhibitors.

Comment 21. How do you plan to prioritize the compounds for further experimental testing, given the computational predictions?
Answer to the comment: Yes, the compounds will be further subjected to in vitro studies and later to in vivo studies.

Competing Interests: Authors declare that there is no competing interest.

Version 1

Reviewer Report 10 October 2024

https://doi.org/10.5256/f1000research.159332.r302514

Shvetank Bhatt
[1] Dr. Vishwanath Karad MIT World Peace University, Pune, Maharashtra, India
[2] Dr. Vishwanath Karad MIT World Peace University, Pune, Maharashtra, India

The manuscript is well written and can be accepted after minor modifications.
1. Author can highlight the names of drugs which are recently approved for the treatment of AD in introduction section.
2. Results and discussion section is presented well by authors.
3. Author should discuss the epidemiology and pathophysiology of diseases briefly to understand the severity of disease.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: CNS Diosrders, AD, Depression, Anxiety

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 16 May 2025

Sreedhara Ranganath Pai

Comment 1. Author can highlight the names of drugs which are recently approved for the treatment of AD in introduction section.
Answer to the comment: The aducanumab, a medication based on the Aβ theory, in 2021 FDA approved for the treatment of AD. Lecanemab's effectiveness and safety in treating early-stage AD require longer study trials.

Comment 2. Results and discussion section is presented well by authors.
Answer to the comment: We thank the reviewer.

Comment 3. Author should discuss the epidemiology and pathophysiology of diseases briefly to understand the severity of disease.
Answer to the comment: AD is the leading cause of dementia among the elderly, with an estimated 44 million individuals affected globally, a number projected to double by 2050. In the U.S., over 5.5 million are currently diagnosed. The pathophysiology of AD includes several key features: the presence of amyloid plaques, which disrupt neuronal communication and incite inflammatory responses; neurofibrillary tangles formed by hyperphosphorylated tau protein, leading to neuronal dysfunction and cell death; and

neuroinflammation, where activated glial cells further damage neurons and exacerbate the disease's progression, cognitive decline and behavioral changes.

Competing Interests: Authors declare that there is no competing interest.

Reviewer Report 13 September 2024

https://doi.org/10.5256/f1000research.159332.r302510

Jigna Samir Shah
[1] Nirma University, Ahmedabad, Gujarat, India
[2] Nirma University, Ahmedabad, Gujarat, India

1. Abstract can be modified. Also, in conclusion no specific compound was identified.
2. Role of GSK-3β in pathogenesis of AD can be explored more. Mechanism of GSK-3β in AD can be defined briefly in introduction section.
3. Why this PDB ID 6Y9R was selected from 89 IDs as mentioned in results and discussion. It can be elaborated briefly. Also, besides this domain, GSK-3β inhibition might be possible through different binding sites. If possible, it can also be explored.
4. There are grammatical errors and improper sentence formations in manuscript, which can be modified. Also, instead of using “we”, the authors can used indirect speech.
5. Full forms mentioned are not consistent. Mention the full form in first use and thereafter abbreviations can be used.
6. In result sections, “Protein root mean square fluctuation (RMSF) is mostly helpful for predicting changes that occur locally along the enzyme chain.” is repeatedly mentioned in each result. The authors can mention the significance of findings like RMSD, RMSF at the start in a separate paragraph, or only once in initial results. Thereafter it can be understood.
7. In ADME studies, it is mentioned that QPlogBB (predicted brain/blood partition coefficient), was predicted however, in results it is not mentioned. It can be mentioned, to evaluate blood brain barrier permeability. In AD, since the target region for therapy is brain, BBB permeability is a significant parameter.
8. No standard drug or inhibitor is included in MDS studies. If possible, a standard can be taken into consideration.
9. There is no discussion about findings of the article, and conclusion is very vague. At the end of study, there is no comparison of the 9 compounds screened. Better compound should be identified and screened. Biological data to support the findings can be added, however it is not mandatory. Discussion section should be added and findings of each compound should be compared and correlated to previously done studies.
10. Discuss future prospects of this study, elaborate briefly what experimental studies should be done further and state the limitations of this study.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Neurodegenerative diseases, oral cancer, breast cancer

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 16 May 2025

Sreedhara Ranganath Pai

Comment 1. Abstract can be modified. Also, in conclusion no specific compound was identified.
Answer to the comment: Thank you for your comment. The abstract and the conclusion have been modified according to the reviewer's comments in the revised manuscript.

Comment 2. Role of GSK-3β in pathogenesis of AD can be explored more. Mechanism of GSK-3β in AD can be defined briefly in introduction section.
Answer to the comment: The relevant details were incorporated as per the reviewer's comment in the revised manuscript, page number: 10

Comment 3. Why this PDB ID 6Y9R was selected from 89 IDs as mentioned in results and discussion. It can be elaborated briefly. Also, besides this domain, GSK-3β inhibition might be possible through different binding sites. If possible, it can also be explored.
Answer to the comment: The PDB ID 6Y9R was released in 2020 for the protein GSK-3β, the amino acid sequence length is proper without any break in sequence, with good resolution and Ramachandran outliers. The SiteMap tool is generally used to explore the different binding sites of a protein. Based on the results from the SiteMap tool, the ATP binding site was explored to identify ATP-competitive inhibitors to explore it in Alzheimer's

disease.

Comment 4. There are grammatical errors and improper sentence formations in manuscript, which can be modified. Also, instead of using “we”, the authors can used indirect speech.
Answer to the comment: These errors are corrected in the revised manuscript. The authors are thankful to the reviewer for the suggestions.

Comment 5. Full forms mentioned are not consistent. Mention the full form in first use and thereafter abbreviations can be used.
Answer to the comment: These are taken care of in the revised manuscript.

Comment 6. In result sections, “Protein root mean square fluctuation (RMSF) is mostly helpful for predicting changes that occur locally along the enzyme chain.” is repeatedly mentioned in each result. The authors can mention the significance of findings like RMSD, RMSF at the start in a separate paragraph, or only once in initial results. Thereafter it can be understood.
Answer to the comment: According to the reviewer's comment, the data was added in the revised manuscript, see page number 12.

Comment 7. In ADME studies, it is mentioned that QPlogBB (predicted brain/blood partition coefficient), was predicted however, in results it is not mentioned. It can be mentioned, to evaluate blood brain barrier permeability. In AD, since the target region for therapy is brain, BBB permeability is a significant parameter.
Answer to the comment: The data was added in the revised manuscript according to the revised manuscript page number: 10-11.

Comment 8. No standard drug or inhibitor is included in MDS studies. If possible, a standard can be taken into consideration.
Answer to the comment: The standard drug or inhibitor are not used for docking a there are no currently available approved drug acting through GSK-3β.

Comment 9. There is no discussion about findings of the article, and conclusion is very vague. At the end of study, there is no comparison of the 9 compounds screened. Better compound should be identified and screened. Biological data to support the findings can be added, however it is not mandatory. Discussion section should be added and findings of each compound should be compared and correlated to previously done studies.
Answer to the comment: In the revised manuscript, these points are incorporated as per the reviewer’s comments.

Comment 10. Discuss future prospects of this study, elaborate briefly what experimental studies should be done further and state the limitations of this study.
Answer to the comment: This point was taken care of in the revised manuscript. The shortlisted compounds can be subjected to in vitro studies to check the cytotoxicity, and based on those study results, the compound can be studied in an animal model to check the safety and efficacy. However, there are limitations to purchasing. The compound, based on the body weight of the animal, requires a larger amount of compound. Further, for

synthesis, it needed a lot of time to get the correct scaffold and the required structure.

Competing Interests: Authors declare that there is no competing interest.

Reviewer Report 29 July 2024

https://doi.org/10.5256/f1000research.159332.r302509

Qingchun Zhao
[1] Shenyang Pharmaceutical University, Shenyang, China
[2] Shenyang Pharmaceutical University, Shenyang, China

In this paper, the authors used computer simulation technology to select 9 compounds with GSK3 as the target, and then studied ADME prediction and molecular docking through different modules.
Keep reading the paper, three questions are raised.
1. In the abstract, the author describes the method as "we have used different modules that were used in previous studies with a little modification", but in fact, the method used by the author is a routine application, and there is no prominent modification.
2. In the introduction section, is there only one sentence to summarize the article?
3.The content of the whole study is simple, the innovation is poor, and the workload is less, which is not enough to support a research article.
Therefore, it is hoped that the author can modify some contents of the article and add some follow-up experiments to make the article more comprehensive.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Alzheimer's Disease, Cancer , Natural Pharmaceutical Chemistry, Pharmaceutical chemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 13 Aug 2024

Sreedhara Ranganath Pai

Reviewer Comment 1: In the abstract, the author describes the method as "we have used different modules that were used in previous studies with a little modification", but in fact, the method used by the author is a routine application, and there is no prominent modification.
Response to comment 1: We used Maestro, a graphical interface of Schrodinger, for our computational simulation studies. In the present work, we have used different modules such as Protein Preparation Wizard for Protein Preparation, LigPrep for Ligand Preparation, Qikprop for ADME (Absorption, Distribution, Metabolism and Excretion) prediction, Glide for docking studies, Prime for Binding energy prediction and Desmond for Molecular dynamic simulation studies used.

Reviewer Comment 2: In the introduction section, is there only one sentence to summarize the article?
Response to comment 2: For better BBB permeation, the structure was finalized based on a wet lab. So, based on this literature search, we have selected core imidazole scaffold for our study, and from that core imidazole scaffold, we have drawn sub-structures in the enamine database. Then docking studies were done on the compounds and subjected to ADME (Qikprop). A molecular dynamic simulation study was conducted on nine compounds.

Reviewer Comment 3: The content of the whole study is simple, the innovation is poor, and the workload is less, which is not enough to support a research article.
Therefore, it is hoped that the author can modify some contents of the article and add some follow-up experiments to make the article more comprehensive.
Response to comment 3: The reviewer's comment is completely one-sided. We regret to answer this comment, and we completely disagree with the reviewer's suggestion, as this work took 18 months from the conceptualization of the idea. This work is computational modelling, and our objectives are clear, and in our view, the data generated is sufficient to make the conclusion.

Competing Interests: NIL