International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 5, May, 2025
Page No.: 102-107

Review

In silico identification of natural Alzheimer inhibitors targeting GSK-3ß using receptor-based pharmacophore and molecular modeling studies

Abhineet Bajaj*, Jeevan Patra

Amity Institute of Pharmacy, Amity University Uttar Pradesh (Lucknow Campus), Lucknow, Uttar Pradesh, India 226010.

Corresponding Author:
Abhineet Bajaj

Email: *NA*

DOI: *10.62896/ijpdd.2.5.11*

Conflict of interest: *NIL*

Article History
Received: 12/04/2025
Accepted: 04/05/2025
Published: 10/05/2025

Abstract:
The discovery of an ideal, and effective Alzheimer's therapy is urgently required. The main pathological hallmarks of Alzheimer's disease are neurofibrillary tangles, amyloid plaques, inflammation, and neuronal atrophy. Reports suggested Glycogen Synthase Kinase-3β is a promising target for exploring novel therapeutic regimens. The main objective of this study is to explore, and decipher natural inhibitors that can target GSK-3β protein effectively. The receptor-based pharmacophore modeling of GSK-3β protein was developed and screened against CNS-like natural databases. The final survival hits aligning accurately with the pharmacophoric features was assessed for molecular docking studies. Taking standard control of GSK-3β, Batatasin III, and Gigantol were confirmed as the best hits based on their binding free energies, molecular interactions, and optimal ADMET profiles. Our findings represent a promising starting point for developing novel natural regimens however rigorous testament is imperative through biological screening which is still underway.

Keywords: Alzheimer's Disease, Glycogen Synthase Kinase-3β, Binding Affinity, Natural Products, Pharmacophore modeling

1. INTRODUCTION

Alzheimer's disease represents one of the most devastating neurodegenerative disorders currently affecting over 55 million people worldwide (1). Despite this enormous societal impact and decades of research, current therapeutic approaches have shown limited efficacy (2). Presently, FDA-approved medications primarily focus on acetylcholinesterase inhibitors such as donepezil, rivastigmine, galantamine, and NMDA receptor antagonist memantine (3). These medications provide temporary symptomatic relief, but they fail to address the underlying pathological mechanisms driving disease progression. Traditional drugs have shown limited success while higher AD clinical trials fail to demonstrate significant clinical benefits (4). The GSK-3β protein, a serine/threonine kinase, operates through multiple mechanisms in AD pathogenesis (5). GSK-3β phosphorylates tau protein at multiple sites leading to microtubule destabilization, and the formation of neurofibrillary tangles (6). Additionally, it modulates amyloid precursor protein processing through interaction with the γ-secretase complex, influencing Aβ production (7). Its hyperactivation also triggers inflammatory responses via NF-κB pathway activation, promoting the release of pro-inflammatory cytokines such as IL-1β and TNF-α in microglia (8).
Natural Products could be attractive chemotypes for addressing the challenges above due to their plethora of benefits such as structural diversity (9), broader pharmacological bioactivity, lesser toxicity (10), and minimal drug resistance (11) over the synthetic drugs.

Hence, natural products serve as the pivotal source for the development of new regimens in ethnopharmacological research (12). Structure-based virtual screening has been a benchmark in the early-stage drug discovery paradigm. Pharmacophore modeling has been versatile due to its conformational and developed hypothesis features explored from the databases which ease in screening chemical space (13). Virtual screening allows us to explore vast chemical space to acquire for biological evaluations. Utilizing multiple parameters for screening virtual libraries embedded with augmented molecular modeling became a holistic approach for the identification of lead hits in the early-stage drug discovery (14, 15).

Encouraged by the above facts, we pioneered the potential of natural products targeting GSK-3β protein in the present study. By integrating pharmacophore modeling, and structure-based virtual screening, we aim to identify novel chemotypes capable of modulating GSK-3β effectively.

2. COMPUTATIONAL METHODOLOGIES

2.1. Structural Crystallography Preparations

The crystallography structures of GSK-3β protein (PDB ID: 8QJI) (16) was obtained from the RCSB PDB bank. The crystallographic species such as heteroatoms, co-factors, and solvents were removed. The protein was analyzed to fill any missing loops and sidechains and protonated at pH 7.0 ± 2.0. Further, the hydrogens were assigned, optimized, and minimized using Chimera.

2.2. Virtual Database Curation and Preparations

Firstly, we examined the virtual natural chemical space that qualifies CNS-like drug properties (17). In the current study, a natural virtual database was constructed from the ZINC natural products (18), COCONUT (COlleCtion of Open Natural ProdUcTs) (19), LOTUS (20), and SuperNatural 3.0 (21). The common natural compounds, ambiguous structures, and broken fragments were filtered using the RDKit program. The physicochemical and virtual ADMET assessments are performed using ADMETlab 3.0 (22). The qualified natural hits were prepared using the Avogadro program.

2.3. Development of pharmacophore hypothesis and screening

The pharmacophore model was developed using ZINC Pharmer (23). The chemical attributes such as hydrogen bond donor, hydrogen bond acceptor, hydrophobic group, positive, negative, and aromatic ring were used to generate pharmacophore feature sites. The screening of natural hits screened against the GSK-3β pharmacophore model. The qualified screened natural hits were taken for molecular docking simulation studies.

2.4. Receptor Grid Generation and Molecular Docking

The receptor grid of the GSK-3β protein was generated using MzDOCK (24). The grid was coordinated at the co-crystallized ligand of GSK-3β which was centered to generate a three-dimensional box and represented as the active binding site. The screened natural hits were fed for the virtual screening under the default parameters of MzDOCK. For each ligand, three poses were generated and the best-scoring states were considered for post-docking analysis. The binding free energies were computed using g_mmpbsa under a continuous solvent model (25).

3. RESULTS AND DISCUSSIONS

3.1. Structural Crystallography and Pharmacophore Development

The crystallographic structure of GSK-3β (PDB ID: 8QJI) complexed with TW362 was solved at 3.02Å resolution (16) (**Figure 2**). The co-crystal occupies a binding pocket, with its aminopyridine fragment oriented towards critical residues such as Asp133, Tyr134, and Val135 in the hinge region between N-terminal, and C-terminal lobes forming tridentate harboring motif in the adenine binding region (26). The phosphorylation occurs at the adopted catalytic domain with Tyr216 residue supporting A-loop conformation situated with Arg220, and Arg223 residues (27, 28). The phosphate-binding region (Phe67, Lys85, and Asp200 residues) creates the anchoring motif. The structure adopts the characteristic bilobal fold typical of kinases, with a well-defined ATP-binding site located between the N- and C-lobes of the kinase domain. The pharmacophore hypothesis has been developed based on the crystallographic facts. The developed pharmacophore hypothesis comprised four pharmacophoric features HBA, HBD, hydrophobic, and aromatic rings. The co-crystal bounded with GSK-3β determines geometric

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 5, May, 2025
Page No.: 102-107

orientation at the catalytic binding site. The developed pharmacophoric features were drawn from the binding interaction pattern of co-crystal with the hall marker residues situated at the hinge region, adenine binding region, phosphate binding region (catalytic domain), A-loop conformation, and anchoring motif of GSK-3β protein.

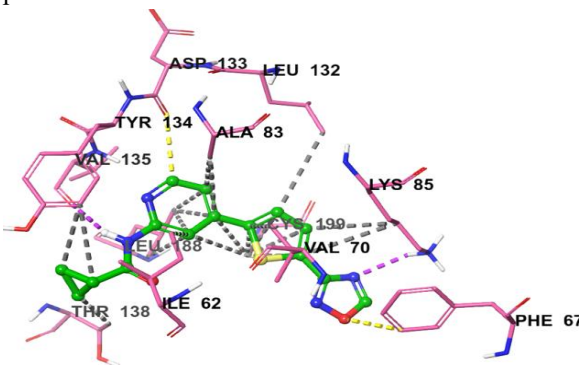

Figure 1: Crystallography structure of GSK-3β and pharmacophore model. The co-crystal and the interacting residues are represented in green, and magenta respectively. The h-bond, aromatic-h, and hydrophobic bond are in magenta, yellow, and grey respectively.

To explore the curated databases and find accurate natural compounds that can bind with the essential residues, the validated pharmacophore hypothesis models were selected for maximum common pharmacophoric features. These commonly aligned features were projected towards the potential residues intended for this study. The screened natural compounds against the GSK-3β hypothesis model produced 626 compounds respectively. The final survived hits were compared to explore natural compounds resulting in eleven compounds (**Table 1**).

Table 1: Best hits screened from pharmacophore model with docking results

Sr. No.	Natural Hits	Align Score	GScore	MMPBSA	NBE
1.	Macelignan	0.109	-6.187	-47.02	-64.14
2.	Cardamonin	0.963	-7.963	-62.27	-75.73
3.	Dehydrodiisoeugenol	1.031	-4.310	-37.63	-77.69
4.	Gigantol	0.180	-9.605	-84.60	-88.37
5.	Isorhapontigenin	0.972	-9.327	-61.14	-70.89
6.	Neobavaisoflavone	0.880	-8.189	-66.24	-71.47
7.	Licoflavone B	0.751	-6.360	-47.94	-61.29
8.	Batatasin III	0.681	-9.657	-74.72	-73.61
9.	Nodakenetin	0.972	-4.488	-22.37	-68.23
10.	Pinostilbene	0.665	-8.119	-63.14	-77.15
11.	S-(+)-Marmesin	0.553	-5.959	-28.45	-60.37
12.	Co-crystal	0.453	-7.637	-58.21	-64.16

Molecular docking simulations were performed for all survival hits to evaluate their molecular binding pattern and interactions. All natural chits achieved accurate docking poses at the lowest RMSD and interacted with the active binding residues. The docking scores ranged from -4.310 kcal/mol to -7.327 kcal/mol. Despite the natural compounds demonstrating the best align score, only two natural hits Batatasin III and Gigantol were found to have higher energies than the co-crystallized ligand. The docking score of best hits were -9.657 kcal/mol, and -9.605 kcal/mol, of Batatasin III, and Gigantol respectively. The molecular docking interactions were found to possess hydrogen bonding with the residues present at the DFG motif of the activation loop comprising the ATP binding site and the hinge region (**Figure 2**). The Adenine pocket comprises Ile62 and Val70 in the glycine-rich loop, demonstrating favorable hydrophobic contacts. Besides these several favorable hydrophobic contacts were formed with Ala83, Lys85, Leu132, Tyr134, Val135, Leu188, and Cys199 residues. Molecular Mechanics Generalized Born Surface Area accurately predicts the Gibbs free energy between the interacting molecules in all protein-ligand complexes. It was noted that the best natural hits had higher binding energies indicating of stronger interactions compared to the co-crystal. The MMGBSA of Batatasin III and Gigantol demonstrated -74.72 kcal/mol, and -84.60 kcal/mol, respectively. ‘

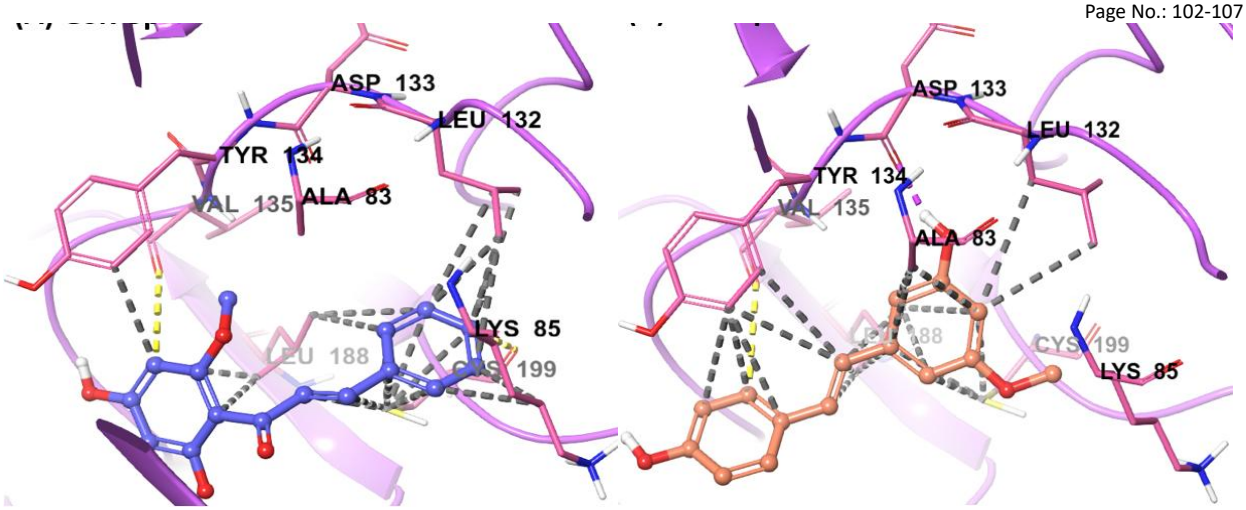

Figure 2: Binding interactions of Batatasin III and Gigantol with GSK-3β protein. Here, hydrogen, aromatic-H, and hydrophobic bonds are represented in magenta, yellow, and grey respectively.

3.2. Physicochemical and ADMET assessment

The best natural hits Batatasin III, and Gigantol were assessed for physicochemical and pharmacokinetic profiling. The hits exhibited predicted solubility within the acceptable limits (0.5 to -4 log mol/L). The partition coefficient shows higher lipophilicity, exceeding the optimal range of 0 – 3 for the selected molecules. All compounds demonstrated favorable Caco-2 permeability. The predicted probability of being a P-gp substrate and inhibitor is within optimum limits. The therapeutic window absorption (human intestinal absorption; HIA) is acceptable within the limits. This correlation can be attributed to the plasma protein binding (PPB) and blood plasma fractions, directly influencing human intestinal absorption. A higher PPB (> 90%) results in a lower volume of drug distribution in blood plasma leading to a lower absorption rate. All compounds showed optimal PPB which led to good drug distribution, and clearance time. The half-life was reasonably lower than others. In terms of toxicity, the hits showed had lower adverse risks.

4. CONCLUSION

In summary, our current study provided strong evidence supporting structure-based drug design for developing potent small-molecule natural inhibitors that can exert against GSK-3β protein. To elucidate the hypothesis, receptor-based pharmacophore modeling and high-throughput virtual screening of natural libraries were undertaken. After thorough screening, two natural hits Batatasin III, and Gigantol emerged as promising candidates, exhibiting better docking scores, MMGBSA, and optimal ADMET profiling. Computational investigations revealed that these hits demonstrated a binding fashion similar to reported crystallography structures. According to the crystal structure, all the hits interacted with the A-loop, harboring motif, and phosphate binding site residues. In comparison with the co-crystal, the screened natural hits effectively targeted GSK-3β suggesting their potential as disease-modifying agents. Since these hits hold promising therapeutic advancement, biological evaluations are still underway.

5. AUTHOR CONTRIBUTIONS

Abhineet Bajaj and **Vaishali Dubey,** performed computational studies, and wrote the original manuscript. **Jeevan Patra,** conceptualized the idea, assisted and analyzed the computational results, reviewed and edited the manuscript.

6. NOTES

The authors declare no competing financial interest.

7. ACKNOWLEDGEMENT

Authors are gratefully thankful to the Amity University Uttar Pradesh Lucknow campus for providing necessity support for conducting this proposed research study.

8. REFERENCES

1. 2024 Alzheimer's disease facts and figures. Alzheimers Dement. 2024;20(5):3708-821.
2. Zhang J, Zhang Y, Wang J, Xia Y, Zhang J, Chen L. Recent advances in Alzheimer's disease: mechanisms, clinical trials and new drug development strategies. Signal Transduct Target Ther. 2024;9(1):211.

3. Singh B, Day CM, Abdella S, Garg S. Alzheimer's disease current therapies, novel drug delivery systems and future directions for better disease management. Journal of Controlled Release. 2024;367:402-24.

4. Kim CK, Lee YR, Ong L, Gold M, Kalali A, Sarkar J. Alzheimer's Disease: Key Insights from Two Decades of Clinical Trial Failures. J Alzheimers Dis. 2022;87(1):83-100.

5. 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.

6. Brion JP, Anderton BH, Authelet M, Dayanandan R, Leroy K, Lovestone S, et al. Neurofibrillary tangles and tau phosphorylation. Biochem Soc Symp. 2001(67):81-8.

7. Llorens-Marítin M, Jurado J, Hernández F, Ávila J. GSK-3β, a pivotal kinase in Alzheimer disease. Front Mol Neurosci. 2014;7.

8. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. Alzheimer's & Dementia: Translational Research & Clinical Interventions. 2018;4(1):575-90.

9. Li C-Q, Lei H-M, Hu Q-Y, Li G-H, Zhao P-J. Recent Advances in the Synthetic Biology of Natural Drugs. Front Bioeng Biotechnol. 2021;9(691152).

10. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. Metabolites. 2012;2(2):303-36.

11. Atanasov AG, Zotchev SB, Dirsch VM, Orhan IE, Banach M, Rollinger JM, et al. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov 2021;20(3):200-16.

12. Beutler JA. Natural Products as a Foundation for Drug Discovery. Curr Protoc Pharmacol. 2009;46:9.11.1-9..21.

13. Schaller D, Šribar D, Noonan T, Deng L, Nguyen TN, Pach S, et al. Next generation 3D pharmacophore modeling. Wiley Interdiscip Rev Comput. 2020;10(4):e1468.

14. Jain AN. Virtual screening in lead discovery and optimization. Curr Opin Drug Discov Devel. 2004;7(4):396-403.

15. Shoichet BK. Virtual screening of chemical libraries. Nature. 2004;432(7019):862-5.

16. Góral I, Wichur T, Sługocka E, Grygier P, Głuch-Lutwin M, Mordyl B, et al. Exploring Novel GSK-3β Inhibitors for Anti-Neuroinflammatory and Neuroprotective Effects: Synthesis, Crystallography, Computational Analysis, and Biological Evaluation. ACS Chem Neurosci. 2024;15(17):3181-201.

17. Ajay, Bemis GW, Murcko MA. Designing libraries with CNS activity. J Med Chem. 1999;42(24):4942-51.

18. Tingle BI, Tang KG, Castanon M, Gutierrez JJ, Khurelbaatar M, Dandarchuluun C, et al. ZINC-22─A Free Multi-Billion-Scale Database of Tangible Compounds for Ligand Discovery. J Chem Inf Model. 2023;63(4):1166-76.

19. Sorokina M, Merseburger P, Rajan K, Yirik MA, Steinbeck C. COCONUT online: Collection of Open Natural Products database. J Cheminform. 2021;13(1):2.

20. Rutz A, Sorokina M, Galgonek J, Mietchen D, Willighagen E, Gaudry A, et al. The LOTUS initiative for open knowledge management in natural products research. eLife. 2022;11:e70780.

21. Gallo K, Kemmler E, Goede A, Becker F, Dunkel M, Preissner R, et al. SuperNatural 3.0—a database of natural products and natural product-based derivatives. Nucleic Acids Res. 2023;51(D1):D654-D9.

22. Fu L, Shi S, Yi J, Wang N, He Y, Wu Z, et al. ADMETlab 3.0: an updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. Nucleic Acids Res. 2024;52(W1):W422-W31.

23. Koes DR, Camacho CJ. ZINCPharmer: pharmacophore search of the ZINC database. Nucleic Acids Research. 2012;40(W1):W409-W14.

24. Kabier M, Gambacorta N, Trisciuzzi D, Kumar S, Nicolotti O, Mathew B. MzDOCK: A free ready-to-use GUI-based pipeline for molecular docking simulations. Journal of Computational Chemistry. 2024;45(23):1980-6.

25. Kumari R, Kumar R, Lynn A. g_mmpbsa—A GROMACS Tool for High-Throughput MM-PBSA Calculations. Journal of Chemical Information and Modeling. 2014;54(7):1951-62.

26. Derewenda ZS. C-H Groups as Donors in Hydrogen Bonds: A Historical Overview and Occurrence in Proteins and Nucleic Acids. Int J Mol Sci [Internet]. 2023; 24(17).

27. Hughes K, Nikolakaki E, Plyte SE, Totty NF, Woodgett JR. Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. Embo j. 1993;12(2):803-8.

28. Grygier P, Pustelny K, Nowak J, Golik P, Popowicz GM, Plettenburg O, et al. Silmitasertib (CX-4945), a Clinically Used CK2-Kinase Inhibitor with Additional Effects on GSK3β and DYRK1A Kinases: A Structural Perspective. J Med Chem. 2023;66(6):4009-24.

*****