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GSK3-TARGETED SMALL MOLECULES AS THERAPIES FOR ALZHEIMER'S DISEASE

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Abstract

Alzheimer's disease (AD) is a progressive neurological disorder that affects memory, thinking, and behavior. It is one of the leading causes of dementia worldwide, and current treatments mainly focus on managing symptoms rather than slowing or stopping disease progression. Research has identified glycogen synthase kinase-3 (GSK3) as a key enzyme involved in abnormal tau phosphorylation, which contributes to the development of Alzheimer's disease. Targeting GSK3 may provide a potential therapeutic approach. In this study, computational methods, including virtual screening and molecular docking, were used to evaluate small molecules as possible GSK3 inhibitors. Docking simulations were performed using SwissDock, and binding affinity was measured using FullFitness scores and estimated Gibbs free energy (ΔG). Several compounds demonstrated strong predicted binding within the enzyme's active site, suggesting stable interactions. These results indicate that certain small molecules may have potential as GSK3-targeted therapies. However, further experimental testing would be required to confirm their effectiveness and safety.

Keywords: *Drug Discovery, virtual screening, Alzheimer's disease, GSK3*

1. Introduction

1.1. Background

The most prevalent cause of dementia is Alzheimer's disease, a neurological condition that gradually deteriorates an individual's memory and cognitive abilities. It can often take five, ten, or even twenty years to show symptoms ("Alzheimer's Disease: Get the Facts." 2024). Beginning with minor memory loss, it could eventually result in the inability to do everyday tasks, have a proper conversation, or interact with the surrounding environment (CDC. "About Alzheimer's." 2024). This disease is fatal; every 65 seconds, someone in the US gets Alzheimer's, but by 2050, it is expected to increase to every 33 seconds. An estimated 500,000 Americans lose their lives to Alzheimer's disease each year, making it the 10th leading cause of death with no cure in the US, as well as one of the most

expensive diseases in the nation. Treatment for AD, which includes healthcare, long-term care, and hospice care, was expected to cost $321 billion in 2022, and by 2050, it is projected to cost more than $1 trillion. Medicare and Medicaid cover about 65% of out-of-pocket medical costs. However, due to an anticipated increase in yearly payments, Alzheimer's is predicted to raise Medicare and Medicaid spending by more than 330% by 2050 (Skaria A. P., 2022).

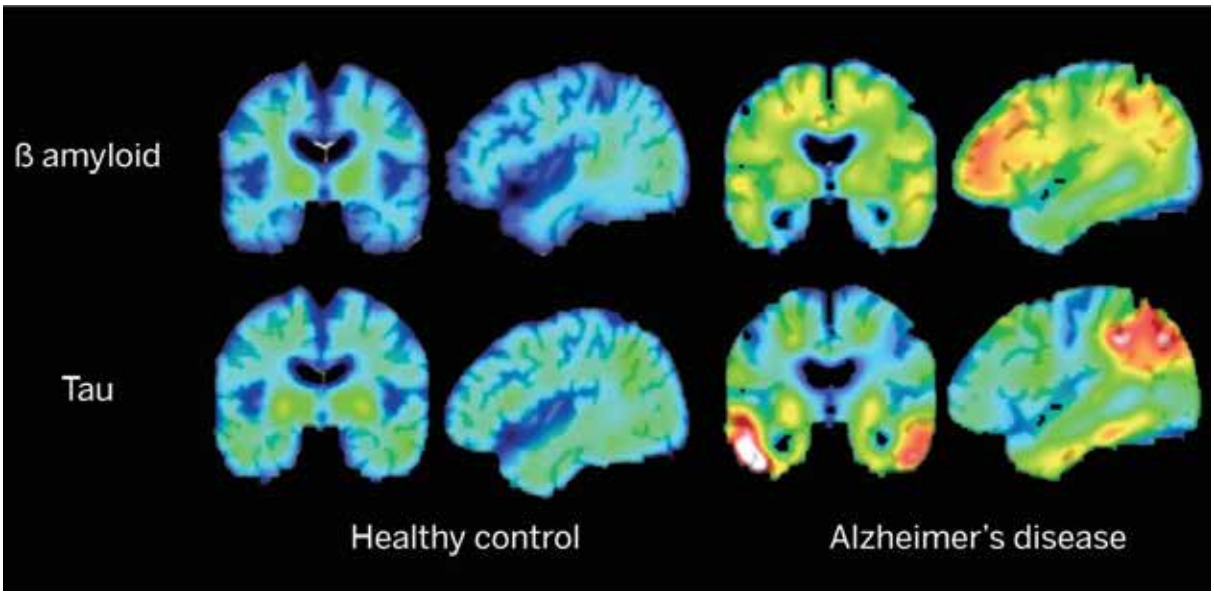
1.2. Acetylcholinesterase theory

AChE is a neurotransmitter that travels through nerve cells to send messages from the brain to the body. It plays a significant role in memory, learning, attention, emotion, and spontaneous muscle activity (Li S., Li A. J., Zhao J., Santillo M. F., Xia M., 2022). Acetylcholinesterase, or AChE, is an enzyme typically found at postsynaptic neuromuscular junctions that breaks down acetylcholine. Acetylcholine is produced when choline and the acetyl group react, facilitated by an enzyme called choline acetyltransferase (Froede H. C., Wilson I. B., Kaufman H., 1986). However, in Alzheimer's disease, damage or death of the cells that produce it leads to the loss of acetylcholine in the brain, resulting in memory loss and confusion. In the brain, AChE usually breaks down acetylcholine. Slowing the breakdown of acetylcholine can be accomplished by blocking AChE, which leaves more acetylcholine available for memory and cognitive functions. This strategy has led to the development of medications known as AChE inhibitors, designed to improve the mental functioning of Alzheimer's patients. By preserving acetylcholine levels, these treatments may enhance nerve-cell communication and reduce specific symptoms associated with the condition (Selkoe D. J., Hardy J., 2016).

1.3. Amyloid theory

Amyloid-beta is a protein naturally produced by the brain. In Alzheimer's disease, this protein forms sticky plaques that build up in the spaces between brain cells. These plaques block communication among brain cells, resulting in memory loss and cognitive impairment. Scientists propose that eliminating amyloid plaques could reduce or potentially prevent Alzheimer's. Aducanumab and lecanemab are two recent medications designed to remove amyloid plaques and improve brain function (Musiek E. S., Holtzman D. M., 2015). This theory suggests that Aβ peptides aggregate, forming plaques, and that these plaques, along with soluble oligomers, disrupt neuronal function and trigger a cascade of events leading to disease progression (DiSabato D. J., Quan N., Godbout J. P., 2016).

Figure 1. *Brain scans comparing β-amyloid and tau levels in a healthy individual and a patient with Alzheimer's disease. The brighter yellow and red areas show greater protein buildup, which is much more visible in the Alzheimer's brain ("Alzheimer's Disease: Get the Facts." 2024)*



1.4. Neuroinflammation

In the brain and spinal cord, neuroinflammation is a complicated inflammatory response that is brought on by a variety of triggers, such as infections, trauma, or neurodegenerative illnesses. It involves the production of inflammatory mediators and the activation of immune and glial cells (Beese, Megan. 2025). Chronic neuroinflammation can harm neurons and impair brain function, although temporary inflammation may be helpful in repair. Numerous neurological disorders, including multiple sclerosis, Parkinson's disease, and the most common, Alzheimer's disease, are associated with this chronic inflammation. Neuroinflammation may exacerbate the accumulation of damaging plaques and tangles in Alzheimer's disease, resulting in cognitive decline and memory loss (Kumar, Anil, et al., 2023).

1.5. FDA-approved drugs for Alzhiemer's disease – Donepezil

Donepezil, one of the five FDA-approved medications for Alzheimer's disease at the moment, is frequently recommended to assist in treating symptoms, including disorientation and memory loss. Although donepezil increases acetylcholine levels, a neurotransmitter crucial for memory and learning, it does not halt the progression of the illness. Although these drugs can offer short-term respite, their effects are usually short-lived. Millions of individuals worldwide are still affected by Alzheimer's (Hooper C., Killick R., Lovestone S., 2008). Therefore, new therapies that address the disease's fundamental causes – such as tau tangles, amyloid plaque accumulation, and neuroinflammation – are desperately needed.

1.6. GSK3 – the connection between GSK3 and Alzheimer's disease

An enzyme called glycogen synthase kinase 3 (GSK3) is involved in metabolism, cell division, and brain development, among other biological processes. GSK3α and GSK3β are its two primary forms, with GSK3β being especially significant in the brain. GSK3 becomes hyperactive in Alzheimer's disease and is closely associated with the formation of tau tangles, one of the main characteristics of the condition. Tau changes form and clumps inside neurons when GSK3 adds too many phosphate groups to the tau protein, a process known as hyperphosphorylation. These tau tangles eventually cause brain cells to die because they prevent them from communicating with one another. Apart from its involvement in tau pathology, GSK3 may also have an impact on amyloid-beta plaque formation and neuroinflammation, both of which are linked to the advancement of Alzheimer's disease (Lauretti E., Dincer O., Praticò D., 2020). GSK3 is being investigated as a potential target for the creation of intriguing and more effective Alzheimer's treatments due to its profound influence on several disease pathways.

1.7. Literature Review

Numerous studies investigating the involvement of Glycogen Synthase Kinase-3 (GSK3) in Alzheimer's disease (AD) have generated a thorough understanding of the illness's fundamental causes and possible treatment approaches. GSK3 is introduced as a central kinase in AD pathophysiology in «The GSK3 Hypothesis of Alzheimer's Disease» (Lauretti E., Dincer O., Praticò D., 2020), which also highlights its role in tau hyperphosphorylation, a crucial step in developing neurofibrillary tangles. Furthermore, by affecting the processing of amyloid precursor protein (APP), GSK3 regulates the generation of amyloid-β, two characteristics characteristic of AD. According to the article, memory, cognitive function, and neurogenesis – all of which are significantly compromised in AD patients – are directly impacted by GSK3 disruption. This theory is expanded upon in another piece, "Glycogen Synthase Kinase-3 Signaling in Alzheimer's Disease" (Lauretti E., Dincer O., Praticò D., 2020), which discusses the interaction between GSK3 and other signaling pathways linked to AD neuropathology. It draws attention to how the kinase affects neurogenesis and synaptic function, indicating that GSK3 activity plays a role in neuronal injury and cognitive decline. The paper describes preclinical efforts to produce GSK3 inhibitors, highlighting GSK3's potential as a beneficial target. However, significant barriers remain to converting these inhibitors into clinical medications, particularly minimizing off-target effects and obtaining effective blood-brain barrier penetration. Based on both papers, GSK3 remains a potential target despite all

of these obstacles, and research is still being done to improve methods for regulating it to slow the progression of AD. According to the pooled research, GAK3 inhibition may have therapeutic potential, but more research is needed to determine its clinical practicality.

2. Methods

2.1 Experiment 1 – Identification of Binding Sites in GSK3

The goal of this experiment was to identify possible binding sites on the GSK3 protein where small molecules could bind. The three-dimensional structure of the GSK3 protein was analyzed using DoGSiteScorer, a computational tool that detects pockets on the surface of proteins.

The protein structure was uploaded into the DoGSiteScorer server. The program scanned the protein surface and identified several potential binding pockets. For each pocket, measurements such as volume, surface area, and drug score were calculated. The drug score estimates how suitable a pocket may be for binding drug-like molecules.

To further analyze the protein, a second tool called PrankWeb was used. PrankWeb predicts ligand binding sites using a machine-learning method that considers factors such as interaction energy and residue conservation. The program ranked the predicted binding pockets based on score, probability, and number of residues involved. The highest ranked pockets were selected as the most promising binding sites.

2.2 Experiment 2 – Virtual Screening

After identifying potential binding pockets, virtual screening was performed to find small molecules that could interact with the GSK3 protein. This step was carried out using the Pharmit platform, which allows large compound libraries to be searched using a pharmacophore model.

A pharmacophore represents the key molecular features required for interaction with a protein. These features can include hydrogen bond donors, hydrogen bond acceptors, and hydrophobic regions. In the Pharmit interface, these features were represented as spheres showing where interactions should occur.

The compound database was screened using the pharmacophore model, and molecules that matched the required spatial and chemical features were selected. After filtering the results, 15 compounds were identified as potential candidates. These compounds were selected for further evaluation in the molecular docking experiment.

2.3 Experiment 3 – Molecular Docking

The selected compounds were further analyzed using molecular docking to evaluate their potential interactions with the GSK3 protein. Docking simulations were performed using the SwissDock server.

The three-dimensional structure of the GSK3 protein and the structures of the selected compounds were uploaded into SwissDock. The program simulated how each molecule could fit into the predicted binding pocket of the protein.

SwissDock generated several possible binding conformations for each compound. For each docking result, two important values were calculated: FullFitness score and estimated Gibbs free energy (ΔG). These values estimate the strength and stability of the interaction between the protein and the ligand. Lower energy values indicate stronger predicted binding interactions.

The docking results were grouped into clusters, and the most favorable binding poses were selected based on the lowest energy values.

3. Results and Discussion

3.1. Experiment 1 – Identification of binding sites in GSK3

The purpose of this experiment was to identify potential binding pockets on the GSK3 protein that could serve as targets for small molecules. These pockets are important because drug molecules must bind to specific regions of a protein in order to affect its activity.

Using DoGSiteScorer, multiple pockets were detected on the surface of the GSK3 protein. Each pocket was evaluated based on its volume, surface area, and drug score. As shown in Table 1, pocket P_0 had the highest drug score (0.81) and the largest volume, suggesting that it may be the most suitable binding site for small molecules. To support these findings, the protein was also analyzed using PrankWeb, which predicts binding sites

using machine learning. Two main binding pockets were identified. The highest-ranked pocket had a probability score of 0.947 and involved 36 residues, indicating a strong likelihood that this region is an important binding site.

The results from both tools identified similar regions of the protein as possible binding pockets. This agreement between the two methods increases confidence in the predicted binding sites and supports their use in later experiments.

3.2 Experiment 2 – Virtual Screening

The goal of this experiment was to identify small molecules that could potentially bind to the GSK3 binding pocket. Virtual screening allows a large number of compounds to be evaluated computationally before laboratory testing. Using the Pharmit platform, a pharmacophore model was created to represent the key interaction features required for binding to the protein. The program then searched a large compound database for molecules that matched these features.

After screening the compounds, 15 molecules were selected because they best matched the pharmacophore model. These compounds are considered promising candidates because their structures are compatible with the predicted binding site. The selected molecules were then used in the molecular docking experiment to further evaluate their interactions with GSK3.

3.3 Experiment 3 – Molecular Docking

The purpose of this experiment was to evaluate how strongly the selected compounds bind to the GSK3 protein. Molecular docking simulations were performed using SwissDock, which predicts how small molecules interact with a target protein.

Each compound was docked into the predicted binding site of the GSK3 protein. The docking program generated several possible binding orientations and calculated energy values for each interaction. Multiple compounds showed strong predicted binding interactions. For example, compound Z785802866 had one of the lowest estimated ΔG values at –9.73 kcal/mol, suggesting a strong and stable interaction with the protein. Other compounds, including Z29354210 and Z5129929917, also showed favorable binding energies.

Lower ΔG values indicate stronger predicted binding between the molecule and the protein. These results suggest that several of the screened compounds may have potential as GSK3 inhibitors. However, because these results are based on computational predictions, further laboratory experiments would be required to confirm their effectiveness.

Experiment No. 1 – How to identify binding sites in proteins?:

Geometric methods

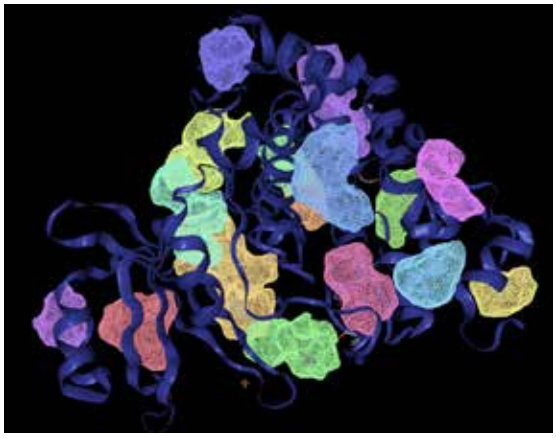
Using DoG Site Scorer

Table 1. *Binding sites identified in GSK3 using the geometric method, DoGsitescorer. Drug Scores listed below represent the most promising binding sites based on the volume and the surface area*

Name	Key (colors)	Volume A	Surface A	Drug Score
P_0		656.9	758.87	0.81
P_1		338.37	559.27	0.61
P_2		302.85	512.29	0.46
P_3		267.01	324.01	0.45
P_4		261.82	346.28	0.51
P_5		241.15	359.06	0.65
P_6		216.32	390.32	0.65
P_7		189.89	263.5	0.52
P_8		175.1	242.16	0.39
P_9		152.26	312.59	0.34

Name	Key (colors)	Volume A	Surface A	Drug Score
P_10		147.78	348.77	0.27
P_11		136.51	363.97	0.3
P_12		132.8	294.27	0.18
P_13		117.89	436.94	0.17
P_14		107.26	313.09	0.21
P_15		102.72	106.27	0.42

Figure 2. *Three-dimensional structure of the GSK3 protein with predicted binding pockets highlighted in different colors. Each colored region represents a potential binding site identified using DoGSiteScorer*



Explanation:

In this experiment, the size of the binding pockets is used to determine possible binding sites in proteins, specifically GSK3, using the geometric method. If a protein has openings or additional areas on its surface that are the proper size to hold tiny molecules, this technique can help identify them. This approach enables us to determine regions where a small molecule could fit by examining the sizes of these pockets. In the early phases of drug discovery, this method is essential since it helps identify possible binding sites for further research. Comprehending the dimensions and form of these pockets can help with the creation of targeted inhibitors or treatments for GSK3.

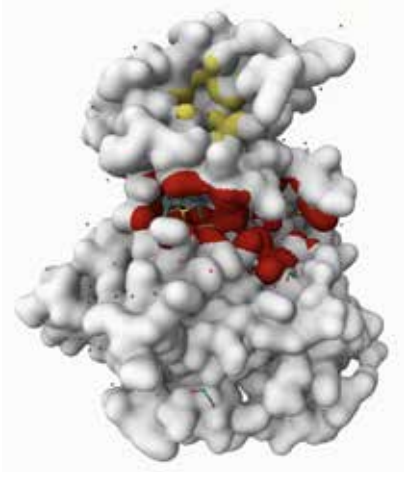
Energetic-based method

Using PrankWeb:

Table 2. *Predicted GSK3 binding sites identified using the energetic-based method PrankWeb. Binding pockets are ranked by score and probability, with Pocket 1 showing the highest likelihood of being a ligand binding site*

Rank	Key	Score	Probability	No. of residues	Avg conservation
1		36.76	0.947	36	2.075
2		2.17	0.051	10	0.444

Figure 3. *Binding sites colored in red and yellow in GSK3, as identified by the machine learning method, Prankweb*



Explanation:

In this experiment, the energy-based approach evaluates the binding energy to investigate the interactions between small compounds and protein binding sites. This method examines hydrogen bonding, hydrophobic forces, and electrostatic interactions to determine how firmly a chemical binds after finding possible sites.

It also considers how protein mutations may change the energy and attraction at the binding site. This approach helps with drug design and understanding protein regulation, particularly for targets like GSK3, by predicting how well small molecules interact with the protein by combining size, energy, and possible mutations.

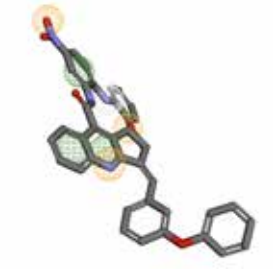
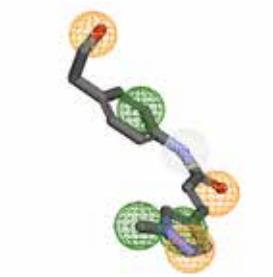
Figure 4. *Pharmacophore model used for virtual screening of potential GSK3 inhibitors on the Pharmit platform. The colored spheres represent key interaction features, such as hydrogen-bond donors, hydrogen-bond acceptors, and hydrophobic regions. The table on the right shows the compounds that matched the pharmacophore model along with their RMSD, mass, and number of rotatable bonds*

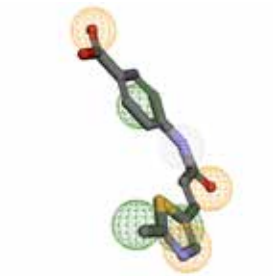
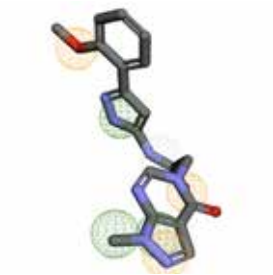
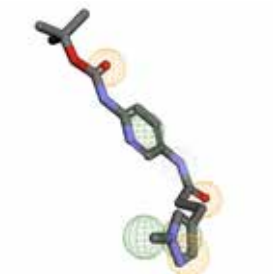
In this experiment, virtual screening was performed using a pharmacophore model on the Pharmit platform to identify small molecules that best fit the GSK3 protein. A pharmacophore is defined by three key elements: the number of interactions, the distance between those interactions, and the types of interactions, each represented as spheres. These features help predict how well a compound might bind to the protein. This phase aimed to filter down the chemical compounds to 15 proteins. This process is crucial in early drug discovery as it helps identify promising molecules for further testing.

Below are the 15 compounds used in this experiment:

Table 3. *Structures of the 15 compounds selected from virtual screening. The table shows the chemical structures of each compound along with their corresponding compound IDs used for molecular docking analysis with the GSK3 protein*

Compound Name	Image:
Z293542101	

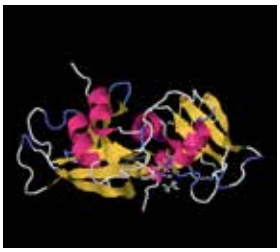
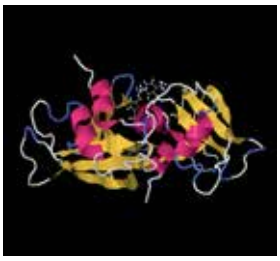
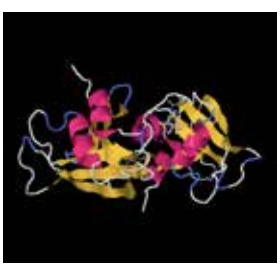
Compound Name	Image:
Z5129929917	
Z5129929917	
Z5129929917	
Z5129929917	
Z5129929917	
Z785802866	

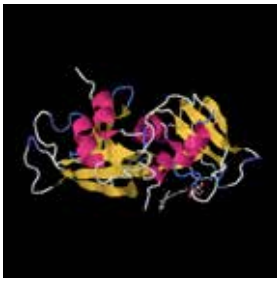
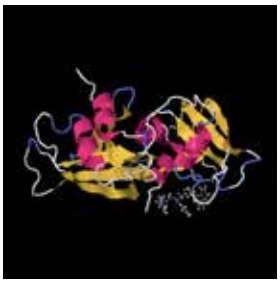
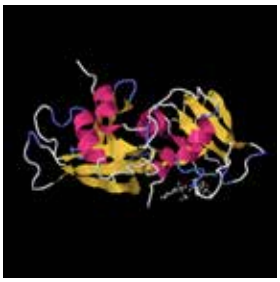
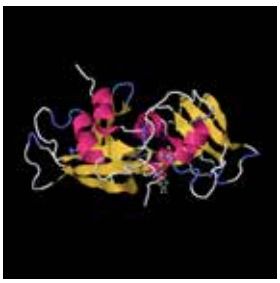
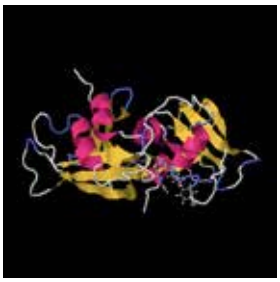
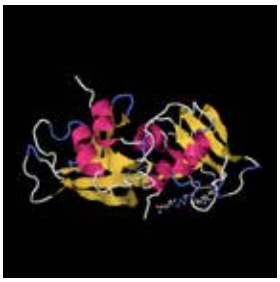
Compound Name	Image:
Z1443864055	
Z5129929917	
Z367619374	
Z927972308	
Z334849748	
Z1067608598	

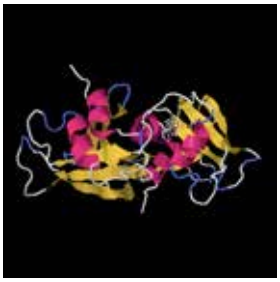
Compound Name	Image:
Z19182681	
Z1001918578	

Next Experiment: Using SwissDock
Molecular Docking:

Table 4. *Molecular docking results for selected compounds interacting with the GSK3 protein using SwissDock. The table shows the docking cluster, element number, FullFitness score, and estimated Gibbs free energy (ΔG) for each compound, along with images of the predicted binding poses within the protein. Lower ΔG values indicate stronger predicted binding interactions*

Name	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)	Picture
Z367619374mol2	10	13	−1243.79	−8.72	
Z19182681mol2	5	0	−1425.42	−7.59	
Z334849748mol2	0	8	−1537.07	−8.83	

Name	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)	Picture
Z785802866mol2	0	0	–1315.58	–9.73	
Z29354210mol2	18	3	–1367.05	–9.41	
Z927972308mol2	19	0	–1383.14	–7.25	
Z1001918578mol2	2	0	–1316.44	–8.75	
Z1443864055mol2	0	4	–1332.86	–8.39	
Z5129929917mol2	3	0	–1385.06	–9.23	

Name	Cluster	Element	FullFitness (kcal/mol)	Estimated ΔG (kcal/mol)	Picture
Z1067608598mol2	2	0	–1413.98	–7.55	

Conclusion:

This study investigated the potential of small molecules as inhibitors of the GSK3 protein, which is strongly associated with the progression of Alzheimer's disease. Computational methods were used to identify possible drug candidates. First, potential binding pockets on the GSK3 protein were identified using geometric and energetic prediction tools. Next, virtual screening using a pharmacophore model was performed to narrow a large compound library down to fifteen candidate molecules that matched the required interaction features. These compounds were then evaluated using molecular docking simulations with SwissDock to predict how strongly they bind to the protein. Several compounds showed favorable binding energies, suggesting stable interactions with the GSK3 binding site. These findings suggest that some of the screened compounds may have potential as GSK3 inhibitors and could serve as starting points for future Alzheimer's disease drug development, although additional experimental testing would be needed to confirm their effectiveness.

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