



Design and Identification of Dual-Target AChE/GSK-3 β Inhibitors for Alzheimer's Disease Through Structure-Based Pharmacophore Modelling, Virtual Screening, and RDKit-Based Lead Optimization

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder in which cholinergic deficit, amyloid- β aggregation, tau hyperphosphorylation, neuroinflammation and oxidative stress act as an interconnected network, limiting the benefit of single-target drugs such as donepezil and memantine. This study applied a multi-target-directed ligand (MTDL) strategy to identify small molecules capable of inhibiting both acetylcholinesterase (AChE; PDB 4EY7, reference ligand donepezil) and glycogen synthase kinase-3 β (GSK-3 β ; PDB 1Q5K, reference ligand AR-A014418). Ligands were prepared using Meeko, while protein preparation, visualization, and complex generation were performed using UCSF ChimeraX. Docking was conducted using AutoDock Vina, followed by protein-ligand interaction analysis using ChimeraX, BIOVIA Discovery Studio, and PLIP and the resulting ligand-interaction coordinates were used to build a four-point dual-target pharmacophore in Pharmit, combining the GSK-3 β hinge (Val135) and hydrophobic (Val110, Tyr134) features with an AChE aromatic anchor (Tyr86). Screening of the ZINC, Molecule and MolPort libraries returned 500 hits, from which five candidates with complementary AChE/GSK-3 β binding were shortlisted. The synthetically accessible lead Z636067564 was modified using RDKit to generate benzimidazole and p-cresol analogues. The benzimidazole analogue showed balanced dual-target docking scores (GSK-3 β -8.166 kcal/mol; AChE -10.544 kcal/mol), zero Lipinski, GSK or PAINS violations, and a favourable synthetic accessibility score of 2.0, and was selected for experimental synthesis. High predicted hERG and hepatotoxicity probabilities for both analogues indicate that further structural optimisation and experimental validation are required.

Keywords: Alzheimer's disease; multi-target-directed ligand (MTDL); acetylcholinesterase; glycogen synthase kinase-3 β ; pharmacophore modelling; molecular docking; virtual screening; ADMET

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia, accounting for roughly 60–80% of cases, and is marked by progressive, irreversible loss of memory, cognition and executive function [1,2]. More than 55 million people live with dementia worldwide, a number projected to nearly triple by 2050, and AD ranks among the leading causes of death in many developed countries [3].

The pathology of AD is multifactorial. Loss of basal-forebrain cholinergic neurons, reduced choline acetyltransferase activity and overactive acetylcholinesterase (AChE) lower acetylcholine levels; AChE also accelerates amyloid- β (A β) fibrillisation through its peripheral anionic site (PAS) [4,5]. A β peptides released from amyloid precursor protein by BACE1 and γ -secretase form oligomers and plaques that disrupt synapses and trigger neuroinflammation [6,7]. Hyperphosphorylated tau detaches from microtubules and forms neurofibrillary tangles that collapse axonal transport [8]. Chronically activated microglia release TNF- α , IL-1 β and IL-6 [9], mitochondrial electron leak and metal-catalysed Fenton chemistry generate reactive oxygen species [10,11], and impaired brain insulin signalling ("type 3 diabetes") adds a further energy deficit [12].

Approved drugs remain symptomatic: donepezil inhibits AChE and memantine blocks NMDA-receptor-mediated excitotoxicity, but neither halts A β accumulation or tau pathology [13,14]. Because AD behaves as an interconnected network with compensatory pathways, single-target agents — including BACE-1 inhibitors and amyloid-only antibodies — have shown limited clinical translation [15,16]. Limitations of single-target approaches have encouraged the development of multi-target-directed ligands (MTDLs) to modulate multiple pathological targets simultaneously. A multi-target-directed ligand (MTDL) is a single molecule that carries pharmacophoric features for two or more targets of the same disease, offering potential advantages over drug cocktails in efficacy, side effects and patient compliance [17,18].

This work targets both AChE and glycogen synthase kinase-3 β (GSK-3 β). AChE inhibition addresses the cholinergic deficit, while PAS inhibition may reduce A β aggregation. GSK-3 β is a major tau kinase and also contributes to BACE1 activity, microglial activation, and impaired long-term potentiation

[19,20]. Pharmacophore-based design narrows the chemical space by mapping essential features, merging pharmacophores, and enabling virtual screening [21]. However, combining two pharmacophores can increase molecular weight, requiring careful optimization of linker length and CNS drug-likeness.(Table 1) [22,23].

Table 1: Physicochemical Parameters for CNS-Active Drugs

Parameter	Target Range	Rationale
LogP	2.0 – 3.5	Optimal balance for membrane permeability
Molecular Weight (MW)	< 450 Da	Higher MW significantly reduces diffusion rates across the BBB
Polar Surface Area (PSA)	< 90 Å ²	High PSA prevents lipid-membrane crossing

2. Materials and Methods

2.1 Targets and Reference Ligands

The AChE catalytic anionic site (CAS; Trp84/Trp86, Ser203, His447, Glu334) and peripheral anionic site (PAS; Trp286, Tyr72, Tyr124, Tyr341) were treated respectively as the symptomatic and disease-modifying targets [24]. For GSK-3 β , the hinge residues Val135 and Asp133, the catalytic residue Lys85 and the gatekeeper Leu132 were the key ATP-pocket interaction points [25]. Donepezil (C₂₄H₂₉NO₃, ~379.5 g/mol), a gorge-spanning inhibitor that binds both the CAS and PAS, and AR-A014418 (C₁₂H₁₂N₄O₄S, ~308.3 g/mol), an ATP-competitive GSK-3 β inhibitor, were used as reference ligands (Figure 1) [26,27].

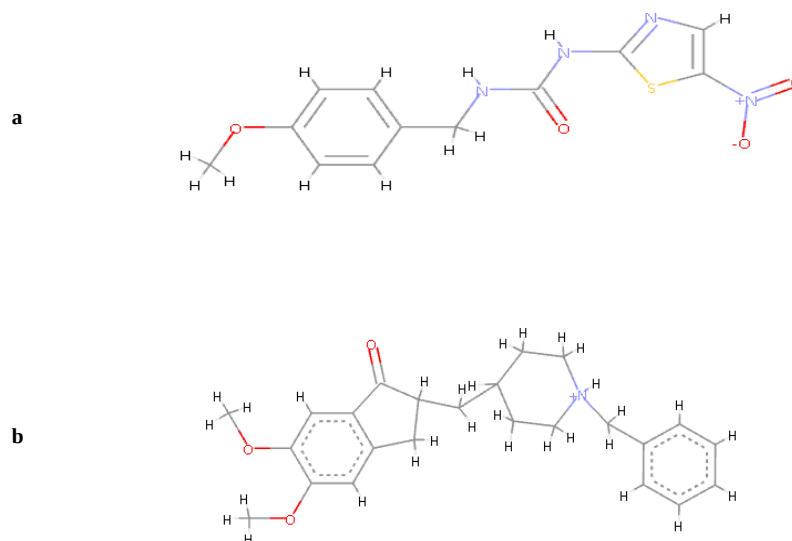


Figure 1. Structures of the reference ligands: (a) AR-A014418 (GSK-3 β) and (b) donepezil (AChE).

Table 2: Reference Ligands Used for Pharmacophore Modelling

Ligand	Target	Molecular Formula	MW (g/mol)
AR-A014418	GSK-3 β	C ₁₂ H ₁₂ N ₄ O ₄ S	~308.3
Donepezil (E20)	AChE	C ₂₄ H ₂₉ NO ₃	~379.5

2.2 Structure Preparation, Docking and Interaction Profiling

AChE (PDB 4EY7) and GSK-3 β (PDB 1Q5K) were prepared in UCSF ChimeraX by removing water, ions and co-crystallised ligands and adding hydrogens and charges [28]. Ligands were converted to PDBQT format using RDKit/Meeko [29]. Molecular docking was performed with AutoDock

Vina using a $22 \times 22 \times 22$ Å grid box centred on each active site and an exhaustiveness of 16; the best-affinity pose was retained for each complex [30]. Docked complexes were analysed with PLIP and BIOVIA Discovery Studio Visualizer, and the ligand interaction coordinates (LIGCOO) were recorded for downstream pharmacophore construction [31].

2.3 Pharmacophore Generation and Virtual Screening

Structure-based pharmacophores comprising four to five features (hydrogen-bond donor/acceptor, aromatic, hydrophobic) were built in Pharmit from the PLIP coordinates, using tolerance radii of 1.0 Å for hydrogen-bond features and 1.2–1.5 Å for aromatic/hydrophobic features, together with shape and exclusion-volume constraints. Compound libraries (ZINC, PubChem, ChEMBL, MolPort, Molecule, ChemDiv) were screened using the physicochemical filters in Table 3 [32].

Table 3: Physicochemical Filtering Criteria for Pharmit Screening

Parameter	Criterion
Molecular Weight	150–500 g/mol
LogP	1.5–3.5 (single-target); 2–5 (dual-target)
Rotatable Bonds	≤ 10
TPSA	≤ 90 Å ²
H-bond Donors / Acceptors	$\leq 2 / \leq 8$
Aromatic Rings	1–3

To build the dual-target model, the GSK-3 β -hit complex and the native AChE-donepezil complex were superimposed in ChimeraX (matchmaker command), the aligned ligand was re-imported into Pharmit, and a four-point dual-target pharmacophore query was defined (Figure 2).

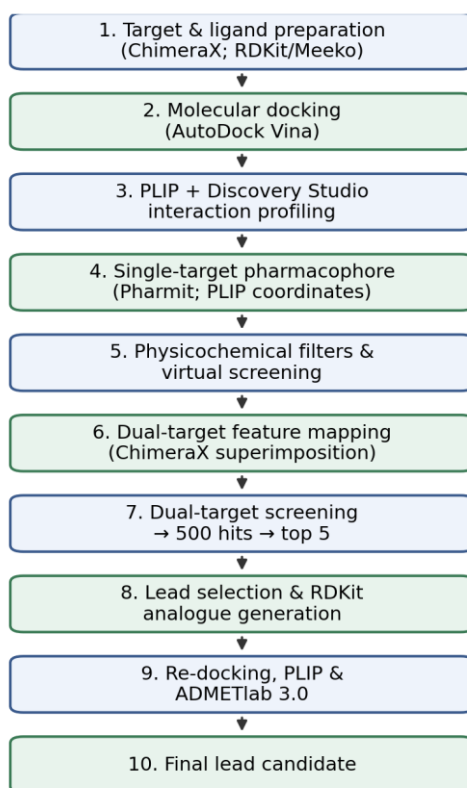


Figure 2. Workflow of structure-based dual-target pharmacophore modelling and virtual screening.

2.4 Lead Selection, Analogue Generation and ADMET

Virtual-screening hits were ranked based on pharmacophore fit, shape similarity, docking score, protein–ligand interactions, and physicochemical properties. Structural analogues of the selected lead were generated in RDKit using SMARTS-based fragment replacement, bioisosteric substitution, and linker modification, followed by physicochemical profiling and re-docking using the same workflow [33]. ADMET and toxicity profiles of the lead and analogues were predicted using ADMETlab 3.0 [[34].

3. Results

3.1 Docking of Reference Ligands

Donepezil docked into AChE with a predicted binding affinity of -11.8 kcal/mol, forming π -stacking interactions with Trp86 (CAS) and Trp286 (PAS) and a hydrogen bond with Phe295. AR-A014418 docked into GSK-3 β at -7.22 kcal/mol, forming a hinge hydrogen bond with Val135 and hydrophobic/ π -alkyl contacts with Val70, Ala83, Leu132, Leu188 and Cys199 (Figure 3). The PLIP-derived ligand-interaction coordinates from these two complexes were used to position the pharmacophore spheres for subsequent modelling.

a) donepezil–AChE (4EY7)

b) AR-A014418–GSK-3 β (1Q5K)

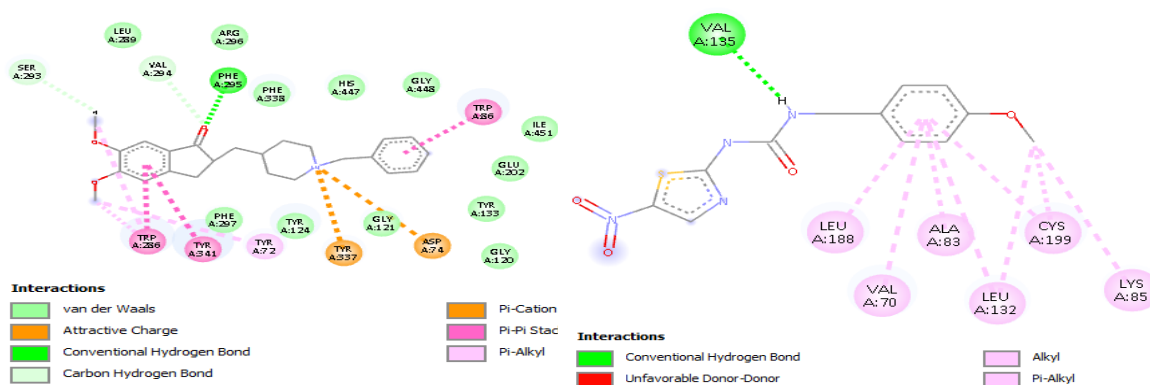


Figure 3. Two-dimensional docking interaction diagrams of (a) donepezil–AChE (4EY7) and (b) AR-A014418–GSK-3 β (1Q5K).

3.2 Single-Target Pharmacophore and Hit Selection (GSK-3 β)

Table 4:PLIP Hydrophobic Interactions of 1Q5K/AR-A014418

Residue No.	Residue Type	Distance (Å)	Ligand Coordinates (LIGCOO)
70	VAL	3.52	20.349, 24.785, 8.515
83	ALA	3.47	20.008, 24.088, 6.236
110	VAL	3.89	18.983, 25.030, 6.095
132	LEU	3.95	18.983, 25.030, 6.095
188	LEU	3.65	20.008, 24.088, 6.236

Table 5:PLIP Hydrogen Bonds Interactions of 1Q5K/AR-A014418

Residue No.	Residue Type	Protein is Donor (PROTISDON)	Distance H-A (Å)	Distance D-A (Å)	Angle (°)	Ligand Coordinates (LIGCOO)
135	VAL	False	2.35	3.22	146.04	23.087, 23.424, 7.128
200	ASP	True	2.70	3.59	150.93	17.659, 26.809, 7.165

For GSK-3 β , a reference ligand (AR-A014418) guided pharmacophore screening of CNS libraries in Pharmit. A four-point GSK-3 β pharmacophore model (Val135 hydrogen bond; Val110, Val70 and Leu132 hydrophobic/ π features) yielded four MolPort hits. and one (MolPort-046-790-851) was selected and merged with the receptor to form a complex .

Table 6: Structure of four MolPort hits

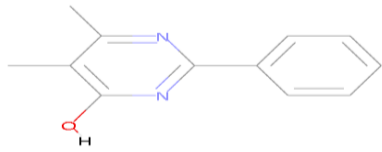
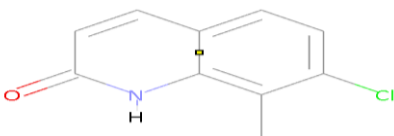
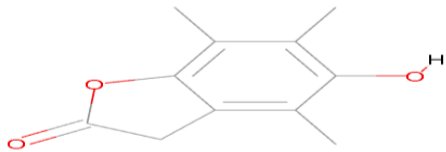
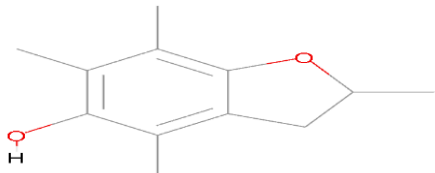
1	MolPort-003-811-558	
2	MolPort-015-143-598	
3	MolPort-022-377-756	
4	MolPort-046-790-851	

Table7: PLIP Hydrophobic Interactions of MolPort-046-790-851

Residue No.	Residue Type	Distance (Å)	Ligand Coordinates (X, Y, Z)
62	ILE	3.79	-1.588, 8.427, 29.551
70	VAL	4.00	0.667, 5.434, 23.197
83	ALA	3.45	-2.161, 3.814, 27.811
85	LYS	3.67	0.667, 5.434, 23.197
110	VAL	3.54	-2.161, 3.814, 27.811
132	LEU	3.92	-2.161, 3.814, 27.811
132	LEU	3.89	-0.852, 4.852, 25.132
134	TYR	3.86	-1.588, 8.427, 29.551
188	LEU	3.47	-2.161, 3.814, 27.811
188	LEU	3.83	-1.414, 7.474, 28.430

Table8:PLIP Hydrogen Bonds Interactions of MolPort-046-790-851

Residue No.	Residue Type	Protein is Donor (PROTISDON)	Distance H-A (Å)	Distance D-A (Å)	Angle (°)	Ligand Coordinates (LIGCOO)
135	VAL	True	2.15	3.10	163.51	-2.414, 5.812, 29.867

MolPort-046-790-851 was selected because it formed a hinge hydrogen bond with Val135 (H-A 2.15 Å; D-A 3.10 Å; 163.5°) together with ten hydrophobic contacts (Ile62, Val70, Ala83, Lys85, Val110, Leu132, Tyr134, Leu188; 3.45–4.00 Å), filling the ATP pocket with good lipophilic complementarity.

Table9:PLIP Hydrophobic Interactions of 4EY7/Donpezil

Residue No.	Residue Type	Distance (Å)	Ligand Coordinates (X, Y, Z)
86	TRP	3.95	-9.712, -45.273, 31.649
286	TRP	3.91	-15.542, -42.927, 26.897
337	TYR	3.79	-13.303, -46.116, 28.443
338	PHE	3.51	-13.762, -44.686, 26.418
338	PHE	3.88	-12.146, -43.907, 28.189
341	TYR	3.57	-16.679, -42.698, 25.951
341	TYR	3.64	-16.364, -43.020, 24.637
341	TYR	3.87	-13.762, -44.686, 26.418
341	TYR	3.32	-13.303, -46.116, 28.443

Table 10:PLIP Hydrogen Bonds Interactions of 4EY7/Donpezil

Residue No.	Residue Type	Protein is Donor (PROTISDON)	Distance H-A (Å)	Distance D-A (Å)	Angle (°)	Ligand Coordinates (X, Y, Z)
295	PHE	True	1.82	2.81	177.71	-14.425, -43.865, 23.587

Table 11:PLIP pi-Stacking Interactions of 4EY7/Donpezil

Residue No.	Residue Type	Centroid Distance (Å)	Angle (°)	Offset (Å)	Ligand Coordinates (X, Y, Z)
86	TRP	3.71	4.87	0.33	-8.344, -45.119, 31.783
286	TRP	4.18	13.85	1.92	-17.614, -42.566, 24.929

For AChE, the donepezil-bound native structure (4EY7) served as the AChE complex. PLIP interaction analysis was then run on the AChE-donepezil complex and the GSK-3β-MolPort hit complex to identify the key interactions and ligand feature coordinates.

3.3 Dual-Target Pharmacophore Model

The GSK-3β-MolPort-046-790-851 complex and the AChE-donepezil complex were superimposed in ChimeraX (RMSD 0.928 Å over three pruned atom pairs; Figure 4), and the aligned ligand was re-mapped in Pharmit. The final model retained three GSK-3β anchors and one AChE anchor (Table 4, Figure 5). Screening of ZINC using this model returned 500 hits (MW ≤ 500 Da; LogP 2–5; TPSA ≤ 90 Å²; rotatable bonds ≤ 8).

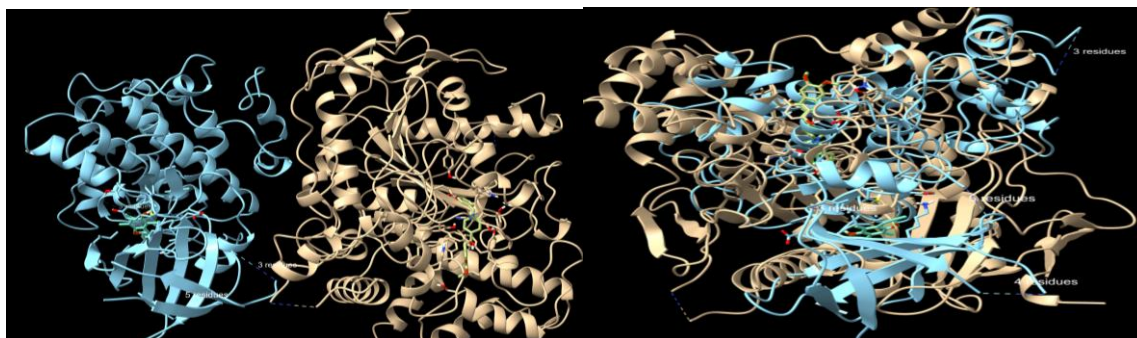


Figure 4: Superimposed GSK-3 β (blue) and AChE (tan) complexes used for dual-target feature mapping.

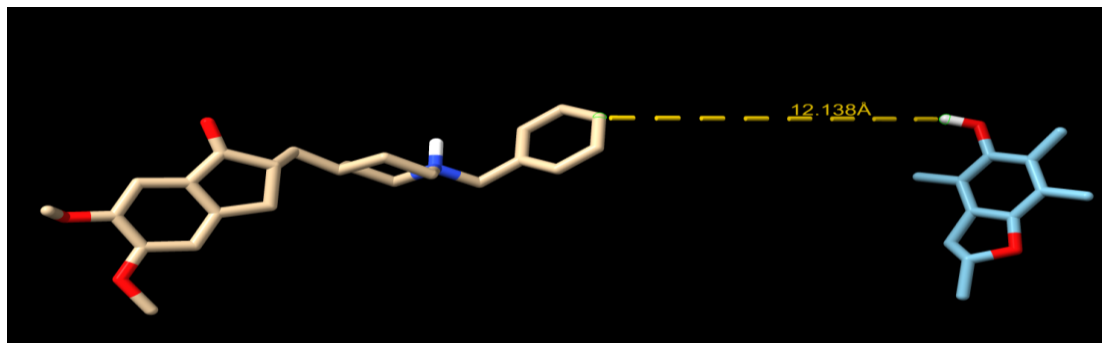


Figure 5: The isolated ligand from Superimposed GSK-3 β and AChE complexes

The isolated ligand was imported into Pharmit to generate a 3D structure-aligned pharmacophore querydual-Target Pharmacophore model by Pharmit

Table 12: Four-Point Dual-Target (AChE/GSK-3 β) Pharmacophore Model

Target	Residue	Feature	Radius	Ligand Coordinates (X, Y, Z)
GSK-3 β	Val135	H-bond	1.0–1.5 Å	-2.414, 5.812, 29.867
GSK-3 β	Val110	Hydrophobic	1.5–2.0 Å	-2.161, 3.814, 27.811
GSK-3 β	Tyr134	Aromatic/Hydrophobic	1.5–2.0 Å	-1.588, 8.427, 29.551
AChE	Tyr86	Aromatic/Hydrophobic	1.5–2.0 Å	-8.344, -45.119, 31.783

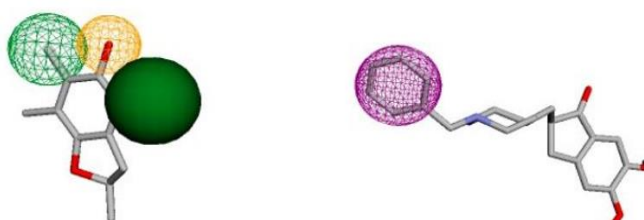


Figure 6: Four-point dual-target pharmacophore features mapped onto the GSK-3 β –MolPort-046-790-851 ligand (left) and AChE–donepezil (right).

3.4 Five Dual-Target Candidates

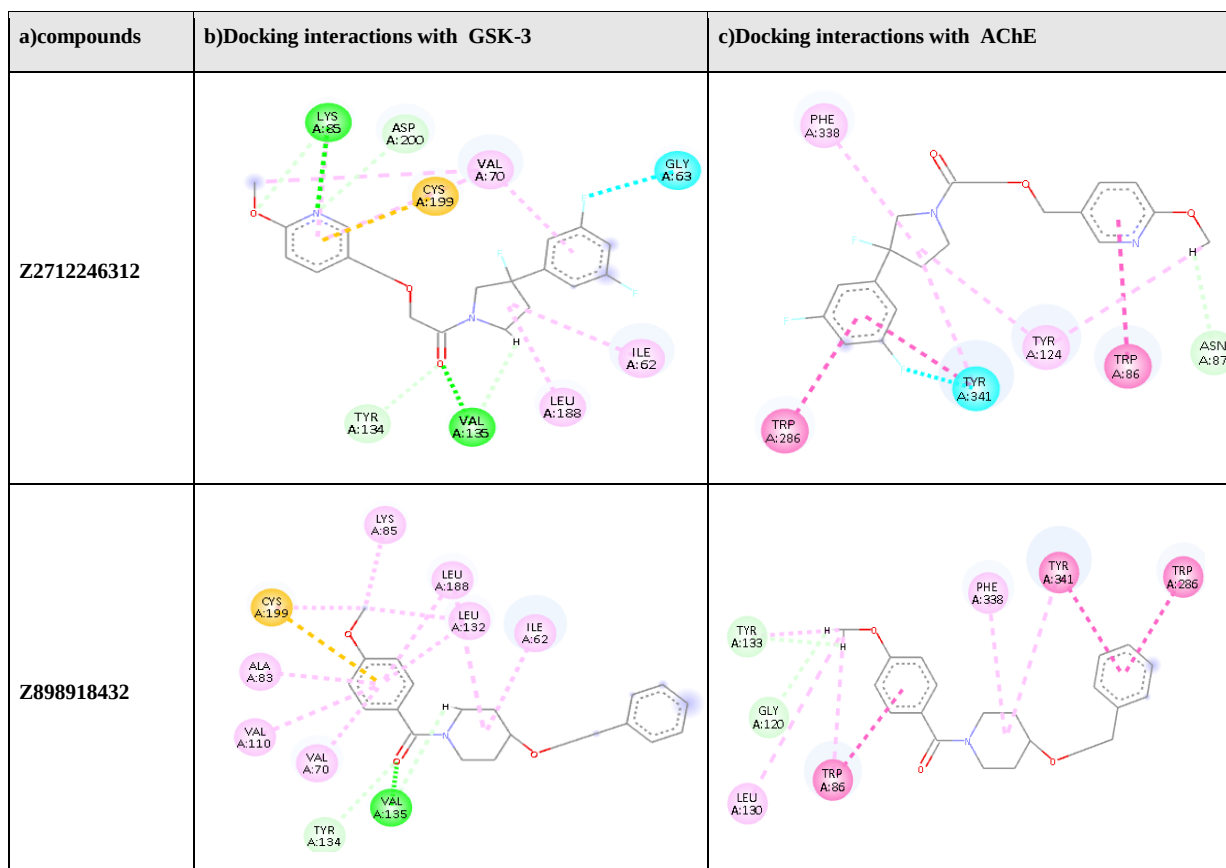
Five hits showing complementary CAS/PAS and ATP-pocket interactions were prioritised (Table 5). All five contacted Val135 in GSK-3 β and Trp86/Trp286 in AChE. Z2712246312 gave the best GSK-3 β score (–8.352 kcal/mol) and Z898918432 the best AChE score (–10.211 kcal/mol). Z636067564 was selected as the lead for further optimisation on the basis of its favourable scaffold, commercially available starting materials and economical synthetic feasibility.

Table 13: Docking Scores of the Five Candidate Dual-Target Inhibitors

Compound	GSK-3 β (kcal/mol)	AChE (kcal/mol)	Key Interactions
Z636067564	-6.980	-9.313	Val135/Lys85 H-bonds; Trp86/Trp286 π
Z31493750	-7.027	-9.580	Val135/Cys199; CAS/PAS π -stacking
Z4493824109	-6.879	-8.886	Val135/Thr138 H-bonds; Trp86/Trp286 π
Z2712246312	-8.352	-10.120	Val135/Lys85 H-bonds; strong Trp86/Trp286 stacking
Z898918432	-7.687	-10.211	Val135 H-bond; strong CAS/PAS π -stacking

Table 14:a) Lead compounds,2D docking interactions of Lead compounds with (b) GSK-3 β and (c) AChE.

a)compounds	b)Docking interactions with GSK-3	c)Docking interactions with AChE
Z636067564		
Z31493750		
Z4493824109		



Among the five initially identified lead compounds, Z636067564 (ZINC000048782262) was selected as the lead/hit compound for further structural optimization. The compound was selected due to its favorable structural features, commercially available starting materials, and economical synthetic feasibility

3.5 Lead Analogue Generation and Evaluation

Replacing the 2-methylimidazole moiety of Z636067564 with benzimidazole or p-cresol fragments gave two analogues by RDKit structural optimization (Figure 7). The benzimidazole analogue scored -8.166 kcal/mol against GSK- 3β (Arg141 hydrogen bond; Val135 and Asp200 carbon–hydrogen contacts; Cys199 π -sulfur interaction; hydrophobic contacts with Ile62, Val70, Ala83, Lys85, Met101, Val110, Leu132 and Leu188) and -10.544 kcal/mol against AChE (π - π stacking with Trp286, Tyr341 and Trp86; π -cation interaction with Tyr337; Figure 8). PLIP confirmed hydrogen bonds to Val135 (2.56 Å) and Ile62 (2.54 Å) in GSK- 3β , and to Tyr124, Ser125 and Phe295 in AChE. The p-cresol analogue scored -7.377 kcal/mol (GSK- 3β) and -11.00 kcal/mol (AChE), with hydrogen bonds to Arg141 in GSK- 3β and to Tyr124 and Ser125 in AChE.

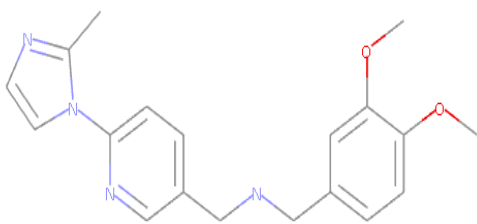


Figure7:Structure of lead compound Z636067564

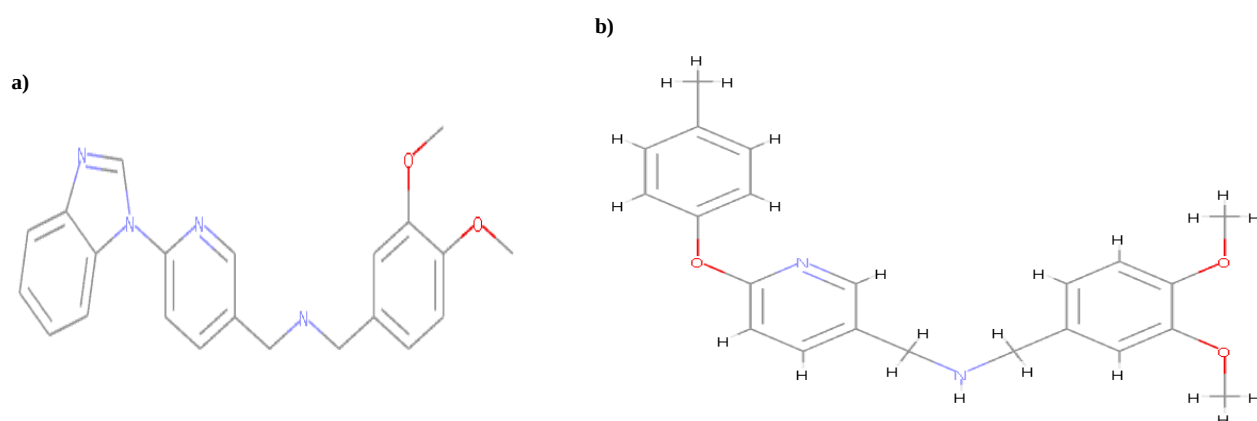


Figure 8: RDKit-generated analogues of Z636067564: (a) benzimidazole analogue and (b) p-cresol analogue.

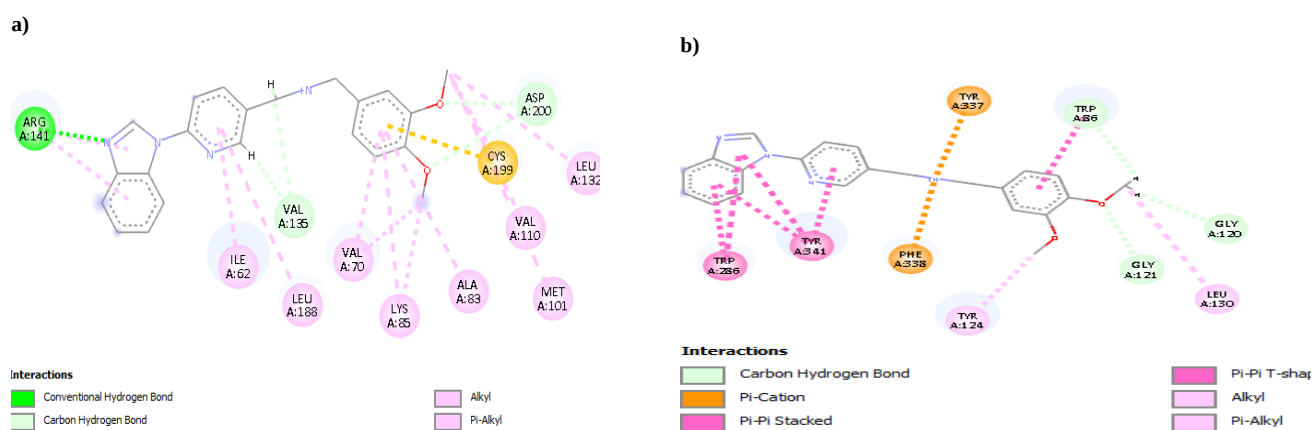


Figure 9: 2D interactions of the benzimidazole analogue with (a) GSK-3β and (b) AChE.

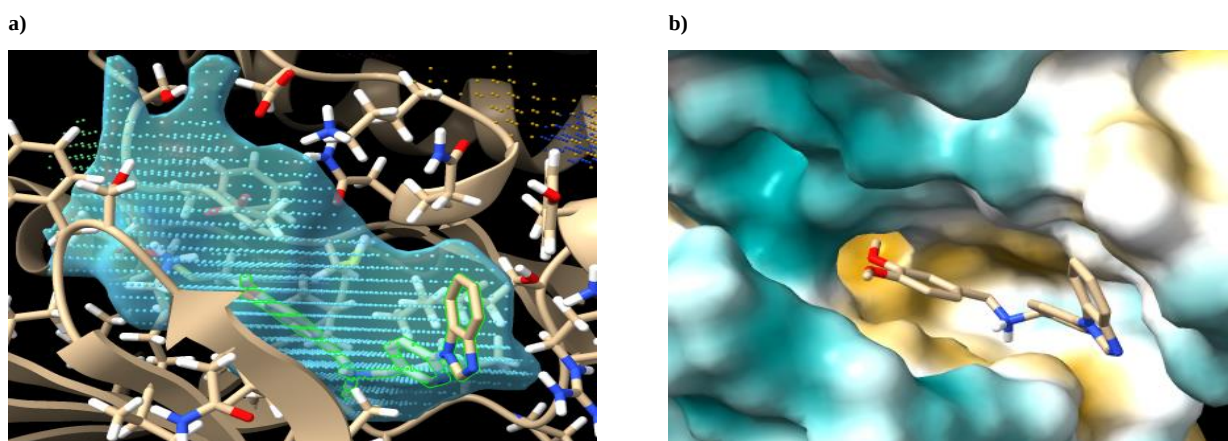


Figure 10: visualization of the GSK-3β with benzimidazole analogue(a) binding cavity; (b) MLP map showing the ligand-protein lipophilic complementarity

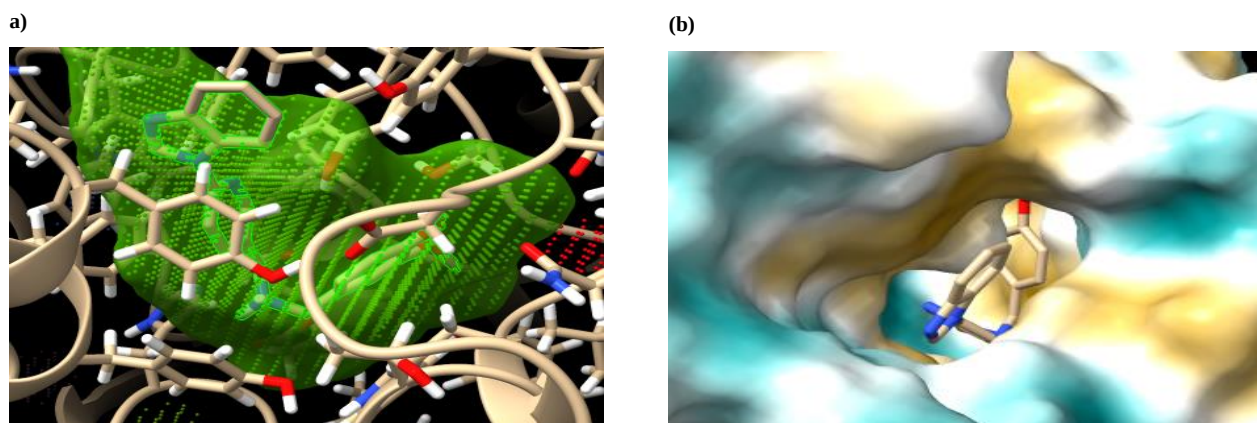


Figure 11: visualization of the AchE with benzimidazole analogue(a) binding cavity; (b) MLP map showing the ligand–protein lipophilic complementarity

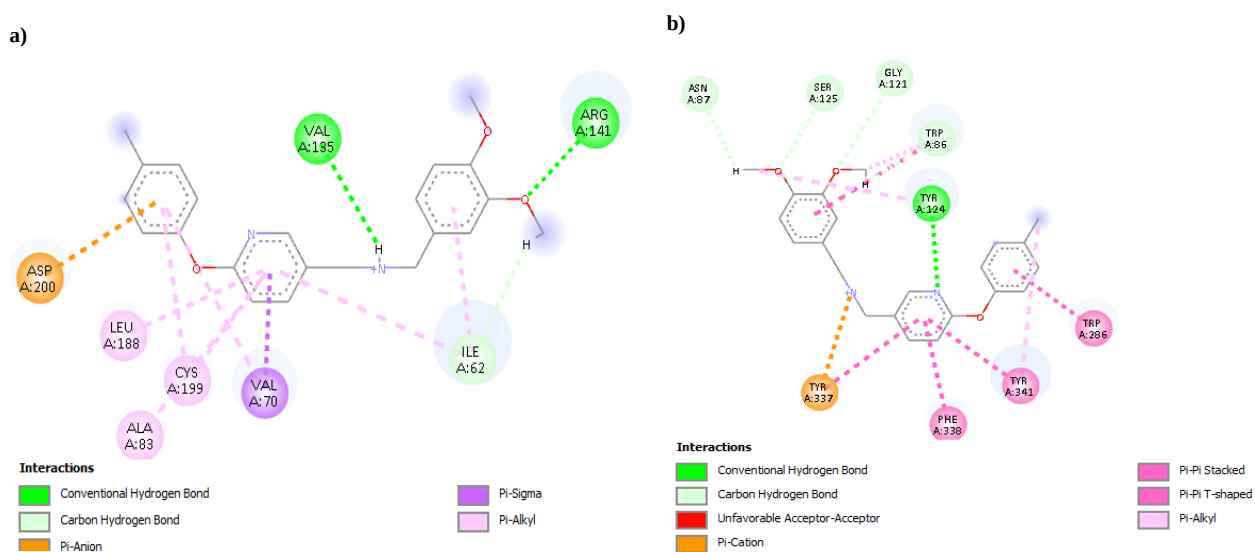


Figure 12:2D interactions of the p-cresol analogue with (a) GSK-3β and (b) AchE

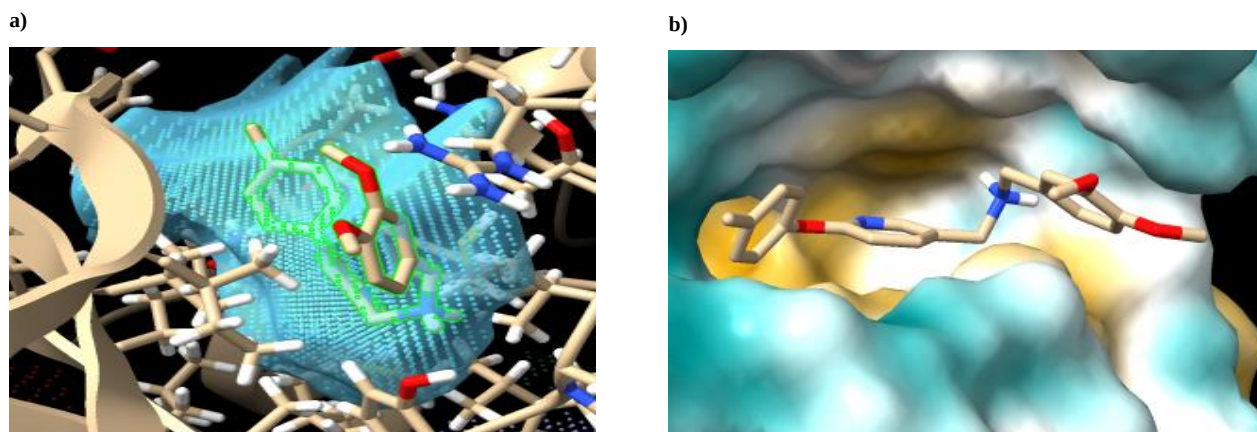


Figure 13: visualization of the GSK-3β with p-cresol analogue (a) binding cavity; (b) MLP map showing the ligand–protein lipophilic complementarity

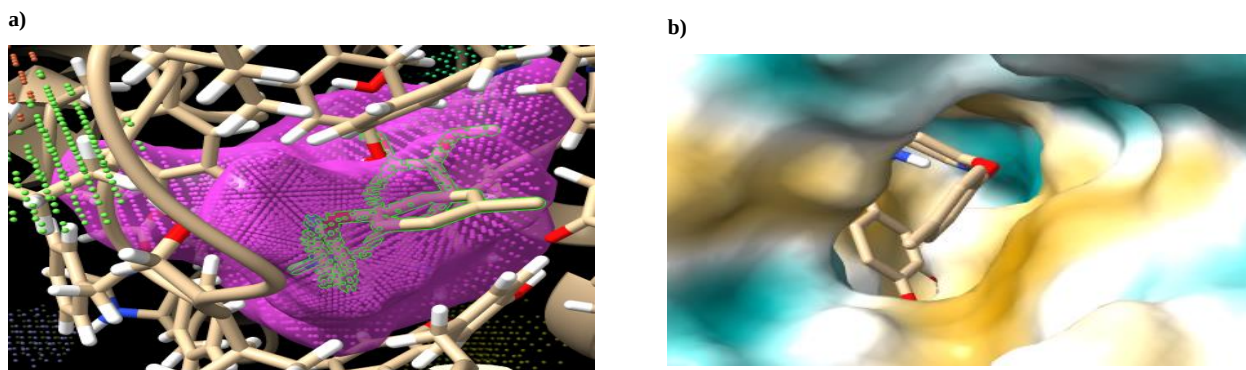


Figure 14: visualization of the AchE with p-cresol analogue (a) binding cavity; (b) MLP map showing the ligand-protein lipophilic complementarity

3.6 ADMET and Drug-Likeness Comparison

Both analogues satisfied Lipinski, GSK and Golden Triangle rules with zero PAINS alerts (Table 6). The p-cresol analogue showed better QED, synthetic accessibility, BBB probability, P-gp substrate probability and several toxicity endpoints, but its plasma protein binding (92.4%) exceeded the <90% criterion and its unbound fraction was low (7.5%). The benzimidazole analogue showed the better GSK-3 β docking score, a lower LogP and lower plasma protein binding, and a higher unbound fraction (18.4%). Both analogues showed elevated predicted hERG, hepatotoxicity and respiratory toxicity probabilities, and the benzimidazole analogue additionally showed high genotoxicity (0.977) and AMES mutagenicity (0.588) probabilities, indicating a need for further optimisation.

Table 15: Docking, Drug-Likeness and ADMET Comparison of the Analogues

Parameter	Benzimidazole Analogue	p-Cresol Analogue
Docking score – GSK-3 β (kcal/mol)	-8.166	-7.377
Docking score – AChE (kcal/mol)	-10.544	-11.000
MW (Da) / LogP / TPSA ($\text{\AA}^2$)	374.17 / 2.707 / 61.2	364.18 / 3.16 / 52.61
QED / Synthetic Accessibility	0.534 / 2.0	0.638 / 1.0
Lipinski / GSK / PAINS violations	0 / 0 / 0	0 / 0 / 0
BBB permeability probability	0.533	0.677
Caco-2 permeability (log unit)	-4.897	-4.867
PPB (%) / Fu (%)	81.8 / 18.4	92.4 / 7.5
P-gp substrate probability	0.039	0.006
hERG / AMES probability	0.805 / 0.588	0.833 / 0.401
Hepatotoxicity / Genotoxicity probability	0.899 / 0.977	0.810 / 0.420
Carcinogenicity probability	0.182	0.160

4. Discussion

The dual-target pharmacophore reproduces the design logic of donepezil, whose benzylpiperidine and indanone groups span the CAS and PAS, while adding a GSK-3 β hinge hydrogen bond to Val135. All five shortlisted hits engaged Val135 together with Trp86/Trp286, supporting the model's value as a filter for genuine dual binders. Predicted AChE affinities (-8.9 to -10.2 kcal/mol) were consistently more favourable than GSK-3 β affinities (-6.9 to -8.4 kcal/mol), consistent with AChE's deep, narrow gorge versus the comparatively shallow GSK-3 β ATP cleft.

Structural optimisation of the lead compound improved binding at both targets: the benzimidazole analogue increased the GSK-3 β score from -6.980 to -8.166 kcal/mol and the AChE score from -9.313 to -10.544 kcal/mol relative to the parent lead, while gaining new anchoring contacts with Val135 and Arg141. It was prioritised for synthesis on the basis of this balanced dual-target improvement, a favourable LogP, a higher unbound plasma fraction, and practical synthetic accessibility, even though the p-cresol analogue performed better on predicted BBB penetration and several toxicity endpoints.

The ADMET prediction results indicated an overall favorable drug-like profile for both analogues, with acceptable physicochemical and pharmacokinetic characteristics supporting their potential as dual-target candidates. The predicted hERG, hepatotoxicity and genotoxicity profiles provide useful guidance for further structural optimisation and future experimental validation. The docking results further supported favorable interactions of the analogues within the binding sites of AChE and GSK-3 β , providing computational evidence for their potential dual-target activity. These findings collectively support the selected compounds for further medicinal chemistry optimisation and experimental evaluation.

5. Conclusion

A structure-based, PLIP-guided dual-target pharmacophore combining essential AChE and GSK-3 β features was used to screen public compound libraries and identify five candidate dual inhibitors. RDKit-based optimisation of the lead compound Z636067564 produced a benzimidazole analogue with balanced, improved dual-target docking scores, zero Lipinski/GSK/PAINS violations and high synthetic accessibility, supporting its selection for experimental synthesis. Future work should prioritise chemical synthesis, in vitro AChE and GSK-3 β inhibition assays, cell-based cytotoxicity and neuroprotection studies, and structural modification to mitigate the predicted hERG and hepatotoxicity liabilities.

Acknowledgements: The authors sincerely acknowledge the support, guidance, and research facilities provided by the Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, Madurai, Tamil Nadu, India, for carrying out this research work.

Author Contributions: Mrs. Tamilarasi G.¹ provided supervision and overall guidance throughout the research work. Ajin T.S.² carried out the major experimental and computational work, including structure-based pharmacophore modelling, pharmacophore-based virtual screening, molecular docking, plip interaction, Discovery Studio-assisted interaction analysis, RDKit-based lead compound optimization. Hameedhash R.³, Archana A.⁴, Priya M.⁵, and Srimathi D.⁶ contributed to computational analysis, literature review, data interpretation, experimental work, and manuscript preparation. All authors contributed to the interpretation and discussion of the results, critically reviewed the manuscript, and approved the final version.

Conflict of Interest: The authors declare that there is no conflict of interest associated with this research work

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