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Generating an efficient BACE1 inhibitor for the treatment of Alzheimer's disease based on AI-powered ADMET analysis, Molecular docking and Molecular dynamics studies

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Alzheimer's disease (AD) is an early stage of dementia due to neurodegenerative disorder that affects the cognitive functions, memory patterns, and learning skills. BACE1 (beta-site amyloid precursor protein cleaving enzyme 1) is a crucial protein involved in the progression of AD. There are many clinical trials being carried out targeting BACE1 for the treatment of AD. However, they critically face limitations to succeed as approved drugs. Hence, this work is aimed to identify novel BACE1 inhibitors using AI-driven drug development processes. Using WADDAICA tool, 300 similar ligands based on the structural features of Atebecestat, AZD3839, LY2811376 are generated and then ADMET analysis was done. The molecular docking studies with BACE1 protein complexes (PDB ID: 7DCZ, 4B05, 4YBI) were helpful to identify 3 ligands as promising BACE1 inhibitors having low-binding energy, and by conducting 100 ns molecular dynamic simulation study, a minimal fluctuations was demonstrated with the considerable duration. Finally, an efficient BACE1 inhibitor M6 {O=C(N(C1CC1)Cc1cccc(c1)c1cccnc1)c1cccc(c1)n1nncc1} with good binding affinity, potency (-7.83 Kcal/mol, 1.83 μM), and high BBB permeability for the treatment of AD is sub-selected from the huge volume of chemical spaces, which will be helpful to narrow down the time factor and can pave ways for subsequent in-vitro studies.

Keywords: BACE1, Frontotemporal dementia (FTD), Molecular docking, Pharmacokinetics

Neurodegenerative diseases are disorders that progressively harm and deteriorate parts of the nervous system, particularly brain. These conditions typically develop over time, with symptoms and effects becoming noticeable in later stages of life[1-3]. Degenerative brain diseases encompass several key types, including dementia-related conditions (such as Alzheimer's disease (AD), frontotemporal dementia (FTD), chronic traumatic encephalopathy (CTE), Lewy body dementia, and limbic predominant age-related TDP-43 encephalopathy (LATE)), Parkinsonism-related disorders, motor neuron diseases (like amyotrophic lateral sclerosis and progressive supranuclear palsy), and prion diseases[4]. BACE1 (beta-site amyloid precursor protein cleaving enzyme 1) is a crucial protein involved in the progression of Alzheimer's disease. It is a major target for AD treatments as it catalyses the initial step in the production of amyloid-β (Aβ)[5,6]. As a result, significant research and development efforts have been focused on creating potent BACE1 inhibitors.

As per the literature[7-10], many state-of-the-art works have been reported for an alternative solution, but, there are very limited clinical successes to the BACE1 inhibitors due to their inability to cross the blood-brain-barrier (BBB)[11-13]. The recent developments of BACE1 inhibitors have reached phase I-III clinical trials (Atabecestat, AZD3839, Elenbecestat, LY2811376, LY-2886721). However, LY-2886721 was terminated due to liver toxicity and Elenbecestat was dropped due to cognitive decline. AstraZeneca released AZD3839 as a pre-clinical research compound, which was evaluated as brain-permeable inhibitor. In a similar manner, based on randomized preclinical study of the non-peptidic BACE1 inhibitor, LY2811376 is available for research purpose. Eli Lilly in their trial have evaluated a nonselective oral β-secretase inhibitor, called Atabecestat[14], however it was associated with trend

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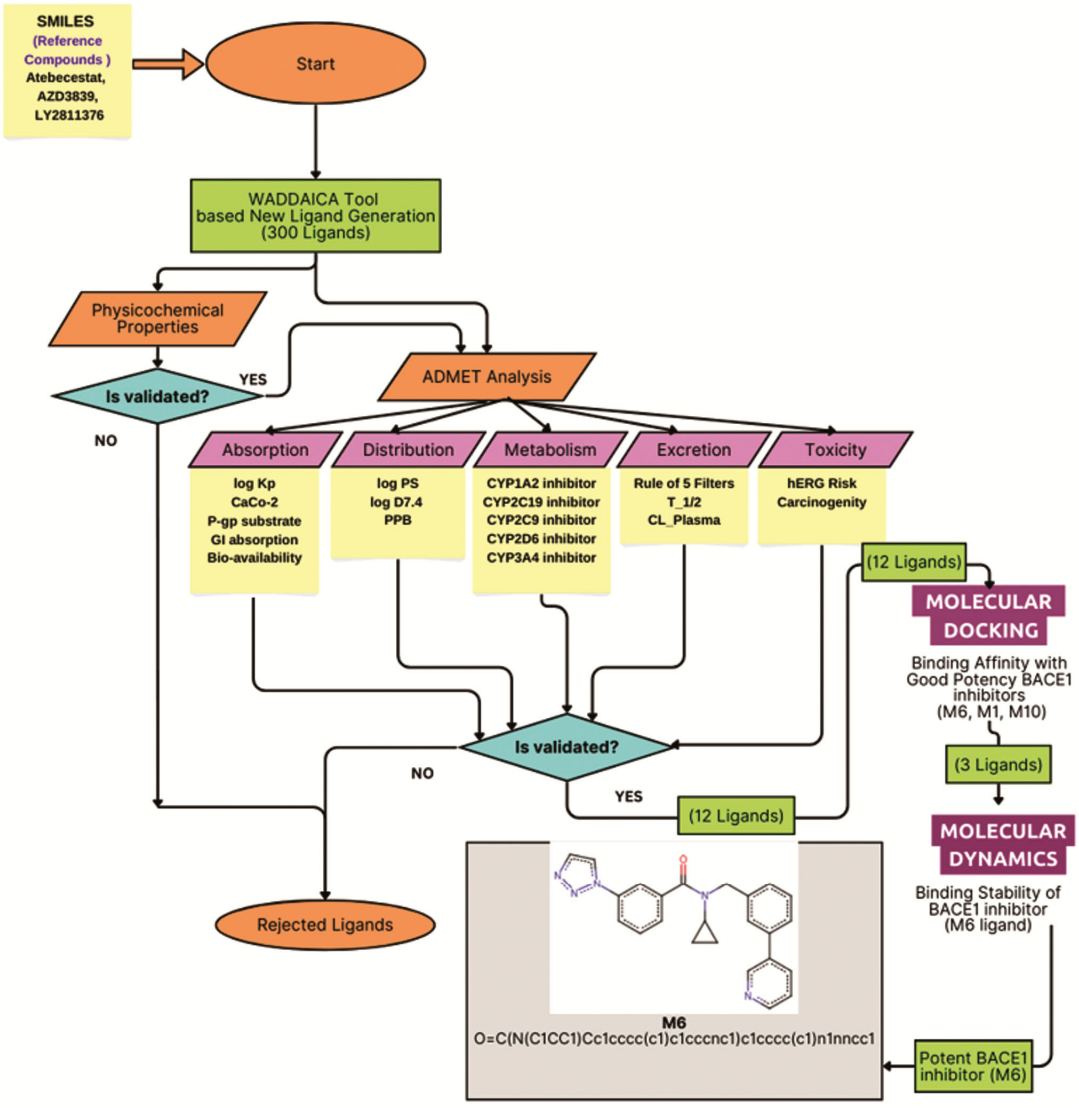

Graphical abstract

toward declines in cognition, and elevation of liver enzymes.

So, the development of BACE1 inhibitors with more activity and lesser side effects than that of the existing clinical trials (Atabecestat, AZD3839, LY2811376) is aimed in this work. This work entails to employ AI-driven *in silico* studies. Initially Onion-Net based new ligands are generated. Based on the physicochemical properties and ADMET analysis, the generated ligands are validated for their drug-likeliness. Molecular docking helps to identify the potential drug by predicting the binding affinity, and their electrostatic, hydrophobic and hydrogen bond interaction of the ligand with the protein, which is fundamental for many biological processes. Based on the literature, the proteins identified for the analysis with the clinical trial compounds, Atabecestat, AZD3839, LY2811376 are 7DCZ, 4B05, 4YBI respectively. The binding interactions are further studied through Molecular Dynamics (MD) simulations, which provide the information about the movement and behavior of the ligands over time.

Materials and Methods

AI based drug design

The molecular generative models[15-20] using graph-based learning approaches have the research potential to enhance the efficiency of drug discovery by leveraging their ability to magnify insights obtained from existing drug-related datasets[21-25]. Using the "Drug design by AI" module of WADDAICA tool[26],

by providing the SMILES strings, for each reference compounds, 100 similar ligands are generated.

Desirable physicochemical, ADMET properties for BACE1 inhibitors

The SwissADME[27] online tool powered by Chemaxon[28] and ADMETLab 3.0[29] are used to assess the physicochemical and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of the generated ligands. Lipophilicity is an important physicochemical parameter (XLOP3) for any BACE1 inhibitor, to link the membrane permeability and drug absorption with the route of clearance. So, the XLOGP3 should be in the range from 3.0 to 5.0, to assure high permeability and moderate to high solubility and metabolism. The log Kp value or skin permeation coefficient, ranging from -10.92 cm/s to -2.08 cm/s, is a measure of how well a chemical can penetrate the human skin. Ligands with logPS values of >-2.0 are considered to be BBB+ (permeable). CYP1A2 is mainly involved in the metabolism of anti-Alzheimer therapies. If a ligand adheres to all of the Lipinski's "rule of five" criteria with zero violations, it is likely to exhibit good oral bioavailability. Also, the ligands should exhibit non-toxic and non-carcinogenicity properties.

Procedure for Molecular Docking studies

Using Autodock-v4.2.6 software[30], by following the user guide's instructions provided by the Scripps Research Institute, the protein - ligand docking is carried out. The top-ranked BACE1 inhibitors can be identified based on the lowest inhibition constant values in the micromolar range and are chosen for binding affinity analysis using Schrödinger's GLIDE module in XP mode[31], including the assessment of their binding interaction patterns. The docked structures are further analyzed using LigPlot+v.2.2.8 tool[32] for 2D binding interactions; and visualized through Discovery Studio Visualizer-v21.1.0.20298[33] for 3D binding interactions.

Procedure for Molecular Dynamics simulation

To validate the movement and behaviour of the ligands over time, molecular dynamics simulation studies[34-36] are conducted using BACE1 enzymes (7DCZ, 4B05, and 4YBI) alongside reference compounds (Atabecestat, AZD3839, and LY2811376). The simulations are performed using the Desmond module of the Maestro Schrödinger Suite[31], with 100 ns simulation duration at 300 K and 1.01325 bar of pressure.

Results and Discussion

AI based virtual screening results

Initially, using WADDAICA web tool, 300 new ligands are generated similar to SMILES of the three of the reference compounds (namely Atabecestat, AZD3839, LY2811376). Using SwissADME tool and ADMET Lab3.0, only 12 out of 300 generated ligands are validated (refer Suppl. Table S1), those satisfy the required physicochemical properties.

Physicochemical properties generated ligands

As per Table 1, these ligands (M1-M12) are found suitable for further performing the molecular analysis as effective BACE1 inhibitors. From Table 1, it was observed that 12 of these ligands (M1-M12) are having molecular weight between 275.32 and 398.46; with maximum of 30 heavy atoms and number of H-Bond donors and acceptors are within limit as ≤ 3 & ≤ 6 respectively. Also, for all 12 ligands, MR has acceptable range from 78.07 to 115.22 and TPSA varies as 41.05 $Å^2$ to 77.05 $Å^2$ along with satisfied XLOP3 range of 1.92 to 3.80 lipophilicity scores. Based on this validation of physicochemical properties, all these 12 ligands (M1-M12) were confirmed to be validated ligands for upcoming stages of ADMET analysis. As per Table 1, based on the assessment of absorption characteristics, six ligands (M1, M4-M6, M8-M9) are identified as 'water soluble' with their log S values >-4.0 mol/L. Also, the remaining six ligands (M2, M3, M7, M10-M12) are identified as 'moderately soluble' with the preferable range >-5.0 mol/L to <-4.0 mol/L.

Analysis of ADMET properties of ligands

Using both SwissADME and ADMETLab 3.0 tools, for the M1-M12 ligands, their pharmacokinetic properties are assessed to ensure their drug-likeness to be identified as BACE1 inhibitors. The results of ADMET analysis is stated in (Table 2) below.

Validation of Absorption properties

The CaCo-2 cell permeability of all 12 ligands ranges from 20.95 x 10^{-6} cm/s to 56.98 x 10^{-6} cm/s, indicating the membrane permeability. Additionally, all 12 ligands show high GI absorption with values above 70%. In terms of high skin permeability, M10, M6, M1 ligands have the highest log Kp values (-6.80 cm/s, -6.29 cm/s, -5.95 cm/s), which is a most important factor for the identification of effective BACE1 inhibitor. The rest of the 9 ligands (M2-M5, M7-M9, M11-M12) exhibit moderate log Kp values (ranges from -4.14 cm/s to -2.75 cm/s) and are having

Table 1 — Physicochemical properties of reference compounds and generated ligands (M1-M12)

Compound ID	MW	No. of Hetero atoms	No. of Aromatic heavy atoms	No. of Rotatable bonds	No. of H-bond acceptors	No. of H-bond donors	Molar Refractivity (MR)	TPSA (Å)	XLogP3 Lipophilicity	Water solubility log S (mol/L)
Atabecestat	367.09	8	12	7	6	3	101.92	104.16	1.97	-3.16
M1	385.18	5	16	8	5	2	113.89	71.09	3.80	-3.1
M2	339.19	5	12	6	5	1	99.69	54.46	2.67	-3.41
M3	321.15	5	12	6	5	2	91.15	71.09	2.46	-4.18
M4	345.15	5	16	4	5	2	102.74	71.09	3.02	-3.97
AZD3839	431.14	8	24	7	5	2	116.68	77.05	3.22	-3.9
M5	374.17	7	23	7	6	1	102.87	68.52	2.78	-3.16
M6	395.17	6	23	6	6	0	114.47	63.91	3.41	-3.53
M7	362.15	6	21	7	5	2	103.12	64.78	2.82	-3.03
M8	398.17	6	23	4	6	1	115.22	71.25	3.28	-4.08
LY2811376	275.15	6	11	4	5	1	74.16	57.70	2.43	-3.67
M9	307.15	6	11	5	4	2	78.07	64.94	3.27	-3.54
M10	305.17	7	11	4	6	1	80.05	66.93	1.92	-3.09
M11	301.16	5	11	2	4	2	82.76	60.91	2.49	-3.43
M12	292.15	6	10	5	4	1	85.23	41.05	2.94	-3.34

sufficient skin permeability. According to P-gp substrate properties, these 12 ligands (M1-M12) can be considered as BACE1 inhibitors, as they are positive P-gp substrates.

Validation of Distribution properties

Based on log PS parameter, all ligands have above -2.0 values, ranging from -1.88 to -0.45. Based on log $D_{7.4}$ distribution coefficient, 10 ligands (M1-M6, M8-M10, M12) fall within the valid range from 2.57 to 3.39. However, M7 & M11 ligands are having lower distribution coefficient as 2.23 and 1.8 respectively, Regarding PPB, only seven ligands (M1-M2, M4, M6-M8, M10) met out criteria for high PPB values, ranging from 92.74% to 98.13%. Based on these distribution properties, only these seven ligands (M1-M2, M4, M6-M8, M10) are validated for further consideration.

Validation of Metabolism, Excretion and Toxicity properties

Except for M9, M11, and M12, the remaining 9 compounds (M1-M8, M10) are supportive of being effective inhibitors of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 enzymes against BACE1. Additionally, none of the ligands violate the Rule of 5 (Lipinski, Ghose, Veber, Egan, Muegge) filters, which further enhances the excretion rate of all the ligands. Regarding the toxicity of the ligands, all are non-toxic; however, M9 has a lower hERG risk, while the rest exhibit medium hERG risks. Based on the validation of ADMET properties, finally, only M1, M6 and M10 ligands are considered for molecular docking and molecular dynamics simulation studies.

BOILED-Egg plot-based drug-likeness

The Brain or Intestinal EstimateD penetration method (BOILED-Egg) uses the surface plot of TPSA (Polarity) versus the WLOGP (Lipophilicity), to predict the passive gastrointestinal absorption (HIA) and BBB permeation. The yellow region (yolk) of the BOILED-Egg shows the physicochemical space of ligands with the highest likeliness of ligands towards permeating to the brain. As per Figure 1, from the BOILED-Egg surface plot, the points in the yellow ellipse (i.e. the yolk) are for compounds with high probability to permeate through the BBB to access the CNS. It is observed that, all the ligands (M1-M12) are reported here as positively P-gp substrate (blue dots) and high BBB permeability (within yolk region) and thus conditioning the neurotoxicity. However, among the 3 reference compounds, only LY2811376 satisfy the polarity and lipophilicity. Other 2 reference compounds are away from yolk region, which means they have lesser BBB permeability and also, Atabecestat is marked as negatively P-gp substrate (red dot).

Based on the detailed discussions in sections 3.1 - 3.3, related to physicochemical & ADMET properties, all the smaller molecular ligands (M1-M12) are identified as satisfying the drug-likeliness and are considered to undergo molecular docking studies to sub-select the most promising ligands that have potential BACE1 inhibition activities and finally

Table 2 — ADMET properties of reference compounds and generated ligands (M1-M12)

	Absorption					Distribution			Metabolism					Excretion			Toxicity	
ADMET Parameters	CaCo2 permeability (cm/s)	GI absorption (>70%)	Skin permeability (log Kp) (cm/s)	P-gp substrate	Bioavailability (>=0.55)	BBB permeability (log PS)	Distribution coefficient (log $D_{7.4}$)	Plasma_Protein_Binding	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	"Rule of 5" #Violations	T_1/2	CL_Plasma	hERG Risk	Carcinogenity (Rat, Mice)
Antabecestat	-4.94		-7.14	No	0.55	-1.35	2.20	93.61	No	No	Yes	No	Yes	0	1.05	3.54		
M1	-4.83		-5.95	Yes	0.55	-1.88	3.39	98.13	Yes	Yes	Yes	Yes	Yes	0	1.01	1.81		
M2	-5.20	High	-3.54	Yes	0.55	-0.45	3.01	92.74	Yes	Yes	Yes	Yes	Yes	0	1.41	10.1	Medium	
M3	-4.81		-4.14	Yes	0.55	-1.28	3.07	82.99	Yes	Yes	Yes	Yes	Yes	0	1.24	1.27		
M4	-4.83		-3.53	Yes	0.55	-1.38	2.89	97.73	Yes	Yes	Yes	Yes	Yes	0	0.65	5.03		
AZD3839	-4.99		-6.65	Yes	0.55	-1.27	1.89	98.26	No	Yes	Yes	Yes	Yes	0	0.66	4.63		
M5	-5.24		-3.29	Yes	0.55	-0.72	2.79	87.13	Yes	Yes	Yes	Yes	Yes	0	0.44	5.68		Negative
M6	-4.87	High	-6.29	Yes	0.55	-1.03	2.57	97.53	Yes	Yes	Yes	Yes	Yes	0	0.54	3.91	Medium	
M7	-5.08		-3.95	Yes	0.55	-1.17	2.23	96.90	Yes	Yes	Yes	Yes	Yes	0	0.29	7.75		
M8	-4.97		-2.75	Yes	0.55	-0.9	3.24	97.50	Yes	Yes	Yes	Yes	Yes	0	0.47	4.51		
LY2811376	-5.54		-6.25	Yes	0.55	-1.02	2.72	71.70	Yes	Yes	Yes	Yes	Yes	0	0.83	7.51	Low	
M9	-5.24		-3.94	Yes	0.55	-0.47	2.83	72.81	No	Yes	No	Yes	No	0	0.62	6.19		
M10	-5.54	High	-6.80	Yes	0.55	-1.82	2.71	92.92	Yes	Yes	Yes	Yes	Yes	0	0.87	7.89	Medium	
M11	-5.15		-3.72	Yes	0.55	-0.47	1.80	64.94	No	No	No	Yes	No	0	1.07	4.45		
M12	-4.86		-3.86	Yes	0.55	-0.5	2.72	66.06	No	No	No	Yes	No	0	0.27	7.45		

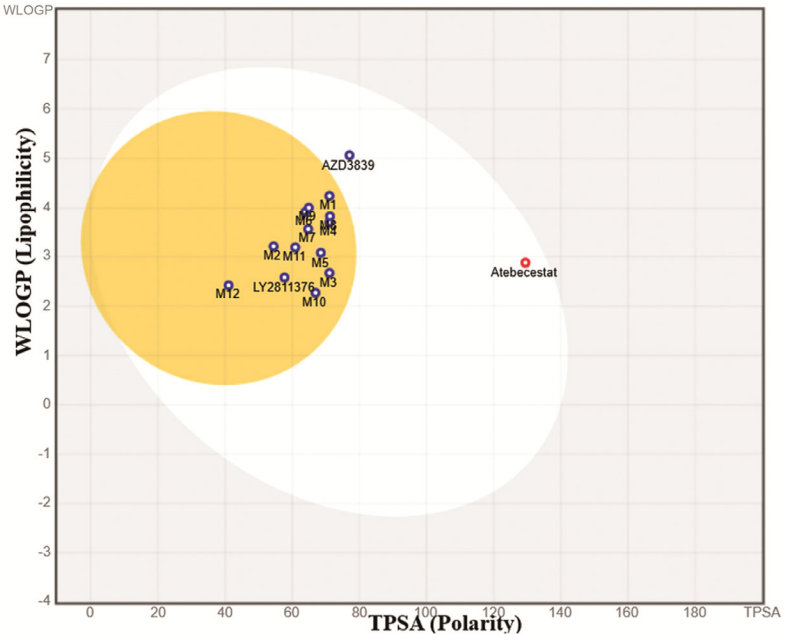

Fig. 1 — BOILED-Egg surface plot for the leading drug-like ligands (M1-M12) and reference compounds (Atebecestat, AZD3839, LY2811376)

during molecular dynamics simulation studies, only those sub-selected ligands are considered for further binding stability analysis.

Analysis of BACE1 inhibitors using Molecular Docking studies

During docking studies using Autodock-v.4.2.6, where groups of validated drug-like ligands (M1-M4,

Table 3 — Trials based calculated binding energy and inhibition constant of ligands (M1-M12) and reference compounds (at the temperature 298.15K) with Protease (7DCZ, 4B05, 4YBI)

Compounds	Protein	Results of molecular docking			
		Trial 1 (300 Runs, Population size=1500, Mutation rate=0.02, Crossover rate=0.8)		Trial 2 (400 Runs, Population size=2000, Mutation rate=0.05, Crossover rate=0.9)	
		Free Binding Energy (Kcal/mol)	Inhibition Constant (μM)	Free Binding Energy (Kcal/mol)	Inhibition Constant (μM)
Atebecestat		-5.13	172.72	-4.82	293.48
M1		-6.47	18.12	-5.87	49.55
M2	7DCZ	-5.46	99.98	-4.63	404.95
M3		-5.79	57.17	-5.23	145.93
M4		-6.18	29.6	-5.78	57.95
AZD3839		-5.73	62.55	-5.70	65.85
M5		-7.22	5.06	-7.06	6.63
M6	4B05	-7.83	1.83	-7.26	4.75
M7		-6.65	13.37	-6.53	16.25
M8		-6.64	13.55	-6.54	16.67
LY2811376		-5.3	131.25	-5.05	199.37
M9		-5.82	54.38	-5.47	98.39
M10	4YBI	-5.93	45.01	-5.53	88.68
M11		-5.35	120.26	-5.35	120.26
M12		-4.68	373.54	-4.55	462.24

M5-M8, M9-12) and reference compounds are docked with their respective BACE1 enzymes (7DCZ, 4B05, and 4YBI). The interaction activities in the binding sites are characterized by free binding energy and inhibition constant values, which are presented in (Table 3).

From Table 3, it seems that the docking studies have identified the ligands M6 < M1 < M10 are comparatively having more binding affinities (-7.83 Kcal/mol < -6.47 Kcal/mol < -5.93 Kcal/mol) and potency (1.83 μM < 18.12 μM < 45.01 μM) than their respective reference compounds (AZD3839, Atabecestat, and LY2811376). But, M1 and M10 have higher Ki values, indicating weaker potency in comparison to M6. Both the ADMET analysis and docking studies support to derive the strong conclusion that, M6 ligand has the most potent inhibition among the three ligands, with lowest binding energy -7.83 Kcal/mol and Ki value of 1.83 μM, which indicates M6 as a strong inhibitor of BACE1.

To support the binding interaction analysis, the electrostatic, hydrogen bond and hydrophobic interactions of M6, M1, and M10 ligands with their respective BACE1 protein enzymes are described in (Suppl. Table S2). This table provide information about bond types and bond lengths between the ligands and nearby amino acid residues. Additionally, as shown in Figure 2, Schrodinger software is utilized to visualize the binding potency of BACE1 inhibitors, by depicting their mode of bindings, interacting atoms and catalytic site residues. A recent literature review highlighted that the interactions with catalytic dyad residues Asp32 and Asp228 are play a vital role for regulating the optimal acidic pH and sequence specificity required for BACE1 enzymatic activity[37].

As detailed in Table 4, in this study, the strong interaction with the catalytic dyad residues, including Asp32 and Asp228, contributes to the high inhibitory activity of M1 and M6 ligands (Refer Fig. 2b & Fig. 2d) against respective BACE1 enzymes as like their clinical trial compounds (Refer Fig. 2a & Fig. 2c). However, M10 ligand (Refer Fig. 2f) has limited interactions only with Asp32 catalytic dyad residue than LY2811376 (Refer Fig. 2e).

As outlined in Suppl. Table S2, the interaction analysis of M1, M6, and M10 ligands with other residues in the active site-gorge of the BACE1 enzyme are conducted. Ligand M1 forms strong hydrogen bonds, acting as a backbone acceptor to Gly74 (2.41 Å) and Phe108 (3.66 Å), and as a backbone donor to Gly230 (1.98 Å, score: 96%). Additionally, M1 exhibits Pi-Cation interactions (hydrophobic bonds) with Tyr71 and Phe108 (5.57 Å, 5.22 Å) and alkyl interactions with Ile110 and Ile118 (4.39 Å, 4.81 Å). Ligand M6 establishes a strong hydrogen bond with Gly289 (3.77 Å) and Pi-Donor hydrogen bonds with Glu290 and Ile352 (3.06 Å,

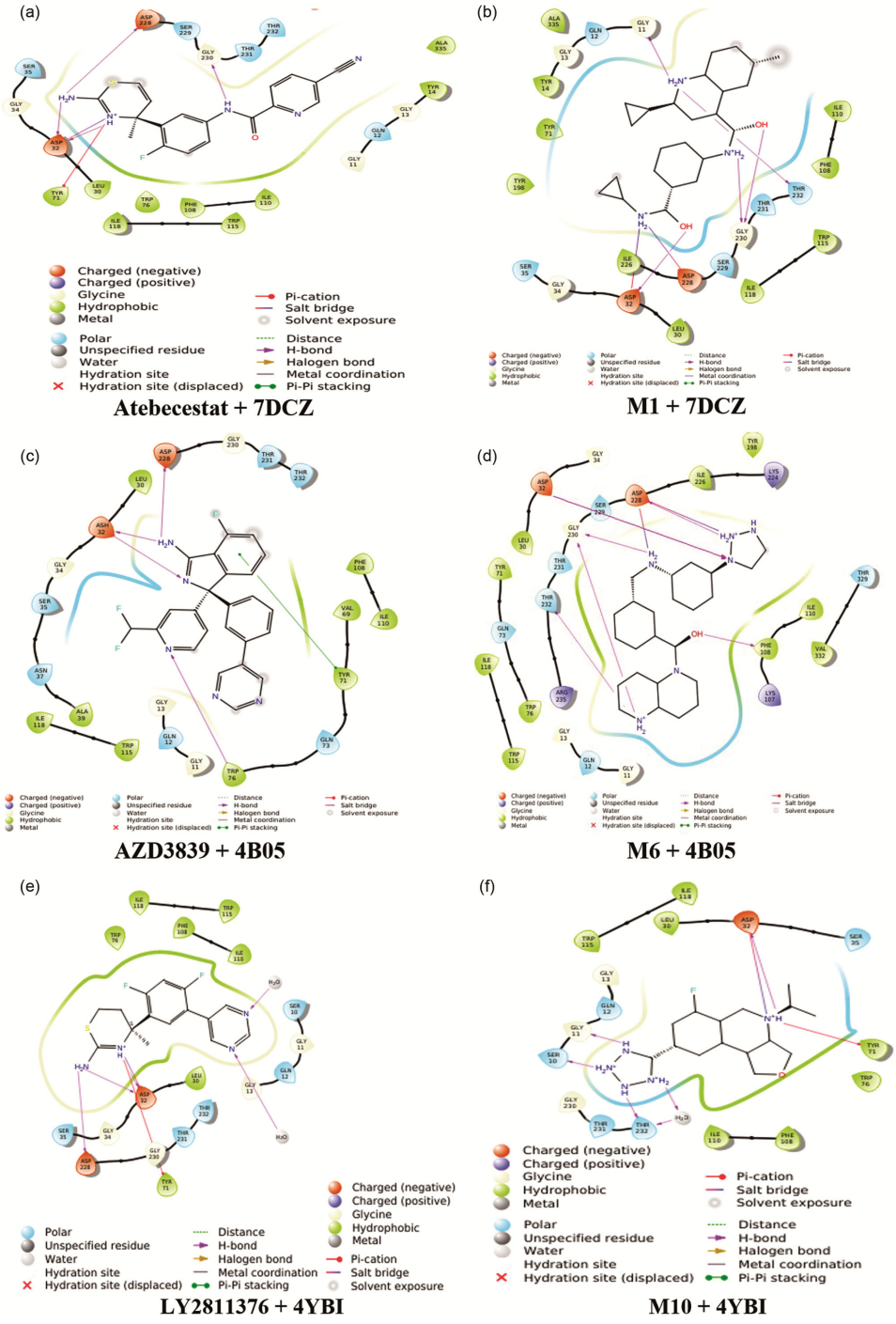

Fig. 2 — Interactions of reference compounds and generated ligands with 7DCZ, 4B05 and 4YBI

3.17 Å). It also forms potent Pi-Cation interactions with Phe296 and Arg351 (4.34 Å, 4.99 Å). Ligand M10 demonstrates numerous strong hydrogen bonds with Ser57 (1.85 Å), Trp277 (2.13 Å), and Ser22 (2.17 Å), along with a halogen bond with Glu364 (3.05 Å). Additionally, M10 shows Pi-Cation bonds with Thr275 (3.6 Å) and a Pi-Anion bond with Glu364 (3.05 Å). These interaction profiles reveal that all three ligands M1, M6, and M10 play a significant role in enhancing drug specificity, metabolism, and adsorption, making them promising candidates for acting as BACE1 inhibitors.

Table 4 — Binding Interactions of M1, M6 and M10 with respective Protease (7DCZ, 4B05, 4YBI)

Compound	Protein ID	Nearby Interaction Residues		
		Electrostatic	Hydrophobicity	Polar
Atebecestat	7DCZ	GLY11, GLY13, GLY34, GLY230, ASP32, ASP228	ALA335, ILE110, ILE118, LEU30, PHE108, TRP71, TRP76, TRP115, TYR14	GLN12, SER35, SER229, THR231, THR232
AZD3839	4B05	GLY11, GLY13, GLY34, GLY230, ASH32, ASP228	ALA39, ILE110, ILE118, LEU30, PHE108, TRP71, TRP76, TRP115, VAL69	GLN12, GLN73, ASN37, SER35, THR231, THR232
LY2811376	4YBI	ASP32, ASP228, GLY11, GLY13, GLY34, GLY230	TRP71, TRP115, TYR71, LEU30, PHE108, ILE110, ILE118	GLN12, SER10, SER35, THR231, THR232
M1	7DCZ	GLY11, GLY13, GLY34, GLY230, ASP32, ASP228	ALA335, ILE110, ILE118, ILE226, LEU30, PHE108, TRP115, TYR14, TRP71, TYR198	GLN12, SER35, SER229, THR231, THR232
M6	4B05	GLY11, GLY230, GLY34, GLY13, ASP32, ASP228, ARG235, LYS107, LYS224	ILE110, ILE118, ILE226, LEU30, PHE108, TRP76, TRP115, TYR71, TYR198, VAL332	GLN12, GLN73,SER229, THR231, THR232, THR329
M10	4YBI	ASP32,GLY11,GLY13, GLY230	ILE110, ILE118, LEU30, PHE108, TRP76, TRP225, TYR71	GLN12, SER10, SER35, THR231, THR232

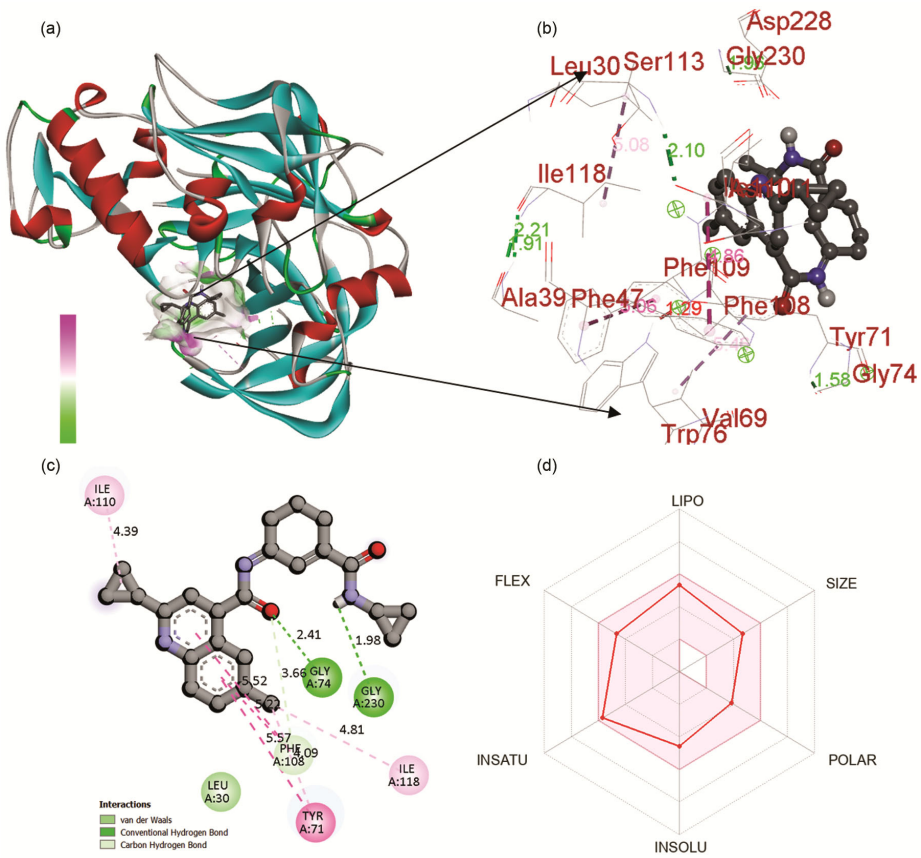

Fig. 3 — (a) Docking pose of M1 Ligand with BACE1 enzyme-7DCZ; (b & c) Interactions with amino acid residues at the binding site of 7DCZ; and (d) Drug like-likeliness of M1 ligand based on bio-availability radar diagram

Evaluation of the *in silico* studies of BACE1 inhibitors

This section outlines the process used to assess the reliability and suitability of the co-crystallized docking poses of the ligands, as well as to confirm their drug-likeliness through a bio-availability radar plot. As shown in Figures 3a-c, the M1 ligand exhibits significant interactions at the binding pockets of the BACE1 enzyme (7DCZ). Also, the bio-availability

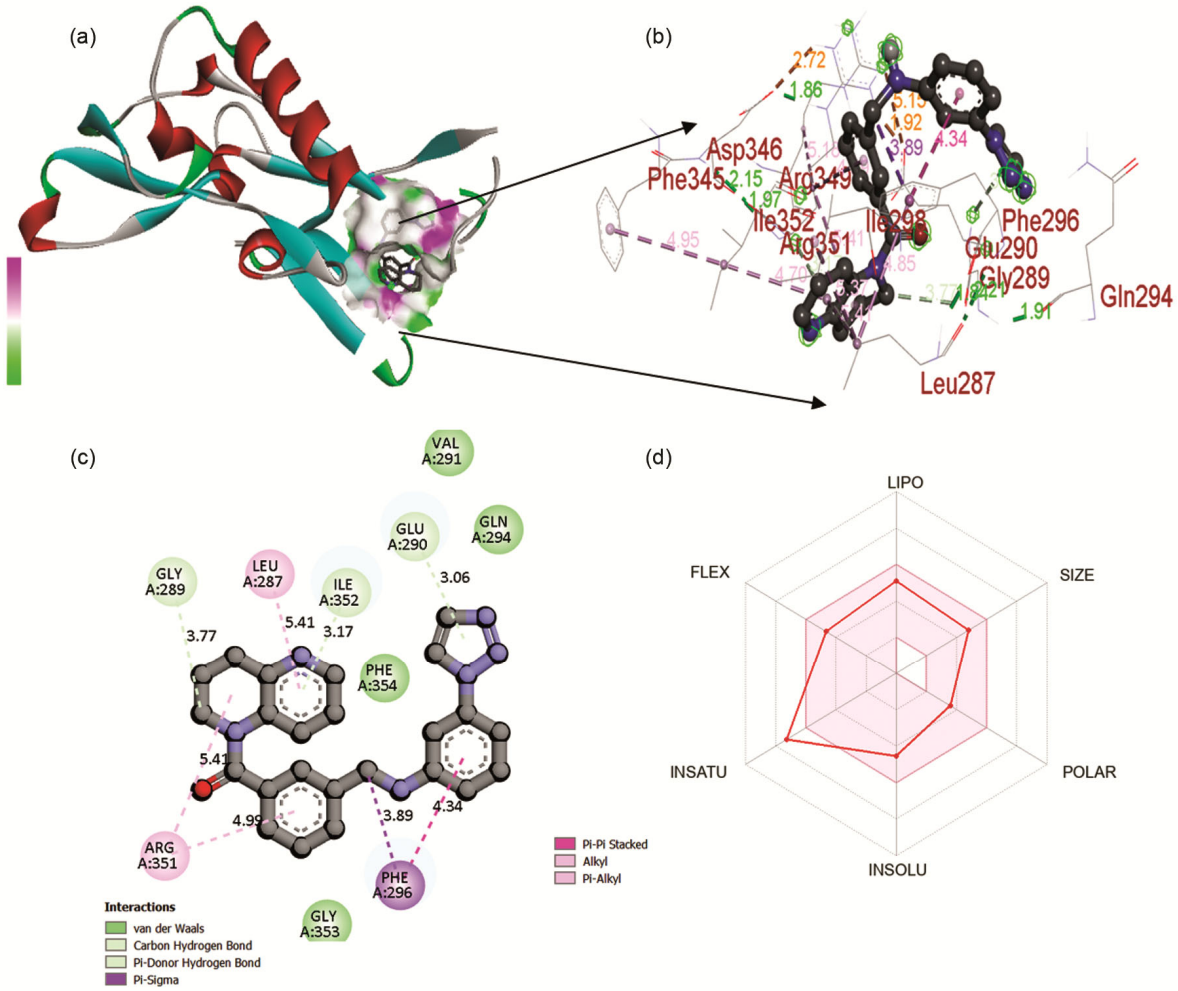

Fig. 4 — (a) Docking pose of M6 Ligand with BACE1 enzyme-4B05 (b & c) Interactions with amino acid residues at the binding site of 4B05; and (d) Drug like-likeliness of M6 ligand based on bio-availability radar diagram

radar plot (Fig. 3d) is helpful to strongly assure the drug-likeliness and potential of M1 ligand for oral bioavailability. For more 2D and 3D visualization of docking results like electrostatic, hydrophobic interactions and 2D binding plot of M1 with 7DCZ refer Suppl. Figs. S1-S3).

While performing the evaluation of M6 ligand, as shown in Figure 4(a-c), the M6 remained stably bound at the binding pockets of the BACE1 enzyme (4B05). Also, the bio-availability radar plot (Fig. 4(d)) is helpful to further assure the drug-likeliness and potential of M6 ligand for oral bioavailability. For more 2D and 3D visualization of docking results like electrostatic, hydrophobic interactions and 2D binding plot of M6 with 4B05 (refer Suppl. Figs. S4-S6).

As depicted in Figure 5a-c, the M10 ligand exhibits limited interactions only at the binding pockets of the BACE1 enzyme (4YBI). Also, the bio-availability radar plot (Fig. 5d) is helpful to accept the drug-likeliness and potential of M10 ligand for oral bioavailability. For more 2D and 3D visualization of docking results like electrostatic, hydrophobic interactions and 2D binding plot of M10 with 4YBI (refer Suppl. Figs. S7-S9).

Molecular Dynamics Simulation studies with ligands

Recent advancements in drug development paved way to utilize molecular dynamics (MD) simulations for the behavioural analysis of BACE1 inhibitors with atomic-level accuracy and high temporal resolution. The RMSD of the protein offers valuable insights into its structural conformation throughout the simulation for 100 ns simulation with temporal boundary conditions using the Desmond module. Interactions between the proteins 7DCZ, 4B05, and 4YBI with Atabecestat and M1, AZD3839 and M6, and

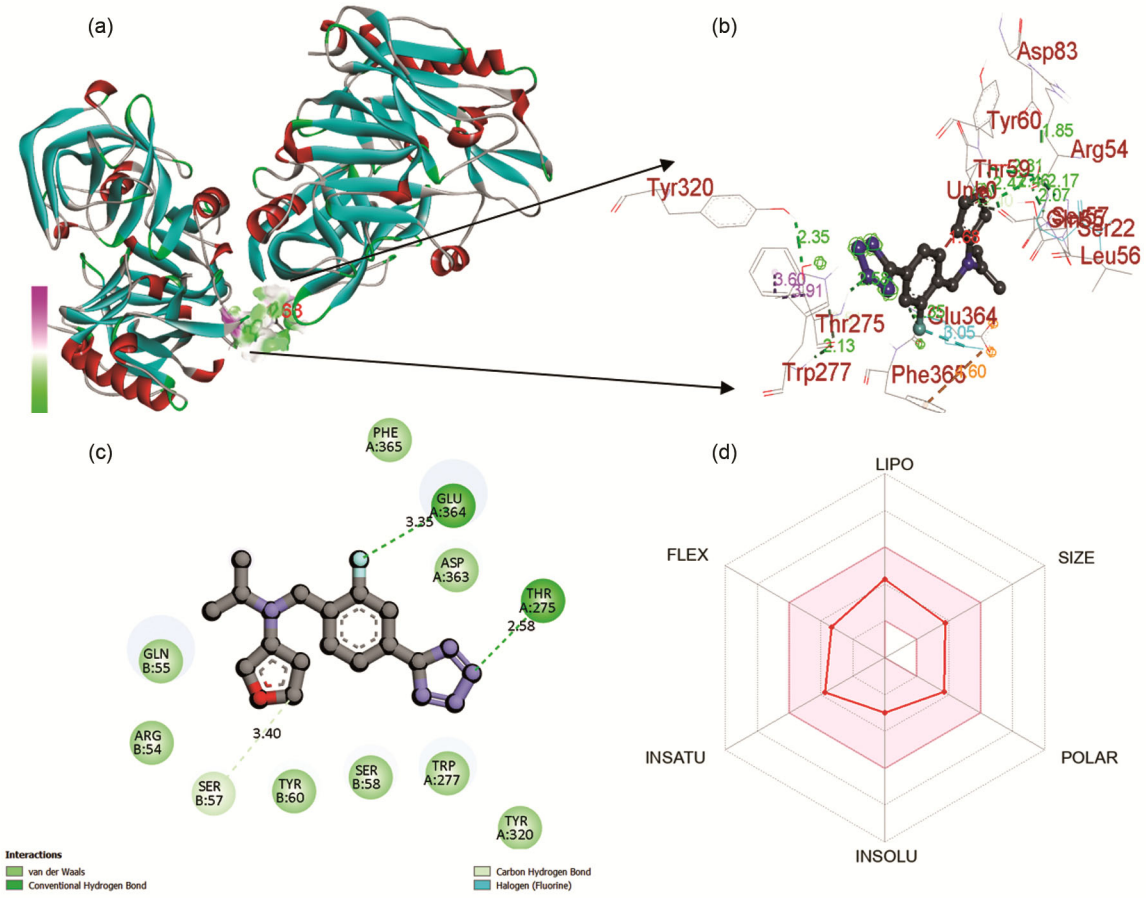

Fig. 5 — (a) Docking pose of M10 Ligand with BACE1 enzyme-4YBI; (b & c) Interactions with amino acid residues at the binding site of 4YBI; and (d) Drug like-likeliness of M10 ligand based on bio-availability radar diagram

LY2811376 and M10, respectively were observed. As depicted in Figure 6, for the Atabecestat compound, the root means square deviation (RMSD) of the 7DCZ protein structure fluctuated between 1.8 Å and 2.4 Å after an initial 25 ns equilibration period, stabilizing from 25 ns to 100 ns. These fluctuations suggest that the 7DCZ protein maintained a stable conformation with minimal structural changes. Similarly, the RMSD of the M1 ligand fluctuated between 1.25 Å and 1.73 Å throughout the simulation, with a variation of approximately 0.48 Å. This indicates that M1 remained stably bound to the 7DCZ site without significant displacement.

As shown in Figure 7, for the AZD3839 compound, the RMSD of the 4B05 protein structure fluctuated between 1.8 Å and 2.8 Å after an initial 28 ns period of equilibration, stabilizing from 28 ns to 100 ns. These fluctuations indicate that the 4B05 protein maintained a stable conformation without significant structural changes. Similarly, for the M6 ligand, after an initial 8 ns period of limited fluctuation, the RMSD of the M6 structure fluctuated minimally, ranging between 1.42 Å and 1.81 Å throughout the simulation, with a variation of approximately 0.39 Å. This suggests that M6 strongly remained stably bound to the 4B05 site without significant displacement.

As shown in Figure 8, for the LY2811376 reference compound, the RMSD of the 4YBI protein structure fluctuated between 1.5 Å and 2.1 Å after an initial 18 ns period of equilibration, stabilizing from 18 ns to 100 ns. These fluctuations suggest that the 4YBI protein maintained a stable conformation with minimal structural changes. Similarly, for the M10 ligand, after an initial 31 ns period of fluctuation, the RMSD of the M10 structure fluctuated between 1.52 Å and 2.23 Å throughout the simulation, with a variation of approximately 0.71 Å. This indicates that M10 remained stably bound to the 4YBI site without significant displacement.

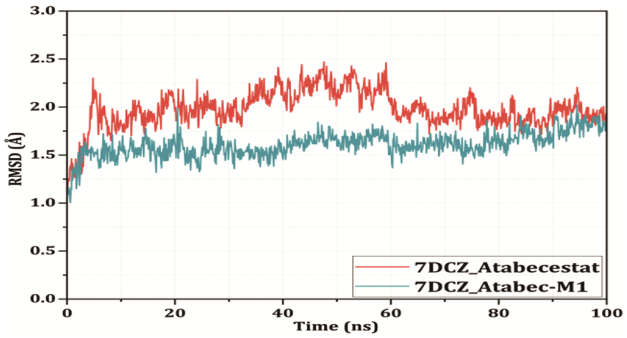

Fig. 6 — RMSD values for 7DCZ protein and ligands (Atebecestat, M1)

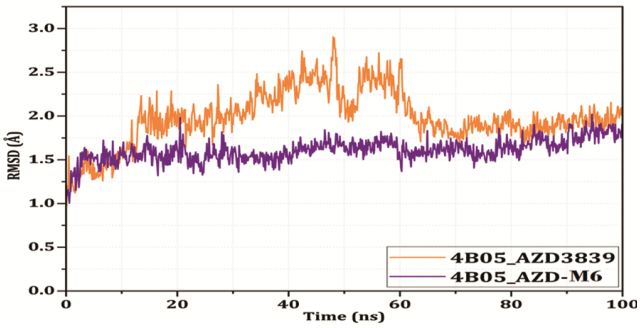

Fig. 7 — RMSD values for 4B05 protein and ligands (AZD3839, M6)

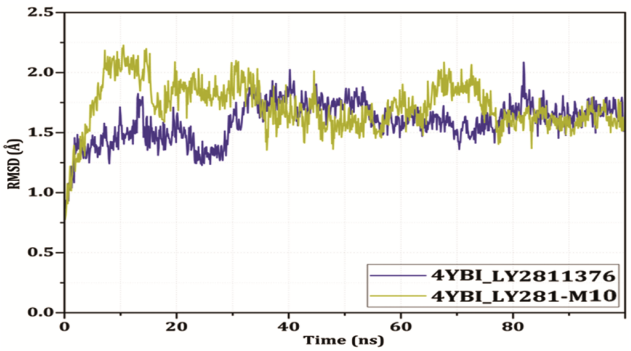

Fig. 8 — RMSD values for 4YBI protein and ligands (LY2811376, M10)

The RMSD analysis reveals that, among M1, M6, and M10, the M6 ligand undergoes a brief period of initial fluctuations before reaching a state of convergence and equilibrium. Over the course of the 100 ns simulation, the RMSD for M6 fluctuated between 1.42 Å and 1.81 Å, indicating stable binding to the 4B05 protein. The convergence of RMSD values highlights that M6, as a BACE1 inhibitor, consistently maintained its interaction throughout the entire molecular dynamics simulation. Also, both the ADMET analysis and docking studies conclude that M6 ligand has the most potent inhibition among the

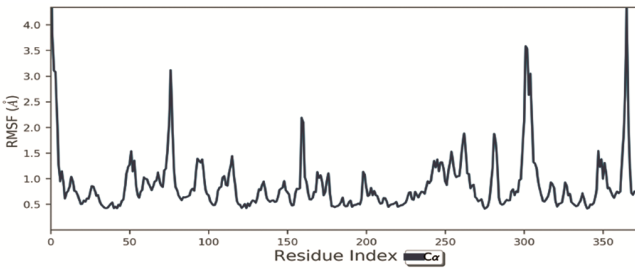

Fig. 9 — RMSF plot for Cα of BACE1 residues with M6

three ligands, with lowest binding energy of -7.83 Kcal/mol and good inhibition potency with Ki value of 1.83 µM, which indicates M6 as a strong inhibitor of BACE1 enzyme.

To further validate the potency of the M6 ligand as a BACE1 inhibitor, the analysis includes the root mean square fluctuation (RMSF), protein-ligand contacts, and time-dependent interactions such as hydrogen bonds, hydrophobic interactions, ionic bonds, and water bridges. Additionally, various profiling factors, including the radius of gyration (rGyr), intra-molecular hydrogen bonds (intraHB), molecular surface area (MolSA), and polar surface area (PSA), are presented to comprehensively illustrate the ligand's effectiveness.

Analysis of effectiveness of M6 ligand

As plotted in Figure 9, the RMSF is a valuable tool for characterizing local changes along the protein chain, providing a distance measure that quantifies the average deviation of each atom. The peaks highlight regions of the BACE1 binding site in the 4YBI protein that exhibit the most fluctuation during the simulation. Figure 10a illustrates the specific residues involved in the interactions observed throughout the simulation, with Figure 10b showing that the most significant interactions occur with Asp32, Asp228, Thr232, Lys107, and Gly230 for more than 70% of the simulation time.

The plot further highlights other types of interactions involving M6, such as water bridging, hydrophobic interactions, and ionic bonding. Figure 11 illustrates various ligand interaction parameters of M6 with the 4YBI protein residues over the 100 ns MD simulation at the ligand-active site. The RMSD fluctuated between 0.5 Å and 1.5 Å, maintaining an average distance of around 0.8 Å. The extent of the M6 ligand, measured as rGyr, ranged from 4.50 to 5.10 Å, with an equilibrium value of approximately 4.8 Å. The molecular surface area (MolSA), equivalent to the vander Waals surface area,

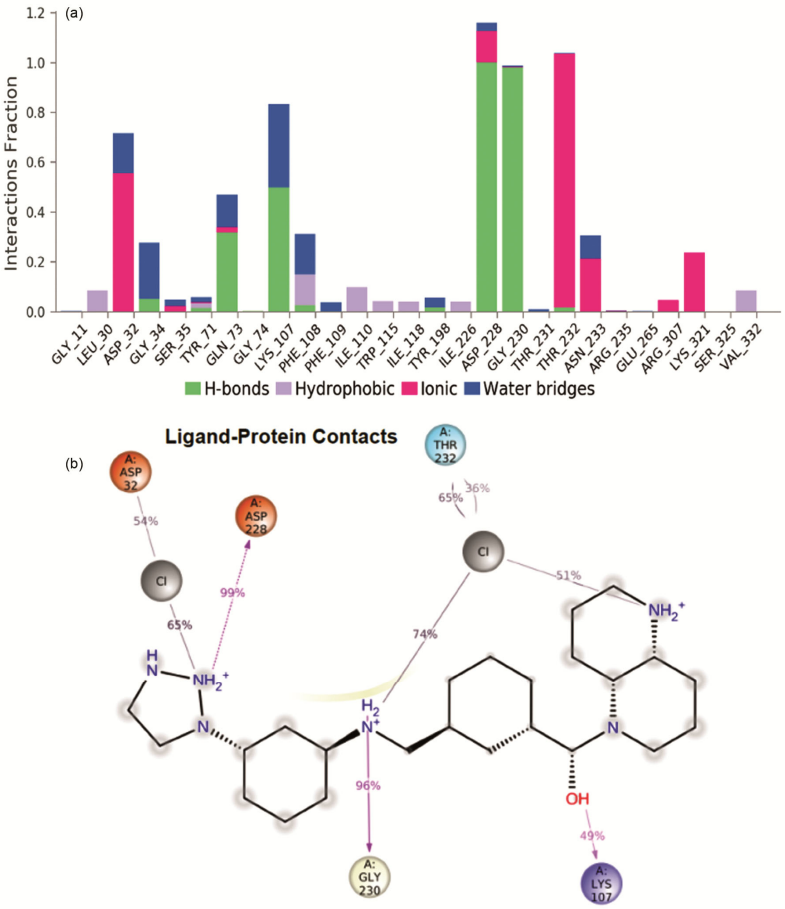

Fig. 10 — (a) Protein-ligand interactions of M6; and (b) Interactions > 50% of simulation time

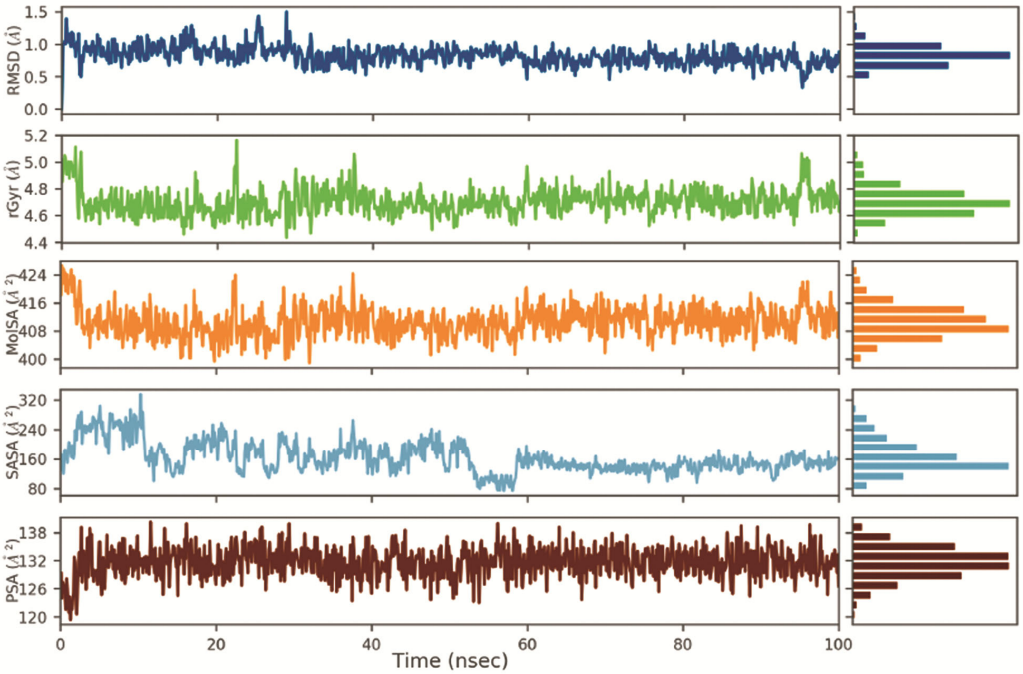

Fig. 11 — Properties of M6 observed during MD simulation

fluctuated between 400 and 425 Å², with an average of around 410 Å². The solvent-accessible surface area (SASA) showed significant fluctuations, ranging from approximately 90 to 300 Å², while the polar surface area (PSA) fluctuated between 120 and 138 Å².

Considering the importance of hydrogen-bonding properties in drug design due to their strong influence on drug specificity, metabolism, and adsorption, the effectiveness of the M6 ligand as a BACE1 inhibitor is clearly supported.

Conclusion

This study primarily focused on designing of ligands for inhibiting the BACE1 enzyme, which plays a crucial role in the formation of Aβ plaques in the human brain, a key factor in the development of Alzheimer's disease. Through AI-driven drug design and ADMET analysis, the drug-likeliness of novel compounds was validated. Among 12 such ligands, molecular docking studies identified M6 as the most potent BACE1 inhibitor demonstrating a strong binding score (-7.83 Kcal/mol) with excellent binding affinity (1.83 μM). Further molecular dynamics simulation (MDS) analysis of binding interactions revealed that M6 formed 4 strong hydrogen bonds, 3 hydrophobic bonds, and 8 ionic bonds with key BACE1 residues, maintaining active and stable interactions with catalytic dyad residues such as Asp32 and Asp228. However, to fully confirm the potential of the M6 ligand, further laboratory and clinical studies are necessary.

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Conflict of interest

The authors declare no conflict of interest.

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