ORIGINAL ARTICLE

In silico docking and comparative ADMET profile of different glycogen synthase kinase 3 beta inhibitors as the potential leads for the development of anti-Alzheimer drug therapy

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ABSTRACT

Glycogen synthase kinase 3 beta (GSK3 β) plays a key role in pathologic hyper phosphorylation of tau and plays an important role in the pathogenesis of Alzheimer's disease. In the present study, we have screened a set of potential hits in *in silico* platform to gain insight regarding binding profile with the target (GSK3 β) from molecular docking, ADME/T, and molecular dynamics (MD) simulations. The three screened compounds 6-BIBEO, 6-BIO, and SB216763 topped the docking score chart when subjected to hard scoring function extraprecision of GLIDE. The active site dynamics study through MD simulations provides insights on residues Asp133, Val135, and Ile62 which are in a state of minimum deviation from their mean special position while they interact with the respective ligands. The same molecules also displayed favorable pharmacokinetic profile, negative Ames test and falls correctly within drug-likeliness rules. These agents can be taken forward further for the development of anti-Alzheimer's drug therapy.

Key words: Glycogen synthase kinase 3 beta, *in silico*, molecular docking, molecular dynamics

INTRODUCTION

Alzheimer's disease (AD), the most prevalent form of dementia in the aging world population usually in the age group of 65 years or above.[1] Glycogen synthase kinase 3 beta (GSK3 β) mediated hyperphosphorylation of tau plays a major role in the pathogenesis of AD.[2] GSK3 β is a serine/threonine kinase.[3] Phosphorylation on tyr216 residue generates the active conformation of GSK3 β.[4] GSK3 β favors phosphorylation of prephosphorylated substrate. The primed phosphorylation of residues Ser235 and Ser404 of the tau-protein by other kinases such as CDK5, subsequently aids phosphorylation by GSK-3 on residues Thr231 and Ser400 on tau protein.[5] In this study, we have evaluated 10 different GSK3 β inhibitors (NSC69386, 6BIO, TCG24, Bio-acetoxime, CHIR98014, 6-BIBEO, 6-BIDECO, 6-BIMYEO, LY2090314, SB216763, and SB415286) in *in silico* platform for the development of potential leads for the treatment of AD.

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METHODOLOGY

Retrieval of target structure: The structure of GSK-3 beta, which is target receptor protein of human, was retrieved from protein data bank server (1UV5).[6] The detail of aminoacid sequence is shown in Figure 1.

Ligands: We have evaluated 10 different ligands (6-BIO, bio-Acetoxime, TC-G24, CHIR98014, NSC693868, 6-BIBEO, 6-BIDECO, 6-BIMYEO, SB216763, SB415286 and LY2090314). The structure of ligand molecules was retrieved from PubChem[7] [Figure 2]. Since, LY2090314[8] is already in preclinical phase,[9] it was taken as controls.[10,11]

Molecular docking: All *in silico* evaluations were carried out using Schrödinger Maestro suite 2019.

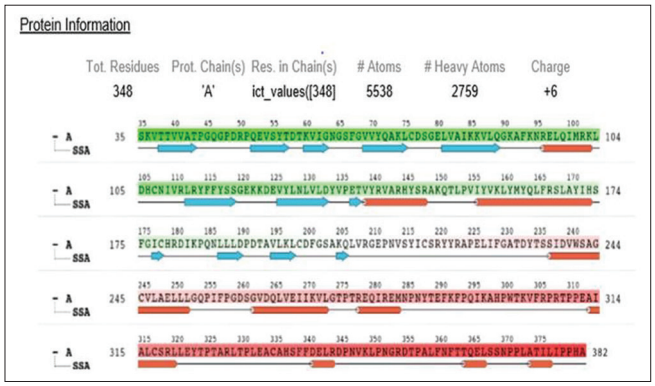

Figure 1: Amino acid residues in glycogen synthase kinase 3 beta

Pharmacokinetics properties of ligands

Admet SAR,[12] AMDET labs[13] and Swiss ADME server[14] were used for the evaluation of pharmacokinetic properties.

Drug-likeliness

Ligands were evaluated for drug likeness using Lipinski, Ghose, Veber, Egan, Muegge criteria using Swiss ADME software.

Molecular dynamics simulations

The overall three best performers and the control were further evaluated in molecular dynamics (MD) studies using a three step process of "system building," minimization, and MD simulation using the Desmond module of Schrodinger Inc (simulation time-50 ns, ensemble class nonproliferation treaty, temperature-300K and pressure = 1 bar).

RESULTS AND DISCUSSION

Chemical structure

Ligand serial number, name, and their chemical structure are illustrated in Figure 2.

Docking profile of the ligands

According to docking score 7, compound shown good binding profile in docking (6BIBEO > 6BIO > CHIR98014 > SB415286>NSC693868 > LY2090314 > SB216763 > TC-G24). Docking score data are showed in Table 1 and Figures 3-6.

ADMET profile

Comparative ADMET profiles of the different agents are showed in Table 2.

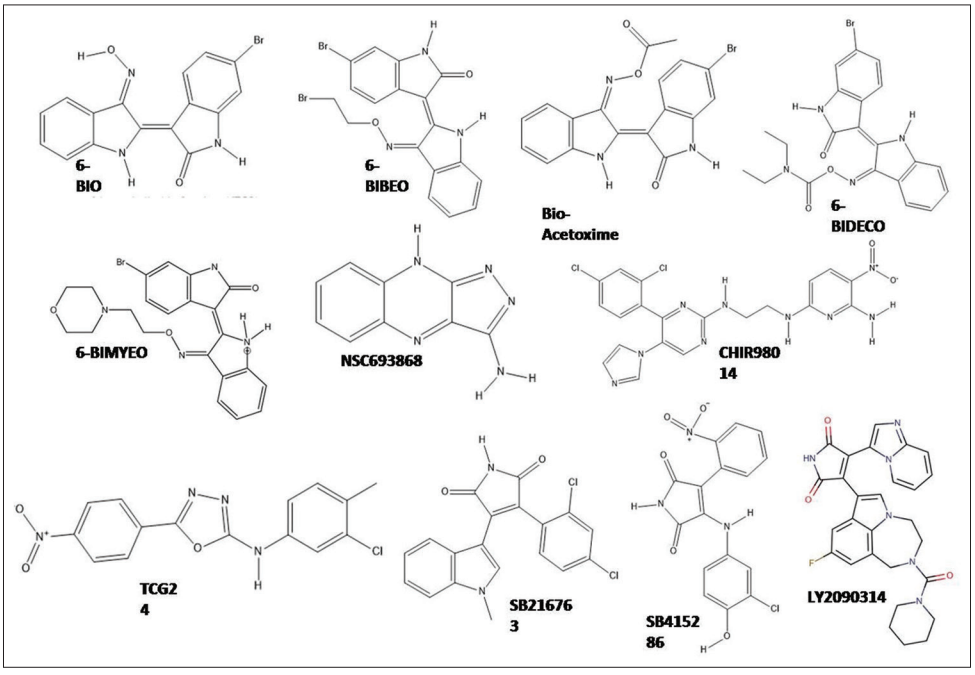

Figure 2: Chemical structure of the compounds under evaluation

Physicochemical properties

Log *P* value (distribution coefficient P)

In our study, all the compounds had Log *P* value below 5. The compound NSC693868 had poor lipid bi-layer permeability (Log *P* = 0.558) compared to other ligands (6BIO, 6BEBIO, BIOACETOXIME, 6-BIDECO, CHIR98014, TC-G24 and SB216763).

LogD7.4

logD7.4 value was in the low in case of NSC693868 and SB415286, highlighting their hydrophilicity. Apart from these two ligands, LogD7.4 value was between 2 and 3.2. None of the compounds had LogD7.4 value higher than 3.5 [Table 2].

Table 1: Docking profile of all the ligands (maestro)

Number	Name	Docking score	Glide score	Glide energy
1	6-BIO	−10.451	−10.452	−52.985
2	6-BIBEO	−10.929	−10.929	−57.439
3	BIO-Acetoxime	−2.213	−2.213	−33.331
4	6-BIDECO	−2.597	−2.597	−31.628
5	6-BIMYEO	−3.136	−3.201	−35.878
6	NSC693868	−7.477	−7.477	−31.533
7	CHIR98014	−8.223	−8.226	−58.705
8	TCG24	−7.009	−7.009	−38.324
9	SB216763	−7.101	−7.118	−24.252
10	SB415286	−8.186	−8.369	−36.635
11	Control (LY2090314)	−7.435	−7598	−61251

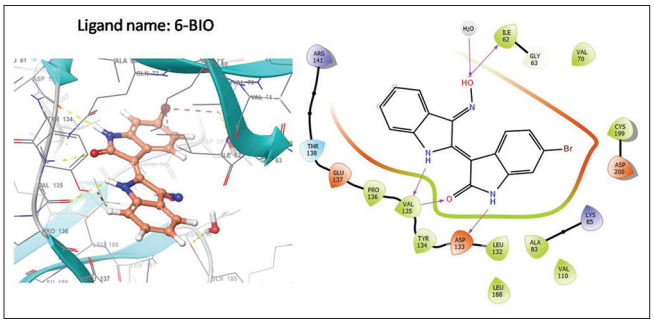

Figure 3: The diagram of docking and ligand protein interaction of the test ligand 6-BIO

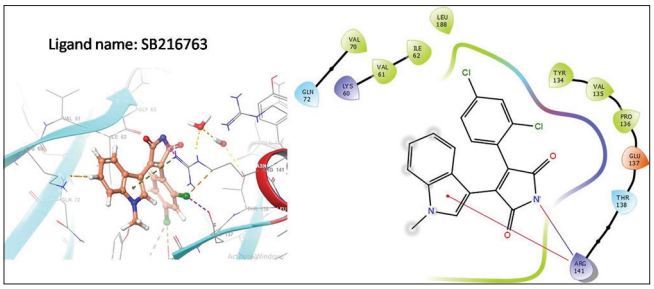

Figure 5: The diagram of docking and ligand protein interaction of the test ligand SB216763

Absorption

All the ligands were found to be positive for human intestinal absorption. However, only 3 of the ligands (NSC693868, TC-G24, and SB216763) were permeable through Caco-2. None of the ligands were substrate of P-gp; however, most of the drugs were P-gp inhibitor except NSC693868, CHIR98014, and SB415286. None of the ligands inhibited renal organic cation transporter [Table 2].

Distribution

These compounds were distributed in three main sub-cellular regions that are plasma membrane, lysosome, and mitochondria. 6-BIO and BIOACETOXIME showed distribution in plasma membrane, NSC693868 in lysosome and other remaining drugs in mitochondria. The plasma protein binding were <80% in case of NSC6938, 80%–90% in case of 6-BIO, 6-BIMYEO, CHIR98014 and LY2090314 and was more than 90% in case of 6-BIBEO, Bio-acetoxime, 6-BIDECO, TCG-24, SB216763, and SB415286. All of the ligands were permeable through blood–brain barrier except 6 BIDECO and SB415286 [Table 2].

Excretion

The half-life of all the ligands was in between 0.8 and 1.9 h. The highest $T_1/_2$ life is 1.932 h was in case of SB216763, and

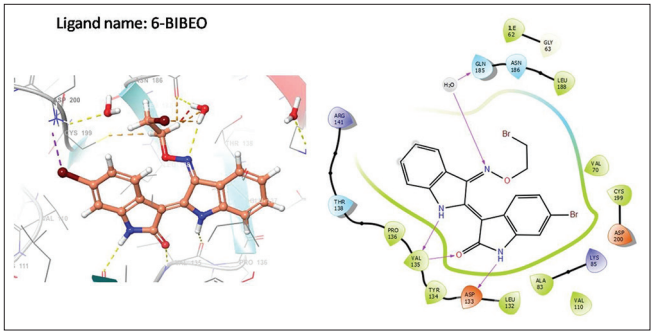

Figure 4: The diagram of docking and ligand protein interaction of the test ligand 6-BIBEO

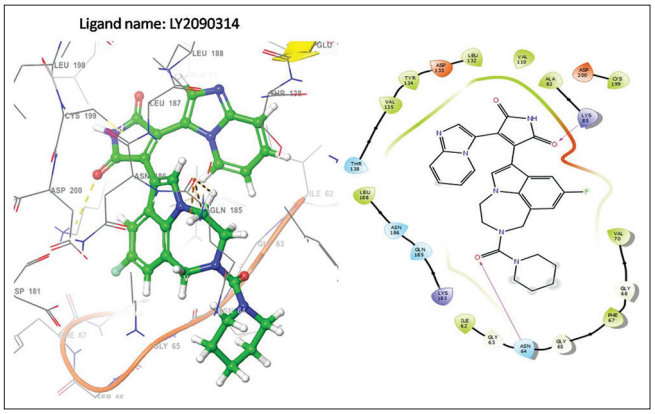

Figure 6: The diagram of docking and ligand protein interaction of the test ligand LY2090314

Table 2: Pharmacokinetic profile (ADME)

	6-BIO	6-BIBEO	BIO-Acetoxime	6-BIDECO	6-BIMYEO	NSC693868	CHIR98014	TCG24	SB216763	SB415286	Control (LY2090314)
Physicochemical properties											
Molecular weight (ADMETLab)	356.17	463.129	398.216	455.312	470.347	185.19	486.323	330.731	371.223	359.725	512.545
LogP (ADMETLab)	3.416	4.354	3.505	4.421	2.074	0.558	4.046	4.35	4.052	2.433	3.417
LogD7.4 (ADMETLab)	2.954	3.24	3.009	3.067	2.393	0.275	2.645	2.798	2.499	0.219	2.417
Absorption											
HIA (admetSAR)	+	+	+	+	+	+	+	+	+	+	+
CACO-2 (admetSAR)	-	-	-	-	-	+	-	+	+	-	-
P-gp-S (ADMETLab)	--	--	---	---	---	---	---	---	-	---	-
P-GP-I (ADMETLabs)	++	++	++	++	+++	---	---	+	+	-	++
ROCT (admetSAR)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	I
Distribution											
Subcellular distribution (admetSAR)	PM	M	PM	M	M	L	M	M	M	M	M
PPB (ADMETlab)	87%	95% (s)	94%	95%	90%	54%	88%	94%	94%	92%	87%
BBB (admetSAR)	+	+	+	-	+	+	+	+	+	-	+
Excretion											
T1/2 (ADMETlabs), h	1.691	1.819	1.677	1.683	1.594	0.823	1.626	1.402	1.932	0.899	1.929
CL (ADMETlabs), ml/min/kg	0.662	0.825	0.848	1.117	0.867	1.87	1.087	0.955	0.695	0.586	1.42
Toxicity											
hERG inhibition (admetSAR)	WI	WI	WI	WI	WI	WI	SI	WI	WI	WI	WI
Ames test (admetSAR)	NT	NT	T	NT	NT	T	T	T	NT	T	NT
Carcinogen (admetSAR)	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
Fish toxicity (admetSAR)	High	High	High	High	High	High	High	High	High	High	High
TP toxicity (admetSAR)	High	High	High	High	High	High	High	High	High	High	High
Honey bee toxicity (admetSAR)	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
Skin senzitizaation (ADMETLab)	No	No	No	No	NO	No	Yes	Yes	No	Yes	No
Human hepatotoxicity (ADMETLab)	++	++	+++	++	+	-	++	++	+++	+++	+
DILI (ADMETLab)	++	++	++	++	++	+	++	++	++	+	+
LD50 mol/kg (admetSAR)	2.4574	2.5050	2.4051	2.6018	2.5731	2.5882	2.5418	2.5510	2.6588	2.4132	2.5286

+ represent the presence of ADME profile, +++ represent the highest level, ++ represent the medium level, – represent the less level, --- represent the very less. WI: Weak-inhibitor, NT: Nontoxic, TP: Tetrahymena pyriformis, NI: Inhibitor, S: Significant, PM: Plasma membrane, M: Mitochondria, L: Lysosome

highest clearance rate was 1.87 ml/min/kg which was found with NSC693868 [Table 2].

Toxicity

All the ligands that were evaluated were weak hERG channel inhibitors, except CHIR98014, which showed strong inhibition. Five ligands showed toxicity in Ames test (BIOACETOXIME, NSC693868, CHIR98014, TC-G24, and SB415286), whereas the rest of the compounds did not show toxicity. None of the ligands were potential carcinogens. All the ligands showed high fish toxicity and TP toxicity and low honey bee toxicity. Skin sensitization was seen in case of CHIR98014, TCG24. Predicted rat LD50 of the compounds ranged from 2.4 to 2.6 mol/kg [Table 2].

Metabolism profile

All of the ligands were CYP3A4 substrate except compound NSC693868 and CHIR98014, which were non substrates. None of the ligands were substrate of CYP2C9 and CYP2D6. Only six ligands (6-BIO, 6-BIBEO, BIOACETOXIME, 6-BIDECO, SB216763, and LY2090314) were inhibitors of CYP3A4, whereas rest were noninhibitors [Table 3].

Drug-likeliness of the ligands

All the selected ligands followed these rules expect ligand CHIR98014 (violated all 5 rules) and LY2090314 (violated Lipinski rule and Ghose rule) [Table 4].

Selection of ligand for further molecular dynamics simulations

Upon comparing and integrating the knowledge acquired from docking and ADMET score, 3 ligands (6-BIO, 6-BIBEO, and SB216763) were selected out to be good hit and were taken forward for MD simulations. LY2090314 was taken as control.

Molecular dynamic simulation

Root mean square deviation (RMSD): The respective RMSD observed were 1.8Å for 6-BIO, 1.9Å for 6-BIBEO, 1.75Å for SB216763, and 2.0 for LY2090314. These RMSD values were well-within the acceptable range of 0–3Å and also RMSD progression equilibrates when it approaches the end of trajectory hence implying to a stable protein-ligand complex formation which can be inferred for positive interaction of 1UV5 with all three ligands (6BIO, 6BIBEO, SB216763, and LY2090314) [Figure 7b, e, h and k]. Details of RMSF values are showed in Figure 7a, d, g and J.

Table 3: Metabolism profile (admetSAR)

CYP 450 substrate			CYP 450 inhibitor				
2C9	2D6	3A4	1A2	2C9	2D6	2C19	3A4
NS	NS	S	I	I	NI	I	I
NS	NS	S	I	I	NI	I	I
NS	NS	S	I	I	NI	I	I
NS	NS	S	I	I	NI	I	I
NS	NS	S	NI	NI	NI	I	NI
NS	NS	NS	I	NI	NI	I	NI
NS	NS	NS	I	NI	NI	NI	NI
NS	NS	S	I	I	NI	I	NI
NS	NS	S	I	I	NI	NI	I
NS	NS	S	I	NI	NI	NI	NI
NS	NS	S	NI	I	NI	NI	I

NS: Nonsubstrate, S: Substrate, NI: Noninhibitor, I: Inhibitor

Protein-Ligand Interactions: Residues Asp133 and Val135 were found to be predominantly important residues exhibiting the high percentage of H-bonding with the three selected candidate (6-BIO, 6-BIBEO, and SB216763) compounds. Residue Asp133, Val135, and Ile62 are the major residues involved in the core-binding cavity showing predominant interaction with the ligand. Moreover, compounds 6-BIBEO (Ile62, Val70, Ala83, and Leu188), 6-BIO (Val70, Ala83, and Leu188) SB216763 (Ile62, Val70, Ala83, Val110, Leu132, and Leu188), and LY2090314 (Ile62, Phe67, Val70, Lys85, and Leu188) exhibit some degree of hydrophobic interactions. Moreover, water bridges were observed with core active site residues in 6-BIBEO (Arg141 and Ile62), in 6-BIO (Gln185 and Tyr140) and in SB216763 (Ile62, Tyr134, Pro136, Tyr138, Val135, Thr138, Arg141, Tyr140, and Gln185) and LY2090314 (Lys85, Asp133, Val135, Pro136, Thr138, Arg141, Lys183, Gln185, and Asp200) [Figures 7c, f, i, l and 8].

DISCUSSION

GSK3 β has a key role in hyperphosphorylation of tau and plays an important role in regulation of intra-neuronal hyperphosphorylated tau level.[15] In this study, we have targeted GSK3 β for *in silico* identification of possible hits for the development of anti-Alzheimer's therapy. Among the 10 selected ligands, 6-BIBEO, 6-BIO, CHIR98014, SB415286, NSC693868, LY2090314, SB216763, and TC-G24 showed good binding profile, evaluated in terms of docking score. logD7.4 value was in the lower side in case of NSC693868 and SB415286, highlighting their hydrophilicity. AD being a disease of the central nervous system, BBB permeability is a major factor. In our study, all the ligands were able to pass the BBB except two ligands (6-BIDECO and SB415286).

CHIR98014 was found to be a strong inhibitor to the hERG, whereas hERG inhibitory profiles of other drugs were comparable to control. Ames test was positive for BIOACETOXIME, NSC693868, CHIR98014, TCG24, and SB415286.

Taking in account, the pharmacokinetics result and target binding profile, 3 of the compounds (6-BIBEO > 6-BIO > SB216763) were found to be the most suitable agents for further development process.

After performing MD simulation of these selected compound with the common target GSK3 β, we can

Table 4: Drug likeness (SwissADME)

Chemical serial number	Lipinski's rule	Ghose	Veber	Egan	Muegge
1	Yes, 0 violation	Yes	Yes	Yes	Yes
2	Yes, 0 violation	Yes	Yes	Yes	Yes
3	Yes, 0 violation	Yes	Yes	Yes	Yes
4	Yes, 0 violation	Yes	Yes	Yes	Yes
5	Yes, 0 violation	Yes	Yes	Yes	Yes
6	Yes, 0 violation	Yes	Yes	Yes	No, 1 violation: MW <200
7	Yes, 1 violation, N or O >10	No, 1 violation, MW >480	No, 1 violation, TPSA >140	No, 1 violation, TPSA >131.6	No, 1 violation, TPSA >150
8	Yes, 0 violation	Yes	Yes	Yes	Yes
9	Yes, 0 violation	Yes	Yes	Yes	Yes
10	Yes, 0 violation	Yes	Yes	Yes	Yes
11	Yes, 1 violation	No; 2 violations: MW >480, MR >130	Yes	Yes	Yes

MW: Molecular Weight, MR: molar refractivity, TPSA: topological polar surface area

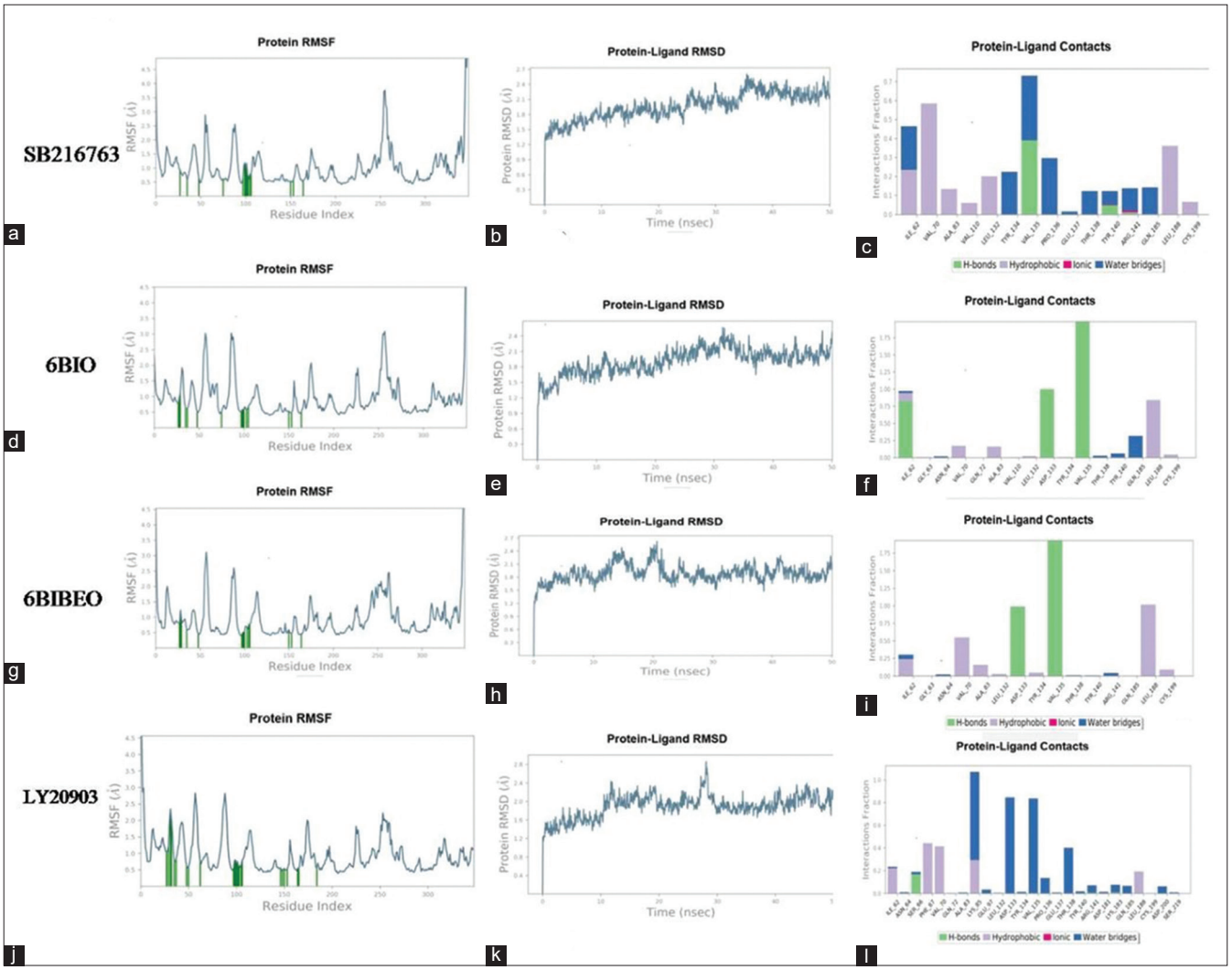

Figure 7: Results from molecular dynamic simulation studies of the selected ligands. Protein root mean square fluctuation (a,d,g,j), protein root mean square deviation(b,e,h,k) and protein ligand contacts in molecular dynamic simulation(c,f,i,l)

interpret from the data providing insight on RMSD of GSK3 β-ligand complex was 1.8Å for 6-BIO, 1.9Å for 6-BIBEO, 1.75Å for SB216763, and 2.0 for LY2090314 with respect to its C-alpha position. The trajectory frames are

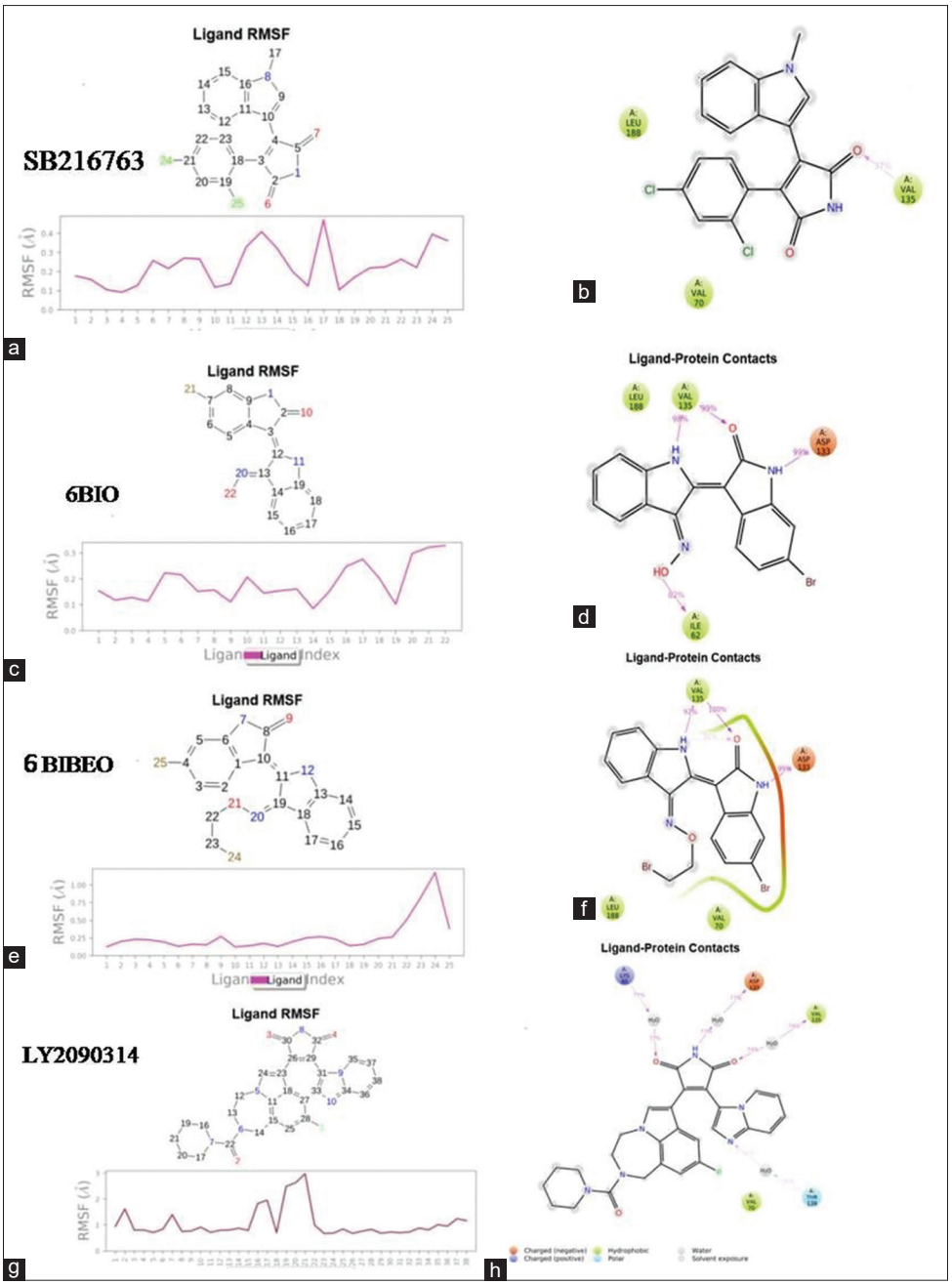

Figure 8: Details of molecular dynamic simulation studies. Ligand root mean square fluctuation (a,c,e,g) and ligand protein contacts in molecular dynamic simulation (b,d,f,h)

well under the scale of 3Å and stabilized as it propagated further. The root mean square fluctuation (RMSF) and ligand-contact ratio of GSK3-Bresidues showing with the respective ligands emphasize that SB216763 shows greater percentage interaction with the core residues of target site, with higher number of water bridge formation, while 6-BIO and 6-BIBEO were dynamically similar in behavior and also formed similar interaction profile with the target site, of which H-bond was a major part. The positive control LY2090314 showed uneven interaction pattern with the GSK3 β residues but showed great affinity by the means of water bridge formation [Figure 7l]. Ligand RMSF details shown in Figure 8, on the basis of which 6-BIBEO emerges out to be most stable at all trajectory frames.

CONCLUSION

Among the ten ligands evaluated, 6-BIO, 6-BIBEO, and SB216763 needs further evaluation as probable anti-Alzheimer's drugs considering the *in silico* ADME parameters, toxicity, blood brain barrier permeability, docking scores, and MD simulation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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