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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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.[2] Glycogen synthase kinase 3 beta (GSK3 β) mediated hyperphosphorylation of tau plays a major role in the pathogenesis of AD.[3] GSK3 β is a serine/threonine kinase.[5] 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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Abstract

Glycogen synthase kinase 3 beta (GSK3 β) plays a key role in pathologic hyperphosphorylation 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
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). 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]. Since, LY2090314 is already in preclinical phase, it was taken as controls. [10,11]

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

Pharmacokinetics properties of ligands
Admet SAR, AMDET labs and Swiss ADME server were used for the evaluation of pharmacokinetic properties.

Drug-likeness
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 1: Amino acid residues in glycogen synthase kinase 3 beta

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, 6BIBEO, BIOACETOXIME, 6-BIDECO, CHIR98014, TC-G24 and SB216763).

LogD$_7.4$
logD$_7.4$ value was in the low in case of NSC693868 and SB415286, highlighting their hydrophilicity. Apart from these two ligands, LogD$_7.4$ value was between 2 and 3.2. None of the compounds had LogD$_7.4$ 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-BIMYE0        | −3.136        | −3.201      | −35.878      |
| 6      | NSC693868       | −7.477        | −7.477      | −31.533      |
| 7      | CHIR98014       | −8.223        | −8.226      | −58.705      |
| 8      | TC-G24          | −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 | −7.598 | −61251 |

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-BIMYE0, CHIR98014 and LY2090314 and was more than 90% in case of 6-BIBEO, Bio-acetoxime, 6-BIDECO, TC-G24, 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...
### Table 2: Pharmacokinetic profile (ADME)

|                  | 6-BIO | 6-BIBEO | BIO-Acetoxime | 6-BIDECO | 6-BIMYE0 | 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 (ADMETLab)   | ++     | ++      | ++            | ++       | +++      | ---        | --         | +     | +        | -        | ++                 |
| 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 (ADMETLab), h  | 1.691  | 1.819   | 1.677         | 1.683    | 1.594    | 0.823      | 1.626       | 1.402  | 1.932    | 0.899    | 1.929            |
| CL (ADMETLab), 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       | NT       | 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-likelihood 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.

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
GSK3β has a key role in hyperphosphorylation of tau and plays an important role in regulation of intra-neuronal hyperphosphorylated tau level. In this study, we have targeted GSK3β 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 compounds. Residue Asp133, Val135, and Ile62 are the major residues involved in the core-binding cavity showing predominant interaction with the ligand.

Table 3: Metabolism profile

| CYP 450 substrate | CYP 450 inhibitor |
|-------------------|-------------------|
| 2C9 | 2D6 | 3A4 | 1A2 | 2C9 | 2D6 | 219 | 3A4 |
| NS | NS | S | I | I | NS | I | I |
| NS | NS | S | I | I | NS | I | I |
| NS | NS | S | I | I | NS | I | I |
| NS | NS | S | I | I | NS | I | I |
| NS | NS | S | I | I | NS | I | I |
| NS | NS | S | I | I | NS | I | I |
| NS | NS | S | I | I | NS | I | I |
| NS | NS | S | I | I | NS | I | I |
| NS | NS | S | I | I | NS | I | I |
| NS | NS | S | I | I | NS | I | I |

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

**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)
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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Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Weller J, Budson A. Current understanding of Alzheimer’s disease diagnosis and treatment. F1000Res 2018 Jul 31;7:1161.
2. Chong FP, Ng KY, Koh RY, Chye SM. Tau Proteins and Tauopathies in Alzheimer’s Disease. Cell Mol Neurobiol 2018;38:965-80.
3. Jantrapirom S, Nimlamool W, Chattipakorn N, Chattipakorn S, Temviriyanukul P, Intachat W, et al. Liraglutide Suppresses Tau Hyperphosphorylation, Amyloid Beta Accumulation through Regulating Neuronal Insulin Signaling and BACE-1 Activity. International Journal of Molecular Sciences. 2020;21:1725.
4. Das TK, Jana P, Chakrabarti SK, Abdul Hamid MRW. Curcumin Downregulates GSK3 and Cdk5 in Scopolamine-Induced Alzheimer’s Disease Rats Abrogating Aβ40/42 and Tau Hyperphosphorylation. J Alzheimers Dis Rep 3:257-67.
5. Hanger DP, Noble W. Functional Implications of Glycogen Synthase Kinase-3-Mediated Tau Phosphorylation. International Journal of Alzheimer’s Disease. 2011;2011:1-11.
6. Meijer L, Skalsounis A-L, Magiatis P, Polychronopoulos P, Knockaert M, Leost M, et al. Gsk-3-Selective Inhibitors Derived from Tyrian Purple Indurubins. ChemBiol. 2003;10:1255.
7. PubChem. PubChem [Internet]. Available from: https://pubchem.ncbi.nlm.nih.gov/. [Last cited on 2020 Sep 30].
8. PubChem. 3-[6-Fluoro-10-(piperidine-1-carbonyl)-1,10-diazatricyclo[6.4.1.04,13]trideca-2,4,6,8(13)-tetraen-3-yl]-4-imidazo[1,2-a]pyridin-3-ylpyrrole-2,5-dione [Internet]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/10029385. [Last cited on 2020 Sep 30].
9. Vasdev N, Garcia A, Stableford WT, Young AB, Meyer JH, Houle S, et al. Synthesis and ex vivo evaluation of carbon-11 labelled N-(4-methoxybenzyl)-N’-(5-nitro-1,3-thiazol-2-yl)urea ([11C]AR-A014418): A radiolabelled glycogen synthase kinase-3β specific inhibitor for PET studies. Bioorganic & Medicinal Chemistry Letters. 2005;15:5270-3.
10. Pandey MK, DeGrado TR. Glycogen Synthase Kinase-3 (GSK-3)-Targeted Therapy and Imaging. Theranostics 2016;6:571-93.
11. Zamek-Gliszczynski MJ, Abraham TL, Alberts JJ, Kulanthaivel P, Jackson KA, Chow KH, et al. Pharmacokinetics, Metabolism, and Excretion of the Glycogen Synthase Kinase-3 Inhibitor LY2090314 in Rats, Dogs, and Humans: A Case Study in Rapid Clearance by Extensive Metabolism with Low Circulating Metabolite Exposure. Drug Metab Dispos 2013;41:714-26.
12. admetSAR @ LMMD [Internet]. Available from: http://lmmd.ecust.edu.cn/admetsar1. [Last cited on 2019 May 30].
13. Home-ADMeLab: ADMET Prediction|ADMET Predictor|QSAR|ADMET Database [Internet]. Available from: http://admet.scbdd.com/ [Last cited on 2020 Sep 30].
14. SwissADME [Internet]. Available from: http://www.swissadme.ch/. [Last cited on 2020 Sep 30].
15. Llorens-Marató M, Jurado J, Hernández F, Ávila J. GSK-3β, a pivotal kinase in Alzheimer disease. Front Mol Neurosci [Internet]. 2014;7. Available from: http://journal.frontiersin.org/article/10.3389/fnmol.2014.00046/abstract. [Last cited on 2020 Sep 30].