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In silico Based Ligand Design and Docking Studies of GSK-3β Inhibitors

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Abstract

Automated docking was performed on a series of thiazolo[5,4-f]quinazolin-9-one derivatives as GSK-3β inhibitors. The docking technique was employed to dock a set of representative compounds within the active site region of 1UV5 using AutoDock 3.05. For these compounds, the correlation between binding free energy (kcal/mol) and IC_{50} (μM) values were examined. The docking simulation clearly predicted the binding mode that is nearly similar to the crystallographic binding mode within 1.0 Å RMSD. Based on the validations and interactions made by R1 and R2 substituents, inhibitor design was initiated by considering simple combinations. For the designed compounds where the interactions and dock scores are being considered for evaluation, compound **17** exhibited large binding energy (-13.14 kcal/mol) against GSK-3β than the remaining. The results help to understand the type of interactions that occur between designed ligands with GSK-3β binding site region and explain the importance of R1 and R2 substitutions on thiazolo[5,4-f]quinazolin-9-one derivatives.

Key Words: AutoDock, automated docking, binding free energy, GSK-3β, ligand design.

Area of Interest: Molecular Docking

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1. Introduction

Glycogen Synthase Kinase-3 (GSK-3), a multifunctional serine/threonine kinase [1][2] has been implicated in the development of insulin resistance [3] and regulation of glycogen synthesis [4]. GSK-3 belongs to the super family of mitogen-activated protein (MAP) kinases [5]. Two isoforms of GSK-3 are reported in mammals: a 51 kDa GSK-3α and a 47 kDa GSK-3β [6]. GSK-3 is highly conserved through evolution and plays a fundamental role in cellular responses [7]. Many GSK-3 substrates require prior phosphorylation by a priming serine/threonine kinase to form the motif S-*X*-*X*-*X*-S(P) (S and *X* indicate serine and any amino acid, respectively) before phosphorylation by GSK-3 [8][9] and three crucial basic residues, Arg96, Arg180, and Lys205 has been identified for the priming phosphate on substrates of GSK-3β.

The activity of GSK-3β is regulated by two different types of mechanisms [10]. The first one being phosphorylation, which can be modulated by diverse pathways including insulin signaling, growth factors, and amino acids. GSK-3β is active in its nonphosphorylated form, and site-specific phosphorylation at Ser9 inhibits its activity mediated by p70 S6 kinase, p90Rsk, PKA, PKB and protein kinase C [11][12]. The second mechanism for GSK-3β regulation is independent of phosphorylation at Ser9 and involves the highly conserved Wingless (Wnt) signaling pathway, which specifies cell fate, proliferation, and differentiation in *Drosophila*, *Xenopus*, and mammals [13].

GSK-3 is involved in many other important signaling cascades which may regulate glucose homeostasis and the development of insulin resistance [4]. Activation of GSK-3 may contribute to neuronal apoptosis. Differentiated cells, including neurons in the CNS, require the presence of survival factors to suppress the intrinsic cell death machinery and thereby avoid apoptosis [14]. Small compound inhibitors of GSK-3 may, therefore, have several therapeutic uses, including the treatment of neurodegenerative diseases, diabetes type II, bipolar disorders, stroke, cancer, and chronic inflammatory disease [15]. Glycogen synthase kinase-3β (GSK-3β) has a central role in Alzheimer's disease [16] and Huntington's disease [17][18].

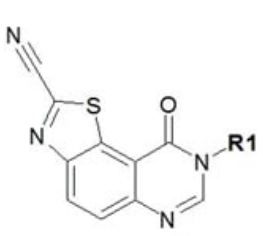
GSK-3β inhibitors are known to inhibit the enzyme in ATP competitive manner. A number of potent GSK-3 inhibitors such as indirubins, indigo, indoxyl, isatin [19][20], Thiadiazolidinones (TDZD) [16], Azaindolylmaleimides [21], AR-A014418, a selective GSK-3 inhibitor as antidepressant [22], L803-mts, a novel antidepressant GSK-3 peptide inhibitor [23], SB-415286 and SB-216763 [24], 3-Imidazo[1,2-a]pyridin-3-yl-4-(1,2,3,4-tetrahydro-[1,4]diazepino-[6,7,1-*hi*]indol-7-yl)pyrrole-2,5-diones [25], thienyl and phenyl alpha-halomethyl ketones [26], acridinyl-thiazolino derivatives [27], bisarylmaleimide [28], aloisines [39], 1-(4-aminofurazan-3-yl)-5-dialkylaminomethyl-1H-[1,2,3]triazole-4-carboxylic acid derivatives [30], N-phenyl-4-pyrazolo[1,5-b]pyridazin-3-ylpyrimidin-2-amines [31], heterocyclic pyrimidyl hydrazones [32], novel bis(indolyl)maleimide pyridinophanes [33], thiazolo[5,4-f]quinazolin-9-ones [34], carbon-11 labelled N-(4-methoxybenzyl)-N'-(5-nitro-1,3-thiazol-2-yl)urea ([11C]AR-A014418) [35], 4-acylamino-6-arylfuro[2,3-d]pyrimidines [36] and 3-(7-azaindolyl)-4-arylmaleimides [37] etc. have been reported. Some inhibitors are found to be specific for GSK-3β and selectively inhibit other kinases, more importantly; CDKs which are homologous to GSK-3. However, due to lack of specificity and low degree of selectivity against CDKs, modulation of these compounds is required to design more potent and highly selective GSK-3β inhibitors. Therefore, in the present study, structure based drug design strategies were employed on thiazolo[5,4-f]quinazolin-9-one derivatives [34] to recognize the different modes of interactions exhibited by these ligands through *in silico* docking procedures.

2. Materials and Methods

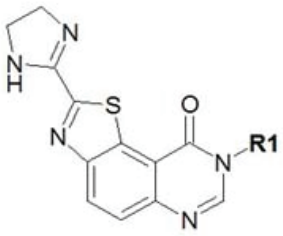
2.1 Data Set

A set of inhibitors listed in Table 1, were taken from the biological data reported by Testard et al. [34].

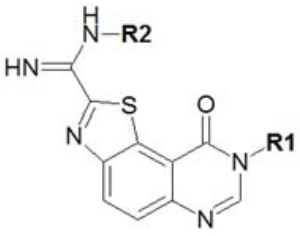
Table 1. GSK-3β inhibitory activity data with their corresponding dock scores against 1UV5.



Ia-d



IIa-d



IIIa-b, IVa-b

Compound No. (from ref. 34)	R1	R2	IC_{50} (μM)	Dock score (kcal/mol)
Ia	CH_2-CH_3	-	6.2	-9.20
Ib	CH_2-CH_2-CH_3	-	6.5	-9.11
Ic	CH_2-CH-$(CH_3)_2$	-	10	-9.29
Id	$(CH_2)_2$-CH-$(CH_3)_2$	-	50	-8.32
IIa	CH_2-CH_3	-	4.2	-9.33
IIb	CH-$(CH_3)_2$	-	1.6	-9.79
IIc	CH_2-CH_2-CH_3	-	1.6	-9.84
IId	CH_2-CH-$(CH_3)_2$	-	4.6	-9.41
IIIa	CH_2-CH_3	$CH(CH_3)_2$	7.2	-9.54
IIIb	CH-$(CH_3)_2$	$(CH_2)_2$-$N(CH_3)_2$	9.4	-9.63
IVa	CH_2-CH_3	$CH(CH_3)_2$	1.3	-9.86
IVb	CH-$(CH_3)_2$	$(CH_2)_2$-$N(CH_3)_2$	0.56	-10.49

2.2 Computational Details

Software AutoDock 3.05 along with a graphical user interface, AutoDock Tools (ADT) was utilized to generate grids, calculate dock score and evaluate the conformers. The structure of compounds were drawn using CAChe 6.1.12.33 [http://www.cache.fujitsu.com] and the energy optimization was performed using MM2 augmented force field with conjugate gradient method to obtain a local minimum structure. Such energy minimized structures are considered for docking and the corresponding pdbq and pdbqs files were prepared in AutoDock.

2.3 Validation

Software validation was performed in AutoDock using PDB protein 1UV5. The X-ray crystal structure of GSK-3β in complex with inhibitor BRW1383 was recovered from Protein Data Bank. The bioactive co-crystallized bound inhibitor 6-bromoindirubin-3'-oxime (BRW1383) was docked within the active site region formed by Ile62, Ala83, Leu132, Asp133, Tyr134, Val135, Pro136, Arg141, Val70, Leu188 and Cys199 residues, respectively and the RMSD of all atoms between these two conformations is 0.62 Å indicating that the parameters for docking simulation are good in reproducing the X-ray crystal structure

Another validation was carried out based on the important interactions made by the bound ligand with the active site residues of all GSK-3β proteins from protein data bank. In order to perform the task, the H-bond interactions formed by various bound co-crystallized ligands of GSK-3β protein structures such as 1UV5, 1Q4L, 1Q41, 1Q3W, 1R0E, 1Q3D and 1Q5K were collected from Ligplot interactions, deposited in PDB summary (PDBsum, http://www.ebi.ac.uk) database. Further study was conducted to identify either similar or novel active site residues that participate in H-bond formation with the designed ligands.

2.4 Molecular Docking

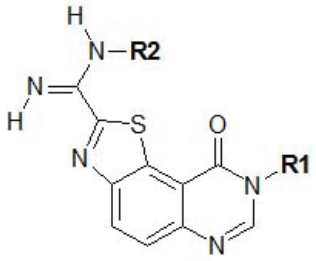
To gain better insight for the interactions between various compounds and GSK-3β, AutoDock 3.0.5 was employed to dock the selected inhibitors listed in Table 1. AutoDock requires the receptor and ligand coordinates in either Mol2 or PDB format. Nonpolar hydrogen atoms were removed from the receptor file and their partial charges were added to the corresponding carbon atoms. The receptor PDB file was transformed into the PDBQS format file containing the receptor atom coordinates, partial charges and solvation parameters. The ligand file was transformed into a PDBQ file, merged nonpolar hydrogen atoms and torsions are defined. The grid calculations were set up and maps were calculated with the program AutoGrid. The grid maps were centered on the ligand binding site and were of dimension 40 × 40 × 40 points. The grid spacing was 0.375 Å and the default AutoDock parameter settings were used for docking.

All docking runs were performed thrice using the Lamarckian genetic algorithm and the obtained dock scores were reported in kcal/mol. The docking protocol utilized in the study consisted of 10 independent runs per ligand, using an initial population of 50 randomly placed individuals, a maximum number of 2.5 x 10^5 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80, and an elitism value of 1. The probability of performing a local search on an individual in the population was 0.06, using a maximum of 300 iterations [38].

2.5 Compound Design

New inhibitor structures were designed based on the compounds selected for docking experiments. Keeping in view on the chemical groups attached to the structures given in Table 1, compound design was done in such a way that simple modifications at R1 and R2 positions should retain the properties associated with the basic scaffold. Also, various R1 and R2 substituents were designed by simultaneously considering the shape of the inhibitor as well as the residues and shape of the active site region of 1UV5. The designed 16 compounds with various substituents are presented in Table 2. Certain chemical rules are utilized to prevent unreasonable structures during molecular design. For instance, structures that include heteroatoms bonded to each other (e.g. O-O, N-N and N-O etc) and eliminating too many heteroatoms bonded to the same carbon atom. Also, certain fragments attached to an aromatic ring possess toxicity. Therefore, in order to recognize such compounds, a forbidden substructure library from the work of Renxiao Wang et al. [39] was used as a filter.

Table 2. New GSK-3β inhibitor design with various R1 and R2 groups



Pattern used to generate new compounds:

aA	**aB**	**aC**	**aD**
bA	**bB**	**bC**	**bD**
cA	**cB**	**cC**	**cD**
dA	**dB**	**dC**	**dD**

R_1 Code	R_1 Side chain	R_2 Code	R_2 Side chain
a	CH_2-CH_3	**A**	CH_2-CH_2-N-$(CH_3)_2$
b	CH-$(CH_3)_2$	**B**	CH_2-CH-(CH_3)-CH_2-NH_2
c	CH_2-CH_2-CH_3	**C**	CH-(NH_2)-CH_2-NH-CH_3
d	CH_2-CH-$(CH_3)_2$	**D**	CH_2-CH_2-CH-$(CH_3)_2$.

3. Results and Discussion

Docking simulations with 1UV5 bound ligand BRW1383 resulted in a dock score of -9.13 kcal/mol and a RMSD value of 0.62 Å showed two major H-bond interactions with Asp133 and Val135 residues, respectively. The correlation between experimental data vs docked scores (Table 1) given in Figure 1 displayed 0.842 r^2 which suggests that the parameters for docking simulation are good in reproducing experimental orientations of these compounds.

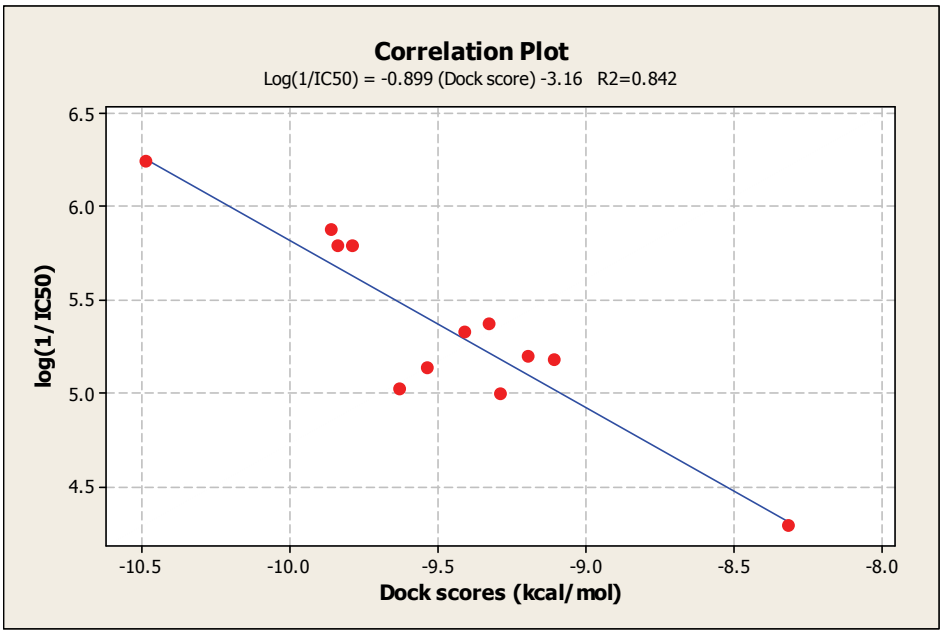


Figure 1. Correlation graph between Log ($1/IC_{50}$) values of compounds from the data set and their corresponding dock scores (kcal/mol)

3.1 Hydrogen bonding interactions

It has been proposed that a group of polar residues located on the binding cavity such as Lys85, Asp200 and Glu97 in GSK-3β and Lys33, Asp144 and Glu51 in CDK5, respectively, play an important role in the ligand - ATP recognition and affinity as Asp200 interacts with the phosphate hydroxyl group of ATP [40]. Our docking results with experimental compounds showed that almost all inhibitors were involved in hydrogen bonding with residues Lys85 and Asp200 in the binding site. The binding modes of R2 position amidino substituted compounds such as IIIa, IIIb and IVb revealed more stable interactions between thaizole ring nitrogen N and the NZ atom of Lys85 as well as the amidine nitrogen N and OD2 atom of Asp200, respectively. Therefore, although other H-bond interactions exist, these hydrogen bonds, in particular, are in fact crucial for the binding activities of thiazolo[5,4-f]quinazolin-9-one series of GSK-3β inhibitors.

Recent crystallographic data of GSK-3β in complex with various non-selective inhibitors like AMP-PNP, staurosporine, hymenialdisine, indirubin-3'-monoxime alsterpaullone and GSK selective inhibitors such as 3-anilino-4-arylmalemide derivatives, 6-Heteroaryl-[3,4-b]pyridine derivatives has provided valuable information on the specific interactions made by the Asp133 and Val135 active site residues as the two key H-bonding interactions [41][42]. Moreover, analysis of PDB records revealed the residues Asp133 and Val135 as predominant and represents important residues for binding diverse range of inhibitors.

Therefore, based on the important residues that participate in H-bond interactions (Lys85 and Asp200) recognized by our studied compounds and to explore the regions occupied by various substitutions at R1 position and R2 in particular, *de novo* design of sixteen compounds (Table 2**)** was carried out. In order to design quite number of compounds, various possible side chains at R1 and R2 positions are considered as given in Table 2. The four R1 substituents remain unchanged in our data set as they appear to interact with Val135 residue, taken from the data reported by Testard et al. [34]. For combinatorial design, R1 substituents are designated with small letters a, b, c and d, whereas the four R2 substituents represent a chemical modification and are designed to explore Lys85, Gln185, Asn186 and Asp200 active site region. They are referred with capital letters A, B, C, D, respectively. The number of atoms that should present in this side chain is limited to five and the attached amine group was varied (Table 2). From these eight different R1 and R2 substitutions, sixteen compounds were generated by simple combinations such as 'R1 a' combines with each of R2 and 'R1 b' with each of R2 and so on.

Further, docking procedures using default parameters on these designed compounds resulted in dock scores up to a maximum of -11.89 kcal/mol, much higher than the active compound IVb (-10.79 kcal/mol) and the interacting residues are given in Table 3. The majority of H-bond interactions formed by these compounds are: Lys85, Asn186, and Asp200 respectively, with few instances being Val135 reported such as compound **10** with a reasonable -11.89 kcal/mol dock score showed five interactions, given in Figure 2.

Table 3. Set of designed compounds displaying various H-bond interacting residues, respective dock scores (kcal/mol) and predicted IC_{50} (nM) values.

Designed Compounds	Combination	Dock scores (kcal/mol)	Predicted IC_{50} (nM)	No. of H-bonds	Interacting Residues
1	aA	-10.73	332	2	Lys 85, Asp 200
2	aB	-11.38	86	3	Lys85, Pro136, Glu137
3	aC	-9.78	2370	3	Asn64
4	aD	-10.80	287	2	Lys85, Asp200
5	bA	-10.53	502	2	Lys85, Asp200
6	bB	-11.45	75	5	Lys85, Gln185, Asn186, Asp200
7	bC	-10.67	376	5	Asn186, Asp200
8	bD	-10.62	417	1	Asp200
9	cA	-11.52	65	4	Lys85, Val135
10	**cB**	**-11.89**	**30**	**5**	**Lys85, Val135, Gln185, Asn186, Asp200**
11	cC	-11.79	37	6	Asn64, Lys85, Asn186, Asp200
12	cD	-11.51	66	3	Lys85, Asn186, Asp200
13	dA	-11.52	65	2	Lys85, Asp200
14	dB	-11.79	37	5	Val135, Pro136, Glu137
15	dC	-11.86	32	6	Asn64, Lys85, Asn186, Asp200
16	dD	-11.52	65	4	Lys 85, Asp 133, Val 135

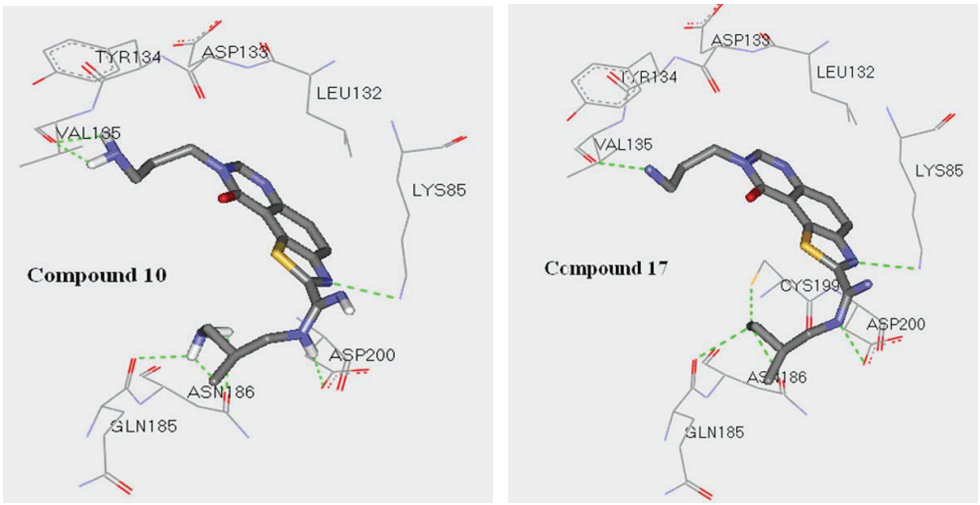


Figure 2. Binding orientations of designed compounds **10** and **17** showing various active site residue interactions.

The data given in Table 3 can be conveniently divided into two sets. First set being combinations resulting from 'R1 a' and 'R1 b' versus R2, where, the reported dock score was less than -11.5 kcal/mol and the remaining compounds represented the second set where the dock score values are between -11.51 to -11.89 kcal/mol, respectively. After careful observations of the residue interactions in the second set, it was recognized that the H-bond interaction with Asn186 was reported in compounds **10**, **11** and **15** with dock scores more than -11.79 kcal/mol because of a conformational change exhibited by groups attached to amidine at R2 position. Hence, compound **10** was further optimized by incorporating the amino group at R1 position of 'R1 c' (Table 2) while keeping the 'R2 B' intact and the resultant compound was named as compound **17** (Table 5). As expected, docking analysis of this compound exhibited a large binding energy value of -13.14 kcal/mol and the image was given in Figure 2.

Table 4. Comparison of dock scores of compounds **IVb, 10,** and **17** against GSK-β, CDK2, CDK5 and CDK6, Values in parentheses indicates RMSD.

Compound	Dock scores (kcal/mol)		
	GSK-3β (1UV5)	CDK2 (2UZO)	CDK5 (1UNH)
Co-crystallized	-9.13 (0.62)	-9.14 (0.83)	-8.73 (0.74)
IVb	-10.49	-9.87	-9.73
10	-11.89	-10.86	-10.52
17	**-13.14**	**-11.21**	**-10.84**

Table 5. New GSK-3β inhibitor designed compound 17 with corresponding dock score, H-bond interacting residues and predicted IC_{50} (nM) value.

R_1 Side chain	R_2 Side chain	Dock score (kcal/mol)	Predicted IC_{50} (nM)	No. of H-bonds	Interacting Residues
CH_2-CH_2-CH_2-NH_2	CH_2-CH-(CH_3)-CH_2-NH_2	-13.14	2	6	Lys85, Val135, Gln185, Asn186, Cys199, Asp200

This is due to the number of interactions made by groups present at both R1 and R2 positions, where a conformational change was adopted by the compound to fit into the Asp133 and Val135 region thereby relaxing the R2 position to involve new residue interactions with Gln185 and Cys199. Also, two of the best designed compounds **10** and **17** reported Gln185 interaction as was not the case with inhibitors reported earlier [34]. To further strengthen the approach employed in new ligand design and testing them *in silico*, the inhibitory values of newly designed compounds were predicted based on the correlation between IC_{50} values and dock scores of compounds Ia to IVb. The inhibitory activities reported in terms of IC_{50} in μM were converted to negative logarithmic values in order to guarantee the linear distribution of data and the relationship between log ($1/IC_{50}$) and the dock scores (compounds Ia to IVb) was established by linear regression technique. According to the regression equation 1, given below, the IC_{50} values of designed compounds are predicted in nM concentrations and are given in Tables 3 and 5.

$$\text{Log}(1/IC_{50}) = -0.8989 \text{ Dock score } -3.16 \quad (1)$$
$$(n = 12, r = 0.918, s = 0.916)$$

Therefore, the *in silico* approach appears to be useful in predicting the key interacting ligand binding residues to understand the type of interactions that occur between designed ligands with GSK-3β binding site region and explain the importance of R1 and R2 substitutions on thiazolo[5,4-f]quinazolin-9-one derivatives. Hence, interactions with Lys85, Val135, Gln185, Asn186, Cys199 and Asp200 are crucial towards binding thiazolo[5,4-f]quinazolin-9-one series of GSK-3β inhibitors. Reports indicate that an excellent similarity exists between various CDKs and GSK-3β [20][43]. Thus, a compound active against GSK-3β might be equally active against CDKs. Hence, specificity of the best experimentally tested and designed compounds were conducted against CDKs.

3.2 Specificity Studies

Studies have been reported that CDK5 and GSK-3β are two main kinases involved in the abnormal hyperphosphorylation of the microtubule-binding protein tau, a diagnostic feature of Alzheimer's disease [20]. Active site differences between CDK2, CDK5 and GSK-3β in complex with various inhibitors showed the residue Leu132 in GSK-β, is replaced by Phe80 in CDK2 and CDK5 [44]. Therefore, ligand specificity studies are conducted on CDKs viz. CDK-2, and -5 proteins (2UZO and 1UNH) extracted from Protein Data Bank. Compounds **IVb**, **10** and **17** are docked in the active site region of three proteins and the data corresponding to RMSD values and the binding energies are presented in Table 4. Comparatively, compound **17** showed a better dock score (-13.14 kcal/mol) than the remaining (-11.21 and -10.84 kcal/mol respectively against CDK2 and CDK5).

4. Conclusion

In this study, thiazolo[5,4-f]quinazolin-9-one derivatives were investigated using AutoDock software to delineate the structural requirements on ligands to recognize the most potent compound for effective GSK-3β inhibitor discovery and design. Docking studies of experimentally identified compounds showed a fairly good correlation between binding free energy (kcal/mol) and IC_{50} (μM) values against GSK-β. The binding modes exhibited by various designed compounds illustrate the importance of specific residue H-bonds within the active site region of 1UV5. From our study it is clear that the efficiency of binding of inhibitors towards GSK-3β would certainly get enhanced when H-bonds are favored with Lys85, Val135, Asn186, Cys199 and Asp200 residues, respectively. Therefore, this study demonstrates the utility of computational tools in inhibitor discovery and design and the presented strategy shall be used as an alternative to the time consuming experiments.

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