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RESEARCH ARTICLE

3D-QSAR AND MOLECULAR DOCKING STUDIES ON ANILINO PYRIMIDINE AND ANILINO QUINAZOLINES AS KINASE INHIBITORS

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ABSTRACT

Three dimensional quantitative structure activity relationship (3D-QSAR) models were developed for anilino pyrimidine and anilino quinazoline derivatives to inhibit the glycogen synthase kinase (GSK-3β) and EGFR tyrosine kinase respectively. The selective glycogen synthase kinase is an important for improved therapeutic profile of gsk3β inhibitors over tyrosine kinase. For this purpose we developed atom based 3D-QSAR for 60 selected compounds. The model showed satisfactory statistical significance; (Regression (r^2) with 0.9738 and Regression coefficient of variation (r^2cv) with 0.6172). These results were found to be more informative in pinpointing the structural basis for the observed quantitative differences of kinase inhibition. The result of the best QSAR model was further compared with structure based investigation using docking studies with the x-ray crystal structure of gsk3β and tyrosine kinase domines. The results helped to understand the nature of substituent's at 2^1 and3^1 positions on aniline ring. Therefore these results will be useful for providing new guidelines for the design of novel inhibitors.

INTRODUCTION

Protein phosphorylation and dephosphorylation are important processes in the control of protein functions; biological phosphorylation mostly occurs on serine, threonine, and tyrosine residues and it is catalyzed by protein kinases whose number is over 500 in the human genome. Given the importance of protein phosphorylation on a main post translation mechanism used by cells to regulate enzyme and other protein (Nigus and Prasad, 2007). Glycogen synthase kinase-3 (GSK-3β) was originally identified and studied for its function in the regulation of glycogen synthase by acting as rate limiting enzyme in glycogen biosynthesis (Michael *et al.,* 2005). It is a serine/ threonine kinase composing two isoforms (α and β) in mammals, these isoforms have same homology (> 90%) at the catalytic domine and are expressed ubiquitously in cellular system and have similar biochemical properties (Han-Cheng *et al.,* 2004). Gsk3β has multiple substrates and plays a crucial role in glucose homeostasis, CNS function, cancer, circadian rhythm, cell death, cell survival and other Karen *et al.,* 2011. EGFR is the closest evolutionary relative of gsk3β and also involved in controlling the cell cycle, apoptosis, neurodegeneration, cancer (Kurup *et al.,* 2001). Drug target EGFR fall in to three main categories depending on the receptor region targeted, extracellular, intracellular and nuclear (Gibson *et al.,* 1997). Small molecule inhibitors which target the intracellular EGFR appear to be the most medicated cancers (Aparna *et al.,* 2005). These molecules act by binding either reversible or irreversible to the c-terminal tyrosine kinase domine of EGFR, there by inhibiting auto phosphorylation of the receptor and therefore activation (Christian *et al.,* 2009). Anilino quinazolines are most developed class drugs that inhibit EGFR and GSk3 intracellularly. These compounds are being studied actively by many researchers groups (Christian *et al.,* 2009) and as a result drug candidates in these groups are in market to cure leukemia's [Fig.1]. In the present study 3D-QSAR studies (atom based 3D-QSAR) were carried out on GSK3β and EGFR kinase inhibitors. In order to provide further insight in to the key structural features required to design potential drug candidates of this class.

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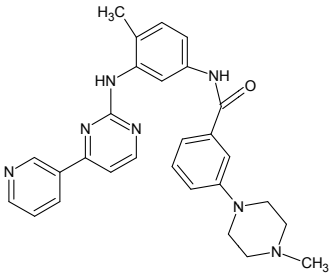
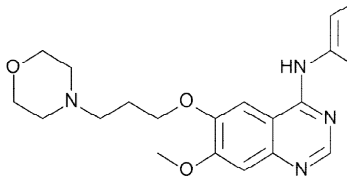
COMPUTATIONAL DETAILS

Data set for analysis

A set of 60 compounds were selected for GSK3β and EGFR tyrosine kinase inhibitors were compiled in Table 1. The compound under study to 3 structurally different families, these groups include anilino pyrimidine derivatives (family A: 25 compounds), anilino quinazolines derivatives (family B: 20 compounds), anilino pyridopyrimidine derivatives (family C: 15 compounds) (Kurup *et al.,* 2001; Gibson *et al.,* 1997; Aparna *et al.,* 2005; Christian *et al.,* 2009; Michael *et al.,* 2010). The biological activities were converted in to the corresponding pIC50 values (-log IC50) about 75% of the 60 compounds were selected as the training set and the remaining 25% were included in the test set.



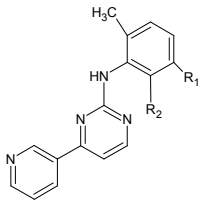
Imatinib (Gleevec)



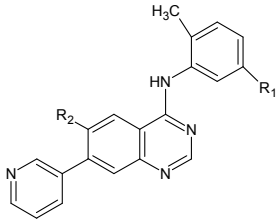
Geftinib (Iressa)

Fig.1. The kinase inhibitors that are in market for cure leukemia's

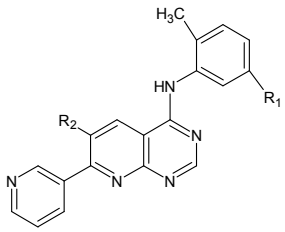
Table 1. Structure of 60 GSk3β inhibitors in this study



A-Anilino pyrimidines
25 compounds

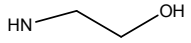
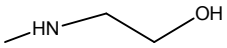
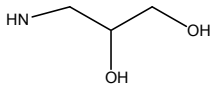
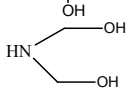
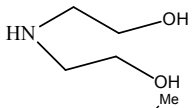
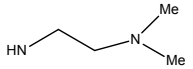
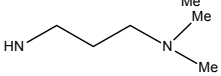
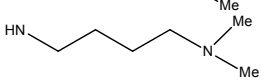
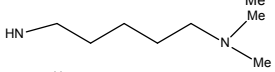
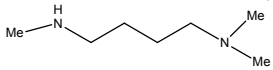
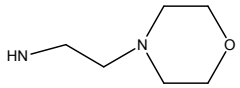
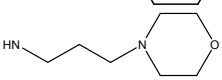
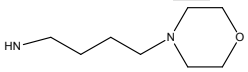
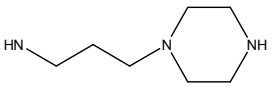
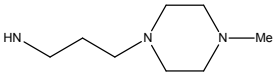


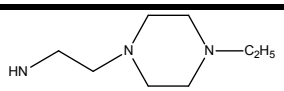
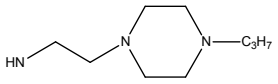
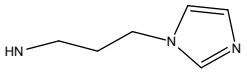
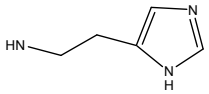
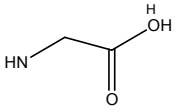
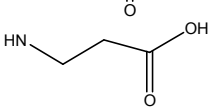
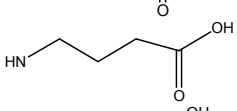
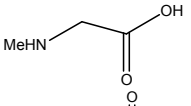
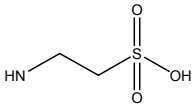
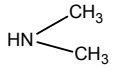
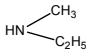
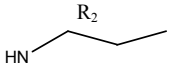
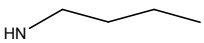
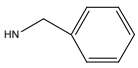
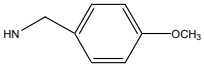
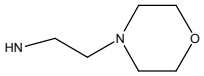
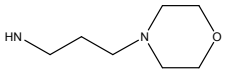
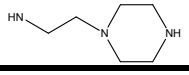
B-Anilino quinazolines
20 compounds



C- Anilino pyrido pyrimidines
15 compounds

Table 1. Structure of 60 GSk3β inhibitors in this study

S.No.	Class	Substitution		pIC50 values
		R_1	R_2	
1	A1	Br	OCH_3	6.45
2	A2	Br		9.61
3	A3	Br		8.58
4	A4	Br		8.49
5	A5	Br		7.85
6	A6	Br		7.92
7	A7	Br		7.34
8	A8	Br		8.05
9	A9	Br		8.13
10	A10	Br		8.07
11	A11	Br		7.39
12	A12	Br		8.49
13	A13	Br		8.72
14	A14	Br		8.26
15	A15	Br		8.26
16	A16	Br		8.29

17	**A17**	**Br**		**8.3**
		R_1	R_2	
18	A18	Br		8.03
19	A19	Br		8.92
20	A20	Br		8.14
21	A21	Br		9.04
22	A22	Br		8.82
23	A23	Br		9.21
24	A24	Br		9.55
25	A25	Br		7.79
26	B1	H	H	6.46
27	B2	Me	H	6.04
28	B3	Cl	H	7.63
29	B4	Br	H	7.56
30	B5	F	H	7.09
31	B6	CF_3	H	6.23
32	B7	Br	NO_2	6.04
33	B8	Br	OCH_3	8
34	B9	Br	OH	9.76
35	B10	Br	NH_2	9.92
36	B11	H	F	7.25
37	B12	H	OCH_3	7.25
38	B13	H	NH_2	6.11
39	B14	CF_3	NH_2	6.24
40	B15	Br	$NHCOOCH_3$	7.92
41	B16	Br	$NHCH_3$	9.16
42	B17	Br		6.79
43	B18	Br		7.92
		R_1	R_2	
44	B19	Br		7.9
45	B20	Br		7.9
46	C1	H	H	6.16
47	C2		Br	8.63
48	C3		Br	8.63
49	C4	$NHCH_3$	Br	7.28
50	C5	Cl	Br	7.08
51	C6	NH_2	Br	6.02
52	C7		Br	6.48
53	C8	OCH_3	Br	6.58
54	C9		Br	6.45
55	C10		Br	7.63
56	C11		Br	8.6

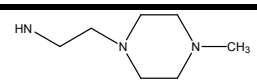
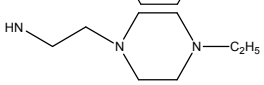
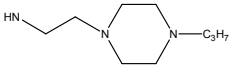
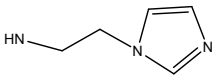
57				
58	C13		Br	7.08
59	C14		Br	7.56
60	C15		Br	6.24

Table 2. Statistical parameters

SD	R^2	R^2 CV	R^2 Scramble	Stability	F	P	RMSE	Q^2	Pearson-r
0.5089	0.9738	0.6172	0.8944	0.666	268.1	2.00E-27	1.95	0.4449	0.6693

Molecular alignments

One of the fundamental assumptions in 3D-QSAR studies are geometrical parallelism. It should exist between the modeled structures and that of the bioactive conformation. The spatial alignment of compounds under study is thus one of the most sensitive and determining factors in obtaining a robust and meaning full model. In the present study the geometry optimized structures were aligned on the respective templates (GSK3β and EGFR tyrosine kinase) by the flexible align database command in glide (Schrodinger 9.3) using the maximum sub structure that is common to all.

Molecular modeling

Molecular modeling studies were performed using a flexible docking method with Glide version 2012 (Schrodinger 9.3). The x-ray crystal structure of GSK3β (pdb i.d. 4acc) and EGFR tyrosine kinase (pdb i.d. 3k5v) was retrieved from the protein data bank. The 3D structures of the molecules were constructed with the chemskech (ACD lab) and chemdraw ultra 8.0. Energy minimization was performed by ligprep using OPLS-2005 force field. The binding affinity of the inhibitors to the protein was evaluated by the total glide docking energies. The 3D QSAR model were developed using atom based QSAR with PLS factor 5 (partial least square).

Partial least square

Partial least square **(**PLS) algorithm was used to quantify the relationship between the structural parameter and the biological activity. The cross validation analysis was performed using leave one out (LOD) method were in one compound is removed from the dataset and its activity is predicted using the model derived from the rest of the dataset. The cross validation r^2 that resulted in optimum number of components and lowest standard error of prediction was taken. To speed up the analysis and reduce noise, a minimum column filtering values (σ) of 1.00 kcal/ mole was used for the cross validation. Final analysis (non cross validation) was performed to calculate conventional r^2 using the optimum number of component obtained from the leave one out cross validation analysis.

Predictive correlation coefficient (r^2 pred)

To further validate the derived model, biological activity of 18 test set molecules were predicted using model derived from the training set. Predictive r^2 value was calculated using formula

$$r^2pred = (SD\text{-}PRESS)/ SD$$

Where SD is the sum of squared deviation between the biological activities of the test set molecules and the mean activity of the training set molecules and PRESS is the sum of squared deviation between the actual and predicted activities of the test set molecules.

Molecular docking

X-ray crystal structure of GSK3β (pdb i.d. 4acc) and tyrosine kinase (pdb i.d. 3k5v) retrieved from protein data bank, docking of the designed molecules in to the binding pocket of gsk3β and tyrosine kinase was carried out using the glide program available within Schrödinger 9.3 package. Glide employs a fast algorithm for flexible docking of small ligands in to fixed protein binding site using an incremental construction process. To further evaluated the docking analysis the xpglide-score values were estimated using the glide modules of Schrodinger 9.3.

RESULTS AND DISCUSSION

The statistical details of the 3D-QSAR model are given in Table 2, the actual and predicted activities obtained from atom based QSAR models of both training and test set molecules are listed in Table 3 and 4 respectively. Scatter plot of actual versus predicted activities for both training and test set molecules obtained from atom based 3D-QSAR (glide, Schrödinger 9.3) are shown in Fig.2 and 3. In the present study the substitutions are made on anilino pyrimidine 2^1 and 3^1 position and bulky pyridine ring at 4^{th} position of pyrimidine nucleus enhance the GSK3β activity and EGFR kinase activity and at 6^{th} position of electron withdrawing group is forward to enhance the GSK3β inhibitory activity. With this structural feature the compound A2, A12, A13, A17, A19, A21, and A24 were shown good predicted activity by best 3D-QSAR model developed using glide (Schrodinger 9.3).

Molecular modeling

For further investigation, the relationship between the virtual receptor ligand interactions of the new compounds with their activity. A molecular modeling studies were performed using the x-ray crystal structure of GSK3β (pdb i.d. 4acc) and tyrosine kinase (pdb i.d. 3k5v) domine from protein data bank that was released during the progress of this work. The most

active molecules (60) were docked in to the active site using glide (Schrodinger 9.3). The active site of protein along with the docked compounds (A24 in gsk3β and A5 in tyrosine kinase) was shown in Fig.4 and 5.

Table 3. Actual and predicted activities of the training set molecules

Ligand Name	Entry ID	Actual activity(pIC50)	Predicted Activity
A1	1	6.45	7.21778
A2	2	9.61	9.44476
A3	3	8.58	8.68293
A4	4	8.49	8.47398
A6	6	7.92	7.74369
A7	7	7.34	6.92431
A8	8	8.05	8.20208
A10	10	8.07	8.08957
A11	11	7.39	7.66875
A13	13	8.72	9.04364
A14	14	8.26	8.05689
A15	15	8.26	8.15408
A16	16	8.29	8.06646
A18	18	8.03	8.35958
A19	19	8.92	8.85073
A20	20	8.14	8.19076
A21	21	9.04	9.17517
A22	22	8.82	8.70447
A23	23	9.21	9.22718
A24	24	9.55	9.22347
A25	25	7.79	7.95185
B4	29	7.56	7.44795
B5	30	7.09	7.22572
B7	32	6.04	6.12877
B10	35	9.92	7.96293
B11	36	7.25	6.97328
B13	38	6.11	7.80419
B14	39	6.24	5.75837
B18	43	7.92	7.89602
B19	44	7.9	7.64181
B20	45	7.9	7.76493
Ligand Name	Entry ID	Actual activity(pIC50)	Predicted Activity
C1	46	6.16	6.16007
C2	47	8.63	9.04312
C4	49	7.28	6.88059
C5	50	7.08	7.26084
C7	52	6.48	6.42322
C8	53	6.58	6.92837
C11	56	8.6	7.55809
C12	57	6.48	6.3564
C13	58	7.08	6.8684
C14	60	7.56	7.44795
C15	61	6.24	6.01209

Table 4. Actual and predicted activities of the test set molecules

Ligand Name	Entry ID	Actual activity(pIC50)	Predicted Activity
A5	5	7.85	7.49508
A9	9	8.13	8.20515
A12	12	8.49	8.36489
A17	17	8.3	8.08448
B1	26	6.46	5.86851
B2	27	6.04	7.42235
B3	28	7.63	7.29194
B6	31	6.23	5.48075
B8	33	8	8.05235
B9	34	9.76	7.4282
B12	36	7.25	7.98513
B15	39	7.92	4.42058
B16	40	9.16	8.21642
B17	41	6.79	5.96031
C3	48	8.63	6.61709
C6	51	6.02	7.56722
C9	54	6.45	7.30008
C10	55	7.63	7.41152

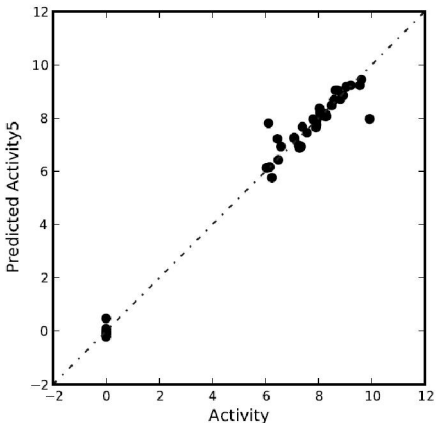


Fig. 2. Scatter-plot of actual verses predicted activity for training set molecules

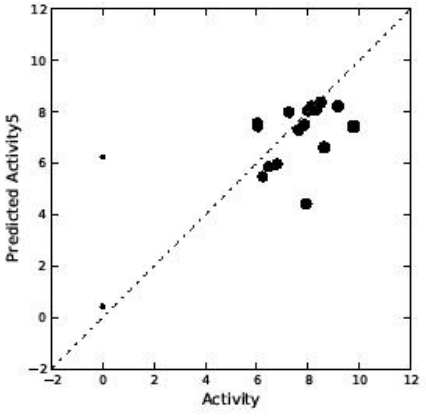


Fig. 3. Scatter-plot of actual verses predicted activity for test set molecules

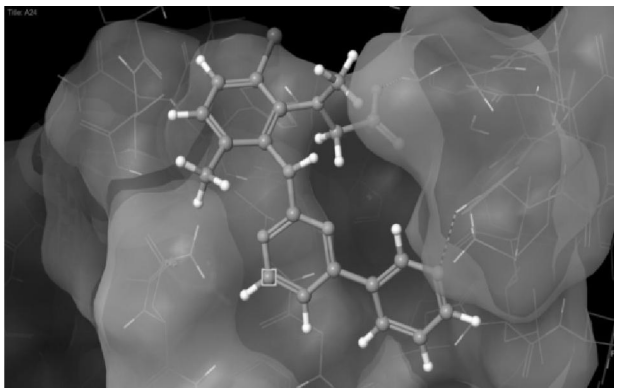


Fig. 4. Docking image of compound a24 in to active site of GSK3β protein (pdb i.d.4acc)

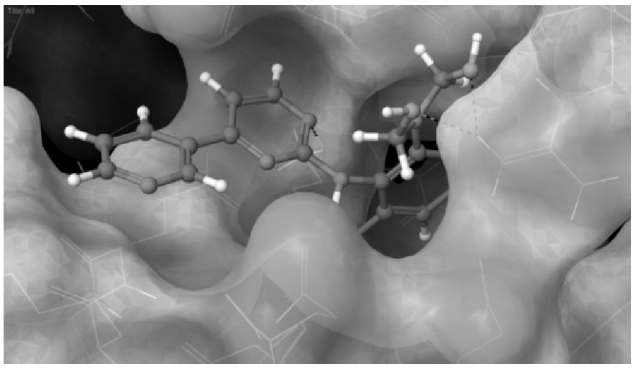


Fig. 5. Docking image of compound a5 in to active site of tyrosine kinase protein (pdb i.d.3k5v)

Docking studies revealed that hydrogen bond formation and hydrophobic interactions were key factors which affect inhibitory action of the compounds on crystal structure of GSK3β and EGFR tyrosine kinase and the binding energy expressed in k.cal/ mols (xp dock-score). It displayed vital hydrogen bond interactions with GLU121, LYS86 and electrostatic interaction with TYR127 residues of GSK3β protein and ARG405, SER 404 electrostatic interaction with LYS209 of tyrosine kinase protein, the respective interaction images of compound A24, A5 are shown in Fig.6 and 7.

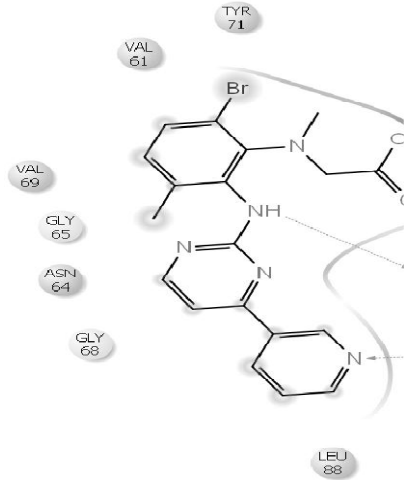


Fig. 6. Interaction image of compound a24 in to active site of GSK3β protein (pdb i.d.4acc)

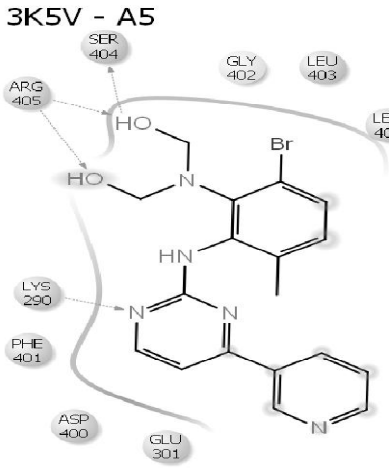


Fig. 7. Interaction image of compound a5 in to active site of tyrosine kinase protein (pdb i.d.3k5v)

Conclusion

Gsk3β represents a promising target and designing gsk3β inhibitor would offer a novel approach to develop potent inhibitors in this class. In the present study we have selected a different class of compounds such as anilino pyrimidine, anilino quinazoline and anilino pyrimidino pyrimidine with different structural features were used for the development of best 3D-QSAR model. Molecular modeling studies revealed that the positioning of a hydrogen bond donor/ acceptor on the quinazoline and pyrimidine moiety play a crucial role in the inhibition of gsk3β and egfr tyrosine kinase.

The reported results are expected to contribute toward deeper insight in to structural activity relationship and would be helped in further designing quinazoline and pyrimidine as potential GSK3β inhibitors.

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