Research Article

The application of a 3D-QSAR Approach for 7-(4H-1,2,4-Triazol-3-yl) benzo[c] [2,6] naphthyridine Derivatives as PIM – 1 Inhibitors

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ABSTRACT

CoMFA (Comparative molecular field analysis) and CoMSIA (Comparative molecular similarity indices analysis) based on Three dimensional quantitative structure activity relationship (3D-QSAR) studies were conducted on a series of (57 molecules) as anti-proliferative agents of 7-(4H-1,2,4-Triazol-3-yl)-benzo[c][2,6] naphthyridine. The best predictions were obtained with a CoMFA model (q^2-0.893, r^2-0.989) and with CoMSIA model (q^2-0.764, r^2-0.985). Both models were validated by a test set of fifteen compounds producing very good predictive r^2 values of 0.752 and 0.702, respectively. CoMFA and CoMSIA contour analysis were then used to analyze the structural features of ligands to account for the activity in terms of positively contributing physiochemical attributes such as steric, electrostatic, hydrophobic, H bond donor and acceptor fields. The resulting contour maps produced by the best CoMFA and CoMSIA models were used to identify the structural features relevant to the biological activity in this series of compounds. FlexX were employed to dock the inhibitors into the active site of the PIM-1 kinase and these docking studies revealed the vital interactions and binding conformation of the inhibitors. The information provided by 3D-QSAR models and the docking interactions may afford valuable clues to optimize the lead and design new potential inhibitors.

Keywords: 3D-QSAR, 7-(4H-1,2,4-Triazol-3-yl)benzo[c][2,6]naphthyridine, CoMFA, CoMSIA, Docking.

INTRODUCTION

PIM – 1 is an oncogene, belongs to a new class of serine/threonine kinases which is involved in the control of proliferation, differentiation, cell growth and apoptosis.[1] Pim-1 kinase induced by a number of growth factors, mitogens, hormones and cytokines. It is also involved in signal transduction.[2] Over expression of Pim kinase diffuse chronic lymphocytic leukemia, B cell lymphoma, postate cancer.[3-5] and also associated with metastasis. Nf-kB[6] and Jak-STAT[7] play important role in activation of pim – 1 kanases. Pim – 1 is more active than other kinases. Pim – 1 includes 3 isoforms Pim – 1, Pim – 2, Pim – 3. These three are implicated in the growth of prostate cancer and hematological malignancies.

In the present study of this novel PIM-1 kinase inhibitors[8] were performed using three dimensional quantitative structure activity relationships and docking approach. Three dimensional quantitative structure activity relationship (3D-QSAR) methods, such as CoMFA[9] (Comparative molecular field analysis) and CoMSIA[10] (Comparative molecular similarity indices analysis), were applied to these inhibitors to gain insights into how steric, electrostatic, hydrophobic, hydrogen bonding interactions influence their activities. FlexX[11] Docking study was performed to explore the binding mode between all of the compounds and the PIM, which produced the bioactive conformer of the whole dataset. Based on the molecular field information of 3D-QSAR tools and molecular docking protocols, a few strategies were proposed to design new molecules with improved activity.

Methodology

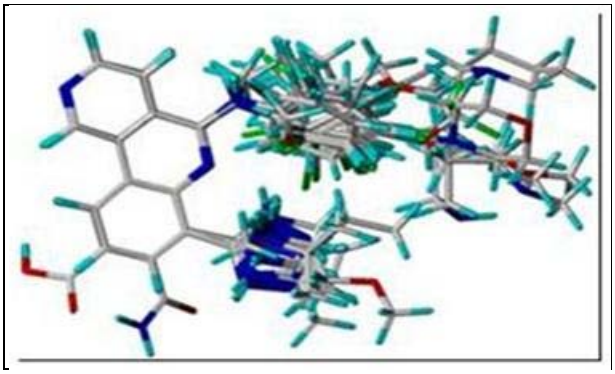
Molecular structures and optimization

Fifty seven molecules selected for the present study were taken from an earlier report[8]. The compound structures and their biological activities are given in table 1. The IC50 values were converted to pIC50 (-logIC50)[12] and used as dependent variables in Comparative molecular field analysis (CoMFA) and Molecular similarity indices analysis (CoMSIA). The 3D-QSAR models were generated using a training set of 42 Pim inhibitors. Predictive power of the resulting models was evaluated suing a test set of 15 molecules (Table 2). The compounds in the test set were selected randomly.

Molecular alignment

Molecular alignment is the most sensitive parameter in three dimensional quantitative structure activity relationship analyses. The quality and predictive power of the model were directly dependent on the alignment rule. CoMFA results are sensitive to a number of factors such as alignment, lattice shifting step size and probe atom type.[13] Structural alignment play important role in prediction of CoMFA models and the reliability of the contour models depend strongly on the structural alignment of the molecules.[13] The molecular alignment was achieved by SYBYL routine align database. The most active compound 55 was used as a template to align the other 56 compounds from the series by common substructure alignment, using the ALIGN DATABSE command in Sybyl 6.7. The common substructure used

for alignment, and the superimposed structure after alignment is presented in Figure 1.



CoMFA studies

Steric and electrostatic fields were calculated using the Lennard-Jones and Coulomb potentials[9] with a distance-dependent dielectric constant at all interactions in a regularly spaced (2Å) grid taking a sp^3 carbon atom as steric probe and a+1 charge as electrostatic probe. The cutoff value was set to 30kcal/mol. The regression analysis was carried out using the fully cross-validated partial least squares (PLS) method (leave one out) [14] with standard options for scaling of variables. The column filtering was set to 2.0 kcal/mol to get better signal to noise ratio by omitting those lattice points whose energy variation was below this threshold.

CoMSIA studies

CoMSIA approach is a substitution to perform 3D-QSAR by CoMFA. Molecular similarity is compared in terms of similarity indices. In Comparative Molecular Similarity Indices Analysis, a distance-dependent Gaussian-type physicochemical function has been adopted to avoid uniqueness at the atomic positions and dramatic changes of potential energy for those grids in the proximity of the surface. The CoMSIA method specifies explicit steric, electrostatic along with hydrophobic, hydrogen bond donor and acceptor fields, were calculated using the sp^3 carbon probe atom with a +1 charge atom and a radius of 1.0 A˚. In CoMFA Steric and electrostatic fields were calculated. Primarily, the intention is to division the different properties into various placements where they play a decisive role in determining the biological activity. In general, similarity indices, $A_{F,K}$ between the compounds of interest were computed by placing a probe atom at the intersections of the lattice points using below equation

$$A^{q}_{F,K}(j) = \sum_{i=1}^{n} W_{probe,k} W_{ik} e^{-a r^2_{iq}}$$

Where q is a grid point, i is a summation index over all atoms of the molecule j under computation, W_{ik} is actual value of the physicochemical property k of atom i, and $W_{probe,k}$ is value of the probe atom.

In the present study, similarity indices were computed using a probe atom ($W_{probe,k}$) with charge +1, radius 1Å, hydrophobicity +1, and attenuation factor a of 0.3 for the Gaussian type distance. The statistical valuation for the CoMSIA analyses was performed in the same manner as described for CoMFA.

Partial least square (PLS) analysis

Partial least square analysis[9] is used to correlate PIM-1 Kinase inhibitor activities with the CoMFA and CoMSIA values. The predictive value of the models was evaluated first by leave-one-out (LOO) cross-validation method in which one compound is removed from the dataset and its activity is predicted using the model derived from the rest of the molecules in the dataset. A minimum column filter value of 2.0 kcal mol^{-1} was used for the cross-validation to speed up the analysis and to reduce the signal-to-noise ratio. The cross-validated coefficient q^2 was calculated according to the following equation:

$$q^2 = 1 - \frac{\sum (Y_{pred} - Y_{actu})^2}{\sum (Y_{actu} - Y_{mean})^2}$$

Where Y_{pred},Y_{actu} and Y_{mean} are predicted, actual and mean values of the target property (pIC50), respectively; and

$$\sum (Y_{actu} - Y_{mean})^2 = PRESS$$

PRESS is the prediction error sum of the squares, derived from the LOO method. The ONC (Optimum number of components) corresponding to the lowest PRESS value was used for deriving the final Partial least square regression models. By using the same number of components performed the Non-cross-validation to calculate conventional r^2.

Molecular Docking

Molecular docking studies were performed using flexX software[15] installed on Silicon Graphics Inc octane2 workstation using the package SYBYL 6.7, to investigate the binding mode between the inhibitors and PIM-1. FlexX is a fragment based method. FlexX handles the flexibility of the ligand by decomposing the ligand into fragments and performs the incremental construction algorithm directly inside the protein active site. This method allows conformational flexibility of the ligand while keeping the protein rigid. The base fragment is selected such that it has most potential interaction groups and the fewest alternative conformations. All the 57 molecules which were used in QSAR studies are taken for molecular docking studies. The crystal structure of PIM-1 (PDB ID: 2XJ1) in complex with XJ1 ((2e)-3-(3-{6-[(Trans-4-Aminocyclohexyl)amino]pyrazin-2-Yl}phenyl) prop-2-Enoic acid) was used in the study. While creating RDF file, active site was defined within a radius 6.5Å of the ligand. Formal charges were assigned to all the molecules and the molecules were docked. FlexX generated 30 different conformations in the active site. All these conformations are ranked according to the FlexX score.

Hardware and software

Sybyl 6.7[16] was used for molecular modeling on a SGI Origin 300 workstation equipped with 4 * 600 MHz R12000 processors.

RESULTS AND DISCUSSION

3D QSAR Studies

CoMFA and CoMSIA 3D-QSAR models were derived using PIM-1 inhibitors. The training molecules with their experimental $pIC_{50,}$ predicted and residual values are given in Table 1, and test set values are given in Table 2.

Table 1: Experimental, Predicted, residual and docking score of PIM-1 inhibitors used in training set

C.No.	PIC50	CoMFA		CoMSIA		Dockscore
		Predicted	Residual	Predicted	Residual	
3	6.10	6.335	-0.238	6.571	-0.474	-31.3
5	6.24	6.543	-0.305	6.544	-0.306	-31.9
7	5.65	6.515	-0.862	6.282	-0.632	-26.3
8	6.47	6.355	0.110	6.017	0.448	-28.7
10	7.28	7.290	-0.031	7.211	0.048	-24.3
12	6.97	7.081	-0.111	6.876	0.094	-26.6
13	6.94	6.737	0.206	7.017	-0.074	-28.7
14	7.57	7.304	0.264	7.349	0.219	-28.4
16	7.89	7.783	0.103	7.812	0.074	-21.6
17	7.60	7.561	0.041	7.512	0.090	-22.3
19	7.28	7.364	-0.081	7.778	-0.495	-21.5
21	7.77	7.937	-0.168	7.871	-0.102	-29.8
22	7.64	7.659	-0.021	7.773	-0.135	-28.9
24	7.55	7.714	-0.162	7.669	-0.117	-29.5
25	7.57	7.751	-0.183	7.801	-0.233	-29.1
26	7.55	7.532	0.020	7.094	0.458	-26.2
27	8.30	8.194	0.107	8.146	0.155	-29.5
28	7.66	7.772	-0.095	7.199	0.478	-25.8
30	7.74	7.761	-0.017	7.881	-0.137	-29.4
31	8.30	7.944	0.357	7.799	0.502	-29.5
32	8.15	8.063	0.091	7.866	0.288	-29.2
33	7.80	8.060	-0.265	7.493	0.302	-28.0
34	8.05	7.903	0.142	8.070	-0.025	-27.3
35	8.00	7.852	0.148	7.877	0.123	-26.4
36	8.10	7.968	0.128	8.185	-0.089	-25.9
37	7.80	7.779	0.016	8.216	-0.421	-27.9
38	7.67	7.542	0.128	7.161	0.509	-25.1
39	7.39	7.638	-0.251	7.731	-0.344	-27.8
41	7.60	7.517	0.085	7.526	0.076	-30.8
42	7.89	8.048	-0.162	7.932	-0.046	-25.8
43	8.30	8.343	-0.042	8.266	0.035	-30.4
45	8.30	8.259	0.042	8.423	-0.122	-30.9
48	8.70	8.348	0.350	8.307	0.391	-30.9
49	8.70	8.393	0.305	8.542	0.156	-28.5
50	8.10	8.266	-0.170	8.280	-0.184	-27.7
51	8.15	8.244	-0.090	8.143	0.011	-29.6
52	8.15	8.113	0.041	8.089	0.065	-29.5
53	8.22	8.217	0.004	8.180	0.041	-28.5
54	8.30	8.300	0.001	8.126	0.175	-30.6
55	8.70	8.256	0.442	8.113	0.585	-28.1
56	7.89	7.893	-0.007	8.054	-0.168	-26.2
58	8.00	7.968	0.032	8.690	-0.690	-27.4

Table 2: Experimental, Predicted, residual and docking score of PIM-1 inhibitors used in training set

C.No.	PIC50	CoMFA		CoMSIA		Dockscore
		Predicted	Residual	Predicted	Residual	
1	7.32	6.95	0.37	6.91	0.41	-30.8
9	7.00	6.74	0.26	6.86	0.14	-29.2
15	6.64	6.83	-0.19	6.29	0.35	-33.6
18	7.41	7.14	0.27	6.97	0.44	-20.9
20	6.71	7.05	-0.34	6.57	0.14	-23.6
23	7.39	7.18	0.21	7.71	-0.32	-22.1
29	7.13	7.17	-0.04	7.09	0.04	-30.3
40	7.62	7.37	0.25	7.44	0.18	-26.4
44	7.59	7.25	0.34	7.26	0.33	-30.8
46	7.59	7.16	0.43	7.38	0.21	-30.9
47	7.35	7.27	0.08	7.84	-0.49	-32.4
57	7.80	7.44	0.36	8.02	-0.22	-30.7
59	8.15	8.03	0.12	8.02	0.13	-32.4
60	8.40	8.13	0.27	7.99	0.41	-31.3
61	8.15	8.32	-0.17	8.31	-0.16	-30.2

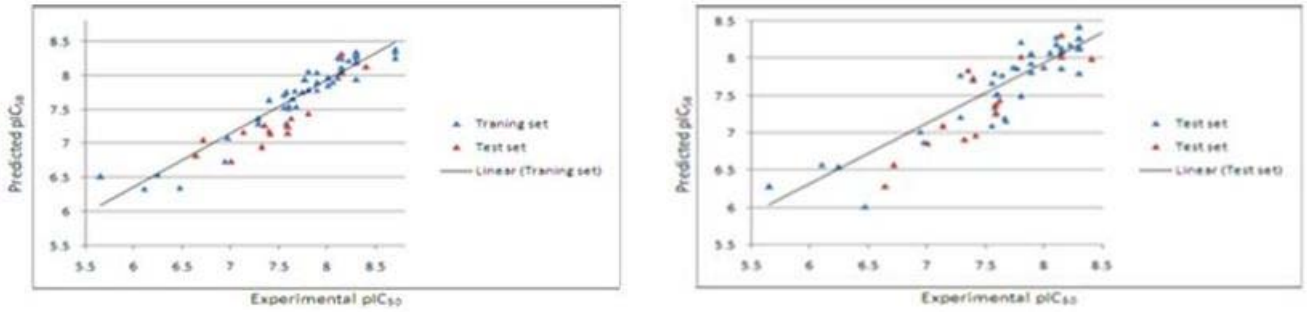


Figure 2: Predicted versus actual inhibitory concentrations for the 57 inhibitors. Blue color indicates Training set (42) and Red color indicates the test set (15) for (A) CoMFA and (B) CoMSIA models. The correlation coefficients (r^2) are 0.989 for the CoMFA model and 0.990 for CoMFA and CoMSIA models

CoMFA analysis

Forty two compounds out of the total fifty seven PIM-1 inhibitors were used as training set and fifteen compounds were used as test set. The test set compounds were selected randomly so that the structural diversity and wide range of activity in the dataset were included. PLS analysis was carried out for the training set and a cross-validated q^2 of 0.893 for five components was obtained. The non cross-validated PLS analysis with the optimum components revealed a conventional r^2 value of 0.989, F value = 638.057 and an estimated standard error of estimate (SEE) 0.076. The steric field descriptors contribution is 47.6 % of the variance, while the electrostatic field contribution is 52.4 % of the variance. 100 runs were carried out for Bootstrap analysis for further validation of the model by statistical sampling of the original dataset to create new datasets. Statistical analyses are given in Table 3.

CoMSIA analysis

The CoMSIA analyses were performed using five descriptor fields: steric, electrostatic, hydrophobic, hydrogen bond donor and acceptor. The CoMSIA study disclosed a cross validated q^2 of 0.764 with optimum number of component 6, a conventional r^2 of 0.985 with a standard error of estimate 0.111 and F value 243.934. The steric field contribution 8.4 % of the variance and, the electrostatic descriptor explains 20.1 %, the hydrophobic field explains 21.7% while the hydrogen bond donor explains 26.4 % of the variance and hydrogen bond acceptor field contribution is 23.4%. For Bootstrap 100 runs was then carried out for model validation by statistical sampling of the original dataset to create new datasets. This yielded higher r^2 bootstrap value 0.990 for CoMSIA with standard error of estimate 0.111 affirming the statistical validity of the developed models. Graphical representation of predicted versus actual activities are given in Figure 2.

3D-QSAR model Validation

The fifteen randomly selected compounds (Table 2) were used as test set and forty two compounds (Table 1) were used as training set to assert the stability and predictive ability of the CoMFA and CoMSIA models. The predicted pIC_{50} with the quantitative structure activity relationship models are in good agreement with the experimental data within a statistically adequate error range, with a predicted correlation coefficient of r^2_{pred} = 0.752 and 0.702 and standard error of estimate value 0.076 and 0.111 for CoMFA and CoMSIA, respectively. The correlation between the testing results indicates that the CoMFA and CoMSIA models can be reliably used in the design of novel PIM-1 inhibitors.

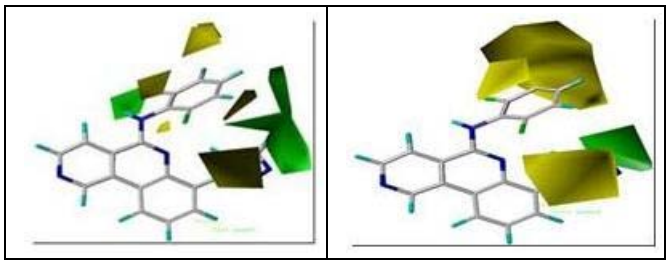
Table 3: Statistical analysis of Pim-1 inhibitors

	CoMFA	CoMSIA
q^2	0.893	0.764
r^2	0.989	0.990
SEE	0.076	0.111
F	638.057	243.934
Cross Validation	0.883	0.784
r^2pred	0.752	0.702
Bootstrap	**Mean**	**Std.dev**
SEE	0.067	0.039
r^2	0.990	0.004
Field contributions		
Steric	47.6	8.40
Electrostatic	52.4	20.1
Hydrophobic	-	21.7
Donor	-	26.4
Acceptor	-	23.4

Contour analysis

The visualization of the results of the CoMFA and CoMSIA models have been performed using the StDev*Coeff mapping option contoured by contribution. The default level of contour with contribution, 80% for favored region and 20% for disfavored region was set during contour analysis.

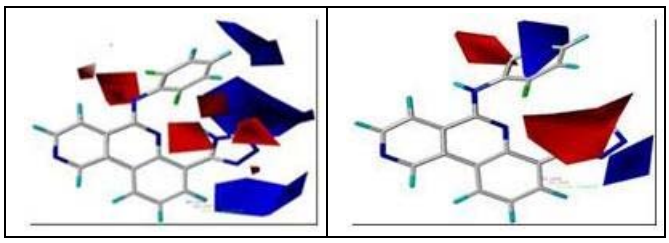
The steric contours of CoMFA and CoMSIA are depicted in Figure 3**.** In CoMFA steric contours, we observed the presence of a prominent green isopleth at the position of the 4H-[1,2,4]-Triazole ring; similar green contour is also present in the CoMSIA steric map indicating that by substituting a bulky steric group at this place will favorably increase the PIM-1 inhibitory activity of the molecule. In CoMFA map we could observe the presence of green contour at NH position, and also two small yellow contours at R^{10} and 3' positions, but in CoMSIA contour map a large yellow contour present at 3' and 4' position.



3(a) 3(b)

Figure 3: CoMFA (3a) and CoMSIA (3b) steric contour maps of Highest active compound

Like the steric CoMFA–CoMSIA contours, CoMSIA electrostatic contour is also comparable to its counterpart electrostatic CoMFA map, three red isopleths present at around 4H-[1,2,4]-Triazole ring, and in CoMSIA a large red contour present around 4H-[1,2,4]-Triazole ring in both the maps indicate that substitution at these regions with electronegative groups will favourably increase the molecules PIM-1 inhibitory activity and like the steric CoMFA contours, the CoMFA electrostatic contours shows areas of red and blue isopleths which are not comprehensible but whereas the CoMSIA counterpart shows well-defined blue isopleths at attachment position of 4H-[1,2,4]-Triazole ring suggesting that replacement of these areas with electropositive groups will increase the molecule's PIM-1 inhibitory activity. Electrostatic contour maps of both CoMFA and CoMSIA are shown in figure 4.



4(a) 4(b)

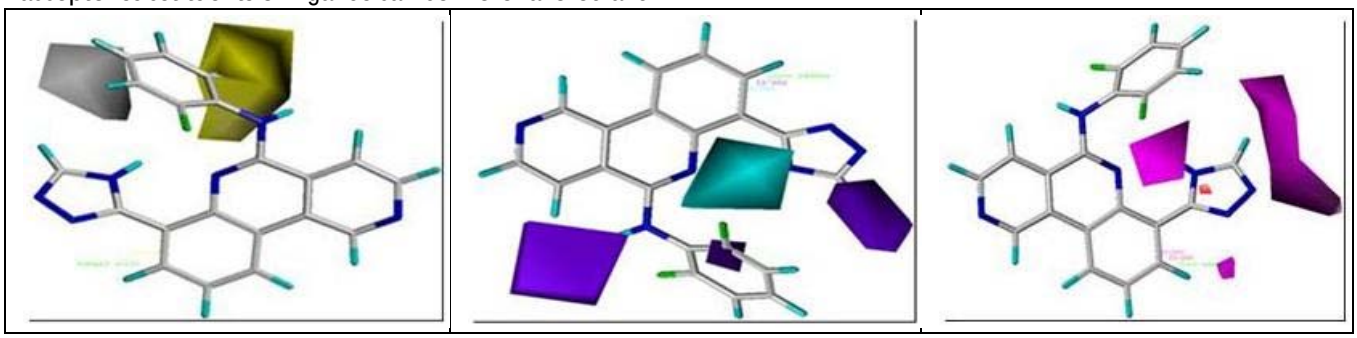
Figure 4: CoMFA (4a) and CoMSIA (4b) electrostatic contour maps of Highest active compound

Figure 5(a) represents the hydrophobic CoMSIA contours which are generally denoted in the yellow and white contours representing favorable and unfavorable hydrophobic group substituting regions, and from the figure we can clearly note large white isopleths nearly 4' and 5' positions of a molecule, indicating that hydrophobic groups substitution in the molecule will drastically decrease its PIM-1 inhibition action but by substituting hydrophilic groups in the molecule can radically increase the molecules PIM-1 inhibition.

Figure 5(b) represents Hydrogen-bond donor isopleths from CoMSIA. H bond donor-favored regions are represented by cyan isopleths and unfavorable regions by purple isopleths. CoMSIA hydrogen-bond donor contour map showed one cyan contour covering –NH substituent at ring in compound 55, 4H-[1,2,4]-Triazole ring suggesting that substitution of H-bond donor groups in

this region can be expected to improve the predictivity of molecule.

Figure 5 (c) represents the hydrogen-bond acceptor isopleths are represented by magenta and red contours. Magenta isopleths indicate regions where hydrogen-bond acceptor substituents on ligands can be more favored and the red ones represent areas where such substituent's on inhibitors may be disfavored. In figure 5(c) two magenta contours are visible which display the importance of the presence of hydrogen-bond acceptor groups for PIM-1 inhibitory activity of 7-(4H-1,2,4-Triazol-3-yl)benzo [c][2,6]naphthyridine inhibitors.



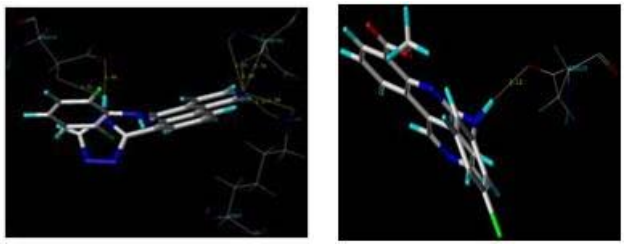
5(a) 5(b) 5(c)

Figure 5(a) represents hydrophobic contour of CoMSIA; **Figure 5(b)** represents donor contour of CoMSIA; **Figure 5(c)** represents acceptor contour of CoMSIA.

Molecular Docking Results

Docking results showed that all the molecules are forming hydrogen bonds with important amino acids Lys67, Asn172 and Asp186 of protein. Highly active molecule 55 is participating in greater number of interactions with important amino acids Asp186, Asp128 and Lys67 than least active molecule. Least active molecule shows only one interaction with Asp128.

The interactions between highly active molecule 55 and amino acids of PIM-1 binding pocket are shown in fig 6. As depicted from figure, receptor and ligand are tightly bound to each other by forming a network of hydrogen bonding interactions. The binding between highest activity compound and PIM-1 amino acids are depicted as 5 hydrogen bonding interactions, for Asp186 it shows three interactions, Asp128 and Lys67 shows two interactions each. Docking interactions of most and least active compounds are shown in Figure 6.



Docking interactions of highest active compound

Docking interactions of lowest active compound

Figure 6: Molecular docking interactions of most and least active compounds.

CONCLUSION

A 3D-QSAR study using CoMFA and CoMSIA methods had been applied for 7-(4H-1,2,4-Triazol-3-yl)benzo[c] [2,6]naphthyridine inhibitors. As a result, the 3D-QSAR models for the 7-(4H-1,2,4-Triazol-3-yl)benzo[c][2,6] naphthyridine with the accessible software package SYBYL 6.7 were successfully constructed. The CoMFA and CoMSIA models were gave good LOO cross validation q^2 and r^2 values respectively. The SEE and the Fisher test value of this model were 0.076 and 638.057 for CoMFA and 0.111 and 243.934 for CoMSIA respectively. All of the constructed models possessed good internal and external validation by showing statistical significance and predictive abilities. Both the predictive evaluation and the contour map analysis accorded well with the experimental interaction mode of the PIM – 1 inhibitor. A combined application of the obtained CoMFA and CoMSIA models was further employed for the design of new PIM – 1 inhibitors.

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