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3D-QSAR STUDY OF BENZOTHIAZOLE DERIVATIVES AS p56^{lck} INHIBITORS

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Keywords:

Benzothiazole, p56^{lck}, 3D-QSAR, CoMFA, CoMSIA, Autodock

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ABSTRACT: 3D-QSAR models of Comparative of Molecular Field Analysis (CoMFA) and Comparative of Molecular Similarity Indices Analysis (CoMSIA) of 44 potent p56^{lck} inhibitors were performed. The conventional ligand-based 3D-QSAR studies were performed for a series of Benzothiazole derivatives as p56^{lck} inhibitors. The model gave q^2 values of 0.710 and 0.642, r^2 values of 0.966 and 0.956 for CoMFA and CoMSIA, respectively. The predictive ability of the models was validated using the external test set of 10 compounds that were not included in the training set of 34 molecules. These results provided better understanding of the relationship between the structural features of p56^{lck} inhibitors and its activities, which should be applicable to design and find new potential p56^{lck} inhibitors.

INTRODUCTION: Heterocyclic compounds and their derivatives have attracted attention due to their different biological and pharmacological properties. [1] The heterocycles are the flexible compounds existing in almost all natural products and synthetic organic compounds. [2] Benzothiazoles are constituents of number of bioactive heterocyclic compounds, having wider range of applications. Benzothiazole compounds has special significance in wide range of chemical applications as well as in clinical applications, because of its anti-mycobacterial properties. [3] Among the heterocycles the benzothiazoles occupy a prominent position. Benzothiazoles usually associated with one or the other biological activity.

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Benzothiazoles are a group of xenobiotic compounds which contains a benzene ring fused with a thiazole ring. The benzothiazoles are manufactured worldwide for a variety of applications. [4] Benzothiazoles are used as fungicides leather production, [5] as an antialgal agents, [6] and also used as chemotherapeutic agents. [7] These applications indicate that Benzothiazole derivatives have a wide spectrum of biological activity. Benzothiazole molecules has been the primary rubber-related chemical found in synthetic grass. Benzothiazole exerts acute toxicity and is a respiratory irritant and skin sensitizer. Genetic toxicity assay shows benzothiazole was positive in Salmonella in the presence of metabolic activation. Benzothiazole metabolism involves ring-opening and formation of aromatic hydroxylamines, metabolites with mutagenic and carcinogenic potential.[8]

CoMFA (Comparative molecular field analysis) [9] and CoMSIA (Comparative Molecular Similarity

Indices Analysis) [10] has emerged as a very important methods in ligand based drug design strategies. Comparative molecular field analysis and comparative molecular similarity indices analysis has a combination of reasonable molecular descriptors, statistical analysis and graphical representation of results. Molecular structures are described with molecular interaction energies as steric and electrostatic fields surrounding the molecules, the statistics is computed by PLS [11] regression analysis and the output is displayed as contour maps superimposed on the molecules. The methodology of comparative molecular field analysis predicts that a suitable sampling of steric and electrostatic fields surround a set of aligned molecules provides all the information necessary for understanding their biological properties. The CoMSIA methodology assumes that a suitable sampling of hydrophobic, hydrogen bond donor and hydrogen bond acceptor along with steric and electrostatic fields.

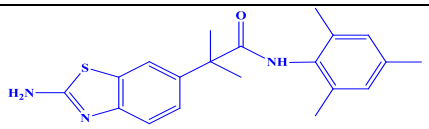
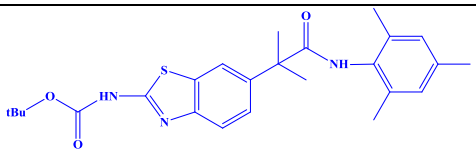
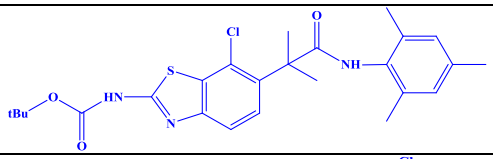
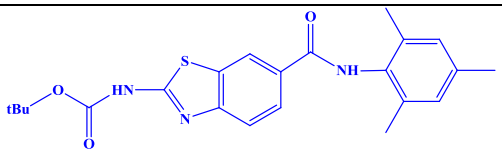
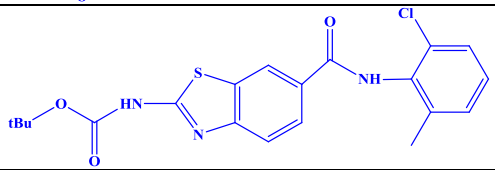
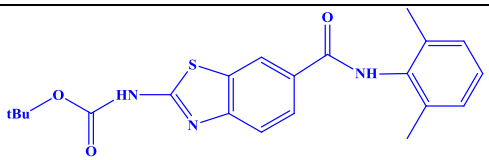
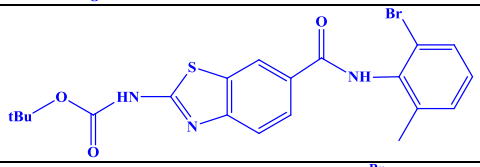
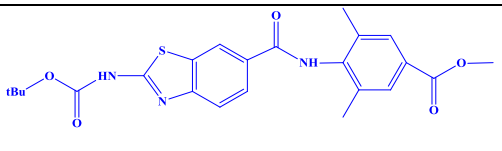
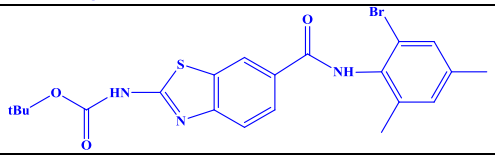
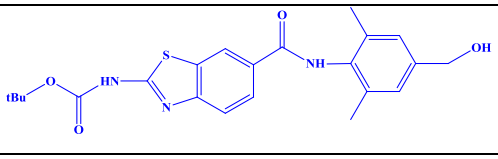
CoMFA is usually employed to increase the binding affinity.[12] When used in a comparative investigation on the same series of compounds acting on multiple targets, such methodology is valuable in identifying the structural basis of the observed quantitative differences in the pharmaco toxicological properties. We developed the 3D QSAR CoMFA and CoMSIA models on $p56^{lck}$ inhibitors in the expectation of getting a model that would account for the quantitative differences in biological activity seen in this series and to capitalize upon the insights to design ligands with pronounced inhibitory potency and selectivity.

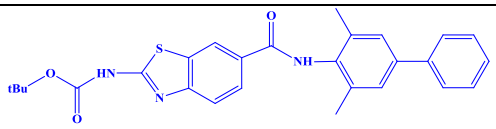
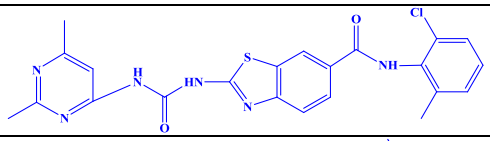
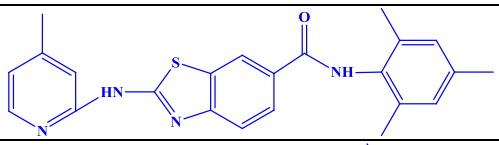
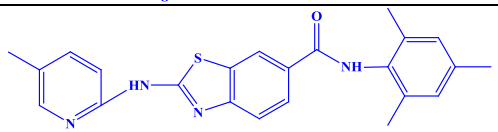
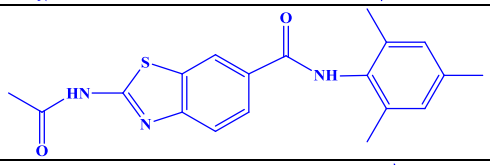
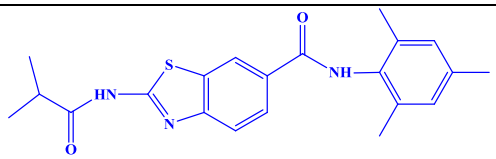
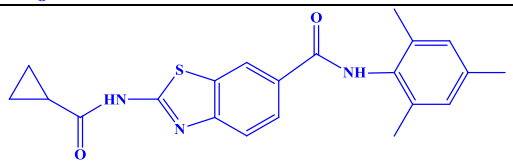
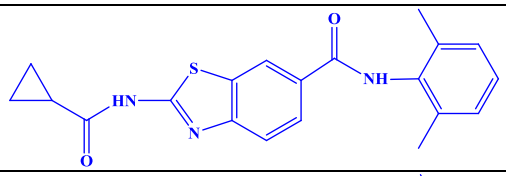
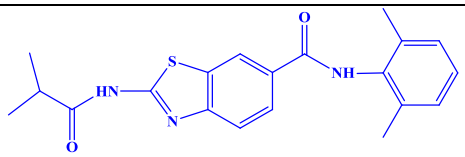
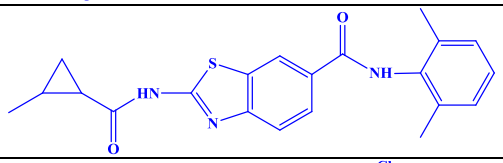
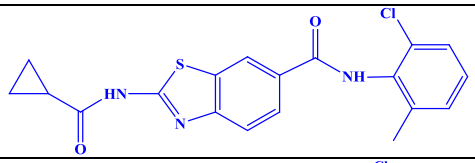
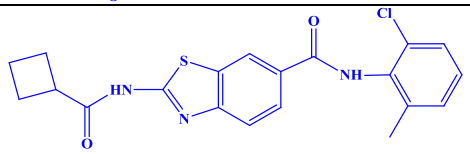
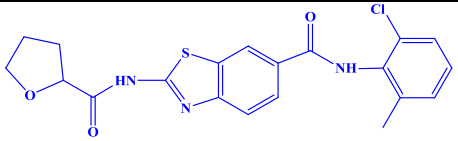
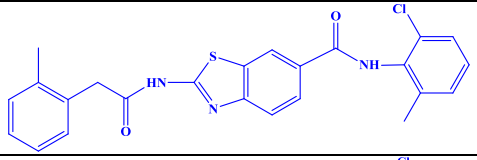
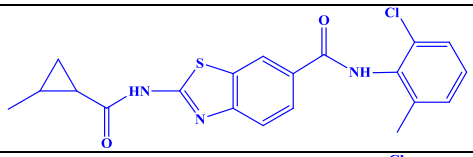
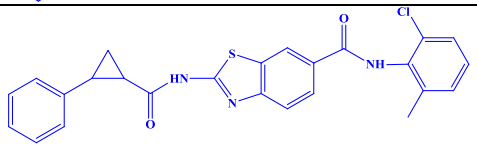
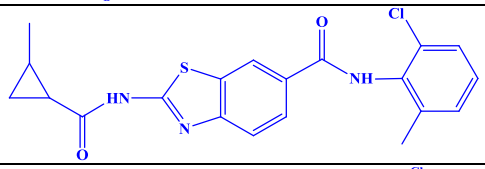
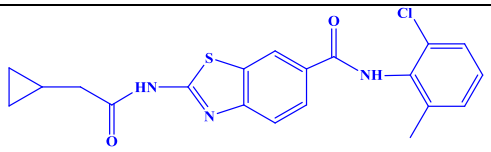
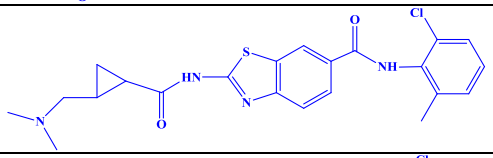
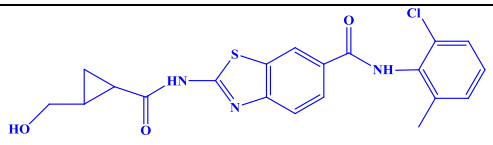
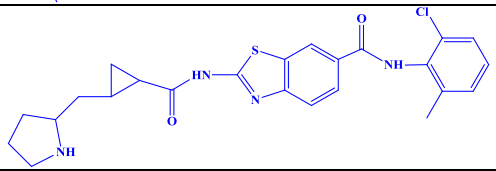
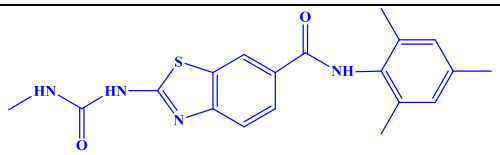
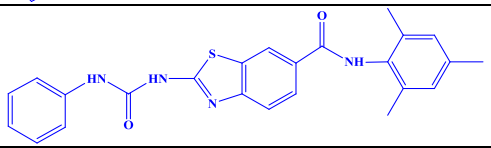
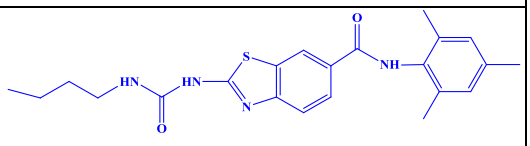
Computational Studies:

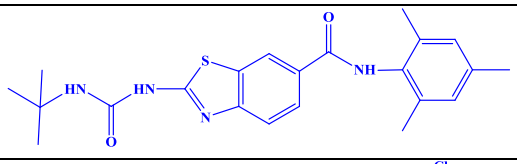
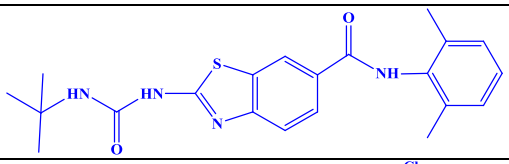
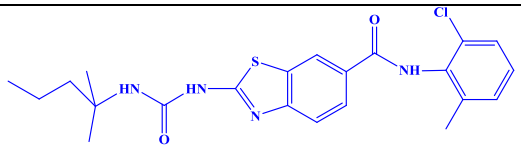
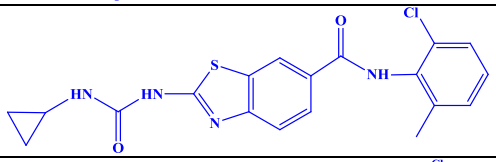
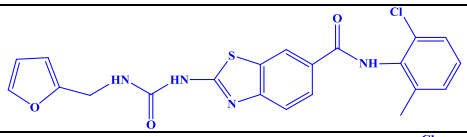
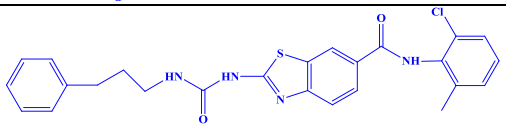
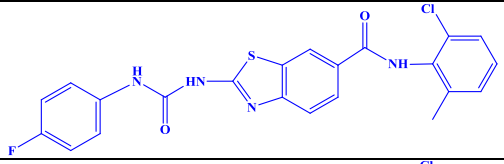
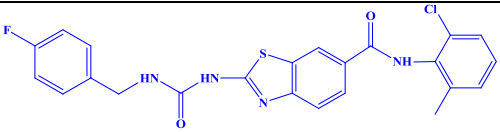
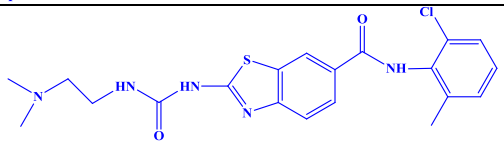
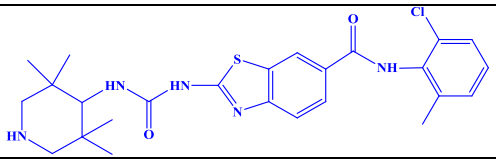
Dataset:

The biological activity data reported as IC_{50} for inhibition of $P56^{lck}$ (ref) by the benzothiazole derivatives as potential $p56^{lck}$ agents was used for the present study. Structures of all the 44 Benzothiazole derivatives are given in table 1. Biological activities were reported in IC_{50} values were then converted into the corresponding pIC_{50} using the formula pIC_{50}=-logIC_{50}. [13] The logarithmic transformation helps to obtain a symmetrically distributed data which is apt for the PLS regression analysis and it is important because log-dose response curve is linear about its middle region.

TABLE 1: STRUCTURES OF BENZOTHIAZOLE DERIVATIVES USED FOR CoMFA AND CoMSIA STUDY

1		2	
3		4	
5		6	
7		8	
9		10	

11		12	
13		14	
15		16	
17		18	
19		20	
21		22	
23		24	
25		26	
27		28	
29		30	
31		32	
33		34	

35		36	
37		38	
39		40	
41		42	
43		44	

Molecular Modeling:

All molecular modeling studies were performed using the molecular modelling package SYBYL6.7 [14] installed on a Silicon Graphics Work station. All the molecules were sketched and minimized by using Gasteiger-Huckel charges. Taken tripos force field and 0.005 kcal/mol cutoff value was used. Later performed geometry optimization using MOPAC[15] interfaced with SYBYL. MMOK, ESP, NOMM was used during geometry optimization and MOPAC partial charges were computed.

Molecular Alignment:

Alignment plays very important role in 3D QSAR studies. One of the important assumptions wherein 3D-QSAR studies are based on that a geometric parallelism should exist between the modeled structures and that of the bioactive conformation of the molecule. It indicates spatial alignment of compounds under study as one of the most sensitive and determining factors in obtaining robustness and meaningful models [16]. In the present study the MOPAC geometry optimized structures were aligned on the template by the routine ALIGN DATABASE command in SYBYL employing the divide and conquer strategy [17] as follows: molecules that have benzothiazole moiety were used as the maximum common substructure for alignments Finally these alignments were combined for subsequent CoMFA study**. Fig. (1)** shows aligned molecules.

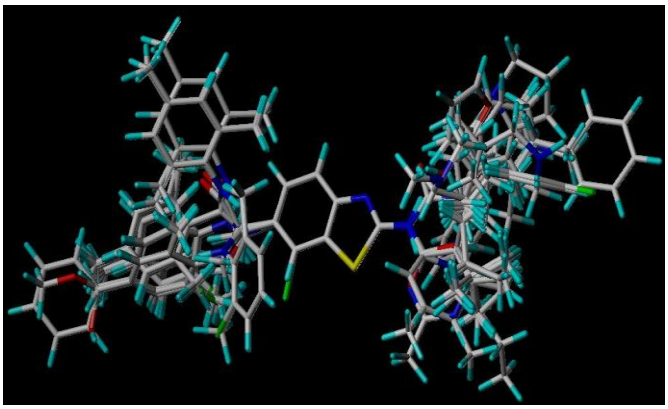


FIG. 1: POSSIBLE BINDING CONFORMATIONS OF BENZOTHIAZOLE DERIVATIVES SUPERIMPOSED ON THE STRUCTURAL TEMPLATE (30). THE ALIGNED MOLECULES ARE SHOWN IN CAPPED STICKS.

CoMFA Interaction Energies:

The comparative molecular field analysis steric and electrostatic potential fields were calculated at each lattice intersection of a regularly spaced grid of 2.0. The van dar Waals potential and Columbic terms, these two represent steric and electrostatic fields respectively, and were calculated using the standard Tripo's force field engine. A distance dependent dielectric constant of 1.00 was used. A sp^3 hybridized carbon atom with +1 charge served as probe atom to calculate steric and electrostatic

fields. +30.0 kcal/mol steric and electrostatic contributions were truncated. [18] The cross-validation analysis was performed using leave-one-out method. The cross-validated q^2 that resulted in optimum number of components and lowest standard error of estimate was taken and also same weights for CoMFA were assigned to steric and electrostatic fields using CoMFA standard scaling option.

To speed up the analysis a minimum column filtering value of 2.00 kcal/mol was used for the cross-validation. Further, final analysis was performed to calculate non cross-validated r^2 using the optimum number of components obtained from the leave one out cross validation analysis. To assess the robustness and statistical confidence we performed bootstrapping analysis by taking 100 runs.

	CoMFA		CoMSIA	
q2	0.710		0.642	
r2	0.966		0.956	
SEE	0.189		0.227	
F value	160.808		97.23	
CV	0.719		0.661	
Bootstrap	Mean	Std.dev	Mean	Std.dev
	0.177	0.128	0.149	0.109
	0.966	0.017	0.977	0.014
Field Contribution (%)				
Steric	69.0		19.6	
Electrostatic	31.0		18.1	
Hydrophobic	-		17.6	
Donor	-		18.0	
Acceptor	-		16.7	

CoMSIA:

In the present study, we analyze the nature of Benzothiazole derivatives using 3D-QSAR (Three-dimensional quantitative structure–activity relationship) analysis. (CoMSIA) Comparative molecular similarity indices analysis was used. In CoMSIA, changes in ligand affinities are directly related to changes in molecular properties. [19] CoMSIA method is good at describing the intermolecular interactions (steric, electrostatic, hydrophobic, hydrogen bond donor and acceptor) present at the molecular binding site. The method has been used to study the ligand–protein interactions before and has proved to be of good predictivity.

Partial Least Square (PLS):

The CoMFA and CoMSIA analyses were performed using the partial least square (PLS) method. PLS regression technique is useful in common cases where the number of descriptors is comparable to or greater than the number of compounds and / or there exist other factors leading to correlations between variables. Biological activity is used as dependent variable and molecular descriptors as independent variable. The column filtering was set to 2.0 kcal/mol, to improve the signal-to-noise ratio. q^2 (conventional r^2) were performed by the Leave-One-Out (LOO) procedure, for the calculation of optimum number of components (N). The cross-validated r^2 resulted in optimum number of components and lowest standard error of prediction were considered for further analysis. No-validation, cross-validation and finally bootstrapping analysis was performed to calculate conventional r^2 using the optimum number of components. Bootstrapping analysis for 100 runs was performed.

TABLE 3: EXPERIMENTAL, PREDICTED AND RESIDUAL VALUES OF BENZOTHIAZOLE DERIVATIVES AS $P56^{lck}$ INHIBITORS USED IN TRAINING SET

C.No	pIC50	CoMFA		CoMSIA	
		Predicted	Residual	Predicted	Residual
1	4.82	4.94	-0.12	4.77	0.05

2	4.90	5.51	-0.61	5.11	-0.21
3	5.36	5.04	0.31	4.79	0.56
5	7.15	7.33	-0.18	7.10	0.04
6	7.15	7.37	-0.22	7.08	0.06
8	5.56	5.77	-0.21	6.35	-0.79
9	6.69	6.09	0.59	5.93	0.76
10	6.51	6.39	0.12	6.08	0.48
11	5.06	5.03	0.02	5.49	-0.43
12	7.27	7.36	-0.09	7.67	-0.40
13	7.49	7.08	0.40	7.21	0.27
14	7.14	7.39	-0.25	7.53	-0.39
15	6.92	7.08	-0.16	7.01	-0.09
18	7.82	7.58	0.23	7.65	0.16
20	7.96	7.51	0.44	7.60	0.35
22	6.18	6.98	-0.80	6.97	-0.79
23	6.56	6.98	-0.42	6.90	-0.34
25	8.15	8.10	0.04	8.06	0.08
26	7.57	7.67	-0.10	8.09	-0.52
27	6.47	7.02	-0.55	6.84	-0.37
28	7.07	7.43	-0.36	7.08	-0.01
29	8.15	7.55	0.59	7.38	0.76
30	5.52	5.09	0.43	5.88	0.36
32	6.88	6.57	0.31	6.99	-0.11
35	7.15	6.70	0.44	6.81	0.33
36	7.00	7.19	-0.19	7.12	-0.12
37	7.09	7.45	-0.36	7.18	0.09
38	7.05	7.53	-0.47	7.23	-0.18
39	7.40	7.30	0.09	7.34	0.05
40	7.72	7.39	0.32	7.33	0.38
41	6.91	7.09	-0.18	6.98	-0.07
42	7.06	7.01	0.04	6.85	0.20
43	7.52	7.43	0.09	7.77	-0.25
44	8.39	7.79	0.59	7.54	0.84

TABLE 4: EXPERIMENTAL, PREDICTED AND RESIDUAL VALUES OF BENZOTHIAZOLE DERIVATIVES AS P56^{lck} INHIBITORS USED IN TEST SET

C.No	pIC50	CoMFA		CoMSIA	
		Predicted	Residual	Predicted	Residual
4	6.54	7.14	-0.60	7.25	-0.71
7	5.37	5.17	0.20	6.05	-0.68
16	6.15	6.02	0.13	6.74	-0.59
17	6.70	6.75	-0.05	6.69	0.01
19	5.74	6.09	-0.35	5.79	-0.05
21	8.04	7.71	0.33	7.57	0.47
24	6.25	6.49	-0.24	6.82	-0.57
31	7.95	7.51	0.44	7.53	0.42
33	6.42	7.08	-0.66	6.56	-0.14
34	6.48	7.15	-0.67	7.05	-0.57

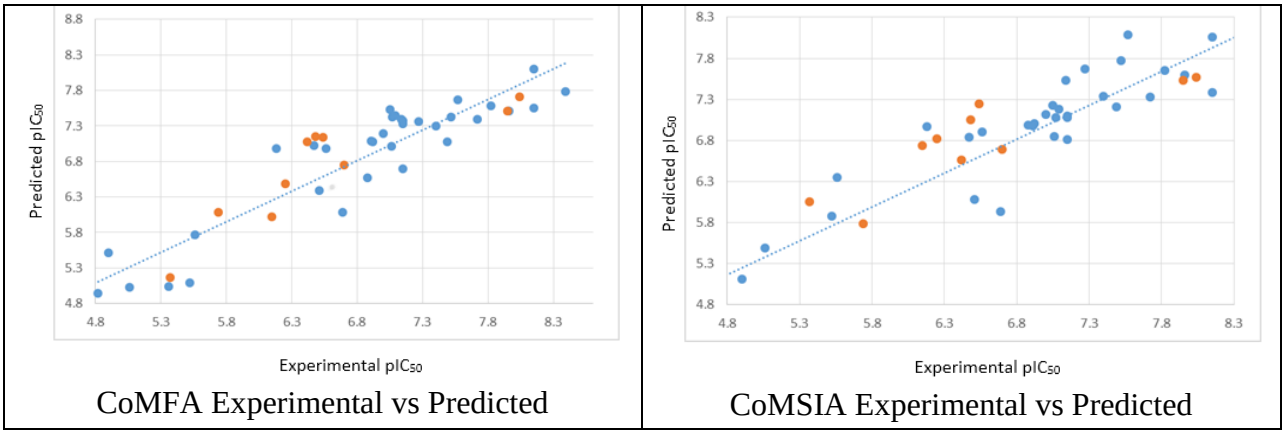


FIG.2: THE PLOTS OF ACTUAL VERSUS PREDICTED PIC$_{50}$ VALUES ARE SHOWN IN FIG. 2: THE CONTOUR PLOTS OF MOST ACTIVE COMPOUND WERE SHOWN IN FIG. 3 AND 4 RESPECTIVELY.

RESULTS AND DISCUSSION:

The 3D-QSAR CoMFA and CoMSIA studies were carried out using Benzothiazole derivatives, which are reported IC_{50} values on $P56^{lck}$. 44 molecules were taken for the present study. All the 44 compounds were partitioned into a training set of 10 and a test set of 34 compounds as 1:3 ratio (1 percent in test set is and 3 percent in training set) were selected randomly. The ambiguity of ligand-receptor interactions in general, a statistically robust models were obtained from the CoMFA and CoMSIA models. Training set and test set Experimental and predicted activities are given in **Table 3** and **4**.

The CoMFA and CoMSIA PLS analysis is summarized in **Table 2**. The cross-validated correlation co-efficient is used as a measure of goodness of prediction whereas the non-cross-validated conventional correlation co-efficient indicates goodness of fit of a QSAR model. F value indicates for the degree of statistical confidence. A cross-validated correlation co-efficient q^2 of 0.710 was obtained using 5 as optimum number of components and 2.0 kcal/mol column filtering was used for the present model. The r^2cv obtained indicates a good internal predictive ability of the models. The models developed also exhibited a wonderful non-cross validated correlation co-efficient r^2 of 0.966. The Test set compounds are used to evaluate the external predictive capabilities of QSAR models. 10 compounds were selected in test set randomly were set-aside during model development. Further, a bootstrapping analysis was done for 100 runs. The r^2bs value obtained 0.966 of bootstrapping by 100 runs which further supports the statistical validity of the developed models and absence of chance correlation. The contributions of steric to electrostatic fields were found to be 69.0% for steric and 31.0% for electrostatic. Steric contribution is more than compared to electrostatic contribution.

The optimum CoMSIA model was derived with the combination of steric, electrostatic, hydrophobic, H-bond donor and H-bond acceptor field contribution using Gasteiger-Hückel charge with 2.0 Å grid space. Leave one out analysis gave the cross-validated q^2 of 0.642 with 6 components and column filtering was set to 1.0 kcal/mol. Non-cross-validated PLS analysis resulted in a correlation coefficient r^2 of 0.977, F= 97.230, with an standard error of estimate 0.221. Later we performed bootstrapping analyses to evaluate the robustness and statistical confidence of the final models (r^2 bootstrapping = 0.977, Std Dev= 0.017). Statistical results obtained from the developed model verified the predictive ability of the model (**Table 1**). The predictive ability of the developed CoMSIA model was assessed by the test set (ten molecules), were excluded during model generation. Predicted, experimental, residual values of all inhibitors are shown in **Table 2**.

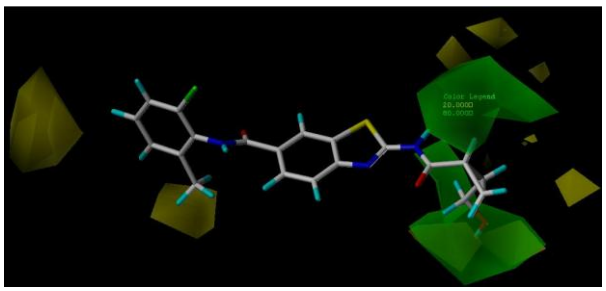
Contour Analysis:

In SYBYL, steric interactions are displayed by green and yellow contours while electrostatic interactions are represented as red and blue contours. Green contours indicate where sterically bulkier groups are anticipated to increase the biological activity whereas the yellow contours are used to decrease the points where bulkier groups

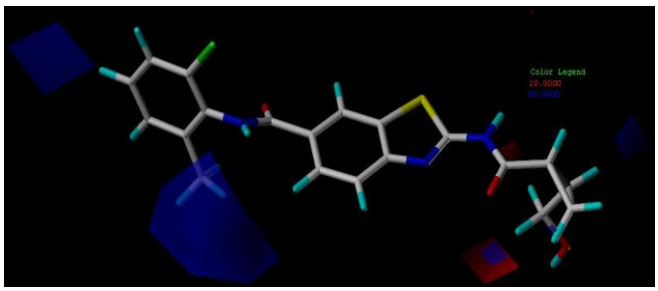
could lower the biological activity. The electrostatic red contours indicates where the presence of a negative charge is expected to increase the biological activity whereas the blue contours indicate where inserting positive charge is expected to better the experimental activity.

CoMFA Contour Analysis:

The steric contour of the CoMFA model is displayed in **Fig. 3(a).** The plot shows two large green contours, where an increase in steric bulkiness is expected to increase the inhibitory potency. This fact is consistent with the better activities of the relatively bulkier compounds. The plot shows one large, five medium sized contours and a small green contour plot present in the highest active compound 30**,** indicating that these areas would prefer a small branch rather than a bulky substituent. These contours are in conformity with the experimental observation.



3(a)



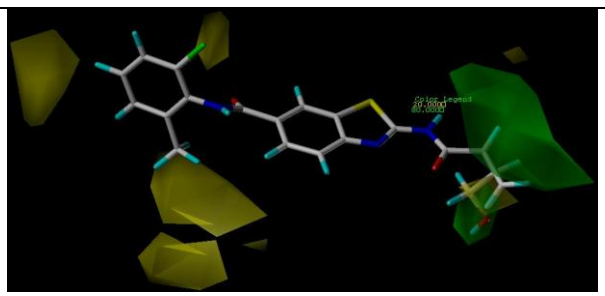
3(b)

FIG. 3: CoMFA Stdv*Coeff. CONTOUR PLOTS (A) THE STERIC FIELD CONTRIBUTION AND (B) THE ELECTROSTATIC FIELD CONTRIBUTION FOR HIGH ACTIVE COMPOUND 30. GREEN CONTOUR MAPS FOR STERICALLY FAVORED AREAS AND STERICALLY DISFAVORED AREAS IN YELLOW. POSITIVE POTENTIAL FAVORED AREAS IN BLUE, NEGATIVE POTENTIAL FAVORED AREAS IN RED.

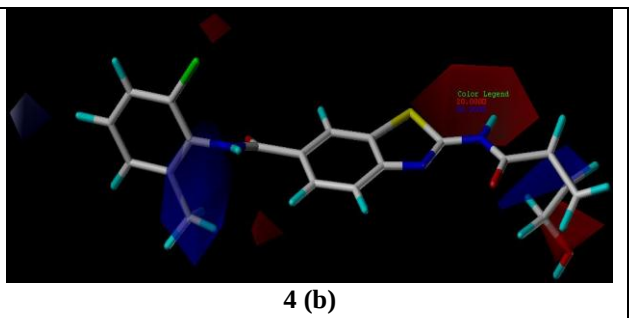
The electrostatic contour map for CoMFA model is depicted in **Fig. 3(b).** In electrostatic contour map a small red contour is present at OH position. And two medium sized blue contours are present. The plot shows red contour at OH position. This plot accounts for the experimentally observed fact that compounds having an electron withdrawing group near this area have a higher inhibitory potency. This is exemplified in the lower activities of compounds. From this, it clearly indicates that electron withdrawing groups near this region are important to enhance biological activity of these inhibitors and these could be usefully employed to design novel inhibitors. The plot also shows two medium blue contours over the methyl group. This seems to explain for the better activity of compound 30 as compared to last active compound. The contours in both cases are generally qualitatively similar.

CoMSIA Contour Analysis:

The contour maps of CoMSIA are represented by color codes shown in **Fig.4**. To aid in visualization, the highly active inhibitor 30 was overlaid in the maps. **Fig. 4 (a**) shows the CoMSIA steric field. A large sized of green contour above the isopropyl ring indicates that bulk substituents are favorable in this region. Green contour region suggests that a bulky substituent is preferred in this position to produce higher inhibitory activity. The yellow contour near the CH_3 position of benzene ring indicates the need for a small substituent in this area to improve the biological activity.



4 (a)



4 (b)

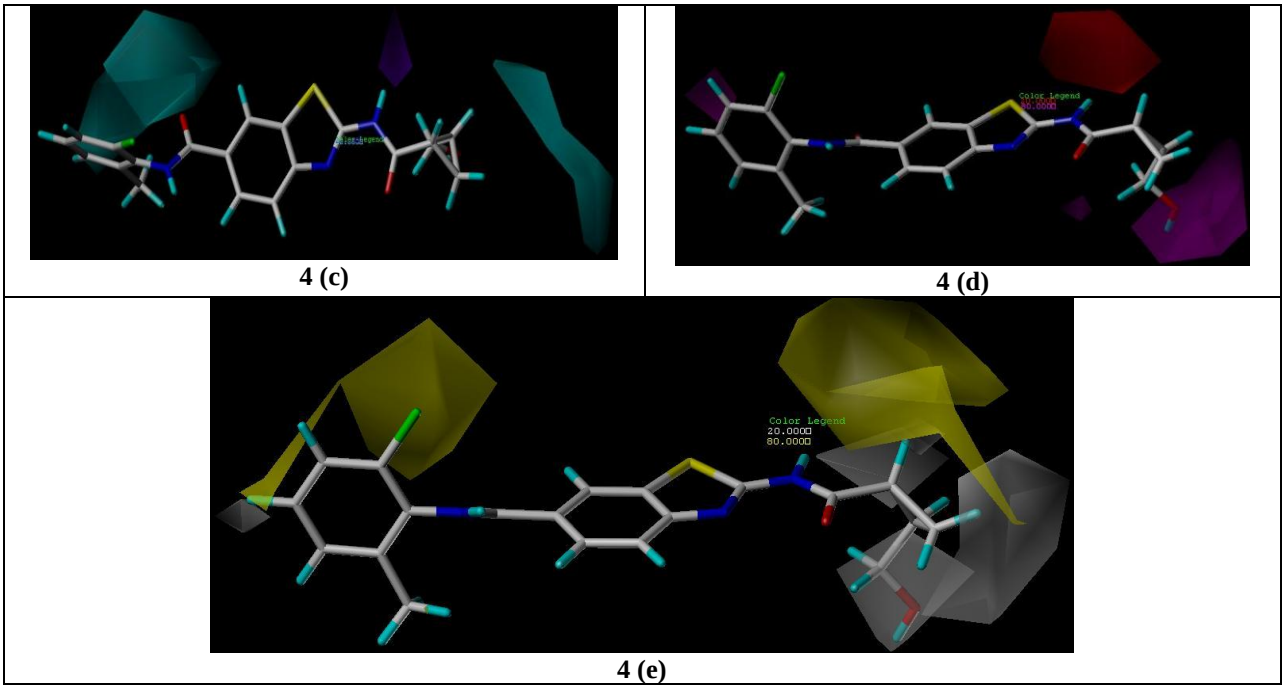


FIG.4 (B) SHOWS THE GRAPHICAL INTERPRETATION OF THE ELECTROSTATIC INTERACTION IN THE COMSIA MODEL. THE RED CONTOUR PRESENT NEAR THE OH POSITION OF THIAZOLE RING INDICATES ELECTRONEGATIVE GROUPS ARE BENEFICIAL TO THE ACTIVITY. ANOTHER RED CONTOUR APPEARS AT ISOPROPYL RING, SHOWING THAT AN ELECTRONEGATIVE GROUP HERE IS IMPORTANT FOR INHIBITORY ACTIVITY. BLUE CONTOUR INDICATES THAT ELECTROPOSITIVE GROUPS WHICH CAN EXTEND TO THIS REGION ADD TO ITS ACTIVITY. A LARGE BLUE CONTOUR PRESENT AT CH_3 POSITION SUGGESTS ELECTROPOSITIVE GROUPS ARE PREFERRED AT THIS POSITION. THEREFORE, WE SUGGEST REPLACING THE HYDROXYL WITH AN ELECTROPOSITIVE GROUP, WHICH MAY BE BENEFICIAL TO THE ACTIVITY.

Fig. 4 (c) shows the graphical interpretation of the hydrogen bond donor interaction in the CoMSIA model. Hydrogen bond donors in the molecule promote or decrease the inhibitory activity. Single purple contour present near the five membered ring suggest that H-bond donor groups are disfavored. The large polyhedra around the isopropyl ring implies that H-bond donor groups could have a positive effect on the inhibitory activity. From the contour analysis, we can accomplish that maybe better for the inhibitory activity if the H atom is transformed to other H-bond donor groups.

Fig. 4 (d) shows the graphical interpretation of the hydrogen bond acceptor interaction in the CoMSIA model. Magenta contours indicate regions where H-bond acceptor group increases biological activity; red contours (20%) represent regions where H-bond acceptor group decreases activity. A big magenta hydrogen bond acceptor favorable contour near the isopropyl ring reveal that these atoms may act as hydrogen-bond acceptor. A small magenta contour present besides the isopropyl ring. These magenta contour maps show that the use of this region would increase the inhibitory activity. In addition, one medium hydrogen bond acceptor unfavorable red contour present near NH group, which suggests the N atom here is very important to the inhibitory activity.

Fig. 4 (e) shows the graphical interpretation of the hydrophobic interaction in the CoMSIA model. Yellow polyhedra regions and white contour region indicate the areas where hydrophobic and hydrophilic properties are preferred. There are two big yellow contours extending towards isopropyl group. One is above the first carbon atom, and the other one is near the isopropyl ring. The two large yellow polyhedra indicates that hydrophobic groups in this region are beneficial to increase the activity. Also, three large white and a small white contours are found near the isopropyl group. These white contour maps reveal that the necessity of the hydrophilic substituent on the isopropyl ring to the activities.

CONCLUSION: The CoMFA and CoMSIA analysis using 44 Benzothiazole derivatives as

$p56^{lck}$ inhibitors was used to build statistically significant models with high correlative and predictive capability for the inhibition of $P56^{lck}$. These models could be usefully employed to prioritize chemicals for synthesis or in search of novel scaffolds from screening of chemical databases. The contours were found to be more or less qualitatively similar in accord with the experimentally observed binding site homology. This is of great aid to design dual inhibitors. The analysis of contours has provided a clue about the structural requirement for the observed biological activity. This analysis could be of help in the rational drug design of potential drug candidates with an enhanced inhibitory potency.

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