SUMMARY

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COX-II is involved in many diseases like cancer, inflammation, arthritis and glaucoma. It was one of our target of choice for carrying out the *insilico* experiments. Till date many selective COX-II inhibitors have been reported and blockbuster drugs of its category 'Rofecoxib' have been withdrawn from the market for its cardiovascular toxicity. Celecoxib is also under scrutiny for its side effects. Hence, it was planned to explore the structural activity relationship for selective COX-II inhibitors using the powerful *in silico* techniques like CoMFA and CoMSIA. Farnesyltransferase was chosen as a second target for the studies due to its involvement in cancer. Most of the farnesyltransferase inhibitors failed in clinical trials. Hence, it was planned to perform *in silico* studies and provide structural insights into the designing of more potent farnesyltransferase inhibitors.

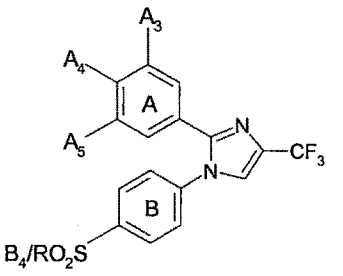
Both, two and three-dimensional drug design methodologies have been employed to identify the structural requirements for COX-II and farnesyltransferase inhibitory activity. In all of the *in silico* experiments, it was tried to use all the information available regarding the target and known inhibitors. Selection of series of compounds for molecular modeling studies was based on the variations in their structures and biological data. Different alignment techniques were employed as alignment rule influences the predictive ability of the CoMFA models.

Comparative molecular field analysis (CoMFA) and Comparative molecular similarity indices (CoMSIA), the most commonly used 3D-QSAR techniques have been applied to generate predictive 3D models and study the structure-activity relationships of COX-II and farnesyltransferase inhibitors. Structure-based information (binding energy) was also incorporated to assess its influence on the CoMFA models. Effect of different partial-charge formalisms on the statistical quality and predictive ability of the CoMFA models for COX-II inhibitors was studied. As all COX-II inhibitors bind to the same active site, a common/universal 3D CoMFA model was developed using series of COX-II inhibitors belonging to diverse chemical classes.

1 Studies on selective COX-II inhibitors

1.1 2D QSAR studies on diarylimidazoles as selective COX-II inhibitors

2D QSAR studies were performed on a series of diarylimidazole derivatives reported as selective COX-II inhibitors. The basic diarylimidazole skeleton was:



The study provided insights into the structural requirements, not only for COX-II inhibitory activity but also for its selectivity towards COX-II enzyme.

The quantitative structure activity relationship data suggested that the COX-II inhibitory activity and selectivity are greatly influenced by the functional groups attached to different positions of the molecule and also by their properties like electrophilicity (Π), hydrophobicity (σ) and vanderWaal's volume.

Several equations were obtained but the best equation for COX-II inhibitory activity is shown below:

$$pIC_{50}\ (COX\text{-}2) = (0.639 \pm 0.173)\ \Sigma\ \Pi\ (A_3 + A_4 + A_5) - (0.3617 \pm 0.077)\ [\Sigma\ \Pi\ (A_3 + A_4 + A_5)]^2 + (0.994 \pm 0.212)\ \sigma\ [A_3 + A_5] - (5.834 \pm 0.648)\ \sigma\ B_4 - (0.020 \pm 0.0041)\ V_w A_3 + (0.6095 \pm 0.105)\ \sigma\ A_4 + (10.928 \pm 0.440)$$

$n = 47,\ r = 0.927,\ S.E = 0.302,\ S.D = 0.279,\ r^2_{cv} = 0.546,\ F_{ratio} = 40.04$

Positive contribution of $\Sigma\ \Pi\ (A_3 + A_4 + A_5)$ and $\sigma\ (A_3 + A_5)$ indicated that increase in the Π and σ value of the substituents at position A_3, A_4 and A_5 would be favorable for COX-II inhibitory activity. The effect of vanderWaal's volume of the substituent at A_3 was significant.

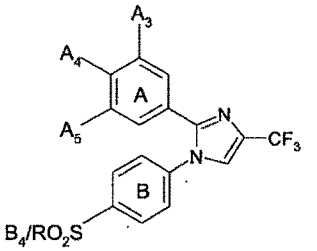
Several equations were obtained for COX-II selectivity but the best equation is shown below:

$$\log (COX\text{-}1/COX\text{-}2) = (2.710 \pm 0.20) - (0.722 \pm 0.230)\ \Sigma\ \Pi\ (A_3 + A_4 + A_5) + (3.44 \pm 0.510)\ \sigma\ [A_3 + A_5] - (0.012 \pm 0.0084)\ V_wA_3 + (2.050 \pm 0.32)\sigma\ A_4$$

$n = 23$, $r = 0.8539$, S.E = 0.40, S.D = 0.4148, $r^2_{cv} = 0.600$

It was observed that whenever the vanderWaal's volume at A_3 is increased the selectivity was decreased. Interestingly, the summed 'Π' contribution at all the three positions in ring-A had negative contribution for COX-II selectivity. Based on the developed QSAR relationships certain compounds were designed (**Table 1**) with very high COX-II selectivity while retaining high inhibitory potency.

Table 1 Designed compounds as COX-II inhibitors



C. N	A_3	A_4	A_5	(Pi)*	[Pi)*]^2	$\sigma(A_3 + A_5)$	V_wA_3	$\sigma\ A_4$	(S)*	pIC_{50} COX-2
D1	Cl	SO_2CF_3	F	1.4	1.96	0.71	22.449	0.93	5.24	7.93
D2	Cl	SCOEt	F	1.49	2.22	0.71	22.449	0.44	4.12	7.54
D3	Cl	SO_2CF_3	Cl	1.97	3.88	0.74	22.449	0.93	4.92	7.72
D4	Br	SO_2CF_3	Cl	2.12	4.49	0.77	31.059	0.93	4.77	7.47
D5	Br	SO_2CF_3	F	1.55	2.4	0.73	31.059	0.93	5.06	7.72
D6	Br	SO_2CF_3	Br	2.27	5.15	0.78	31.059	0.93	4.69	7.36
D7	SMe	SO_2CF_3	F	1.30	1.69	0.49	43.578	0.93	4.35	7.55
D8	SMe	SO_2CF_3	Cl	1.87	3.49	0.52	43.578	0.93	4.02	7.38
D9	SMe	SCOEt	Cl	1.96	3.84	0.52	43.578	0.44	3.95	6.70
D10	SMe	SO_2CF_3	Br	2.02	4.08	0.55	43.578	0.93	4.0	7.32

1.2 3D QSAR CoMFA and CoMSIA studies on furanone derivatives as selective COX-II inhibitors.

A receptor independent 3D-QSAR was established for 5-aryl-2,2-dialkyl-4-phenyl-3(2*H*)-furanones as selective COX-II inhibitors employing the most widely used 3D QSAR techniques, CoMFA and CoMSIA. The compounds pooled for these studies belong to one of the general structures depicted in **Figure 1.**

This study signifies the importance of ligand orientation and selection of the training and test set molecules in the development of statistically significant QSAR models. The statistical comparison of the best models obtained by CoMFA and CoMSIA is shown in **Table 2.**

Table 2 Statistical comparison of the best models obtained by CoMFA and CoMSIA

Technique	r^2_{cv}	SEP	N_c	r^2_{ncv}	SEE	r^2_{bs}	S.D
CoMFA	0.664	0.360	6	0.916	0.180	0.947	0.029
CoMSIA	0.777	0.282	4	0.905	0.185	0.933	0.028

Overall, CoMSIA technique provided better statistical models than CoMFA, which implies the significance of hydrogen bond donor and hydrophobic fields in the selectivity and activity of these ligands, in addition to steric and electrostatic fields.

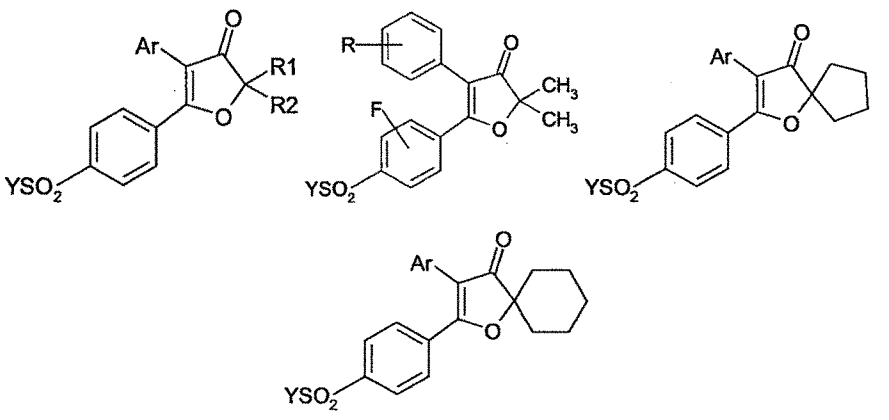


Figure 1 General structures of furanone derivatives used in 3D QSAR studies

The statistical significance and robustness of generated 3D-QSAR models were confirmed using an external set of molecules. The structural requirements identified in the present study can be utilized strategically in the design of novel, potent and selective cyclooxygenase-2 inhibitors belonging to furanone series as anti-inflammatory agents.

1.1.3 Integrating Ligand and Target-Based Information to Assess the Influence of Calculated Enzyme-Inhibitor Binding Affinities and Partial Charges on the Statistical Quality and Predictive Ability of 3D-QSAR CoMFA Models: A Case Study with Selective COX-2 Inhibitors

Enhanced CoMFA models were developed for diarylimidazoles as selective COX-II inhibitors employing different partial charge formalisms and including enzyme-inhibitor binding affinity as an additional descriptor. The 3D-QSAR models developed exhibited good to excellent agreement between the CoMFA predicted and experimentally observed pIC_{50} values, but varied for four different charge formalisms confirming the influence of partial atomic charges on the predictive ability of CoMFA models. Model $AI1_{iv}$ constructed using atom-based alignment (I) and MMFF94 charges, yielded the best results amongst the 24 models developed. All the models yielded acceptable statistical quality (high r^2, q^2 and r^2_{pred} values). In all the models binding energy had some degree of influence on the CoMFA models but not to that extent as expected. The primary objective of this study was to evaluate an efficient scheme for reliable prediction of biological activity by combining the essential features of ligand–based approaches (i.e. 3D-QSAR models) and receptor-based approaches (i.e enzyme-inhibitor binding energies).

Both structure and analog based approaches can be employed separately to yield reasonable predictive models. But it is always better to use the full extent of information provided for the system under study. As in the present case, this information may include knowledge of the three-dimensional structure of the receptor and/or receptor-ligand binary complex. The availability of such structural data is indispensable to exploiting the vast array of receptor-based approaches in

rational drug design. This includes the docking of known ligands (e.g. inhibitors) inside the receptor's binding site and calculation of their binding energies. The present study has demonstrated that calculated binding energies and optimization of partial atomic charges can serve as a reasonable predictive tool for screening a large number of compounds in terms of their relative binding affinity. Taking into account the reliability and reasonable speed of calculation, this approach is more than adequate for the rapid screening of moderately sized compound libraries and even for the prediction of biological activity.

1.1.4 Development of a universal CoMFA model for selective COX-II inhibitors

All the COX-II inhibitors bind to the same target site and exert their inhibitory action. Hence, a universal 3D CoMFA model was derived from a diverse set of 143 inhibitors belonging to different chemical classes. The general structures of these inhibitors are shown in **Figure 2.**

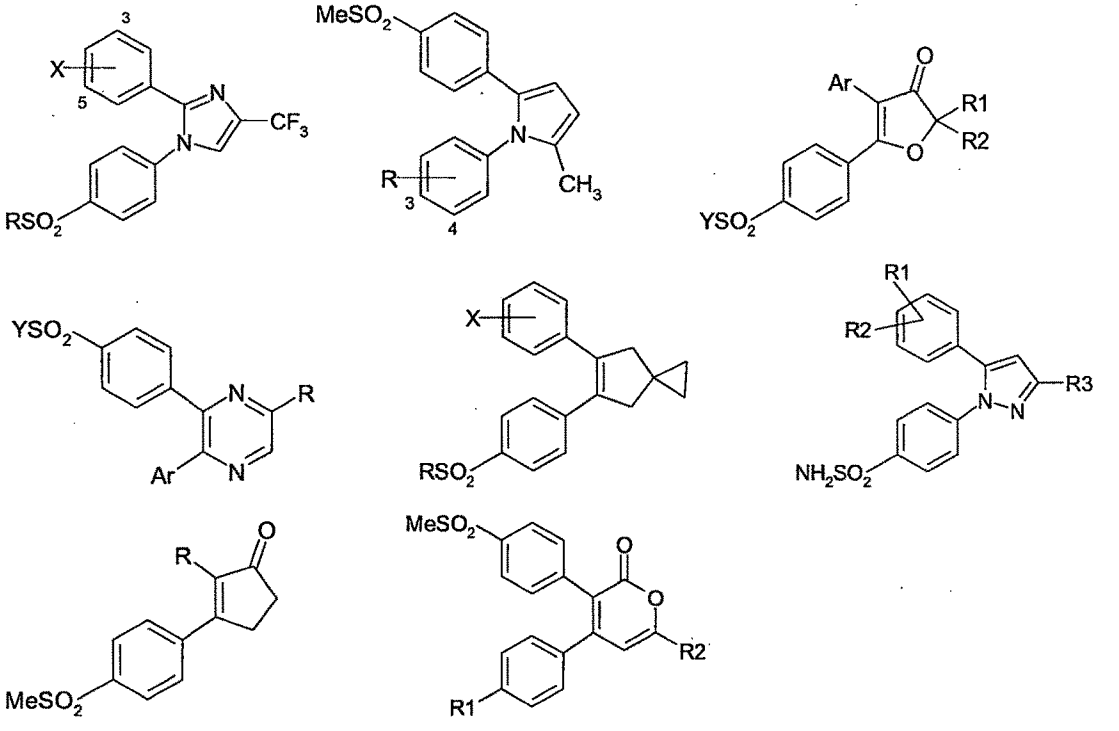


Figure 2 Scaffolds of different classes of compounds used to develop a universal CoMFA model.

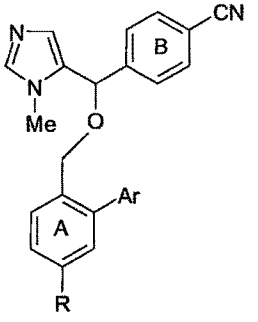
Compounds were selected based on the structural and biological variations to avoid redundancy. Training and test set comprised of 123 and 20 molecules, respectively.

A good correlation was observed within the actual and predicted activities for both training (r^2 0.988) and test set (r^2 0.794) compounds. CoMFA model generated using combination of atom and centroid-based alignment showed cross-validated r^2 0.749 with seven components, non cross-validated r^2 0.962, F value 420.83, low standard error of estimate (0.199), bootstrapped r^2 0.981 and predictive r^2 0.776. The model was further validated by predicting the coxibs which are in clinical use, confirming the utility of this model in designing potent and selective COX-II inhibitors.

2 Studies on farnesyltransferase inhibitors

2.1 3D QSAR studies on benzonitrile derivatives as farnesyltransferase inhibitors using comparative molecular field analysis.

3D-QSAR analysis using CoMFA method was successfully applied to a set of recently synthesized benzonitrile derivatives as farnesyltransferase inhibitors. Compounds chosen for analysis had the general structure as follows:



The database alignment yielded a cross-validated r^2 of 0.619 with three components, non cross-validated r^2 0.991, F value 249.67, bootstrapped r^2 0.998 and predictive r^2 0.770. The steric and electrostatic contributions were 40.1 % and 59.9 %, respectively. The contour plots generated using this model provided many useful insights into relationships between structural features and farnesyltransferase inhibitory activity.

These features could be used to design new lead compounds belonging to benzonitrile class with higher inhibitory activities.

2.2 Understanding the Antitumor activity of novel tricyclicpiperazinyl derivatives as farnesyltransferase inhibitors using CoMFA and CoMSIA.

A 3D-QSAR model using CoMFA and CoMSIA technique was employed to rationalize the farnesyltransferase inhibitory activity of 126 compounds belonging to tricyclicpiperazinyl class. The compounds selected for developing CoMFA model belonged to the general structures depicted below in **Figure 3**.

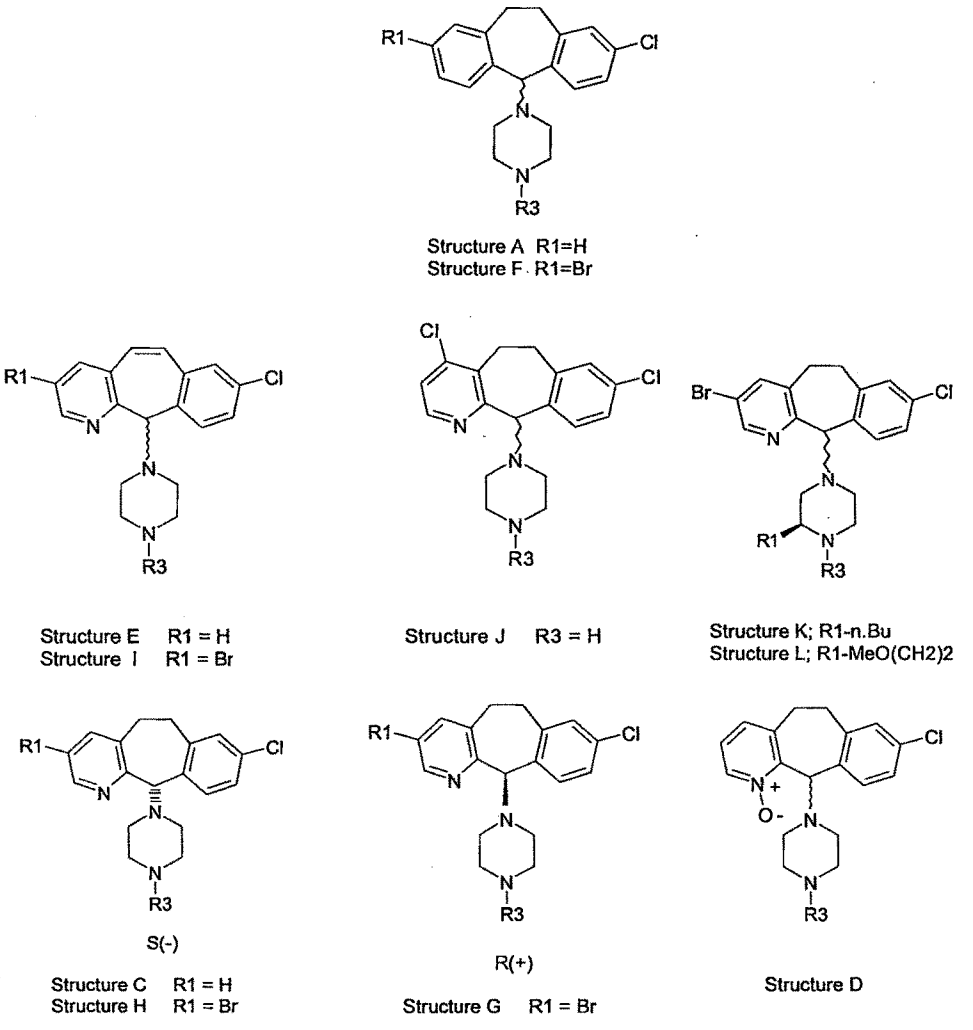


Figure 3 General structures for tricyclicpiperazinyl derivatives.

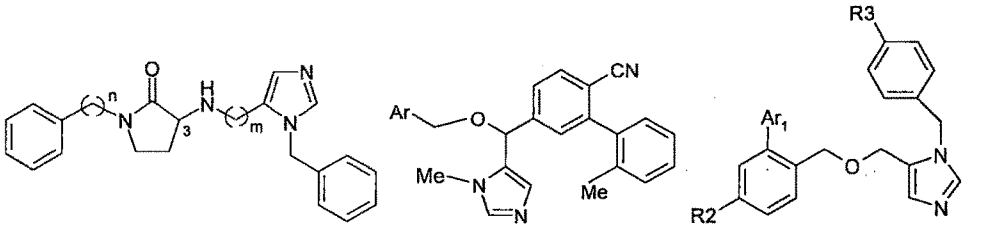
Selection of the training set and test set molecules was done by considering the fact that test set molecules represent a range of biological activity similar to that of the training set. Thus, the test set was the true representative of the training set. The 3D-QSAR obtained using the DATABASE alignment showed a high correlative and predictive ability after the removal of outliers.

Initial analysis yielded a correlation with an r^2_{cv} of 0.399 (eight principal components) and a conventional r^2 of 0.823. This model displayed poor external predictivity with r^2_{pred} 0.275. Thus, in order to increase the predictive power of the derived model, further experiments were performed. Based on the results of QSAR studies from PLS analysis nine molecules of the training set with high residual values were omitted. The CoMFA results obtained after removing outliers and using the training set of 93 compounds showed good confidence level in statisticcs. This analysis showed improved cross-validated r^2 of 0.550, conventional r^2 of 0.969, F value of 121.990, bootstrapped r^2 of 0.947 and predictive r^2 of 0.543. A high bootstrapped r^2 value (0.947) and small standard deviation (0.011) indicated that a similar relationship exists between all compounds. The steric and electrostatic contributions were found to be 46.2 % and 53.8 %, respectively. The higher contribution of electrostatic fields indicated the importance of electrostatic interactions of the molecule with the receptor that could be an important factor for antitumor activity.

Further contour maps were generated with this improved model. The CoMFA contour maps show a good compatibility with the receptor properties even though the conformations and alignments of ligands were not based on receptor structure. CoMSIA studies were performed in which the combination of steric, electrostatic, hydrogen bond acceptor and hydrophobic fields gave best results. The structural requirements of the inhibitors identified through the CoMFA and CoMSIA contour plots would help in designing new farnesyltransferase inhibitors with enhanced activity from this class.

2.3 Insights into the structural requirements of farnesyltransferase inhibitor benzonitriles as potential anti-tumor agents based on 3D QSAR CoMFA and CoMSIA models

Farnesyltransferase inhibitors belonging to (a) 3-aminopyrrolidinone (b) 2-aminonicotinonitriles and (c) 1-aryl-1′-imidazolylmethyl ethers were analyzed using analog based drug design methodologies, CoMFA and CoMSIA. Individual CoMFA and CoMSIA models were developed for each class of compounds.



Atom based alignment approach was employed to calculate the CoMFA and CoMSIA interaction energies for all the three series of compounds. The templates and atoms used for alignment are shown in **Figure 4**.

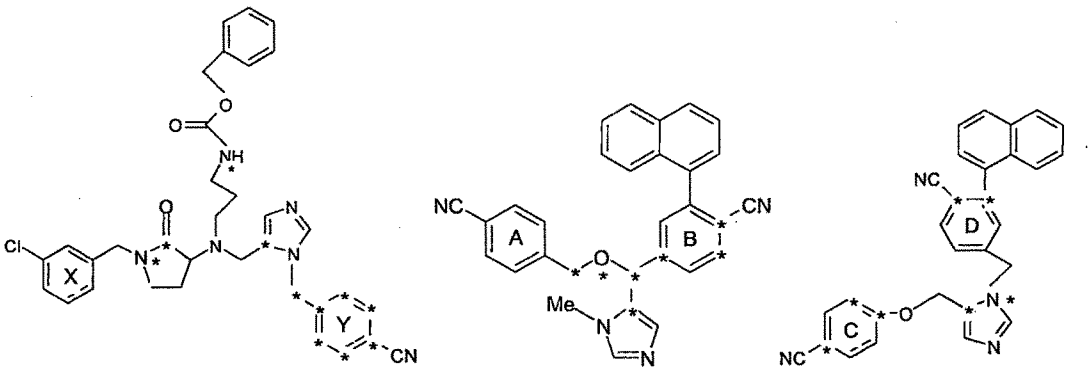


Figure 4 Atoms used for alignment for three classes of compounds

3D QSAR studies yielded stable and statistically significant predictive models indicated by moderate to high cross correlation coefficients. Initially, CoMFA derived models (Analysis A, A′ and A″) showed poor external predictivity (**Table 3**).

Table 3 CoMFA analysis

Statistical parameter	Alignments					
	I[a]		II[b]		III[c]	
	Analysis A	Analysis B	Analysis A′	Analysis B′	Analysis A″	Analysis B″
r^2_{cv}	0.526	0.596	0.403	0.803	0.346	0.479
N_c	7	6	2	4	11	8
SEP	0.615	0.414	0.463	0.285	0.403	0.422
r^2_{ncv}	0.928	0.945	0.893	0.983	0.998	0.993
SEE	0.251	0.224	0.189	0.089	0.024	0.048
r^2_{pred}	0.475	0.790	0.357	0.811	0.289	0.686

[a] 3-aminopyrrolidinones; [b] 2-aminonicotinonitrile; [c] 1-aryl-1′-imidazolylmethyl ethers

All the CoMFA models generated using the atom-based RMS fitting method exhibited statistically significant and predictive power after the removal of outliers (Analysis B, B′ and B″) (**Table 3**). The statistics shown reflects the predictive power of the CoMFA models generated for the three series of farnesyltransferase inhibitors. Models derived from Analysis B, B′ and B″ were used to generate contour maps for 3-aminopyrrolidinone, 2-aminonicotinonitrile and 1-aryl-1′-imidazolylmethyl ether class, respectively.

The steric and electrostatic field contributions revealed relatively higher contributions of electrostatic fields except for the 2-aminonicotinonitrile class, which showed higher contributions of steric fields. The comparison of CoMFA and CoMSIA models obtained using steric and electrostatic fields suggested that CoMFA yielded relatively improved models for 3-aminopyrrolidinones and 1-aryl-1′-imidazolylmethyl ethers. CoMSIA yielded fairly improved models for 2-aminonicotinonitriles and statistically insignificant models for 1-aryl-1′-imidazolyl methyl ethers. Overall CoMFA yielded comparable models for 3-aminopyrrolidinones and 1-aryl-1′-imidazolylmethyl ethers highlighting the significance of steric and electrostatic fields towards FTase inhibitory activity. While CoMSIA models were statistically significant for 2-aminonicotinonitriles indicating the importance of hydrophobic, H-bond donor and acceptor fields towards FTase inhibitory activity. The CoMSIA steric and electrostatic field maps are in accordance with field distribution of CoMFA maps and consistent with structure-activity

relationships. The comparison of 3D QSAR models with the active site of FTase revealed the interactions of amino acid residues with the substructures of molecules with steric, electrostatic, hydrophobic and H-bond donor and acceptor fields. Thus, we have identified the significance of various structural elements binding to active site which may be combined to improve the overall activity. Overall, the 3D QSAR study investigated the indispensable structural features of the different chemical classes of molecules which can be exploited for the structural modifications of these lead molecules in order to achieve improved selective FTase inhibitory activity.