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3D-QSAR studies of farnesyltransferase inhibitors: A comparative molecular field analysis approach

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Dedicated to Maya Puntambekar on her 54th birthday.

Abstract—3D-QSAR analysis has been performed on a series of previously synthesized benzonitrile derivatives, which were screened as farnesyltransferase inhibitors, using comparative molecular field analysis (CoMFA) with partial least-square fit to predict the steric and electrostatic molecular field interactions for the activity. The CoMFA study was carried out using a training set of 34 compounds. The predictive ability of the model developed was assessed using a test set of eight compounds (r^2_{pred} as high as 0.770). The analyzed 3D-QSAR CoMFA model has demonstrated a good fit, having r^2 value of 0.991 and cross-validated coefficient q^2 value as 0.619. The analysis of CoMFA contour maps provided insight into the possible modification of the molecules for better activity.

Inhibition of farnesyltransferase (FT) has been vigorously pursued as a promising target for the treatment of broad spectrum of cancers and several compounds such as SCH66336 (Sarasar™) and R115777 (tipifarnib or Zanestra™) are currently used in advanced human clinical trails.[1–3] Farnesyltransferase is a zinc metalloenzyme which catalyzes the reaction between farnesyl diphosphate (FPP) and the cysteine residue of a polypeptide C-terminal CaaX motif (C is Cys; a is usually an aliphatic amino acid; X is the C-terminal residue, typically Met) in the carboxy terminal of a group of membrane-bound small G-proteins such as Ras, RhoB, RhoE, lamin A and B, and transducin. This post translational processing is essential for the signal transduction function of these proteins since the farnesyl moiety is required to anchor these proteins to the cell membrane.[4]

Apart from being a promising target for developing anti-cancer agents, the enzyme protein farnesyltransferase appears to be a promising 'piggy-back' antimalarial target. Recently, Laxman Nallan et al. reported synthesis and antimalarial activity of tetrahydroquinolone-based analogs.[5] Inhibitor effects of some novel benzonitrile derivatives on farnesyltransferase were investigated.[6,11] To gain further insight into the relationship between the structure and biological activity, we have applied a three-dimensional quantitative structure–activity relationship (QSAR) approach comparative molecular field analysis.[7,8]

CoMFA was introduced by Crammer.[7,8] In this method, a relationship is established between the biological activities of a set of compounds and their steric and electrostatic properties. An 'active conformation' of the ligands is generated and superimposed as per the predefined rules. These molecules are then placed in a box of predefined grid size. The steric and electrostatic interaction energy between each structure and a probe atom of defined size and charge are calculated at each grid point using the molecular mechanics force fields. A multivariate data analysis technique called partial least squares (PLS)[9,10] was used to derive linear equations from the resulting matrices. PLS was used in combination with cross-validation to obtain the optimum number of components. This ensures that the QSAR models were selected on their ability to predict the data rather than to fit the data. The advantages of the CoMFA studies are in the ability to predict the target properties of the compounds and to graphically present the QSAR in the form of coefficient contour maps.

Keywords: 3D-QSAR; CoMFA; Farnesyltransferase inhibitors.
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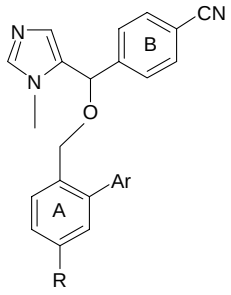
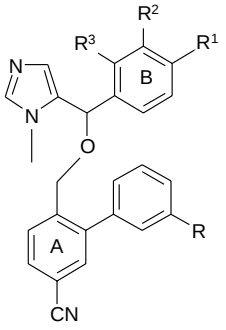
Table 1. Structures and biological activities of the molecules used in the training set



Compound	R	Ar	pIC_{50}
1	Cl	2-Cl–Ph	5.05
2	Cl	3-Cl–Ph	6.09
3	Cl	3-OEt–Ph	5.95
4	CN	Ph	5.88
5	CN	1-Naphthyl	6.02
6	CN	3-Cl–Ph	6.06
7	CN	3-OMe–Ph	6.45
8	CN	3,4-OCH_2O–Ph	6.06
9	CN	3,4-OCF_2O–Ph	5.95
10	CN	3-OEt–Ph	6.16
11	CN	4-OMe–Ph	6.01
12	CN	4-OEt–Ph	5.92
13	CN	3-OCF_3–Ph	6.07
14	CN	4-CH_3–Ph	5.95
15	CN	3,5-DiF–Ph	6.0
16	NO_2	3-OMe–Ph	5.95
17	$NHSO_2CH_3$	3-OMe–Ph	4.45
18	$NHCOCH_2OMe$	3-OMe–Ph	4.95
19	CO_2Me	3-OMe–Ph	4.88
20	CHO	3-OMe–Ph	5.79



Compound	R	R^1	R^2	R^3	pIC_{50}
21	OMe	Cl	H	H	5.69
22	OMe	CN	Cl	H	6.01
23	Cl	CN	F	H	6.35
24	Cl	CN	H	F	6.11
25	Cl	CF3	H	H	5.95

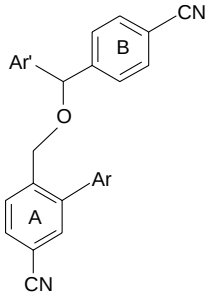
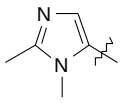
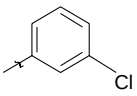
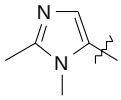
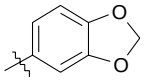
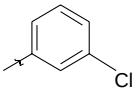
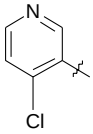
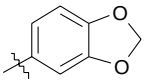
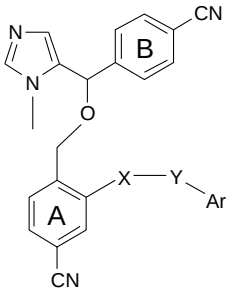
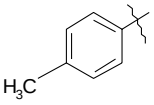
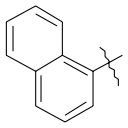
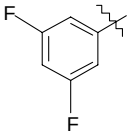
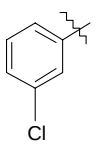


Table 1 (*continued*)

Compound	Ar′	Ar	pIC_{50}
26			5.69
27			5.79
28			5.39
29			4.82
			

Compound	X–Y	Ar	pIC_{50}
30	$NHSO_2$		6.0
31	$NHSO_2$		5.58
32	$NHCH_2$		6.21
33	CONH		5.65
34	CONH		6.12

The identification of the bioactive conformation of ligands under investigation is one of the crucial steps in CoMFA.

We present here, the 3D-QSAR studies using the CoMFA method on a training set of benzonitrile derivatives as farnesyltransferase inhibitors[6] by considering the steric and electrostatic influences. The model deduced from this investigation provides underlying structural requirements and good predictive ability, which could aid new farnesyltransferase inhibitors prior to their synthesis.

Comparative molecular field analysis (CoMFA) technique was used to derive 3D-QSAR models for benzonitrile derivatives as selective farnesyltransferase inhibitors. The in vitro inhibitory activity pIC_{50} was used as dependent variable (Table 1).

The lower energy conformer obtained from MULTISEARCH option in SYBYL[12] was used in the study. All the molecules were aligned employing atom/shape-based RMS fitting and RMSD-based database fitting techniques. Using the training set molecules, 3D-QSAR models were generated and validated with test set comprising of eight molecules (Table 2). The external predictions were used to select the best model.

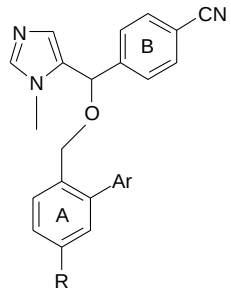
Studies were performed using 34 molecules in training set revealing the significance of CoMFA parameters on final results. PLS analysis was performed using varying σ_{min} values to set an optimum column filtering value (σ_{min}). The comparison of F values indicate that fairly good variations have been obtained using a minimum of 2.0 kcal/mol, therefore a σ_{min} of 2.0 kcal/mol was used for all proceeding calculations as a threshold column filtering value in PLS analysis. In order to study the importance of individual CoMFA fields, PLS analysis was performed and the cross-validated r^2 values of only electrostatic fields were found to be higher than that of only steric field and both steric and electrostatic field analyses, suggesting higher contributions of electrostatic parameters in these series of compounds. The results of PLS analysis are reported in Table 3.

CoMFA results obtained from four different alignments using 34 molecules in training set are shown in Table 3. The CoMFA model generated from shape-based RMS alignment I showed cross-validated r^2 0.408 with two components, noncross-validated r^2 0.965, F value 90.23, bootstrapped r^2 0.982, and predictive r^2 0.450. The steric and electrostatic contributions were 44.5% and 55.5%, respectively.

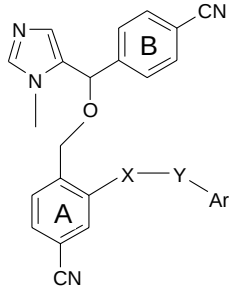
The CoMFA model generated from atom and shape-based RMS alignment II (Table 3) showed cross-validated r^2 of 0.307 with two components, noncross-validated r^2 0.954, F value 73.78, bootstrapped r^2 0.971, and predictive r^2 0.350 with 40.9% steric and 59.1% electrostatic contributions.

The atom-based alignment III yielded (Table 3) cross-validated r^2 0.299 with two components, noncross-validated r^2 0.971, F value 120.64, bootstrapped r^2 0.984,

Table 2. Structures and biological activities of the molecules used in test set



Compound	R	Ar	pIC_{50}
35	Cl	4-Cl–Ph	5.31
36	Cl	3-OMe–Ph	6.04
37	CN	8-Quinolinyl	5.28
38	CN	3-CH_2–OCH_3–Ph	6.72
39	NH_2	3-OMe–Ph	4.39
40	CO_2H	3-OMe–Ph	4.08



Compound	X–Y	Ar	pIC_{50}
41	$NHSO_2$		6.05
42	NHCO		5.30

Table 3. Results of CoMFA using different alignment rules

Alignment	I[a]	II[b]	III[c]	IV[d]
r^2_{cv} [e]	0.408	0.307	0.299	0.619
N_c [f]	2	2	2	3
SEP[g]	0.334	0.324	0.386	0.284
r^2_{ncv} [h]	0.965	0.954	0.971	0.991
SEE[i]	0.091	0.110	0.087	0.049
F value	90.23	73.78	120.64	249.67
Prob $r^2 = 0$	0.0	0.0	0.0	0.0
Contrib. steric	44.5	40.9	40.3	40.1
Contrib. elect	55.5	59.1	59.7	59.9
r^2_{bs} [j]	0.982	0.971	0.984	0.998
SD[k]	0.011	0.016	0.015	0.018
r^2_{pred}	0.450	0.350	0.300	0.770

[a] Alignment by shape-based RMS fit.
[b] Alignment by atom- and shape-based RMS fit.
[c] Alignment by atom-based RMS fit.
[d] Alignment by atom- and shape-based RMS fit.
[e] Cross-validated r^2.
[f] Number of components.
[g] Standard error of prediction.
[h] Noncross-validated r^2.
[i] Standard error of estimate.
[j] From 100 bootstrapping runs.
[k] Standard deviation.

and predictive r^2 0.30. The steric and electrostatic contributions were 40.3% and 59.7%, respectively.

The database alignment IV yielded a cross-validated r^2 of 0.619 with three components, noncross-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.

Based on the predictive ability of the four CoMFA models (Table 3), the model generated with database alignment IV carrying good predictive r^2 0.770 was selected for developing CoMFA contours. The graphs of actual versus fitted and predicted activities for the training and test set of molecules are depicted in Figures 3 and 6, respectively. The field values generated at each grid point were calculated as the scalar product of the associated QSAR coefficient and the standard deviation of all values in the corresponding column of the data table (STD*COEFF) plotted as the percentage contributions to QSAR equation. The CoMFA steric and electrostatic contour maps are shown in Figures 4 and 5, respectively. The green colored regions indicate areas where steric bulk enhances farnesyltransferase inhibitory activity, while the yellow contours indicate regions where steric bulk is detrimental for biological activity. Blue colored regions show areas where electropositive charged groups enhance farnesyltransferase inhibitory activity, while red regions represent where electronegative charged groups improve the activity.

In the present 3D-QSAR studies, CoMFA method was employed for deriving a 3D-QSAR model comprising a training set of 34 benzonitrile derivatives (Table 1), keeping in vitro activity pIC_{50} as dependent variable. We suggest to predict the 3D molecular steric and electrostatic inhibitory interactions between the analyzed compounds and farnesyltransferase enzyme using the well-known CoMFA method (Tables 4 and 5).

The most critical and important part of the QSAR model development is the model validation, where the internal predictive power of the model and its ability to reproduce biological activities of untested compounds are to be established. This essentially depends on the orientation of ligands and selection of training/test set molecules. The alignment defines the putative pharmacophore for the series of ligands and the predictive power of the QSAR models reveals the significance of alignment in the 3D-QSAR model development. The ligand molecules were aligned onto a template structure (compound **7**) employing four different alignment rules (Fig. 1) and all alignments, except for atom-based alignment III, exhibited statistically significant correlative models with an average to good predictivity supporting our choice of atoms/centroids for superimposition. The variations in predictivity from the different alignment rules employed in the present study may be due to the rigidity of ligands in the test set and a slight change in their orientations leads to the placement of functional groups in unfavorable regions supporting the exact superimposition of ligand molecules on the template structure essential for good predictions in 3D-QSAR/CoMFA studies (Figs. 2 and 3). CoMFA model generated from alignment IV, which exhibited good internal and maximum external predictivity, was used in the analysis of CoMFA contours (Figs. 4 and 5). Significant green contours surrounding ring B and the aryl group attached to ring A represent favored steric area to increase inhibition against farnesyltransferase. This is the reason why compounds **5** (Ar-naphthyl), **8** (3,4-OCH_2O–Ph), **10** (3-OEt–Ph), and **38** (3-CH_2OCH_3–Ph) have shown enhanced farnesyltransferase inhibitory activity. Also compounds **26**–**29**,

Table 4. Actual and predicted biological activities and residuals of the training set compounds by the CoMFA model

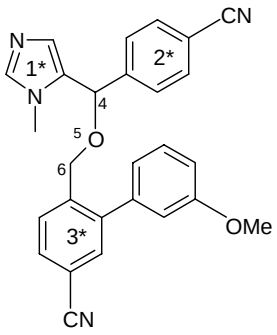
Compound	FTase inhibition actual activity[a]	CoMFA-predicted activity	Residuals
1	5.05	5.11	−0.06
2	6.09	5.94	0.15
3	5.95	6.01	−0.06
4	5.88	5.87	0.01
5	6.02	5.92	0.10
6	6.06	6.08	−0.02
7	6.45	6.42	0.03
8	6.06	6.10	−0.04
9	5.95	6.00	−0.05
10	6.16	6.17	−0.01
11	6.01	6.03	−0.02
12	5.92	6.00	−0.08
13	6.07	6.06	0.01
14	5.95	6.10	−0.15
15	6.00	5.98	0.02
16	5.95	5.93	0.02
17	4.45	4.52	−0.07
18	4.95	5.01	−0.06
19	4.88	4.72	0.16
20	5.79	5.29	0.50
21	5.69	5.60	0.09
22	6.01	6.02	−0.01
23	6.35	6.38	−0.03
24	6.11	6.06	0.05
25	5.95	5.98	−0.03
26	5.69	5.82	−0.13
27	5.79	5.74	0.05
28	5.39	5.50	−0.11
29	4.82	4.90	−0.08
30	6.00	5.98	0.02
31	5.58	5.71	−0.13
32	6.21	6.23	−0.02
33	5.65	5.62	0.03
34	6.12	6.21	−0.09

[a] Farnesyltransferase inhibition activity is expressed as $\log(1/IC_{50})$.

Table 5. Actual and predicted biological activities and residuals of the test set compounds by the CoMFA model

Compound	FTase inhibition actual activity[a]	CoMFA-predicted activity	Residuals
35	5.31	5.40	−0.09
36	6.04	6.15	−0.11
37	5.28	5.22	0.06
38	6.72	6.68	0.04
39	4.39	4.76	−0.27
40	4.08	4.11	−0.03
41	6.05	5.86	0.19
42	5.30	5.35	−0.05

[a] Farnesyltransferase inhibition activity is expressed as $\log(1/IC_{50})$.



Alignments	Atoms/ Centroids
I	1* 2* 3*
II	1* 2* 3* 5
III	4 5 6
IV	1* 2* 3* 4 5 6

Figure 1. Alignment rules with template (molecule **7**).

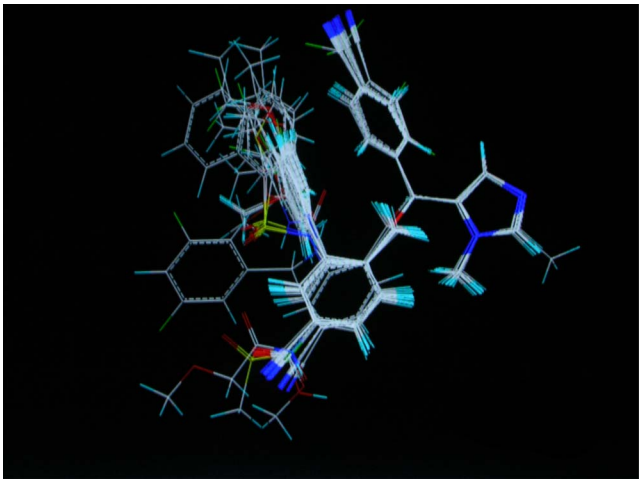


Figure 2. Alignment of the compounds used in the training set of 3D-QSAR analysis.

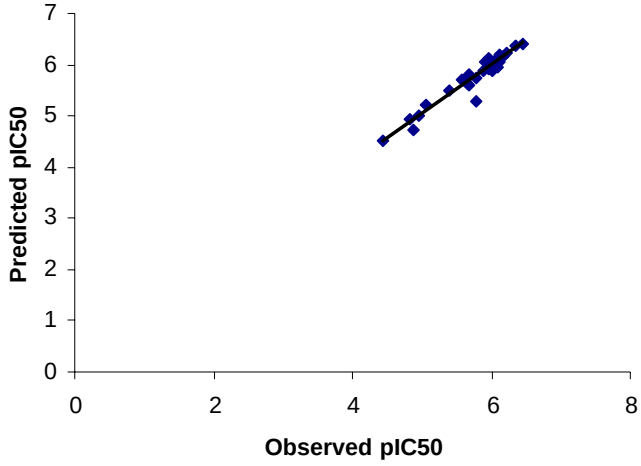


Figure 3. Graph of observed activity versus predicted activities of training set molecules from CoMFA analysis; activity expressed as pIC_{50}.

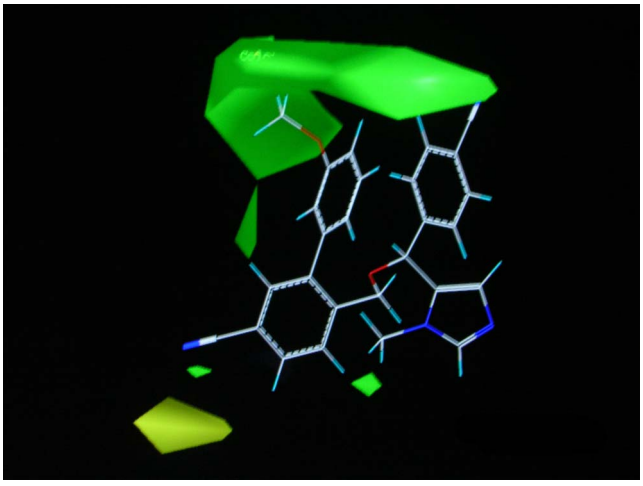


Figure 4. CoMFA standard deviation coefficient steric contour plots with compound **7**; green contours indicate regions where bulky group increases activity, whereas yellow contour indicates regions where bulky group decreases activity.

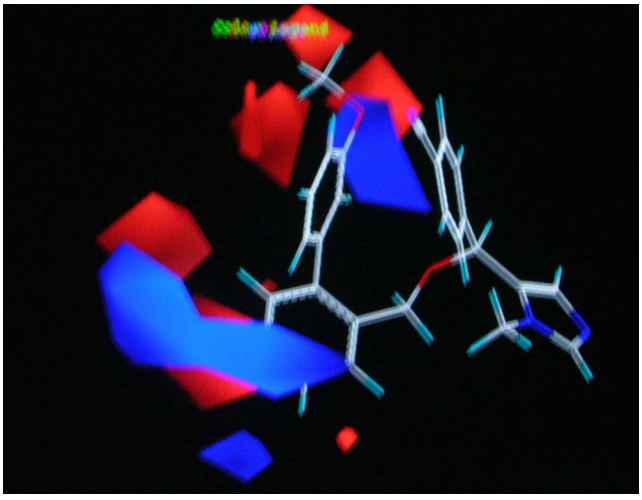


Figure 5. CoMFA standard deviation coefficient electrostatic contour plots with compound **7**; blue contours indicate regions where positively charged groups increase activity, whereas red contours indicate regions where negative charge increases activity.

which have bulky aryl groups attached to ring A, have shown better inhibitory activity. A yellow contour in the vicinity of the cyano group attached to ring A suggests that bulkier substituents on ring A (especially para and meta position which are close to the sterically unfavorable yellow region) are detrimental for FTase inhibitory activity. This is the reason why compounds **17** ($R\text{-}SO_2CH_3$) and **18** ($R\text{-}NHCOCH_2OMe$) exhibit poor inhibitory activities than compounds **2** and **3** having chlorine at R position and compounds (**4**–**15**) which have a cyano group at R position (Fig. 6).

Significant blue and red contours (Fig. 5) surrounding ring A and aryl group attached to ring A represent regions where positively charged and negatively charged substituents favor the farnesyltransferase inhibitory activity. The red contour close to ring A clearly justifies

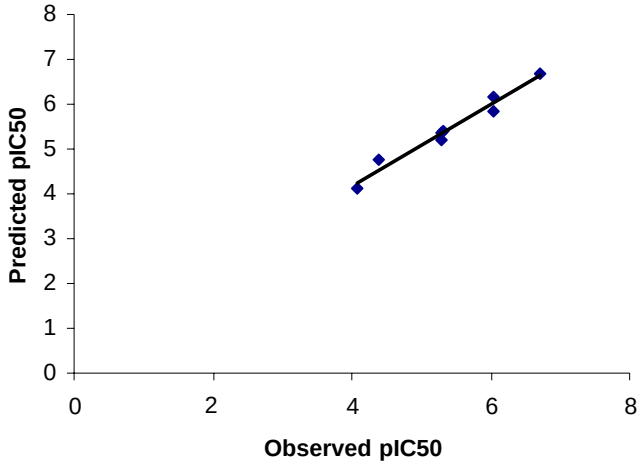


Figure 6. Graphs of actual versus predicted pIC_{50} of test set molecules obtained from a CoMFA model.

the need of having electronegative substituents (as in compounds where R-Cl or CN) at its para position as observed in most of the compounds. Hence, compound **39**, which has a NH_2 group at para position, shows decreased activity. The substitution pattern on the aryl group is important for enzymatic activity.[6] Red contours are observed in the vicinity of meta position of the aryl group indicating the need of electronegative substituents. Hence, compounds **2** and **15** having chlorine and fluorine at meta position, respectively, are more potent than **35** which has chlorine at para position (4-Cl–Ph).

In conclusion, the 3D-QSAR analysis using CoMFA method has been successfully applied to a set of recently synthesized benzonitrile derivatives as farnesyltransferase inhibitors. The contour plots provide many useful insights into relationships between structural features and inhibitory activity. These features could be used to design new lead compounds showing higher inhibitory activities.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.019.

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