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Pharmacophore mapping of diverse classes of farnesyltransferase inhibitors

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Abstract—Protein farnesyltransferase (FTase) is a zinc-dependent enzyme that catalyzes the attachment of a farnesyl lipid group to the sulfur atom of a cysteine residue of numerous proteins involved in cell signaling including the oncogenic H-Ras protein. Pharmacophore models were developed by using Catalyst HypoGen program with a training set of 22 farnesyltransferase inhibitors (FTIs), which were carefully selected with great diversity in both molecular structure and bioactivity for discovering new potent FTIs. The best pharmacophore hypothesis (Hypo 1), consisting of four features, namely, one hydrogen-bond acceptor (HBA), one hydrophobic point (HY), and two ring aromatics (RA), has a correlation coefficient of 0.961, a root mean square deviation (RMSD) of 0.885, and a cost difference of 62.436, suggesting that a highly predictive pharmacophore model was successfully obtained. For the test series, a classification scheme was used to distinguish highly active from moderately active and inactive compounds on the basis of activity ranges. Hypo 1 was validated with 181 test set compounds, which has a correlation coefficient of 0.713 between estimated activity and experimentally measured activity. The model was further validated by screening a database spiked with 25 known inhibitors. The model picked up all 25 known inhibitors giving an enrichment factor of 10.892. The results demonstrate that the hypothesis derived in this study can be considered to be a useful and reliable tool in identifying structurally diverse compounds with desired biological activity.

Inhibition of farnesyltransferase (FTase) has generated much attention recently as a promising target for the treatment of a broad spectrum of cancers due to their reduced intrinsic toxicity as compared to the conventional cytotoxic agents.[1] FTase catalyzes the transfer of a farnesyl moiety from farnesyl pyrophosphate to a cysteine residue found in the tetrapeptide sequence CAAX (C = Cys, A = an aliphatic amino acid, X is typically Met)[2] in the carboxyl terminal of a group of membrane-bound small G-proteins such as Ras, RhoB, RhoE, lamin A and B, and transducin. FTIs can stop protein farnesylation and suppress the growth of Ras-dependent tumor cells. Hence, over the last two decades, several researchers synthesized different classes of FTIs, such as SCH66336 (Sarasar™) and R115777 (tipifarnib or Zanestra™), which are currently in advanced stages of human clinical trials.[3–5] Our literature survey revealed that FTIs in different classes possess 19 different scaffolds (Fig. 1). Thus, quantitative structure–activity relationship (QSAR) analysis of different classes of inhibitors could be utilized for extracting out valuable information for developing new potent FTIs. The pharmacophore mapping is a well-established approach to quantitatively explore common chemical features among a considerable number of structures with great diversity, and qualified pharmacophore model could also be used as a query for searching chemical databases to find new chemical entities.

In the present study, we have generated pharmacophore model using Catalyst[6–8] software for diverse set of molecules of FTIs with an aim to obtain pharmacophore model that could provide a rational hypothetical picture of the primary chemical features responsible for activity. This is expected to provide useful knowledge for developing new potentially active candidates targeting the FTase, which can be useful as cytotoxic agents.

Selection of molecule. Pharmacophore modeling correlates activities with the spatial arrangement of various

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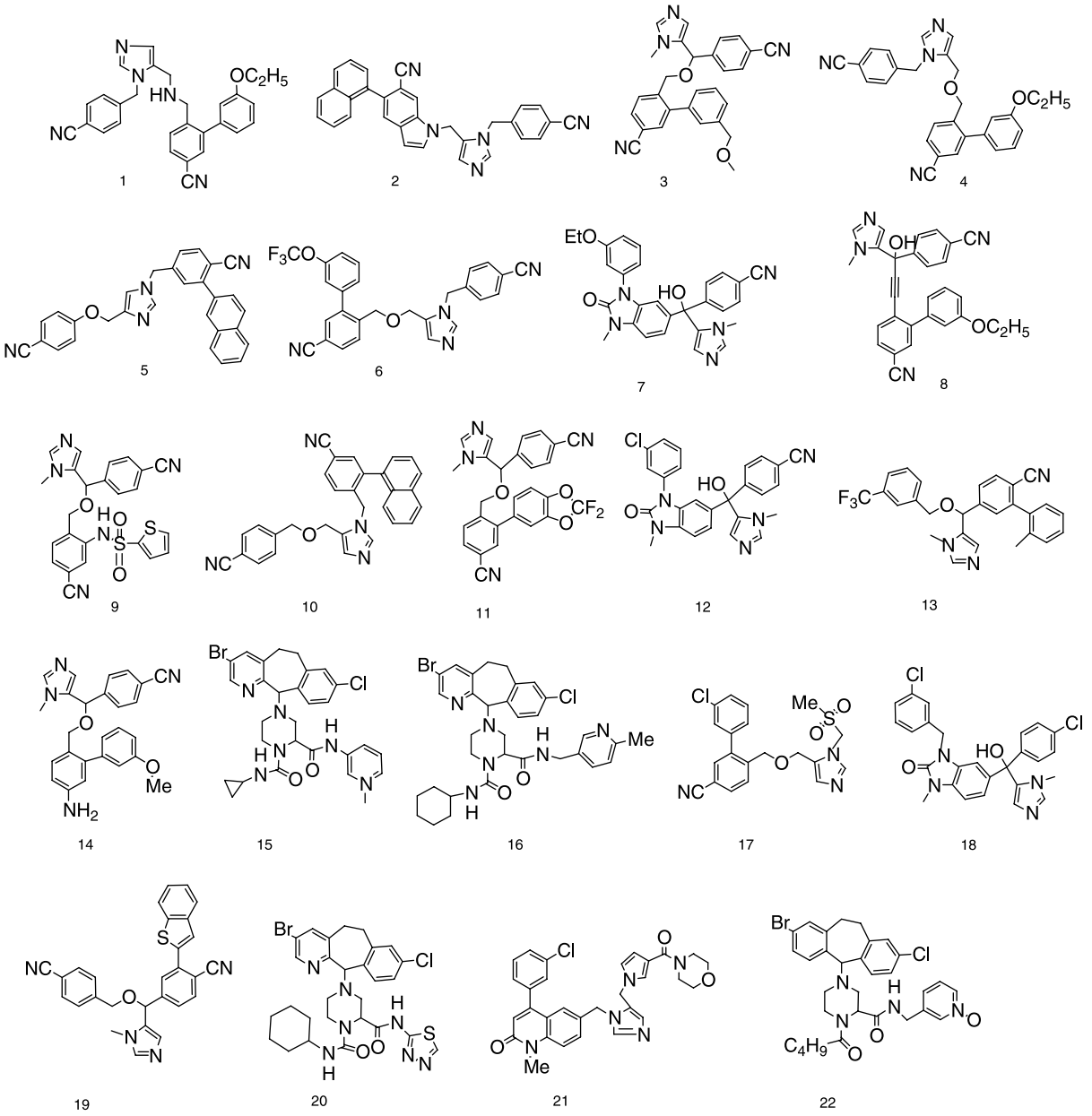

Figure 1. Chemical structures of the 22 training set molecules applied to HypoGen pharmacophore generation.

chemical features. For the pharmacophore modeling studies, a set of 203 farnesyltransferase inhibitory activity data (IC_{50}) spanning over 5 orders of magnitude (from 0.014 to 1800 nM) were selected from the literature.[9–16] The dataset was divided into training set and test set. The training set was selected by considering both structural diversity and wide coverage of the activity range. The most active, several moderately active, and some inactive compounds were also included in order to obtain critical information on pharmacophore requirements. The important aspect of this selection scheme was that each active compound would teach something new to the HypoGen module to help it uncover as much critical information as possible for predicting biological activity. The training set consisted of 22 compounds selected with the above criteria (Fig. 1 and Table 1). To validate our pharmacophore, the other 181 compounds were used as the test set (Table L in Supporting information). The activities (IC_{50}) against FTase are reported to be classified as: highly active (<10 nM), moderately active (10–100 nM), and inactive (>100 nM). All the IC_{50} values were obtained using the same assay method.[17]

Molecular modeling. The structures of all the compounds were built from fragments in Catalyst 4.10. A CHARMM like force field[18] in the Catalyst program was utilized to ascertain the energy-minimized conformations for each structure. Details of the pharmacophore development procedures have been described in the literature.[19,20] Initially, conformational models of all molecules for FTase datasets were generated using the 'best quality' conformational search option within the Catalyst ConFirm module using the 'Poling'

Table 1. Output of the score hypothesis process on the training set

Compound	True IC_{50} (nM)	Estimated C_{50} (nM)	Error factor[a]	Fit value[b]	Activity scale[c]	Estimated activity scale	Mapped features			
							HBA	HY	RA1	RA2
1	0.014	0.039	2.8	12.39	+++	+++	+	+	+	+
2	0.15	0.59	3.9	11.22	+++	+++	+	+	+	+
3	0.19	0.59	3.1	11.21	+++	+++	+	+	+	+
4	0.27	0.32	1.2	11.48	+++	+++	+	+	+	+
5	0.32	0.53	1.6	11.26	+++	+++	+	+	+	+
6	0.49	0.18	−2.7	11.73	+++	+++	+	+	+	+
7	0.50	0.49	−1.0	11.30	+++	+++	+	+	+	+
8	0.73	2	2.7	10.69	+++	+++	+	+	+	+
9	0.88	0.67	−1.3	11.16	+++	+++	+	+	+	+
10	0.96	1.7	1.7	10.76	+++	+++	+	+	+	+
11	1.10	1.5	1.4	10.80	+++	+++	+	+	+	+
12	1.40	2.2	1.6	10.65	+++	+++	+	+	+	+
13	29	18	−1.6	9.72	++	++	+	+	+	+
14	40	21	−1.9	9.67	++	++	+	+	+	+
15	51	120	2.3	8.92	++	+	–	+	+	+
16	61	260	4.3	8.57	++	+	+	+	+	–
17	96	140	1.4	8.85	++	+	+	+	+	–
18	150	51	−2.9	9.28	+	++	+	+	+	+
19	160	40	−4.0	9.38	+	++	+	+	+	+
20	490	250	−1.9	8.58	+	+	+	+	+	–
21	1000	140	−7.0	8.83	+	+	+	+	+	–
22	1800	340	−5.3	8.46	+	+	–	+	+	+

[a] The error factor is computed as the ratio of the measured activity to the activity estimated by the hypothesis or the inverse if estimated is greater than measured.
[b] Fit value indicates how well the features in the pharmacophore overlap the chemical features in the molecule.
[c] Activity scale: +++, <10 nM (highly active); ++, 10–100 nM (moderately active); +, >100 nM (inactive).

algorithm.[21] A maximum of 250 conformations were generated for each compound to ensure maximum coverage in the conformational space within an energy threshold of 20.0 kcal/mol above the global energy minimum. Instead of using just the lowest energy conformation of each compound, all conformational models for each molecule in training set were used in Catalyst for pharmacophore hypothesis generation.

Generation of pharmacophore model. From the structures of the training set compounds and their experimentally determined inhibitory activities against FTase, 10 best pharmacophore (called hypotheses in the program) models were generated using HypoGen module implemented in Catalyst 4.10 software. An initial analysis revealed that three chemical feature types such as hydrogen-bond acceptor (HA), hydrophobic (HY), and two ring aromatic (RA) features could effectively map all critical chemical features of all molecules in the training and test sets. These features were selected and used to build a series of hypotheses with the HypoGen module in Catalyst using default uncertainty value 3 (defined by Catalyst as the measured value being within three times higher or three times lower of the true value). Indeed, Catalyst generates a chemical-feature-based model on the basis of the most active compounds. These compounds are determined by performing a simple calculation based on the activity and uncertainty. As a matter of fact, the activity of the most active compound is multiplied by the uncertainty to establish a comparison number, 'A'. The activity of the next most active compound is divided by the uncertainty, and this results in 'B', which is then compared to A. If B is smaller than A, the compound is included in the most active set; if not, the procedure stops.[22]

In hypothesis generation, the structure and activity correlations in the training set were rigorously examined. HypoGen identifies features that were common to the active compounds but excluded from the inactive compounds within conformationally allowable regions of space. It further estimated the activity of each training set compound using regression parameters. The parameters are computed by the regression analysis using the relationship of geometric fit value versus the negative logarithm of activity. The greater the geometric fit, the greater the activity prediction of the compound. The fit function does not only check if the feature is mapped or not, it also contains a distance term, which measures the distance that separates the feature on the molecule from the centroid of the hypothesis feature. Both terms are used to calculate the geometric fit value.

Pharmacophore validation. The generated pharmacophore model should be statistically significant, should predict activity of the molecules accurately, and should identify active compound from a database. Therefore, the derived pharmacophore map was validated using (i) cost analysis, (ii) test set prediction, and (iii) enrichment factor.

Cost analysis. The HypoGen module in Catalyst performs two important theoretical cost calculations (represented in bit units) that determine the success of any pharmacophore hypothesis. One is the 'fixed cost' (also termed as ideal cost), which represents the simplest

model that fits all data perfectly, and the second one is the 'null cost' (also termed as no correlation cost), which represents the highest cost of a pharmacophore with no features and estimates activity to be the average of the activity data of the training set molecules. A meaningful pharmacophore hypothesis may result when the difference between null and fixed cost value is large; a value of 40–60 bits for a pharmacophore hypothesis may indicate that it has 75–90% probability of correlating the data (Catalyst 4.10 documentation).

The total cost (pharmacophore cost) of any pharmacophore hypothesis should be close to the fixed cost to provide any useful models. Two other parameters that also determine the quality of any pharmacophore hypothesis with possible predictive values are the configuration cost or entropy cost, which depends on the complexity of the pharmacophore hypothesis space and should have a value <17, and the error cost, which is dependent on the root mean square differences between the estimated and the actual activities of the training set molecules. The RMSD represents the quality of the correlation between the estimated and the actual activity data. The best pharmacophore model has highest cost difference, lowest RMSD, and best correlation coefficient.

Test set activity prediction. In addition to estimation of activity of training set molecules, the pharmacophore model should also estimate the activity of new compounds. Therefore, a set of 181 FTIs (Table L), which were not included in training set, was considered as a test set. These molecules are covering wide range of activities spanning from 0.034 to 560 nM. The best pharmacophore (Hypo 1) having high correlation coefficient (r), lowest total cost, and lower RMSD value was chosen to estimate the activity of test set. Test set compounds were classified on the basis of their activity as highly active (<10 nM, +++), moderately active (10–100 nM, ++), and inactive (>100 nM, +).

Enrichment of database. In the lead-discovery studies, the pharmacophore model should identify active leads against FTIs in the database screening. Therefore, in-house database of 1500 molecules was spiked with 25 known inhibitors in order to validate whether the pharmacophore model could identify active compounds. This spiked database (containing 1525 molecules) was screened with the pharmacophore model and the enrichment factor (E)[19] was calculated using Eq. 1.

$$E = \mathrm{Ha}/\mathrm{Ht} \div A/D \quad (1)$$

where Ht = the number of hits retrieved, Ha = the number of actives in the hit list, A = the number of active molecules present in the database, and D = the total number of molecules in the database.

Results and discussion. HypoGen generated 10 alternative pharmacophores describing the FTase inhibitory activity of 22 training set molecules. In this study, the cost of the null hypothesis for all 10 hypotheses was 170.242 and fixed cost of the run was 91.973 with a cost difference of 78.268 bits. All 10 hypotheses showed total cost close to the cost of the fixed hypothesis and having large difference with no correlation cost. As mentioned earlier configuration cost value must be less than 17 for a good pharmacophore and accordingly 16.849 was obtained. The cost values, correlation coefficients (r), RMSD, and pharmacophore features are listed in Table 2.

Out of 10 hypotheses, Hypo 1 had low total cost (107.806), less difference between total and fixed cost (15.833), high cost difference between null cost and total cost (62.436), least RMSD (0.885), and a strong correlation coefficient (0.961) between experimental and estimated activity. These results conclude that Hypo 1 is the best ranking pharmacophore among the 10 hypotheses obtained. This model consists of spatial arrangement of four chemical features: one hydrogen-bond acceptors (HBA), one hydrophobic (HY) feature, and two planar ring aromatic (RA) features (Fig. 2).

Activities were estimated for all compounds based on the best ranking pharmacophore (Hypo 1). The actual and estimated FTI activities of the 22 compounds are listed in Table 1. For 22 molecules, all the active compounds were predicted as active (+++), three moderately active compounds were predicted as inactive (+), and two inactive compounds were predicted as moderately active (++). The difference between the actual and estimated activity observed for the five compounds was only about 1 order of magnitude, which might be an artifact of the program that uses different number of degrees of freedom for these compounds to mismatch the

Table 2. Results of pharmacophore hypothesis generated using training set against farnesyltransferase inhibitors (FTIs)

Hypo No.	Total cost	Cost difference (Null[a] – Total)	Error cost	RMS	Correlation (r)	Features[b]
1	107.806	62.436	82.631	0.885	0.961	HBA, HY, RA, RA
2	113.335	56.907	89.093	1.171	0.925	HBA, HY, RA, RA
3	117.894	52.348	94.041	1.349	0.897	HBA, HBA, HY, RA
4	119.091	51.151	97.672	1.467	0.871	HBA, HY, RA, RA
5	119.478	50.764	100.927	1.564	0.849	HBA, HY, RA, RA
6	122.240	48.002	102.352	1.605	0.841	HBA, HY, HY, RA
7	123.227	47.015	101.813	1.590	0.847	HBA, HBA, HY, HY
8	123.426	46.816	101.824	1.590	0.847	HBA, HY, HY, RA
9	123.502	46.740	100.769	1.560	0.855	HBA, HBA, HY, RA
10	124.563	45.679	103.641	1.641	0.835	HBA, HY, HY, RA

[a] Null cost = 170.242; fixed cost = 91.973; configuration = 16.849. All cost units are in bits.
[b] HBA, hydrogen-bond acceptor; HY, hydrophobic feature; RA, ring aromatic feature.

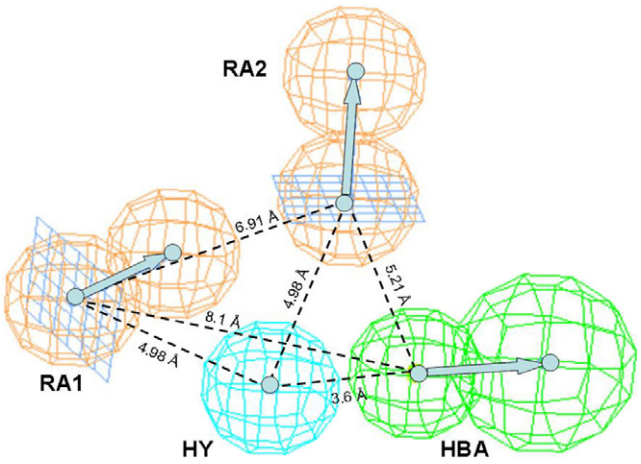

Figure 2. The best hypothesis model Hypo 1 produced by the HypoGen module in Catalyst 4.10 software. Pharmacophore features are color-coded with green, blue, and brown contours representing the hydrogen-bond acceptor feature (HA), hydrophobic feature (HY), and ring aromatic features (RA), respectively. Distance between pharmacophore features is reported in angstroms.

pharmacophore model. Interestingly, in the training set, all highly active compounds map all the features that is hydrophobic (HY), hydrogen-bond acceptor (HBA), and two ring aromatics (RA1 and RA2). With a few exceptions, in moderately active and inactive compounds one feature is missing. All the compounds in the training set map HY and RA1 feature revealing that these two features should be mainly responsible for the high molecular bioactivity, thus, should be taken into account in discovering or designing novel FTIs. The most active compound, **1**, has a fitness score[23] of 12.39 when mapped to Hypo 1 (Fig. 3A) whereas the least active, **22**, maps to a value of 8.46 (Fig. 4A). In **1**, HBA feature corresponds to the CN at C4 position of phenyl ring, which is attached to imidazole ring, whereas in **22**, HBA feature does not map. One HY feature corresponds to phenyl ring, which is attached to imidazole ring. Two ring aromatic features, RA1 and RA2, correspond to imidazole ring and phenyl ring (attached directly to the bridged nitrogen), respectively. All four pharmacophore features are mapped in the training set molecules with IC_{50} value less than 40. For molecules with lesser activity (**15**, **16**, **17**, **19**, **20**, and **21**), at least one feature is missing. For example, **16**, **17**, **20**, and **21** miss the RA2 feature. Pharmacophore superimposed with the five potent compounds (**1**, **2**, **3**, **5**, and **7**) in the training set is shown in Figure 5.

The predictive power of the Hypo 1 was validated with 181 test set compounds. All the compounds were

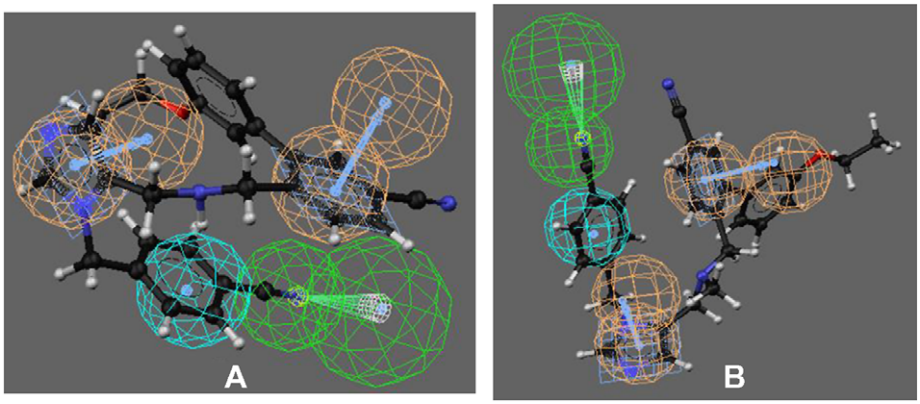

Figure 3. Pharmacophore mapping of the most active compounds on the best hypothesis model Hypo 1. (A) Compound **1** from the training set and (B) compound **172** from the test set. The green, blue, and brown contours represent the hydrogen-bond acceptor feature (HA), hydrophobic feature (HY), and ring aromatic feature (RA), respectively.

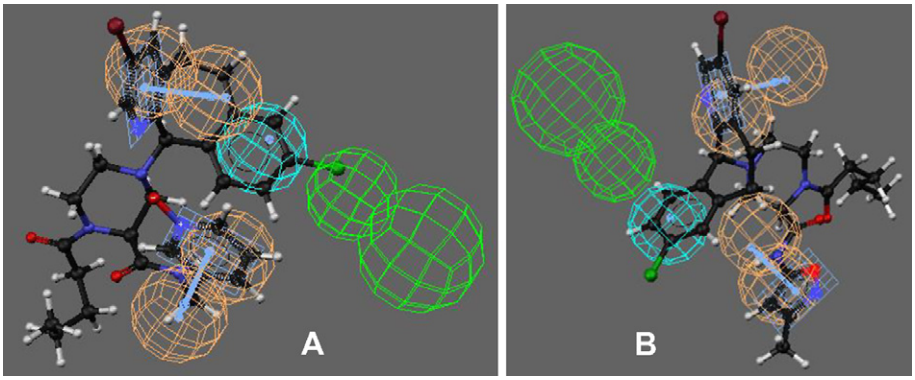

Figure 4. Pharmacophore mapping of the least active compounds on the best hypothesis model Hypo 1. (A) Compound **22** from the training set and (B) compound **47** from the test set. The color contours have the same annotation as for Figure 3.

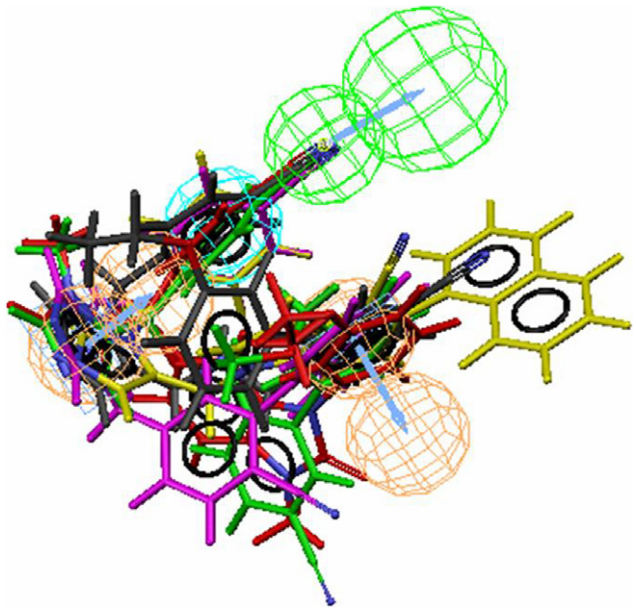

Figure 5. Pharmacophore superimposed with the five potent compounds in the training set using Hypo 1. Compound **1** is shown in gray, compound **2** in green, compound **3** in pink, compound **5** in yellow, and compound **7** in red.

imported into spreadsheet of hypothesis generation workbench and activities were estimated. A correlation coefficient of 0.713 shows a good correlation between the actual and estimated activities (Fig. 6). In detail, 82 of 123 highly active, 30 of 57 moderately active, and 1 inactive compounds were predicted correctly. Thirty-three highly active compounds were underestimated as moderately active and eight highly active compounds were underestimated as inactive; nine moderately active were overestimated as false positive and eighteen underestimated as inactive. The most active compound **172** in the test set had a fitness score of 11.05 when mapped to the Hypo 1 (Fig. 3B) and shows that all the features are being mapped accurately, whereas the least active compound **47**, in which HBA feature is missing, maps to a value of 5.37 (Fig. 4B). In summary, most of the compounds in the test set were predicted correctly for their biological activity.

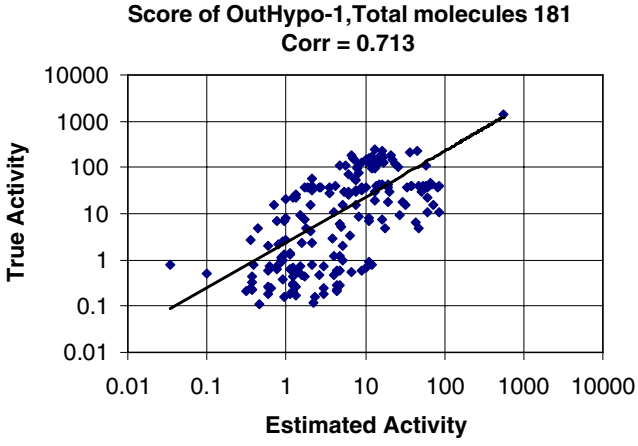

Figure 6. Correlation graph between experimental and Hypo 1-estimated activities of test set.

Hypo 1 was further validated for picking active molecules in the database against FTIs. For this validation experiment, when a spiked database (*D*) having 1525 compounds including 25 (*A*) known inhibitors of FTase was screened with Hypo 1, 140 molecules (Ht) were retrieved as hits. Among these hits, 25 (Ha) molecules were from the 25 known activities. Thus, the enrichment factor (as per Eq. 1) was found to be 10.892, indicating that it is 10 times more probable to pick an active compound from the database than an inactive one.

In conclusion, the work presented in this study shows how chemical features of a set of compounds along with their activities ranging over several orders of magnitudes can be used to generate pharmacophore hypotheses that can successfully predict the activity. The models were not only predictive within the same series of compounds but different classes of diverse compounds also effectively mapped onto most of the features important for activity. A highly predictive pharmacophore model was generated based on 22 training set compounds, which consists of one hydrogen-bond acceptor, one hydrophobic point, and two ring aromatic features. The utility of our pharmacophore model on 181 test set compounds showed that the model is able to accurately differentiate various classes of FTIs. The pharmacophore generated from FTIs can be used (1) as a three-dimensional query in database searches to identify compounds with diverse structures that can potentially inhibit FTase and (2) to evaluate how well any newly designed compound maps on the pharmacophore before undertaking any further study including synthesis. Both these applications may help in identifying or designing compounds for further biological evaluation and optimization. Thus, we hope that our pharmacophore model should be helpful in identifying novel lead compounds with improved inhibitory activity through three-dimensional database searches and useful in designing novel FTIs.

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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.2006.12.087.

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23. Fitness values indicate how well the features in the pharmacophore overlap with the chemical features in the ligand. These are normalized for the number of features in the phramacophore model.