Bioorganic & Medicinal Chemistry Letters 20 (2010) 1013–1018

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Pharmacophore modeling and virtual screening for designing potential 5-Lipoxygenase inhibitors

P. Aparoy, K. Kumar Reddy, Suresh K. Kalangi, T. Chandramohan Reddy, P. Reddanna *

School of Life Sciences, University of Hyderabad, Hyderabad 500 046, India

ARTICLE INFO

Article history:
Received 21 September 2009
Revised 9 December 2009
Accepted 11 December 2009
Available online 21 December 2009

Keywords:
5-LOX
Asthma
Pharmacophore modeling
Docking
Virtual screening
Lipoxygenase inhibitor

ABSTRACT

Inhibitors of the 5-Lipoxygenase (5-LOX) pathway have a therapeutic potential in a variety of inflammatory disorders such as asthma. In this study, chemical feature based pharmacophore models of inhibitors of 5-LOX have been developed with the aid of HipHop and HypoGen modules within Catalyst program package. The best quantitative pharmacophore model, Hypo1, which has the highest correlation coefficient (0.97), consists of two hydrogen-bond acceptors, one hydrophobic feature and one ring aromatic feature. Hypo1 was further validated by test set and cross validation method. The application of the model shows great success in predicting the activities of 65 known 5-LOX inhibitors in our test set with a correlation coefficient of 0.85 with a cross validation of 95% confidence level, proving that the model is reliable in identifying structurally diverse compounds for inhibitory activity against 5-LOX. Furthermore, Hypo1 was used as a 3D query for screening Maybridge and NCI databases within catalyst and also drug like compounds obtained from Enamine Ltd, which follow Lipinski's rule of five. The hit compounds were subsequently subjected to filtering by docking and visualization, to identify the potential lead molecules. Finally 5 potential lead compounds, identified in the above process, were evaluated for their inhibitory activities. These studies resulted in the identification of two compounds with potent inhibition of 5-LOX activity with IC_{50} of 14 μM and 35 μM, respectively. These studies thus validate the pharmacophore model generated and suggest the usefulness of the model in screening of various small molecule libraries and identification of potential lead compounds for 5-LOX inhibition.

Lipoxygenases (LOXs-linoleate: oxygen oxido reductase, EC 1.13.11.12) are a group of closely related non-heme iron containing dioxygenases. Polyunsaturated fatty acids containing a series of cis–cis double bonds act as suitable substrates for LOXs. LOXs are classified according to their positional specificity of arachidonate oxygenation into 5-, 8-, 9-, 11-, 12- and 15-LOXs.[1] LOX metabolites are potent physiological effectors in a variety of cellular responses. Particularly, leukotrienes (LTs), the mediators of allergy and asthma, are produced through the 5-LOX pathway. It has been also reviewed that 5-LOX plays a key role in Gastro Esophageal Reflux Disease (GERD).[2] Elevated levels of LTB_4 have been found in blood and joint fluid from patients with rheumatoid arthritis[3] and in colonic mucosa from patients with ulcerative colitis or Crohn's disease.[4,5] LOX and their products are shown to play important role in tumor formation and cancer metastasis.[6–8] High expression of 5-LOX was found in prostate, lung and other cancer cell lines.[9,10] Inhibitors of the 5-LOX pathway, therefore, have a therapeutic potential in a variety of inflammatory and allergic diseases. These efforts have resulted in the release of Zileuton (5-LOX inhibitor) and Montelukast (LT receptor antagonist) into the market for the treatment of asthma. Recently, the arachidonate 5-LOX gene (*Alox5*) has been identified as a critical regulator of leukemia stem cells (LSCs) in BCR-ABL-induced chronic myeloid leukemia (CML). It has been also reported that the treatment of CML mice with a 5-LOX inhibitor prolonged survival.[11]

As 5-LOX is implicated in many inflammatory disorders, there is growing emphasis by many pharmaceutical companies and academic research groups on the development of effective 5-LOX inhibitors. The novel inhibitors thus developed provide a good basis for elucidating the structure–activity relationship, which will aid in the identification of more potent inhibitors. Lack of crystal structure information of 5-LOX, however, has been an obstacle for the application of structure based drug design strategies. As an alternative homology modeling strategy was employed to generate 3-D models of various LOXs, which were used in various drug design strategies.[12–18] Ligand based drug design is an alternative in such cases. In a ligand-based design, identification of a pharmacophore is one of the most important steps.

Pharmacophore model is widely employed to quantitatively explore common chemical characteristics among a considerable number of structures with great diversity. Such a model could also be used as a query for searching chemical databases and find new chemical entities.[19–22] In this Letter, we identified pharmacophore

* Corresponding author. Tel.: +91 40 23134542; fax: +91 40 23010745.
E-mail addresses: prsl@uohyd.ernet.in, preddanna@yahoo.com (P. Reddanna).

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doi:10.1016/j.bmcl.2009.12.047

Table 1
Statistical parameters of the top 10 hypotheses of 5-Lipoxygenase inhibitors generated by HypoGen program

Hypo No.	Total cost[a]	Cost diff.[b]	RMSD	Correlation (r)	Features[c]
1	108.338	58.281	0.602576	0.974978	AAZR
2	109.789	56.830	0.68082	0.967973	ALZR
3	110.088	56.531	0.755627	0.959451	AZRR
4	110.388	56.231	0.721508	0.963781	ALZR
5	110.805	55.814	0.740654	0.961824	ALZR
6	111.068	55.551	0.769418	0.958449	ALZR
7	111.770	54.849	0.854401	0.947673	ALRR
8	111.882	54.737	0.825159	0.951754	LLZR
9	112.050	54.569	0.711489	0.96675	ALZR
10	112.257	54.362	0.814461	0.953537	AAZR

[a] The total cost value of a hypothesis is calculated by summing three cost factors, a weight cost (data not shown), an error cost (data not shown) and a configuration cost (a constant among all the hypotheses).

[b] The difference between the total cost of a hypothesis and that of the null hypothesis, roughly correlates with significance. The larger the difference between the two, the greater the significance of the hypothesis. A true correlation in the data will very likely be estimated by models that exhibit a cost difference (Null cost - Total cost) (fixed cost = 102.934, configuration cost = 21.0826 and null cost = 166.619). All cost values are in bits.

[c] A, hydrogen-bond acceptor; L, hydrogen-bond acceptor lipid; Z, hydrophobic feature; and R, ring aromatic moiety.

model of the 5-LOX inhibitors. Then the best quantitative pharmacophore model generated was used as a 3D query to screen several commercial databases comprising of compounds which follow Lipinski's rule of five[23] and docking study.[21] Finally, five of the identified potential lead compounds were evaluated for their 5-LOX inhibitory activities.

All the pharmacophore modeling calculations were carried out by using the Catalyst 4.11 software package (Accelrys, San Diego, USA)[24] on SGI workstation. The HipHop and HypoGen modules within Catalyst were used for the construction of qualitative and quantitative models, respectively. Chemical feature based pharmacophore hypotheses can be generated automatically using the HypoGen algorithm within Catalyst, provided that structure–activity relationship data of a well balanced set of compounds are available. A training set of 24 molecules (Fig. 1) with IC_{50}'s ranging from 0.003 μM to 41 μM for 5-LOX were selected from the literature (Table 1).

All structures in the training set were built in 2D/3D Visualizer within Catalyst and minimized to the closest local minimum based on a modified CHARMM force field within the confirm module.[25] Catalyst generated a representative family of conformational models for each compound using a Monte–Carlo-like algorithm

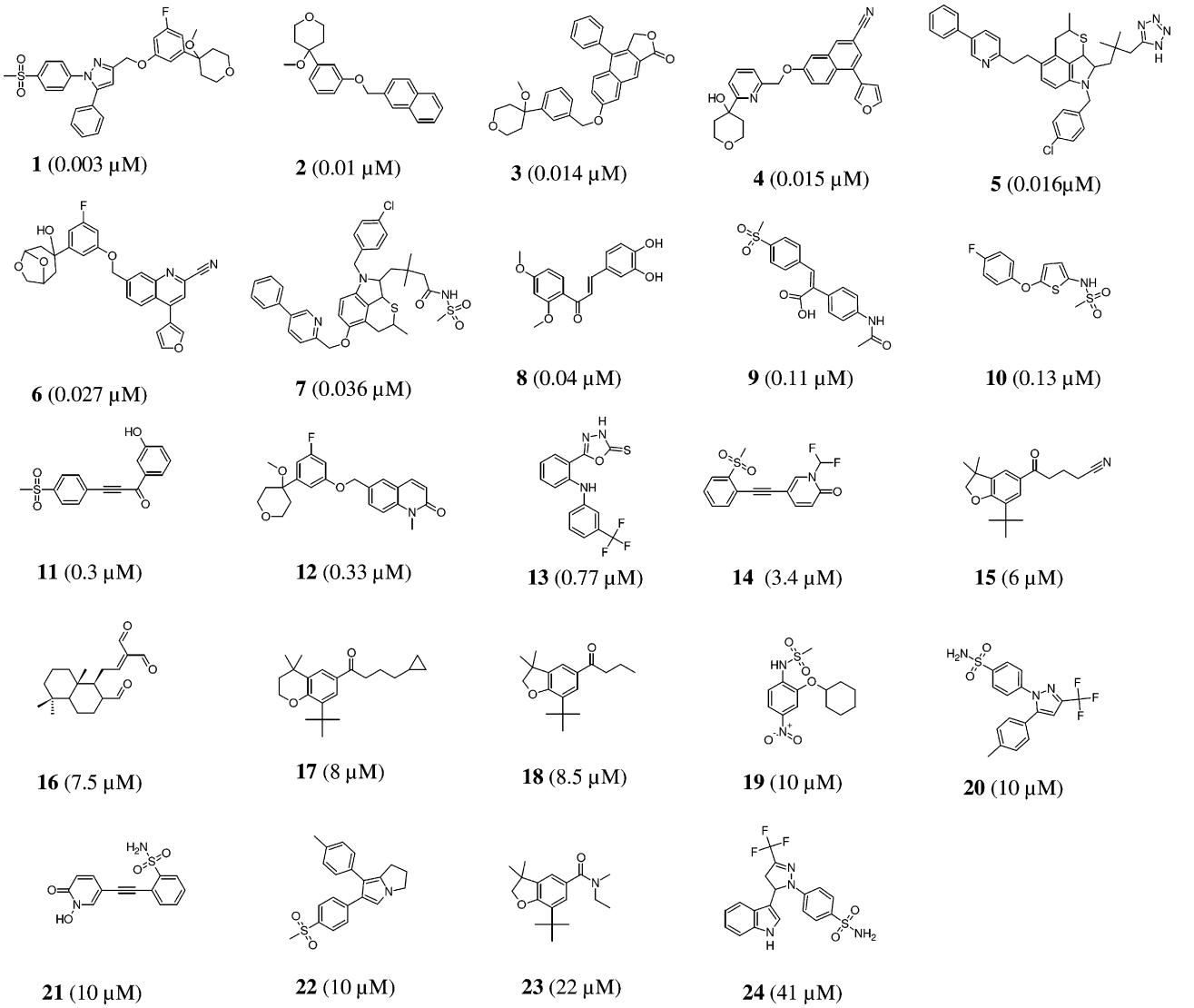

Figure 1. Chemical structures of 5-LOX inhibitors in training set (compounds **1**–**24**) and their biological activity data (IC_{50} values, in parentheses).

together with Poling.[26] Diverse conformational models for each compound were generated using an energy range of 15 kcal/mol of the calculated potential energy minimum. Maximum number of conformers was specified to 250 for each molecule to ensure maximum exploration of the conformational space.

Before performing the quantitative pharmacophore modeling, the qualitative HipHop model was generated based on the five most active compounds (**1**–**5**) in training set, the purpose of which is to identify pharmacophore features necessary for potent 5-LOX inhibitors. In the HipHop run, the most active compound **1** was considered as 'reference compound' specifying a 'principal' value of 2 and a 'MaxOmitFeat' value of 0. The 'principal' and 'Max-Omit-Feat' values were set to 1 for the remaining four compounds. HipHop parameters were kept at their default values. The HipHop pharmacophore hypothesis clearly indicated the importance of hydrogen-bond acceptor, hydrogen-bond acceptor lipid, hydrogen-bond donor, hydrophobic moiety, hydrophobic aliphatic moiety, hydrophobic aromatic moiety and ring aromatic feature. In these trials, it has been observed that taking hydrogen-bond acceptor, hydrogen-bond acceptor lipid, hydrogen-bond donor, hydrophobic moiety and ring aromatic feature generated a good quality pharmacophore model.

The approach we used here is to develop a pharmacophore model using the HypoGen module in Catalyst which can be used to correlate the observed biological activities for a series of compounds with their chemical structures. Default parameters were used in HypoGen. The generated HypoGen models were evaluated according to Debnath in terms of cost functions and statistical parameters, which were calculated by HypoGen module during hypothesis generation. A pharmacophore model should have a high correlation coefficient, lowest total cost and RMSD (Root Mean Square Deviation) values. The total cost should be close to the fixed cost and away from the null cost. The difference between the cost of the generated hypothesis and the cost of the null hypothesis signifies the reliability of a pharmacophore model. A value of 40–60 bits between them for a pharmacophore hypothesis may indicate that it has 75–90% probability of correlating the data.

The top 10 ranked hypotheses as well as their statistical parameters are presented in Table 1. The best pharmacophore model (Hypo1), which was characterized by the lowest total cost value (108.338), the highest cost difference (58.281), contains four features, namely, two hydrogen-bond acceptor, one hydrophobic and one ring aromatic feature. A 'measured' versus 'estimated' activity for the training set exhibited a correlation coefficient (r) of 0.974978 with RMSD of 0.6025. The good score value indicated a reliable ability to predict activities within the training set. The config costs of the runs were high and exceeded the maximum limit of 18. This may be because of the training set compounds, which seem to increase the entropy of the hypothesis. The reasonable cost difference of the hypothesis and high correlation obtained and further evaluation of the resulting model with Fischer randomization test and with test set compounds should surpass any drawbacks related to the less than optimal config cost.

The 3D space and distance constraints of these pharmacophore features are shown in Figure 2A and B. Figure 2C and D present the Hypo1 aligned with the most active compound **1** (IC_{50}: 0.003 μM) and the least active compound **24** (IC_{50}: 41 μM) in the training set, respectively. All features of Hypo1 model were nicely mapped with the corresponding chemical functional groups on compound **1**. By contrast, the compound **24** just mapped three features while the other feature of hydrogen-bond acceptor was not mapped. Table 2 shows the experimental and estimated inhibitory activities of the 24 training set compounds. All the compounds with high activity were predicted correctly. In comparison to other compounds, compound **2** showed higher error value of +6.2. The error costs of all the other compounds in test set were below 3, indicating the correctness of the hypothesis developed.

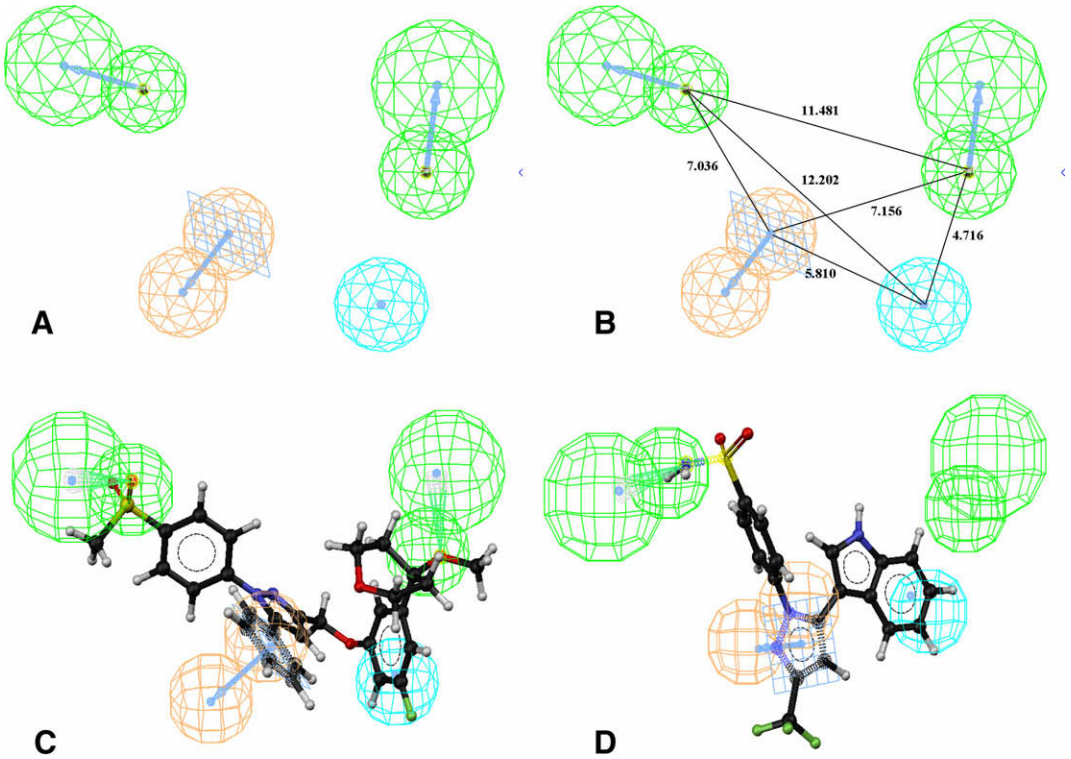

Figure 2. Pharmacophore model of 5-LOX inhibitors generated by HYPOGEN. (A) The best HYPOGEN model Hypo1. (B) 3D spatial relationship and geometric parameters of Hypo1. (C) Hypo1 mapping with the most active compound **1** (IC_{50}: 0.005 μM). (D) Hypo1 mapping with the least active compound **24** (IC_{50}: 41 μM). Pharmacophore features are color-coded with light-blue for hydrophobic feature, orange for ring aromatic feature and green for hydrogen-bond acceptor.

Table 2
Experimental and estimated (by Hypo1) IC_{50} values (μM) together with the error values (defined as the ratio between experimental activity and estimated activity) of the training set compounds **1–24**

Molecule	Exptl. IC_{50}[a] (μM)	Estimated IC_{50}[b] (μM)	Error[c]	Fit value	Reference
1	0.003	0.0019	−1.6	8.62	27
2	0.01	0.062	+6.2	7.1	28
3	0.014	0.018	+1.3	7.63	28
4	0.015	0.005	−2.6	8.13	29
5	0.016	0.034	+2.1	7.36	30
6	0.027	0.028	+1	7.44	31
7	0.036	0.062	+1.7	7.10	30
8	0.04	0.076	+1.9	7.01	32
9	0.11	0.12	+1.1	6.81	33
10	0.13	0.13	−1	6.78	34
11	0.3	0.57	+1.9	6.13	35
12	0.33	0.24	−1.4	6.51	28
13	0.77	0.46	−1.7	6.23	36
14	3.4	1.4	−2.5	5.75	37
15	6	10	+1.7	4.87	38
16	7.5	6.4	−1.2	5.08	39
17	8	11	+1.4	4.83	38
18	8.5	10	+1.2	4.87	38
19	10	10	+1	4.87	27
20	10	5.8	−1.7	5.12	40
21	10	11	+1.1	4.86	41
22	10	11	+1.1	4.85	42
23	22	10	−2.1	4.87	43
24	41	11	−3.8	4.85	44

[a] Exptl. = experimental activity (IC_{50} values, μM).
[b] Predicted activity (IC_{50} values, μM).
[c] The negative value indicates that the experimental IC_{50} is higher than the predicted IC_{50}.

Validation of the pharmacophore model. Fischer randomization test method[45] was used to evaluate the statistical relevance of Hypo1 by using the CatScramble program implemented in Catalyst. The confidence level was set to 95%. Thereby CatScramble program generated 19 random spreadsheets to construct hypotheses using exactly the same conditions as used in generating the original pharmacophore hypothesis. The total costs of pharmacophore models obtained in the 19 HypoGen runs as well as the original HypoGen run are presented in Figure 3. From Figure 3, one can see that the original hypothesis is better than those of the 19 random hypotheses generated. These results validate the pharmacophore model generated. An independent test set which contains 65 external compounds (shown in Supplementary data) was used to validate the established model (Hypo1). The experimental and predicted activities of the test set compounds are shown in Table S1 of Supplementary data. Further, a fairly good correlation coefficient of 0.85 was observed for regression analysis of the experimental and predicted inhibitory activity values for the test set compounds (Fig. 4). The high correlation coefficient value denotes the predictive capability of the model.

Virtual screening. The validated hypothesis Hypo1 was used as a 3D structural query for retrieving potential inhibitors from Maybridge and NCI databases available in Catalyst and compounds from Enamine Ltd, Ukraine. Compounds that follow the Lipinski's rule of five were obtained from the drug like databases. A total of 15,162 compounds showed very good mapping with the Hypo1 of which the top 1000 compounds were selected for further study.

Docking study. To further refine the retrieved hits, the 1000 compounds were docked into the inhibitor binding site of 5-LOX by using GOLD program.[46] As the crystal structure of 5-LOX is not yet available, the homology model of 5-LOX reported earlier by us was used in the study.[12] Docking was performed and the molecules were even scored using Ludi and Ligandfit in Accelrys Discover Studio.[47] Since there is no generally applicable scoring function so far, the compounds which were commonly scored top by various applications were ranked higher. After screening by visualization of protein–ligand interactions, potential compounds were identified and fifteen of them are shown in Figure 5. Of them, five compounds (**11**–**15**) were procured from Enamine Ltd, Ukraine and tested for their 5-LOX inhibition studies in vitro.

In vitro 5-LOX inhibitory assay. 5-LOX from potato tubers was purified and assayed as per the method described by Reddanna et al.[48] Enzyme activity was measured using polarographic method with a Clark's oxygen electrode on Strathkelvin Instruments, model 782, RC-300. Typical reaction mixture contained 50–100 μl of enzyme and 10 μl of substrate (133 μM of arachidonic acid) in a final volume of 3 ml with 100 mM phosphate buffer pH 6.3. Rate

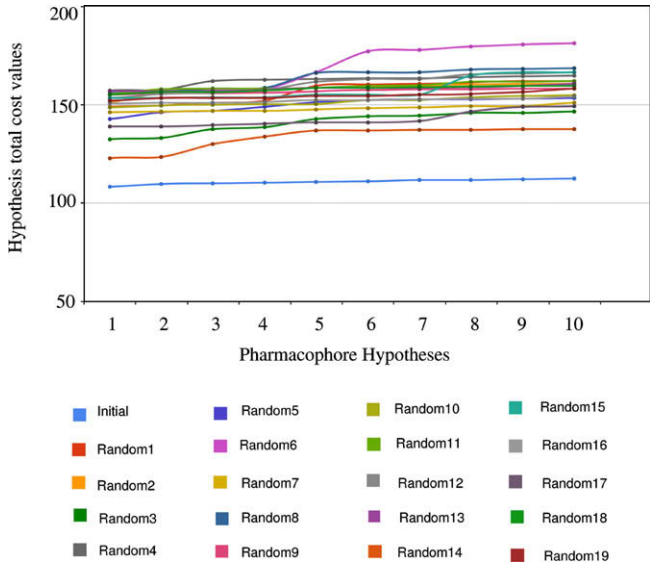

Figure 3. The difference in total cost of hypotheses between the initial spreadsheet and 19 random spreadsheets after CatScramble run.

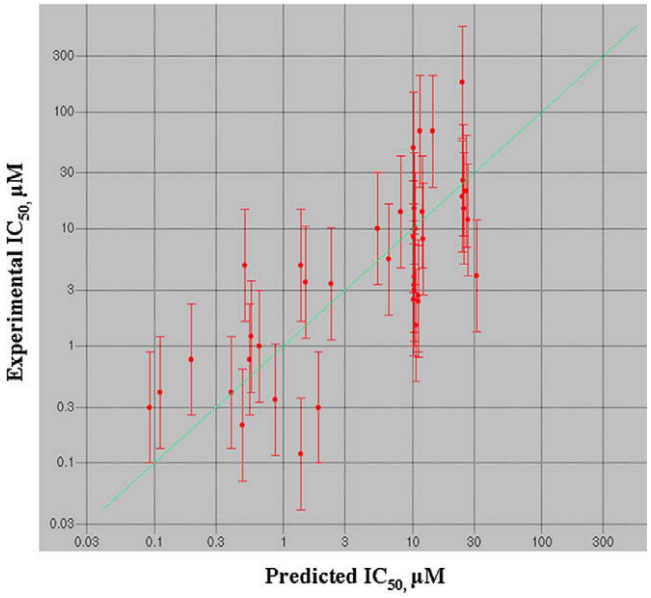

Figure 4. Plot of the correlation (r) between the experimental activity (μM) and the predicted activity (μM) by Hypo1_II for test set molecules (in red). The vertical bars are a plot of the uncertainty values. The regression line (green line) is the best fit line for the dots on the graph and the equation of the line is y = x. The dots closer to the regression line represent data with minimum variance.

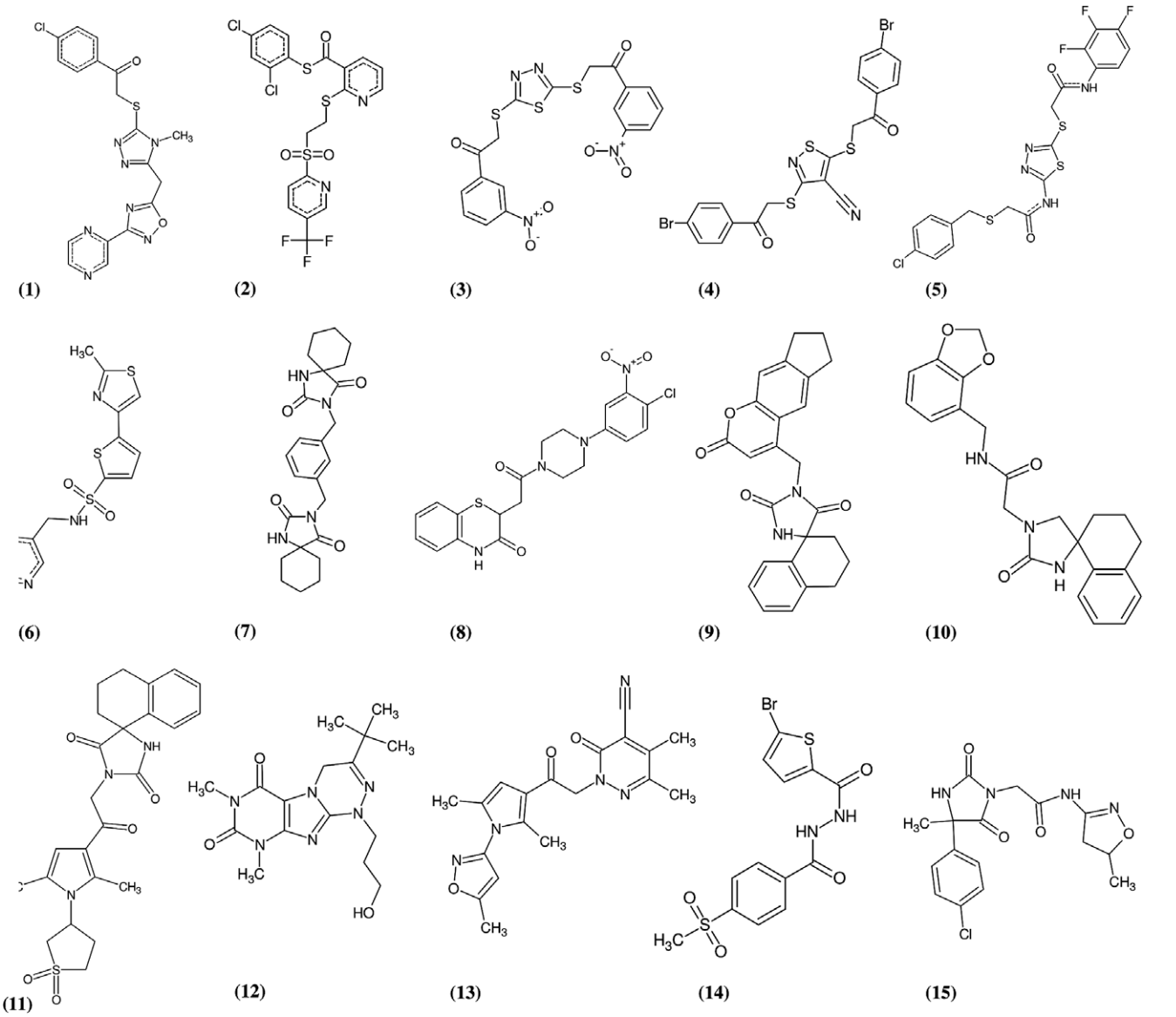

Figure 5. Potential molecules retrieved from the Enamine and Maybridge databases.

of decrease in oxygen concentration was taken as a measure of enzyme activity. Stock solutions of test compounds, prepared immediately before use, were dissolved in DMSO. Various concentrations of test drug solutions were added and the LOX reaction was initiated by the addition of substrate. The reaction was allowed to proceed at 25 °C and the maximum slope generated was taken for calculating activity. Percent inhibition was calculated by comparison of LOX activity in the presence and absence of inhibitor. The concentration of the test compound causing 50% inhibition (IC_{50}, μM) was calculated from the concentration-inhibition response curve. Each assay was repeated thrice. Of the tested compounds, 11 and 13 showed inhibition with an IC_{50} of 14 μM and 35 μM, respectively. The other compounds didn't show inhibition up to 100 μM.

In conclusion, in this study, chemical feature based pharmacophore modeling of inhibitors of 5-LOX have been carried out by using HypoGen module within Catalyst program package. The best HypoGen model, which was characterized by the lowest total cost (108.338), the lowest RMSD (0.602576) and the best correlation coefficient (0.974978), consists of two hydrogen-bond acceptors, one hydrophobic and one ring aromatic feature. Both test set and cross validation methods have been used to validate the pharmacophore model, Hypo1. Results obtained by using the test set method show a fairly good correlation between the experimental and predicted IC_{50} values, indicating a good predictive ability. The statistical confidence of Hypo1 has also been confirmed by using CatScramble program within Catalyst. In our study, the Hypo1 was used as a 3D query to screen various databases. The hit compounds were subsequently subjected to docking studies. Of the five potential lead compounds identified, two compounds showed inhibition of 5-LOX activity with IC_{50} of 14 μM and 35 μM, respectively. These studies thus provide a pharmacophore model, which will be helpful in designing novel 5-LOX inhibitors. This study assumes importance in the light of key role played by 5-LOX in various pathological manifestations and lack of crystal structure for use in molecular modeling and design studies.

Acknowledgments

We thank Centre for Modelling, Simulation and Design (CMSD), University of Hyderabad for permitting us to use the SGI workstation and Catalyst facilities. We duly acknowledge Council of

Scientific and Industrial Research (CSIR), Govt. of India for providing senior research fellowship to P.A.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.047.

References and notes

1. Rapoport, S. M.; Schewe, T.; Wiesner, R.; Halangk, W.; Ludwig, P.; Janicke-Hohne, M.; Tannert, C.; Hiebsch, C.; Klatt, D. *Eur. J. Biochem.* **1979**, *96*, 545.
2. Chen, X.; Li, N.; Wang, S.; Hong, J.; Fang, M.; Yousselfson, J.; Yang, P.; Newman, R. A.; Lubet, R. A.; Yang, C. S. *Carcinogenesis* **2002**, *23*, 2095.
3. Davidson, E. M.; Rae, S. A.; Smith, M. J. *Ann. Rheum. Dis.* **1983**, *42*, 677.
4. Sharon, P.; Stenson, W. F. *Gastroenterology* **1984**, *86*, 453.
5. Rask-Madsen, J.; Bukhave, K.; Laursen, L. S.; Lauritsen, K. *Agents Actions* **1992**, *Spec No*, C37.
6. Nie, D.; Che, M.; Grignon, D.; Tang, K.; Honn, K. V. *Cancer Metastasis Rev.* **2001**, *20*, 195.
7. Honn, K. V.; Tang, D. G.; Gao, X.; Butovich, I. A.; Liu, B.; Timar, J.; Hagmann, W. *Cancer Metastasis Rev.* **1994**, *13*, 365.
8. Kelavkar, U.; Glasgow, W.; Eling, T. E. *Curr. Urol. Rep.* **2002**, *3*, 207.
9. Anderson, K. M.; Seed, T.; Vos, M.; Mulshine, J.; Meng, J.; Alrefai, W.; Ou, D.; Harris, J. E. *Prostate* **1998**, *37*, 161.
10. Avis, I. M.; Jett, M.; Boyle, T.; Vos, M. D.; Moody, T.; Treston, A. M.; Martinez, A.; Mulshine, J. L. *J. Clin. Invest.* **1996**, *97*, 806.
11. Chen, Y.; Hu, Y.; Zhang, H.; Peng, C.; Li, S. *Nat. Genet.* **2009**, *41*, 783.
12. Aparoy, P.; Reddy, R. N.; Guruprasad, L.; Reddy, M. R.; Reddanna, P. *J. Comput. Aided Mol. Des.* **2008**, *22*, 611.
13. Du, L.; Zhang, Z.; Luo, X.; Chen, K.; Shen, X.; Jiang, H. *J. Biochem.* **2006**, *139*, 715.
14. Charlier, C.; Henichart, J. P.; Durant, F.; Wouters, J. *J. Med. Chem.* **2006**, *49*, 186.
15. Hammarberg, T.; Provost, P.; Persson, B.; Radmark, O. *J. Biol. Chem.* **2000**, *275*, 38787.
16. Bindu, P. H.; Sastry, G. M.; Sastry, G. N. *Biochem. Biophys. Res. Commun.* **2004**, *320*, 461.
17. Hemak, J.; Gale, D.; Brock, T. G. *J. Mol. Model.* **2002**, *8*, 102.
18. Werz, O.; Tretiakova, I.; Michel, A.; Ulke-Lemee, A.; Hornig, M.; Franke, L.; Schneider, G.; Samuelsson, B.; Radmark, O.; Steinhilber, D. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13164.
19. Xie, H. Z.; Li, L. L.; Ren, J. X.; Zou, J.; Yang, L.; Wei, Y. Q.; Yang, S. Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1944.
20. Taha, M. O.; Atallah, N.; Al-Bakri, A. G.; Paradis-Bleau, C.; Zalloum, H.; Younis, K. S.; Levesque, R. C. *Bioorg. Med. Chem.* **2008**, *16*, 1218.
21. Wang, H. Y.; Cao, Z. X.; Li, L. L.; Jiang, P. D.; Zhao, Y. L.; Luo, S. D.; Yang, L.; Wei, Y. Q.; Yang, S. Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4972.
22. Lu, A.; Zhang, J.; Yin, X.; Luo, X.; Jiang, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 243.
23. Lipinski, C. A. *J. Pharmacol. Toxicol. Methods* **2000**, *44*, 235.
24. CATALYST 4.11; Accelrys: San Diego, CA, 2005, http://www.accelrys.com.
25. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187.
26. Smellie, A.; Teig, S. L.; Towbin, P. *J. Comput. Chem.* **1995**, *16*, 171.
27. Barbey, S.; Goossens, L.; Taverne, T.; Cornet, J.; Choesmel, V.; Rouaud, C.; Gimeno, G.; Yannic-Arnoult, S.; Michaux, C.; Charlier, C.; Houssin, R.; Henichart, J. P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 779.
28. Ducharme, Y.; Brideau, C.; Dube, D.; Chan, C. C.; Falgueyret, J. P.; Gillard, J. W.; Guay, J.; Hutchinson, J. H.; McFarlane, C. S.; Riendeau, D., et al *J. Med. Chem.* **1994**, *37*, 512.
29. Hamel, P.; Riendeau, D.; Brideau, C.; Chan, C. C.; Desmarais, S.; Delorme, D.; Dube, D.; Ducharme, Y.; Ethier, D.; Grimm, E.; Falgueyret, J. P.; Guay, J.; Jones, T. R.; Kwong, E.; McAuliffe, M.; McFarlane, C. S.; Piechuta, H.; Roumi, M.; Tagari, P.; Young, R. N.; Girard, Y. *J. Med. Chem.* **1997**, *40*, 2866.
30. Hutchinson, J. H.; Riendeau, D.; Brideau, C.; Chan, C.; Delorme, D.; Denis, D.; Falgueyret, J. P.; Fortin, R.; Guay, J.; Hamel, P., et al *J. Med. Chem.* **1993**, *36*, 2771.
31. Dube, D.; Blouin, M.; Brideau, C.; Chan, C. C.; Desmarais, S.; Ethier, D.; Falgueyret, J. P.; Friesen, R. W.; Girard, M.; Girard, Y.; Guay, J.; Riendeau, D.; Tagari, P.; Young, R. N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1255.
32. Nakamura, C.; Kawasaki, N.; Miyataka, H.; Jayachandran, E.; Kim, I. H.; Kirk, K. L.; Taguchi, T.; Takeuchi, Y.; Hori, H.; Satoh, T. *Bioorg. Med. Chem.* **2002**, *10*, 699.
33. Moreau, A.; Chen, Q. H.; Praveen Rao, P. N.; Knaus, E. E. *Bioorg. Med. Chem.* **2006**, *14*, 7716.
34. Kirchner, T.; Argentieri, D. C.; Barbone, A. G.; Singer, M.; Steber, M.; Ansell, J.; Beers, S. A.; Wachter, M. P.; Wu, W.; Malloy, E.; Stewart, A.; Ritchie, D. M. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 1094.
35. Rao, P. N.; Chen, Q. H.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4842.
36. Charlier, C.; Michaux, C. *Eur. J. Med. Chem.* **2003**, *38*, 645.
37. Chowdhury, M. A.; Abdellatif, K. R.; Dong, Y.; Rahman, M.; Das, D.; Suresh, M. R.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 584.
38. Janusz, J. M.; Young, P. A.; Scherz, M. W.; Enzweiler, K.; Wu, L. I.; Gan, L.; Pikul, S.; McDow-Dunham, K. L.; Johnson, C. R.; Senanayake, C. B.; Kellstein, D. E.; Green, S. A.; Tulich, J. L.; Rosario-Jansen, T.; Magrisso, I. J.; Wehmeyer, K. R.; Kuhlenbeck, D. L.; Eichhold, T. H.; Dobson, R. L. *J. Med. Chem.* **1998**, *41*, 1124.
39. Abe, M.; Ozawa, Y.; Uda, Y.; Morimitsu, Y.; Nakamura, Y.; Osawa, T. *Biosci. Biotechnol. Biochem.* **2006**, *10*, 2494.
40. Chowdhury, M. A.; Abdellatif, K. R.; Dong, Y.; Das, D.; Suresh, M. R.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6138.
41. Chowdhury, M. A.; Chen, H.; Abdellatif, K. R.; Dong, Y.; Petruk, K. C.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4195.
42. Ulbrich, H.; Fiebich, B.; Dannhardt, G. *Eur. J. Med. Chem.* **2002**, *37*, 953.
43. Janusz, J. M.; Young, P. A.; Ridgeway, J. M.; Scherz, M. W.; Enzweiler, K.; Wu, L. I.; Gan, L.; Chen, J.; Kellstein, D. E.; Green, S. A.; Tulich, J. L.; Rosario-Jansen, T.; Magrisso, I. J.; Wehmeyer, K. R.; Kuhlenbeck, D. L.; Eichhold, T. H.; Dobson, R. L. *J. Med. Chem.* **1998**, *41*, 3515.
44. Reddy, M. V.; Mallireddigari, M. R.; Cosenza, S. C.; Pallela, V. R.; Iqbal, N. M.; Robell, K. A.; Kang, A. D.; Reddy, E. P. *J. Med. Chem.* **2008**, *51*, 86.
45. Fischer, R. *The Design of Experiments*; Hafner Publishing: New York, 1966. Chapter 2.
46. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. *J. Mol. Biol.* **1997**, *267*, 727.
47. Accelrys Discovery Studio 1.7; Accelrys: San Diego, CA, 2006. http://www.accelrys.com.
48. Reddanna, P.; Whelan, J.; Maddipati, K. R.; Reddy, C. C. *Methods Enzymol.* **1990**, *187*, 268.