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CoMFA and CoMSIA studies on 5-hydroxyindole-3-carboxylate derivatives as 5-lipoxygenase inhibitors: Generation of homology model and docking studies

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ABSTRACT

In this study, comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) were performed on a series of 2-substituted 5-hydroxyindole-3-carboxylate derivatives as potent 5-LOX inhibitors with IC_{50} values ranging from 0.031 to 13.4 μM. Two datasets of same molecules were prepared with two different partial atomic charges; one with Gasteiger–Huckel and another with the ESPFIT charges obtained from the GAUSSIAN package. CoMFA and CoMSIA models were generated for both the datasets and the results were analysed. With regard to the non-cross validated r^2 values (r^2_{ncv}) and cross-validated q^2 values (q^2_{cv}) of the resulting QSAR models, the dataset with ESPFIT charges yielded higher values; hence it was further used in the study. The CoMFA and CoMSIA models have been further validated for their stability and robustness using group validation and bootstrapping techniques and for their predictive abilities using an external test set of ten compounds. The predictive power of the CoMSIA model was higher than the CoMFA model, the high predictive r^2 values of the test set reveals that the models prove to be useful tools for activity prediction of newly designed 5-LOX inhibitors. The ESPFIT-derived charges yielded better models than those based on charges calculated from Gasteiger–Huckel charges. We generated a homology model for human 5-LOX and identified the key residues at the binding site. The 3D-QSAR models were compared with the interactions at the active site to further elucidate the accuracy of the models. The data generated from 3D-QSAR study was used to design potential 5-LOX inhibitors.

Lipoxygenases (LOXs) (linoleate: oxygen oxido reductase, EC 1.13.11.12) are a group of closely related non-heme iron containing dioxygenases. These enzymes catalyze the addition of molecular oxygen into Poly Unsaturated Fatty Acids (PUFAs) containing cis, cis 1–4 pentadiene structures to give their hydroperoxy derivatives.[1] LOXs are classified according to their positional specificity of arachidonate oxygenation into 5-, 8-, 9-, 11-, 12- and 15-LOXs.[2] One of the LOX pathways of arachidonic acid metabolism, the 5-LOX pathway, is the source of potent pro-inflammatory mediators.[3] LOX metabolites are potent physiological effectors in a variety of cellular responses, associated with normal host defense and inflammation. In particular, leukotrienes (LTs), the mediators of allergy and asthma, are produced through the 5-LOX pathway. Products of the 5-LOX pathway are thus important mediators of inflammation. Inhibitors of the 5-LOX pathway, therefore, have therapeutic potential in a variety of inflammatory and allergic diseases. 5-LOX plays a key role in gastroesophageal reflux disease (GERD), rheumatoid arthritis and Crohn's disease.[4] High expression of 5-LOX was found in prostate, lung and other cancer cell lines.[5–8] Recently it has been shown that 5-LOX (ALOX5) is critical regulator for leukemia cancer stem cells (LSCS) in chronic myeloid leukemia (CML).[9] Currently an emerging strategy of therapeutic value consists of creating molecules with specific 5-LOX inhibition activity.

In our earlier studies, a theoretical 3D model of potato 5-LOX was elaborated by homology modeling.[10] The 5-LOX active site was then characterized from a structural point of view and used to study the docking of selected inhibitors. This shed new light on the binding features of the enzyme. In a more recent study, chemical feature based pharmacophore modeling of inhibitors of 5-LOX have been carried out by using HypoGen module within Catalyst program package.[11] The fact that 5-LOX inhibitors can be successfully identified by employing pharmacophore based virtual screening explains its usefulness in predicting activities of large datasets of molecules. Thus, our earlier studies provided homology and pharmacophore models which help in designing the novel 5-LOX inhibitors.

In this study, we have performed three dimensional quantitative structure–activity relationship (3D-QSAR), using the comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) techniques.[12,13] The study

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has been carried out on 2-substituted 5-hydroxyindole-3-carboxylate derivatives to determine the influence of steric, electrostatic and hydrophobic fields of these compounds on their 5-LOX inhibitory activity. Furthermore, these fields have been mapped onto binding pocket of human 5-LOX model that has also been generated in this study to better understand the interactions.

Fourty compounds (Table 1) with IC_{50}s ranging from 0.031 to 13.4 μM for 5-LOX were selected from the literature.[14] The IC_{50} values were converted into corresponding pIC_{50} values by the formula in Eq. (1). The calculated pIC_{50} values ranged from 4.87 to 7.50.

$$pIC_{50} = -\log IC_{50} \quad (1)$$

The initial set of compounds has been randomly divided into training set (30 compounds) and test set (10 compounds). The structures of the molecules in training set and test set are included in Supplementary data. All molecular modeling calculations were performed using SYBYL program package version 8.0 (Tripos Associates Inc.) on a Linux operating system.[15] Molecular building was done with a molecular sketch program.

Two datasets of the compounds with different partial charges were prepared; first dataset with charges calculated by the Gasteiger-Hückel method and the second dataset with ESPFIT charges.

For the first dataset the molecular geometry of each compound was first minimized using a standard Tripos molecular mechanics force field with a 0.001 kcal/mol Å energy gradient convergence criterion, and their charges were calculated by the Gasteiger-Hückel method. Partial atomic charges were assigned to each atom and then energy minimization of each molecule was performed by using steepest descent method 500 steps and Conjugate gradient method until molecule converged using Tripos standard force field with a distance-dependent dielectric function.

For the generation of second dataset GAUSSIAN 03 package was used. All the molecules were optimized using HF/6-31G* basis set and the partial atomic charges for each of them were obtained with ESP fitting (HF/6-31G* OPT ESP).

A proper alignment of the structures is critical for obtaining valid 3D-QSAR models.[16,17] Further, to obtain a reliable and consistent alignment, the lowest energy conformation of the most active molecule, compound **24** (Fig. 1) was used as the template. The atoms used for alignment were the common substructure of each molecule as shown in the Figure 1B. An automatic alignment method was carried out by using Database alignment. The conformations of all aligned molecules of the training set are shown in Figure 1C.

The standard CoMFA procedure implemented in SYBYL8.0 was used in the present study. A 3D cubic lattice with a grid spacing of 2 Å was created automatically by the program to encompass all the aligned ligands. CoMFA[12] steric and electrostatic fields were calculated using Tripos force field taking a sp3 carbon probe atom with a Van der Waals radius of 1.52 Å and a charge of +1.0 to generate steric (Lennard–Jones 6–12 potential) field energies and electrostatic (Coulombic potential) fields with a distance-dependent dielectric constant at each lattice point. Steric and electrostatic fields generated were scaled by the CoMFA-Standard method in SYBYL with default cut-off energy of 30 kcal/mol. The minimum column filtering was set to 2.0 kcal/mol to improve the signal-to-noise ratio by omitting those lattice points whose energy variation was below this threshold. CoMSIA similarity indices were derived according to Klebe et al.[13]

The 3D-QSAR models of CoMFA and CoMSIA descriptors were derived using PLS regression method[18] as implemented in the SYBYL package. The cross-validated coefficient, q^2, was calculated using Eq. (2).

$$q^2 = 1 - \frac{\sum(Y_{predicted} - Y_{observed})^2}{\sum(Y_{observed} - Y_{mean})} \quad (2)$$

Where $Y_{predicted}$, $Y_{observed}$, and Y_{mean} are predicted, actual, and mean values of the target property (pIC_{50}), respectively. $\sum(Y_{predicted} - Y_{observed})^2$ is the predictive sum of squares (PRESS).

Table 1
Observed/experimental and CoMFA/CoMSIA predicted 5-LOX inhibitory activity (IC_{50}) of 2-substituted 5-hydroxyindole-3-carboxylate derivatives in the training set

S. No.	Exptl. IC_{50} (μM)	pIC_{50}	CoMFA predicted activity (error)		CoMSIA predicted activity (error)	
			Gasteiger–Huckel	ESPFIT	Gasteiger–Huckel	ESPFIT
1	8.1	5.0915	5.075 (0.1158)	5.147 (0.0555)	5.074 (0.0175)	5.089 (0.0025)
2	9.8	5.0088	4.893 (0.1158)	4.891 (0.1178)	4.953 (0.0558)	4.941 (0.0678)
3	13.4	4.8729	4.957 (−0.0841)	4.878 (0.0051)	4.976 (0.1031)	5.026 (0.1531)
4	8.3	5.0809	5.142 (−0.0611)	5.144 (0.0631)	5.142 (0.0611)	5.109 (0.0281)
5	6.9	5.1612	5.138 (0.0232)	5.095 (0.0662)	5.121 (0.0402)	5.158 (0.0032)
6	3.5	5.4559	5.367 (0.0889)	5.53 (−0.0741)	5.28 (0.1759)	5.416 (0.0399)
7	0.3	6.5229	6.061 (0.4619)	6.112 (0.4109)	6.054 (0.4689)	5.95 (0.5729)
8	2	5.699	5.789 (−0.09)	5.873 (−0.174)	5.778 (−0.079)	5.833 (−0.134)
9	1.6	5.7959	5.893 (−0.0971)	5.879 (0.0831)	5.871 (0.0751)	5.785 (0.0109)
10	1.2	5.9208	5.847 (0.0738)	5.81 (0.1108)	5.946 (0.0252)	5.862 (0.0588)
11	4.8	5.3188	5.769 (−0.4502)	5.734 (0.4152)	5.819 (0.5002)	5.724 (0.4052)
12	0.7	6.1549	6.178 (−0.0231)	6.164 (0.0091)	6.155 (0.0001)	6.226 (0.0711)
13	7.3	5.1367	5.11 (0.0267)	4.975 (0.1617)	5.143 (0.0063)	5.075 (0.0617)
14	0.24	6.6198	7.093 (−0.4732)	7.025 (0.4052)	7.171 (0.5512)	7.118 (0.4982)
15	0.086	7.0655	6.993 (0.0725)	7.033 (0.0325)	6.872 (0.1935)	7.029 (0.0365)
16	0.097	7.0132	7.014 (−0.0008)	7.105 (0.0918)	6.985 (0.0282)	6.978 (0.0352)
17	0.084	7.0757	7.049 (0.0267)	6.975 (0.1007)	7.05 (0.0257)	7.042 (0.0337)
18	0.096	7.0177	7.052 (−0.0343)	7.017 (0.0007)	7.019 (0.0013)	7.013 (0.0047)
19	0.098	7.0088	7.039 (−0.0302)	7.004 (0.0048)	7.081 (0.0722)	7.058 (0.0492)
20	0.13	6.8861	6.9 (−0.0139)	6.887 (0.0009)	6.893 (0.0069)	6.931 (0.0449)
21	0.15	6.8239	6.796 (0.0279)	6.771 (0.0529)	6.822 (0.0019)	6.845 (0.0211)
22	0.045	7.3468	6.97 (0.3768)	7.04 (0.3068)	6.913 (0.4338)	6.924 (0.4228)
23	0.067	7.1739	7.136 (0.0379)	7.184 (0.0101)	7.149 (0.0249)	7.137 (0.0369)
24	0.031	7.5086	7.5 (0.0086)	7.451 (0.0576)	7.502 (0.0066)	7.551 (0.0424)
25	0.049	7.3098	7.347 (−0.0372)	7.336 (0.0262)	7.313 (0.0032)	7.257 (0.0528)
26	0.13	6.8861	6.816 (0.0701)	6.843 (0.0431)	6.869 (0.0171)	6.849 (0.0371)
27	0.65	6.1871	6.242 (−0.0549)	6.212 (0.0249)	6.188 (0.0009)	6.179 (0.0081)
28	0.11	6.9586	6.979 (−0.0204)	6.923 (0.0356)	6.954 (0.0046)	6.853 (0.1056)
29	0.17	6.7696	6.719 (0.0506)	6.812 (0.0424)	6.786 (0.0164)	6.877 (0.1074)
30	1.2	5.9208	5.928 (−0.0072)	5.943 (0.0222)	5.911 (0.0098)	5.957 (0.0362)