



ORIGINAL INVESTIGATION

ISSN: 2320-1304

International Journal of Pharmamedix India

April 2013, Volume-I, Issue-II.



Jain A, Singh A; International Journal of Pharmamedix India, 2013, 1(2), 317-326

“Hansch Analysis of benzoxazole derivatives as 5-lipoxygenase inhibitors”.

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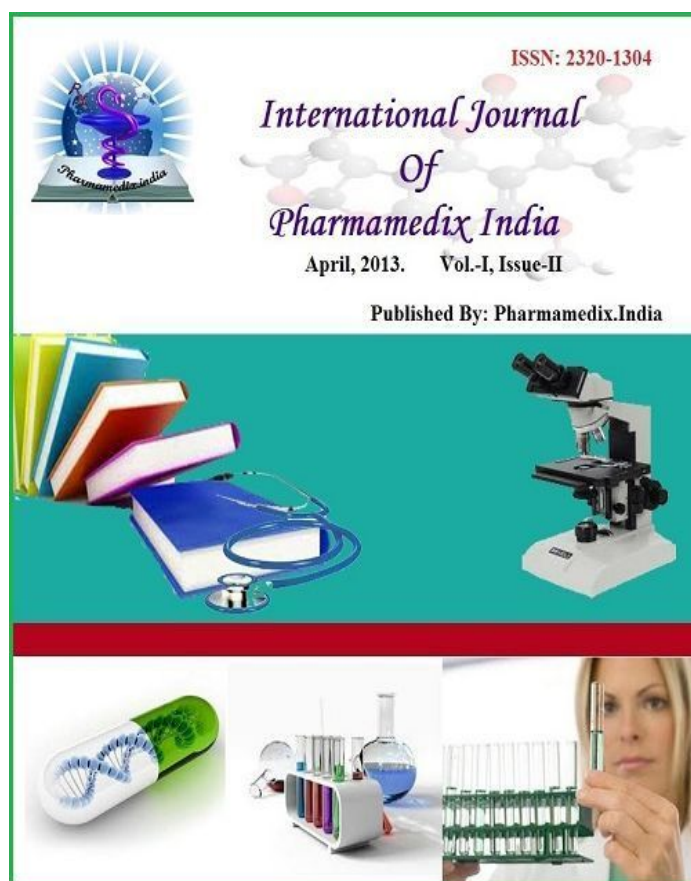
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Abstract:

A QSAR study on benzoxazole derivatives as 5-lipoxygenase inhibitory agents was performed with 14 (11 training + 3 test) compounds. Molecular modeling studies were performed using Chemoffice 6.0 supplied by cambridgesoft. The sketched structures were subjected to energy minimization and the lowest energy structure was used to calculate the physiochemical properties. The regression analysis was carried out using a computer program called VALSTAT. The best models were selected from the various statistically significant equations. The substitution on benzoxazole ring affected the 5- LOX inhibition. The introduction of nitro group on 5-position of benzoxazole clearly decreased the activity ($2e < 2b$). However, 5 chloro benzoxazoles 2c and 2d showed almost same activity compared to their 5-H congeners 2a and 2b. The electron donating groups, such as 5-methyl and 5-methoxy, increased the activity ($2f > 2a$, $2g > 2b$, and $2k > 2a$). This observation could be explained by the proposed affinity of 5-LOX iron to the ligands having the enhanced electron density as a result of the introduction of electron donating X and Y to the aromatic rings. The analysis resulted in QSAR equation, which suggests that, $n=9$, $r=0.923$, $r^2=0.852$, adjusted squared multiple $R=0.711$, Standard error of estimate(s) = 0.192 & validated $r^2 (q^2) = 0.631$. This study can help in rational drug design and synthesis of new selective 5-lipoxygenase inhibitor with predetermined affinity.

Keywords: QSAR analysis, benzoxazole derivatives, 5-lipoxygenase.

Introduction:

Asthma is a predominantly inflammatory disease of the respiratory tract that has bronchial hyperreactivity and bronchospasm as a result. Increased number of inflammatory cells such as mast cells, eosinophils, neutrophils, and lymphocytes can be found in the bronchial mucosa from asthmatic patients^[1-3]. The novel 5-lipoxygenase (5-

LOX) inhibitor, was investigated for its effect on exercise-induced bronchoconstriction in asthmatic subjects. 5-Lipoxygenase (5- LOX) is potential target in the treatment of asthma, atherosclerosis, prostate cancer, and allergy, because 5-LOX is required for the production of leukotrienes which are potent bronchocontraction and proinflammatory mediators^[4-9]. Mammalian lipoxygenases

constitute a heterogeneous family of lipid-peroxidizing enzymes, and the various isoforms are categorized with respect to their positional specificity of arachidonic acid oxygenation into 5-, 8-, 12-, and 15-lipoxygenases. Structural modeling suggested that the substrate binding pocket of the human 5-lipoxygenase is 20% bigger than that of the reticulocyte-type 15-lipoxygenase; thus, reduction of the active site volume was suggested to convert a 5-lipoxygenase to a 15-lipoxygenating enzyme species. 5-LOX belongs to a family of lipid peroxidising enzymes, which are expressed in both the vegetal and animal kingdoms. These dioxygenases catalyse the oxygenation of polyunsaturated fatty acids containing a 1,4-cis,cis-pentadiene moiety to produce hydroperoxy derivatives. They require one non-heme iron atom per molecule, which oscillates between Fe^{2+} (inactive enzyme) and Fe^{3+} (active form) during the catalytic cycle^[10]. Although the detailed mechanism of the LOX reaction still remains controversial, its radical nature is commonly agreed upon. It consists of three consecutive steps: (1) stereo-selective hydrogen abstraction from a doubly allelic methylene group, (2) radical rearrangement, and (3) stereo-specific insertion of molecular oxygen and reduction of hydroperoxy-radical intermediate to the corresponding anion. The knowledge about the biological roles of 12- and 15-LOX is limited and needs to be investigated

further^[11]. 5-LOX, in contrast, has been widely studied and it might be biologically the most important LOX. It is involved in the biosynthesis of potent inflammatory mediators, the LTs (leukotrienes), the second major metabolic pathway of amino acid. 5-LOX has been characterized from various mammalian species. It is a monomeric protein of 75- 80 kDa, containing about 673 amino acids. While its 3D structure has not been determined yet, several structures of LOXs (two isomer forms from soybeans, LOX-1 and LOX-3 and one from rabbit, 15-LOX have been reported. In regard to its characteristics and mechanism of action, different strategies have been developed to inhibit the 5-LOX pathway. Direct approaches, on the one hand, involve (i) redox inhibitors or antioxidants, which interfere with the redox cycle of 5-LOX, (ii) iron-chelator agents, and (iii) non-redox competitive inhibitors, which compete with AA to bind the enzyme active site. On the other hand, indirect inhibitors, namely 5-LOX activating protein (FLAP) inhibitors, can prevent 5-LOX from interacting with FLAP and, therefore, inhibit the leukotrienes biosynthesis. Rational design of iron-chelator inhibitors, widely described by hydroxamic acids, one of the most powerful metal ligand groups and N-hydroxyurea derivatives, has been more fruitful. Zileuton was the first 5-LOX inhibitor brought to market for the treatment of bronchial asthma. This compound, however, has shown a variety of

adverse effects, including hepatic toxicities and drug interactions. Further studies yielded potent analogues with better pharmacokinetics. Atreleuton, for instance, has entered phase III clinical trials [12-13]. Although the positional specificity of arachidonic acid oxygenation is decisive for mammalian LOX classification, the structural reasons for the variation of this enzyme property remain unclear. The crystal structures of two plants and one mammalian LOX suggest a U-shaped hydrophobic substrate-binding pocket containing the catalytic non-heme iron. Site-directed mutagenesis studies identified Phe-353, Ile-418 and Met-419 as well as Ile-593 as sequence determinants of the reticulocyte-type 15-LOXs and similar experiments on various 12-LOX isoforms confirmed these results. More recently, Tyr-603 and His-604 have been identified as critical amino acids for the positional specificity of the murine epidermis 8S- and the human epidermis-type 15S-LOX isoforms. Unfortunately, neither specificity-related mutagenesis data nor x-ray coordinates are currently available for the pharmacologically most relevant human leukocyte 5-LOX [14-15].

Experimental Section

Data Set

In QSAR analysis, it is imperative that the biological data be both accurate and precise to develop a meaningful model. The overall

performance of the current method used for QSAR study is critically depends on the selection of compounds for series used to build the classifier model. The most critical aspect of the construction of the series is to warrant a great molecular

Diversity in this data set. The 5-lipoxygenase inhibitors activity data of benzoxazole derivatives were taken from the reported work of Hyunmin Song, 2010. The list of reported compounds with their IC_{50} values was reported in table 1. The biological activity data (IC_{50} in μM) was converted to negative logarithmic dose (IC_{50} in moles) for QSAR analysis. For the external validation of QSAR models, the molecules were rationally divided into training having 9 and test set having 5 compounds on the basis of structural diversity and cover the complete range of variations in inhibitory activity as the guidelines for dividing into training and test sets. The observed and calculated biological activity values of training set compounds and observed and calculated biological activity values of test set compounds was reported in table 2 & table 3 respectively.

Molecular Structure Generation

The studies of benzoxazole derivatives were performed using Chemoffice 2003 version 6.0 supplied by Cambridge Software Company, USA. All the molecules were sketched using Chem Draw Ultra module. The two-

dimensional (2D) structures were transformed into three dimensional (3D) structures by using the Chem3D Ultra module. The resulting 3D structures were then subjected to an energy-minimization by using the molecular mechanics (MM2) method. The energy minimized molecules were re-optimizing using molecular orbital package (MOPAC). The numerical descriptors are responsible for encoding important features of the structure of the molecules and can be categorized as electronic, steric, and thermodynamic characters. The thermodynamic, spatial, electronic, and topological descriptors were calculated for QSAR analysis. The thermodynamic parameters describe free energy change during drug receptor complex formation. Spatial parameters were quantified for steric feature of drug molecules required for its complimentary fit with the receptor. Electronic parameters describe weak non-covalent bonding between drug molecules and the receptor.

Division of Test and Training Set

It is proven that the only way to estimate the true predictive power of a model is to test it on a sufficiently large collection of compounds from an external test set. The test set includes three compounds, whose activities and structure must cover the range of activities and structures of compounds

from the training set. This application is necessary for obtaining trustful statistics for comparison between the observed and predictive activities for these compounds. In this series 3 compounds were selected as a test set. This set used for the validation of model.

Statistical Analysis

Statistical methods are an essential component of QSAR work. They help to build models, estimate a model's predictive abilities, and find relationships and correlations among variables and activities. The contribution of descriptors to biological activity (BA) was studied using simple linear regression analysis by VALSTAT Software and, due to the problem of collinearity among descriptors, different combinations of descriptors were subjected to sequential and stepwise multiple regression analysis. The regression methods are used to build a model in the form of an equation that gives relationship between dependent variable (usually activity) and independent variable ("descriptors"). The model can then be used to predict activities for new molecules.

Results and Discussion

When data set of 14 compounds was subjected to stepwise multiple linear regression analysis, in order to develop QSAR

model, several model were obtained. The final set of equation was obtained using 11 compounds and the best equation was obtained by using the optimal combination of descriptors. Descriptors were selected for the final equation having intercorrelation coefficient below 0.5 were considered. The best QSAR model has characters of large F, low error S, low p-value, r^2 and q^2 value close to 1, as well as $P < 0.001$. The large F means proposed regression model fits the data well. The low error means less standard deviation of the sampling distribution associated with the estimation method. The lower the p-value, more "significant" the result is, in the sense of statistical significance. The r^2 and q^2 value close to 1 means model explained well the activity variations in the compounds.

The stepwise development of model along with changes in statistical qualities on gradual addition of descriptors was done.

Model 1

BA = [0.375564 (± 0.692891)] +BE [0.0304004 (± 0.0195427)] +VDW [-0.0497677(± 0.0583655)] +DE [-0.236217(± 0.12585)]

n=9, $r=0.84307$, $r^2=0.710767$, $r^2_{adj}=0.537227$, std = 0.268773, F=4.0957

Model 1 explains only 71.0% variance in the lipoxxygenase inhibitory activity. It shows that descriptor bending energy (BE) contribute positively; whereas Van der Waals Energy (vdW) and dipole energy (DE) contribute

negatively towards lipoxxygenase inhibitory activity. It is not a very good significant equation, therefore new model required for good explained variance.

Model 2

BA = [2.57591(± 0.841908)] +HOF [-0.0143185(± 0.00911936)] +VDW [-0.203354(± 0.0688653)] +DE [-0.208376(± 0.121798)]

n=9, $r=0.844113$, $r^2=0.712527$, $r^2_{adj}=0.540043$, std = 0.267955, F=4.13097

Model 2 explains only 71.2% variance in the lipoxxygenase inhibitory activity. It shows that descriptor heat of formation (HOF) contribute positively; whereas Van der Waals Energy (vdW) and dipole energy (DE) contribute negatively towards lipoxxygenase inhibitory activity. It is not a very good significant equation, therefore new model required for good explained variance.

Model 3

BA = [0.835113(± 0.277734)] +ST E [0.0523565 (± 0.0131288)] + NON VDW [0.346521(± 0.177004)] +DE [-0.306948(± 0.107449)]

n=9, $r=0.894972$, $r^2=0.800974$, $r^2_{adj}=0.681558$, std = 0.222955, F=6.70745

Model 3 explains only 80.0% variance in the lipoxxygenase inhibitory activity. It is not a very good significant equation, therefore new model required for good explained variance. In this equation stretch energy (ST E) & Non-

vander Waals Energy (NVDW) contribute positively, where as dipole energy (DE) contribute negatively towards lipoxxygenase inhibitory activity.

Model 4

$$BA = [0.0388579(\pm 0.230759)] + BE [0.0655056(\pm 0.0150785)] + NON VDW [0.348897(\pm 0.164378)] + DE [-0.310773(\pm 0.100613)]$$

$n=9$, $r=0.908697$, $r^2=0.82573$,
 $r^2_{adj}=0.721168$, $std = 0.208629$, $F=7.89702$

Model 4 explains only 82.5% variance in the lipoxxygenase inhibitory activity. It is not satisfactory significant equation, therefore new model required for good explained variance. Eq. shows bending energy (BE) and Non-vander Waals Energy (NVDW) contribute positively, where as dipole energy (DE) contribute negatively towards lipoxxygenase inhibitory activity.

Model 5

$$BA = [14.5327 (\pm 3.76513)] + HOF [-0.0695172(\pm 0.0188886)] + TOR [0.248581(\pm 0.07699)] + VDW [-0.978699(\pm 0.250614)]$$

$n=9$, $r=0.923182$, $r^2=0.852265$,
 $r^2_{adj}=0.763624$, $std = 0.19209$, $QF=4.80599$,
 $PE=0.03283$, $F=9.61479$

The r^2 -value accounts for 85.2% variance in observed activity value. Therefore model 5 is the best equation in the QSAR study. The r^2 value can be easily increased by increasing the number of descriptors in the model, so

cross validated correlation coefficient (q^2) was used as a parameter to select the optimum number of descriptors. Model shows that HOF is a thermal descriptor, which explains the significance of orientation and conformation rigidity of the molecule. The positive coefficients of this descriptor suggest the presence of bulky substituent oriented towards X-axis of the molecules will give better activity. The Lipophilic parameter, partition coefficient (P), denotes direct relationship to solubility in aqueous phase, to membrane permeation, and its entropic contribution to binding & it is positively correlated means groups which are lipophilic nature have enhance the activity of the compound. Heat of Formation (HOF) is responsible for the stability of the compounds and it is positively correlated. Anything which can affect the bond properties and strength of the bonds in the molecule can affect the value of HOF of that molecule of them, the number of atoms and number of the bonds and order of the bonds, and number of non-organic elements (heavy atoms) in a molecule directly affect on the value of HOF. Number of atoms which are commonly existed in all molecules such as oxygen and fluorine atoms, and even heavy atoms affect HOF of a molecule. Decrease in the number of these atoms in a molecule, increases HOF of that molecule. The torsional energy (TOR) & van der Waals energy (VDW), a thermodynamic property, denotes the sum of the angle-bending terms of

the force-field equation, & it is negatively correlated, which is indicative of deformation of the structure. The developed QSAR model can be utilized for the further designing of new compounds having 5-LOX inhibitory activity. It interacts with a small membrane protein designated 5-LOX activating protein (FLAP). This protein transfers arachidonic acid to 5-LOX and this interaction between the enzyme and FLAP being crucial for cellular leukotriene biosynthesis, as it reveals the various physico-chemical parameters that play important roles in exhibiting potential 5-LOX inhibitory activity.

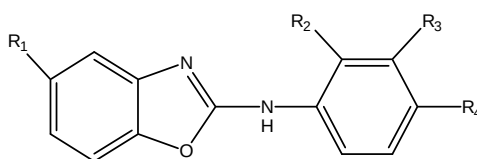
Conclusion

It was observed from the selected QSAR models that biological activities of derivatives are governed by thermodynamic, electronic and steric properties of the molecules. The models also suggest about the groups that responsible to increase the activity. This information can be explored for the designing of new molecules having better antiasthmatic activity.

Acknowledgement

Authors are thankful to institute for providing the necessary facilities & guidance to carry out this research.

Table 1: Structure and in vitro 5-LOX inhibition of synthesized compounds



Compounds	R ₁	R ₂	R ₃	R ₄	IC ₅₀	B.A.
2a	H	H	H	H	8.91	0.9499
2b	H	H	H	CH ₂ CH ₃	1.21	0.0828
2c	Cl	H	H	H	4.50	0.6532
2d	Cl	H	H	CH ₂ CH ₃	0.95	-0.0223
2e	NO ₂	H	H	CH ₂ CH ₃	23.88	1.3780
2f	CH ₃	H	H	H	4.60	0.6628
2g	CH ₃	H	H	CH ₂ CH ₃	0.12	-0.9208
2h	CH ₃	CH ₂ CH ₃	H	H	5.66	0.7528
2i	CH ₃	H	H	SCH ₃	7.31	0.8639
2j	CH ₃	H	O CH ₃	O CH ₃	6.83	0.8344
2k	OCH ₃	H	H	H	7.88	0.8965
2l	OCH ₃	H	H	CH (CH ₃) ₂	2.64	0.4216
2m	OCH ₃	H	H	SCH ₃	2.77	0.4425
2n	OCH ₃	H	H	H	2.03	0.3075

Table 2: Observed and calculated biological activity values of training set compounds

Compounds	Calculated BA	Observed B.A.
2a	0.7675	0.9499
2d	0.4937	-0.0223
2e	1.3412	1.3780
2f	0.6193	0.6628
2h	0.9276	0.7528
2j	0.9811	0.8344
2k	0.6669	0.8965
2l	0.4133	0.4216
2n	-0.0305	0.3075

Table 3: Observed and calculated biological activity values of test set compounds

Compounds	Calculated BA	Observed B.A.
2b	0.2086	0.0828
2c	0.9718	0.6532
2g	0.0676	-0.9208
2i	-0.1977	0.8639
2m	0.2536	0.4425

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