**2D and 3D QSAR Analysis of Imidazole Derivatives as Heme Oxygenase Inhibitor**

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**ABSTRACT**

Selective inhibition of heme oxygenase is an important strategy in design of potent inhibitors of enzyme for the treatment of neonatal jaundice, cancer and many more. QSAR analysis is employed for a given set of compounds containing imidazole pharmacophore in order to establish a relationship between the biological activity and related descriptors, which provides us an idea to gain a potent inhibitor with lesser side effects. In this paper we present results of 2D and 3D QSAR studies of series of 26 molecules containing imidazole pharmacophore as selective heme oxygenase inhibitor using V Life MDS 3.5 Software. The 2D QSAR studies was performed using Partial Least Square Regression method and the 3D QSAR studies was performed using k- Nearest Neighbor Molecular Field Analysis(kNN- MFA) method. The analysis has produced good predictive and statistically significant QSAR models. 2D QSAR studies produced good statistical model with $r^2$ value 0.8487, cross validated $r^2$ value 0.6553 and pred_r$^2$ value 0.7478 by PLSR method while 3D QSAR model gave statistical value of cross validated $r^2$ value 0.5493 and pred_r$^2$ value 0.3358. The results of the QSAR analysis suggested that the 2-D descriptor viz. physicochemical and alignment independent played an important role for heme oxygenase inhibition and the 3-D descriptors electrostatic and steric revealed the relative positions and range for substitution in a molecule. In 3D model, grid suggested that a positive electrostatic potential is favorable for increase in biological activity and the steric field with negative range and the negative range indicates that negative steric potential is favorable for increase in the activity. Thus, the descriptors generated by 2-D and 3-D QSAR analysis were useful in designing of potent molecules.

**Key words:** Heme Oxygenase Inhibitor; HO-1; 2D QSAR; 3D QSAR; kNN-MFA

**1. INTRODUCTION**

Heme Oxygenase (HO) (EC 1.14.99.3) catalyses the first and rate-limiting step in the oxidative breakdown of heme to carbon monoxide (CO), biliverdin (which is rapidly reduced to bilirubin), and ferrous iron.\(^1\)\(^-\)\(^4\)\(^,\)\(^5\) Heme Oxygenase Inhibitor is highlighten in case of neonatal jaundice,\(^6\)\(^-\)\(^8\) intracerebral hemorrhage,\(^9\)\(^-\)\(^11\) as an anticancer agent\(^12\)\(^-\)\(^14\) and many more. Various Heme Oxygenase inhibitors are being made till date like metalloporphyrins\(^15\)\(^-\)\(^17\) and imidazole-dioxolane compounds.\(^18\)\(^-\)\(^21\) Our present focus is to make a potent inhibitor which is helpful to us in the neonatal jaundice which is a severe pathological condition exhibited in neonates, and can also lead to drastic condition of brain damage i.e.‘kernicterus’. Also, our main emphasis is to examine potential applications of pharmacologic inhibitors of HO activity as therapeutic agents in the context of disease processes associated with excessive activation of heme oxygenase system. So, there is a need to design and screen heme oxygenase inhibitors with higher bioactivities. There is a need to analyze the correlation between heme oxygenase inhibitor activity and physico-chemical parameters of each category of compounds using the Quantitative Structure Activity Relationship (QSAR) methods because the quantitative analysis of such molecules can be utilized for increasing the potency and minimizing the side effects.
Imidazole is an organic compound with the formula C₅H₅N₂. This aromatic heterocyclic is classified as an alkaloid. Imidazole ring system is present in important biological building blocks such as histidine, and the related hormone histamine. It has a wide range of pharmacological activity like antifungal, anticytobacterial, antiprotozoal, analgesic, antancer, angiotensin antagonist, antidepressant, antihistaminic, and as heme oxygenase inhibitor. Various researches are being going on imidazole-dioxolane compounds as heme oxygenase inhibitor, so QSAR analysis will help us to predict some newer potent molecule in order to study and establish a correlation between structure and biological activity of imidazole-dioxolane, as heme oxygenase inhibitors.

2. MATERIALS AND METHODS

2.1 Data Set

The Heme Oxygenase Inhibition of imidazole-dioxolane, figure 1 has been reported by Vlahakis et al., in terms of inhibitory concentration 50% of enzyme Heme Oxygenase-1 [HO-1] (IC₅₀ in micromoles). One of the compounds of the series has no well defined activity, so the QSAR study was performed on a set of 25 molecules. The enzyme inhibition data were converted to negative logarithmic pIC₅₀ to reduce skewness of dataset and then used for subsequent QSAR analysis as dependent variables. The structures of all imidazole analogues with HO-1 inhibitory activity are presented in Table no-1. All computational studies were performed using V-Life Molecular Design Software Version 3.5. The sketched structures were then exported to three dimensional structures (3D). The geometries of generated 3D structures were optimized using Merck Molecular Force Field (MMFF) fixing Root Mean Square Gradients (RMS) to 0.01 Kcal/mol Å as implemented in the V-Life MDS 3.5.

2.2 2D QSAR

The QSAR models were generated by using biological activity as dependent variable and descriptors as independent variables. The series of compounds were divided into test and training set for the generation of models. In present study, manual selection method is applied, using Partial Least Square [PLS], with forward- backward variable selection method. The program employs a stepwise technique, i.e., only one parameter at a time was added to a model and always in the order of most significant to least significant in terms of F-test values. Statistical parameters were calculated subsequently for each step in the process, so the significance of the added parameter could be verified. The goodness of the correlation is tested by the regression coefficient (r²), the cross-validated squared correlation co-efficient (q²), the F-test and the standard error of estimate (SEE). The correlation coefficient values closer to 1.0 represent the better fit of the model. The F-test reflects the ratio of the variance explained by the model and the variance due to the error in the model (i.e., the variance not explained by the model). High values of the F-test indicate that the model is statistically significant. The predictive r² (r²pred) was calculated for evaluating the predictive capacity of the model. The value of prdr² ≥ 0.5 indicates the good predictive capacity of the QSAR model. It has been observed that the values of statistical parameters like q² ≥ 0.5 and prdr² ≥ 0.5 was not achieved in models generated, also from the fitness plot of the generated model, 2 molecules were considered outliers, so it has been removed from the data set and further the models were regenerated. After removal of outliers, a good statistical result was observed.

2.3 3D QSAR

Molecular alignment utility allows alignment of two or more molecules in a dataset with respect to selected template or with respect to a particular set of atoms explicitly selected in every molecule of the data set. The resulting set of aligned molecules can then be used in the 3D-QSAR for building quantitative models to predict new molecules having the similar template or set of atoms. Molecular alignment is also used in visualizing the structural diversity in the given set of molecules. Template Based alignment method was used for the generation of model. Template Based Alignment feature performs alignment of set of molecules based on given template. A reference molecule is selected for defining coordinates to align rest of the molecules. The aligned molecules are automatically stored in the reference folder. Set of molecules lacking common template can also be aligned based on a set of atoms selected in same order in all the molecules of the set. In the present study imidazole template was considered (figure 2) and the alignment was observed (figure 3).

Aligned molecules were used to calculate 3-D descriptor with biological activity as dependent variable. Before calculation of 3-D descriptors following values and parameters are to be fixed like field type as electrostatic, steric and hydrophobic. Charge type set as Gasteiger- Marsili, dielectric constant as 1.0 with distance dependent dielectric function. A sp3 carbon atom and +1.0 charge was served as the probe atom to calculate steric and electrostatic fields. The grid setting is given below (Table 2).

The QSAR models were generated using k-nearest neighbor method (kNN) of V Life molecular design suite. The test set of 7 molecules and a training set of 16 molecules were subjected to 3D QSAR analysis. The kNN-MFA model provided direction for the design of new molecules in a rather convenient way. The kNN-MFA model show the grid which shows the point contributes stepwise kNN-MFA. The range of property values for the chosen points may aid in the design of new potent molecules.
The range is based on the variation of the field values at the chosen points using the most active molecule and its nearest neighbor set.

3. RESULTS & DISCUSSION

3.1 2D-QSAR studies

QSAR analysis on a series of imidazole-dioxolane was performed by using V-Life software. The physiochemical descriptors and inhibitory activity was taken as independent and dependent variables respectively. Correlations were established between the biological activity and calculated molecular physiochemical descriptors through Partial Least Square regression (Stepwise forward-backward). The summary of model is given below:

**Equation:** PIC50 = -1.2583 T_N_O_7 -0.0075 Mol.Wt. + 0.1232 SsNH2E-index + 0.5912 T_O_F_5 + 3.0766

**Statistics:** n= 18; r² = 0.8487, q² = 0.6553, prd r² = 0.7478, r²se = 0.2055, q²se = 0.3102, prd r² se = 0.1988, F test = 42.0756.

The statistics of the generated model explains 84.87% (r² = 0.8487) of the total variance in the training set as well as it has internal (q²) and external (prd r²) predictive ability of 65% and 74% respectively. The low standard error r²se = 0.2055, q²se = 0.3102 and prd r²se = 0.1988 demonstrates accuracy of the model. The F test = 42.0756 shows the statistical significance of 99.99% of the model which means that probability of failure of the model is 1 in 10000.

The descriptors: mol. wt. signifies molecular weight of compounds, SsNH2E-index signifies Electro topological state indices for number of –NH₂ group connected with one single bond, T_N_O_7 signifies the count of number of nitrogen atom separated from oxygen atom by 7 bonds in a molecule, T_O_F_5 signifies the count of number of oxygen atom separated from fluorine atom by 5 bonds in a molecule. The developed PLSR model reveals that the descriptors T_N_O_7, Mol. Wt., SsNH2E-index and T_O_F_5 were highly correlated to biological activity (Figure 4). The descriptor T_N_O_7 (i.e. the count of number of nitrogen atom separated from oxygen atom by 7 bonds in a molecule) plays most important role (-39.28%) and is inversely proportional to the biological activity. A negatively correlated descriptor Mol. Wt. (-24.68%) shows that a decrease in it will lead to increase in activity. The descriptor SsNH2E-index (22.59%) is directly proportional to the activity; this descriptor signifies electro topological state indices for number of –NH₂ group connected with one single bond.

Finally descriptor T_O_F_5 (13.45%) is an influential descriptor and is directly proportional to the activity which signifies the count of number of oxygen atom separated from fluorine atom by 5 bonds in a molecule.

The plot of observed versus predicted activity (Figure 5) showed that the model is able to predict the activity of training set quite well (all points close to the regression line) as well as external test set (all points close to the regression line) providing confidence in predictive ability of the model.

Unicolumn Statistics is a method to analyze the descriptor data to check data spread by calculating mean and standard deviation for both training dataset as well as test set. The result of Unicolumn statistics for model is given in Table No. 3.

The min and max values in both training and test should be compared in a way that:

- The max of the test should be less than max of training set.
- The min of the test should be greater than min of training set.

Unicolumn statistics of model shows that the test set is interpolative i.e. derived within the min-max range of the training set. The mean and standard deviation of the training and test set provides insight to the relative difference of mean and point density distribution (along mean) of the two sets. Also, a relatively higher standard deviation in training set indicated that training set has widely distributed activity of the molecules as compared to the test set. Correlation matrix showed the measure of dependence between the two descriptors (Table No.4). The actual and predicted activity with residual of model is shown in Table 5.

3.2 3D-QSAR studies

3-D QSAR analysis was performed using kNN – MFA with stepwise forward-backward variable selection method. The model summary is given in Table 6.

The model explains internal (q² = 0.5493) as well as external (prd r² = 0.3418) model validation and prediction. The model consisted of two electrostatic descriptor and one steric descriptor with kNN (k=2). The electrostatic and steric descriptors at the grid showed the relative position and ranges in the model providing guidelines for new molecule design (Figure- 6,7). Positive range in the electrostatic descriptor means that a positive electrostatic potential is favorable for biological activity. Negative range in the steric descriptors means that a less bulky substitution is favorable for biological activity. The actual and predicted activities with residual values are shown in Table 7.
Table 1. Imidazole analogues with HO-1 inhibitory activity

![Chemical structure of imidazole dioxane](image)

| S.No. | R                      | IC$_{50}$ (µM) of HO-1 | pIC$_{50}$ of HO-1 |
|-------|------------------------|------------------------|--------------------|
| 01.   | Phenyl sulphanyl       | 1.03                   | -0.01283           |
| 02.   | 4-aminophenyl sulphanyl| 0.33                   | 0.48148            |
| 03.   | 2-aminophenyl sulphanyl| 4                      | -0.60205           |
| 04.   | 3-aminophenyl sulphanyl| 4                      | -0.60205           |
| 05.   | Pyridin-4-yl sulphanyl | 2.5                    | -1.39794           |
| 06.   | 4-hydroxyphenyl sulphanyl| 1.59                  | -0.20139           |
| 07.   | 4-bromophenyl sulphanyl| 2.1                    | -0.32221           |
| 08.   | 4-methoxyphenyl sulphanyl| 0.7                   | 0.15490            |
| 09.   | 4-chlorophenyl sulphanyl| 2.8                    | -0.44715           |
| 10.   | 4-fluorophenyl sulphanyl| 2.2                    | -0.34242           |
| 11.   | 4-nitrophenyl sulphanyl| 6                      | -0.77815           |
| 12.   | (5-trifluoromethyl)pyridin-2-yl sulphanyl| 2.1                    | -0.32221           |
| 13.   | cyclohexyl sulphanyl   | 0.94                   | 0.02687            |
| 14.   | naphthalene-2-yl sulphanyl| 0.9                   | 0.04575            |
| 15.   | 3-bromophenyl sulphanyl| 5                      | -0.69897           |
| 16.   | 2-bromophenyl sulphanyl| 6                      | -0.77815           |
| 17.   | 4-aminophenoxyl         | 1.4                    | -0.14612           |
| 18.   | 4-hydroxyphenoxy       | 1.8                    | -0.25527           |
| 19.   | Phenoxy                | 0.59                   | 0.22914            |
| 20.   | 4-bromophenoxy         | 3.5                    | -0.54406           |
| 21.   | 4-fluorophenoxy        | 0.28                   | 0.55284            |
| 22.   | Biphenyl-4-yl oxy      | 2                      | -0.30102           |
| 23.   | 4-methoxyphenoxy       | 1.33                   | -0.12385           |
| 24.   | 4-iodophenoxy          | 9                      | -0.95424           |
| 25.   | 4-cyanophenoxy         | 0.67                   | 0.17392            |
Table 2. Grid Settings.

| Grid Setting | From         | To           | Interval |
|--------------|--------------|--------------|----------|
| X            | -4.881500    | 20.057900    | 2.0000   |
| Y            | -7.056700    | 16.736500    | 2.0000   |
| Z            | -7.959000    | 8.692700     | 2.0000   |

Table 3. Unicolumn Statistics

| Column name | Average | Max   | Min   | Std Dev. | Sum |
|-------------|---------|-------|-------|----------|-----|
| Training    | pIC₅₀   | -0.2811 | 0.5528 | -1.3979  | 0.4962 | -5.0592 |
| Test        | pIC₅₀   | -0.4011 | 0.1739 | -0.7782  | 0.3725 | -2.0056 |

Table 4. Correlation matrix of Descriptors

| Mol. Wt.       | Mol. Wt.       | SsNH2E-index | T_N_O_7 | T_O_F_5 |
|----------------|----------------|--------------|---------|---------|
| Mol. Wt.       | 1              | -0.18064     | 1       |         |
| SsNH2E-index   | -0.18064       | 1            |         |         |
| T_N_O_7        | -0.25501       | 0.443648     | 1       |         |
| T_O_F_5        | -0.21914       | -0.08574     | -0.08575| 1       |

Table 5. Actual and predicted activity [pIC₅₀] with residual of model

| Molecule | Actual (pIC₅₀) | Prediction | Residual |
|----------|----------------|------------|----------|
| DG01     | -0.01283       | -0.01565   | 0.00282  |
| DG02     | 0.48148        | 0.588312   | -0.106832|
| DG03'    | -0.60205       | -0.62844   | 0.02639  |
| DG04     | -0.60205       | -0.65409   | 0.05204  |
| DG05     | -1.39794       | -1.28131   | -0.11663 |
| DG06     | -0.20139       | -0.13487   | -0.06652 |
| DG07     | -0.32221       | -0.60358   | 0.28137  |
| DG08     | 0.1549         | -0.2394    | 0.39430  |
| DG09     | -0.44715       | -0.27233   | -0.17482 |
| DG10     | -0.34242       | -0.14971   | -0.19271 |
| DG11     | -0.77815       | -0.35097   | -0.42718 |
| DG12     | -0.32221       | -0.52973   | 0.20752  |
| DG13     | 0.02687        | -0.06071   | 0.08758  |
| DG15     | -0.69897       | -0.60358   | -0.09539 |
Table 6. Statistics value of 3-D model

| kNN = 2 | n = 16 | Degree of freedom = 12 |
|---------|--------|------------------------|
| $q^2 = 0.5493$ | $q^2 se = 0.3557$ |                      |
| Prd $r^2 = 0.3558$ | Prd $r^2 se = 0.3418$ |                      |
| Descriptor Range: E_834 (2.9876, 3.2702) |                       |
|             | E_932 (9.2356, 9.9801) |                       |
|             | S_601 (-0.5045, -0.5044) |                       |

Table 7: Actual and predicted activities [pIC_{50}] with residual values for the 3D-model

| Molecule | Actual (pIC_{50}) | Prediction | Residual |
|----------|-------------------|------------|----------|
| DG01     | 0.01283           | -0.23676   | 0.22393  |
| DG02*    | 0.48148           | -0.10683   | 0.58831  |
| DG03*    | -0.60205          | -0.74143   | 0.139384 |
| DG04*    | -0.60205          | -0.7328    | 0.130754 |
| DG05     | -1.39794          | -0.67523   | -0.722712|
| DG06     | -0.20139          | -0.08955   | -0.111837|
| DG07     | -0.32221          | -0.39752   | 0.075311 |
| DG08*    | 0.1549            | -0.10674   | 0.261642 |
| DG09     | -0.44715          | -0.33222   | -0.114935|
| DG10     | -0.34242          | -0.38733   | 0.044905 |
| DG11     | -0.77815          | -1.13374   | 0.35559  |
| DG12*    | -0.32221          | -0.38462   | 0.062407 |
| DG13     | 0.02687           | 0.189304   | -0.162434|
| DG15     | -0.69897          | -0.77813   | 0.079162 |
| DG16     | -0.77815          | -0.68554   | -0.092608|
| DG18*    | -0.25527          | -0.75054   | 0.495265 |
| DG19     | 0.22914           | -0.10923   | 0.338373 |
| DG20     | -0.54406          | -0.91203   | 0.367971 |
| DG21     | 0.55284           | 0.080772   | 0.472068 |
| DG22*    | -0.30102          | -0.32609   | 0.025066 |
| DG23     | -0.12385          | 0.360731   | -0.484581|
| DG24     | -0.95424          | -0.52521   | -0.429033|
| DG25     | 0.17392           | 0.337069   | -0.163149|

* indicates compounds of test set
**Figure 5:** Fitness plot between actual and predicted activities for model training set (red spots) and test set (blue spots)

**GRID OF 3D- QSAR MODEL:**
Figure 6: Distribution of point in the SW kNN-MFA
4. CONCLUSION

An imidazole-dioxolane compound has emerged as a potential therapeutic agent for Heme Oxygenase inhibition. So the present work focused on the QSAR analysis on some novel imidazole derivatives for the development of potent Heme Oxygenase inhibitor with the help of software. The 2-D QSAR analysis suggested four descriptors, Molecular weight, SsNH2E index, T_N_O_7, and T_O_F_5 contributed to the biological activity. Out of the four descriptors Molecular weight and T_N_O_7 contributed negatively while SsNH2E index and T_O_F_5 contributed positively. The model showed good statistical values and correlation matrix of the descriptors. The 3-D descriptors electrostatic and steric revealed the relative positions and range for substitution in a molecule. In 3D model, grid suggested that a positive electrostatic potential is favorable for increase in biological activity and the steric field with negative range and the negative range indicates that negative steric potential is favorable for increase in the activity. So, with the help of 2D and 3D data, some new molecules can be designed in order to get a potent compound.

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