ISSN 2349-7203

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

HUMAN

Human Journals
Research Article
July 2015 Vol.:3, Issue:4

Predicting the Anti-Inflammatory Activity of Novel 5-PhenylsulfamoyI-2-(2-(Nitrooxy) (Acetoxy)Benzoic Acid Derivative using 2D and 3D-QSAR (kNN-MFA) Analysis

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ISSN 2349-7203
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Dr. Anwar Rafique Shaikh[1]*, Malik Amber[1], Imadulislam[1], Ziyaul Haque[2], Dr. Sanjay J. Kshirsagar[1], Kankate R. S[1], Yogita B. Thombare[1]

[1]Bhujbal Knowledge city, MET's Institute of pharmacy, Adgaon, Nashik-422003

[2]AliAllana College of Pharmacy, Jamia Isalamia Isha-atul Uloom's Ali-Allana College of Pharmacy, Akkalkuwa.

Submission: 26 June 2015
Accepted: 3 July 2015
Published: 25 July 2015

Keywords: anti-inflammatory agent, salicylic acid, 2D QSAR, 3D QSAR, kNN-MFA, VLife MDS

ABSTRACT

2D-QSAR and 3D-QSAR studies by means of MLR, PLS and PCR were performed on a series of salicylic acid derivatives for anti-inflammatory activity using software MDS 4.2 version (VLife Science). This study was performed with 19 compounds (Data set) using random as well as manual data selection methods for the division of the data into training and test set. MLR methodology with stepwise forward-backward variable selection method was used for building the QSAR models. Statistically significant QSAR models were generated. Among them most significant model has squared correlation coefficient (r^2) = 0.9236, cross validated correlation coefficient (q^2) = 0.8360 and predictive correlation coefficient (pred_r^2) = 0.3738 for the anti-inflammatory activity. The idea of the present study is the search for novel salicylic acid analogues that would show promise to be useful in the anti-inflammatory agent.

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1. INTRODUCTION

Most NSAIDs are weak organic acids. Once absorbed, they get bound to serum albumin. Due to increased vascular permeability in localised sites of inflammation, this high degree of protein binding may result in the delivery of higher levels of NSAIDs. They are a chemically diverse group.

The primary effect of NSAIDs is to inhibit COX enzyme, thereby blocking the transformation of arachidonic acid to prostaglandins, prostacyclin and thromboxanes. These result in complex effects on vascular permeability and platelet aggregation, undoubtfully contributing to the overall clinical effects of these compounds (Abadi at all 2005).

COX 1, or prostaglandin synthase H, is a house keeping enzyme that regulates normal cellular functions and is stimulated by hormones and growth factors. It is constitutively expressed in most tissues and is inhibited by NSAIDs in varying degrees. COX 1 is important in maintaining the integrity of the gastric and duodenal mucosa and many of the side effects of NSAIDs on the gastrointestinal tract are attributed to its inhibition (Furst and Ulrich, 2007).

COX 2 or prostaglandin synthase H_2 is an inducible enzyme and is usually not detectable in most tissues. Its expression is increased during states of inflammation or experimentally in response to mitogenic stimuli. Its expression is inhibited by glucocorticoids. COX-2 is also inhibited by all of the presently used NSAIDs, to a lesser or greater degree. Thus, differences in the effectiveness with which a particular NSAID inhibits an isoform of COX may affect with its activity and its potential toxicity. It has been proposed that the ideal NSAID would inhibit the inducible COX-2 alone, without having any effect on COX-1.

Our interest is in developing new effective medications as anti-inflammatory agent because of less toxicity in GIT. Pharmacological evaluation shows the NO_2 release of compound will decrease the GIT toxicity (Bandarage, et al, 2000).

The main objective of proposed plan was to design, synthesize & evaluate newer salicylate derivatives by introduction of nitroxy functional group to reduce the GI side effects (associated with aspirin) and substitution at 5-position to improve the anti-inflammatory profile. Gastrointestinal effects of non steroidal anti-inflammatory drug (NSAID) are still a significant

drawback to its use. To explore this deficiency, our aim was to perform 2D and 3D-QSAR analysis on a series of salicylic acid derivatives of Figure 1.

The purpose of the present study is to investigate the physicochemical parameters responsible for the anti-inflammatory effect of 5-phenylsulfamoyl-2-(2-(nitrooxy) (acetoxy)benzoic acid derivatives, explore the correlation between them and to obtain more information for designing novel substituted 5-phenylsulfamoyl-2-(2-(nitrooxy) (acetoxy)benzoic acid derivatives with potent protective activity. In the present investigation, three widely used techniques, viz. stepwise forward variable selection method, Genetic algorithm and simulated annealing have been applied for descriptor optimization and multiple linear regression analysis, principal component regression and partial least square has been applied for two and three-dimensional QSAR models development. The generated models provide insight into the influence of various interactive fields on the activity and, thus, can help in designing and forecasting the protecting effect of novel 5-phenylsulfamoyl-2-(2-(nitrooxy) (acetoxy)benzoic acid molecules (Bhandari, et al, 2009).

A series of 5-phenylsulfamoyl-2-(2-(nitrooxy) (acetoxy)benzoic acid analogues which were reported are chosen for QSAR study in order to establish quantitative relationship between physiochemical properties and biological activities of the compounds using MDS software (VlifeScience) [22].

2. MATERIALS AND METHODS

All molecular modelling studies (2D and 3D) were performed using the Molecular Design Suite (VLife MDS software package, version 3.5; from VLife Sciences, Pune, India), on a HP computer with a Pentium IV processor and a Windows 7 operating system. Structures were sketched using the 2D draw application and converted to 3D structure
(Figure 1, Table 1).

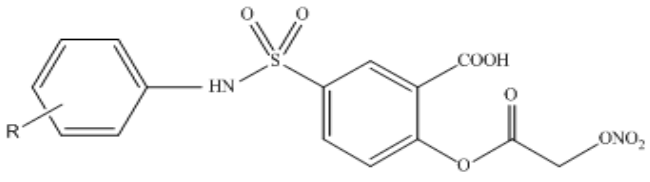


Figure 1: Parent Chemical structure of 5-phenylsulfamoyl-2-(2-(nitrooxy) (acetoxy)benzoic acid derivative

Table 1: **% Release of nitric oxide**

Sr. No.	Compound Code	Activity	
		Clogp[b]	% NO Release
1	F1	1.31	20.86
2	F2	1.05	11.23
3	F3	1.15	14.31
4	F4	1.05	11.26
5	F5	1.11	13.02
6	F6	1.06	11.51
7	F7	1.26	18.20
8	F8	1.25	17.82
9	F9	1.21	16.34
10	F10	1.19	15.74
11	F11	1.08	12.14
12	F12	1.05	11.25
13	F13	0.95	9.00
14	F14	1.15	14.40
15	F15	1.20	15.95
16	F16	1.29	19.90
17	F17	1.17	14.80
18	F18	1.08	12.30
19	F19	1.05	11.31

2.1 Biological data

The anti-inflammatory activity of 5-phenylsulfamoyl-2-(2-(nitrooxy) (acetoxy)benzoic acid derivative were taken from the reported work. The total set of compounds was divided into a training set for generating 2D and 3D QSAR models and a test set for validating the quality of the models. Selection of the training set and test set molecules was done on the basis of structural

diversity and a wide range of activity such that the test set molecules represent a range of biological activity similar to that of the training set; thus, the test set is truly representative of the training set. The biological activity values [IC50 (μM)] reported in micromolar units were converted to their molar units and then further to negative logarithmic scale and subsequently used as the dependent variable for the QSAR analysis. The values of IC_{50} along with the structure of the compounds in the series are listed in **Table 1**.

2.2 Molecular modelling for 2D QSAR

In 2D QSAR analysis, significant methods Multiple linear regression, principal component regression and partial least square were applied to generate the 2D-QSAR model. The 2D structures were converted to 3D structures by sending them to MDS software. Each compound was energy minimized and batch optimized by using Merck Molecular Force Field and charges followed by Austin Model-1. Hamiltonian method was available in MOPAC module with the convergence criterion 0.001 kcal/mol A˚ fixing Root Mean Square Gradients (RMS) to 0.001 kcal/mol A˚. 2D descriptors (physicochemical and alignment independent) were calculated for the optimized compounds on QSAR plus worksheet. The invariable descriptors (the descriptors that are constant for all the molecules) were removed, as they do not contribute to QSAR. Most stable structure for each compound was generated after energy minimization and used for calculating various physicochemical descriptors like thermodynamic, steric and electronic. The energy-minimized geometry was used for the calculation of the various 2D descriptors (Individual, Chi, ChiV, Path count, Chi Chain, ChiV Chain, Chain path count, Cluster, Path cluster, Kappa, Element Count, Estate number, Estate contribution, Semi-empirical, Hydrophilic– hydrophobic, Polar surface area and Alignment independent) and was considered as independent variables in the present study.

QSAR analysis was performed after removal of all the invariable columns, as they do not contribute to the QSAR. The optimal test and training data set were generated using the manual as well as random data selection method. Sphere exclusion method was also adopted for division of training and test set. Sphere exclusion method is used for creating training and test set from the data. This is a rational selection method which takes into consideration both biological and chemical space for division of data set. Dissimilarity value provides handle to vary train/test set size. It needs to be adjusted by trial and error until a desired division of train and test set is

achieved. As a rule, increase in dissimilarity value will lead to increase in number of molecules in the test set. All 13 molecules were subjected to regression analysis using Multiple linear regression analysis, as model building methods coupled with stepward forward backward variable selection method. Regression analysis was carried out for treatment of drug abuse disorders and the best model was cross-validated. Best two dimensional QSAR results obtained by multiple linear regression analysis (using random and manual data selection method), Partial Least Squares and Principal Component Regression are obtained by the following Table 2.1, 2.2 and 2.3 respectively.

Table 2.1: 2D QSAR model optimization by Multiple linear regression analysis (using Random selection method)

Model No. / Parameters	21
Test set	11,13,3,9
N	13
DOF	8
r^2	0.9236
q^2	0.8360
F-test	24.1907
r^2se	0.0290
q^2se	0.0424
pred_r^2	0.3738
pred_r^2se	0.1083

Table 2.2: 2D QSAR model optimization by Partial least square

Model No. / Parameters	25	44
Test set	11,12,6,7	13,3,4,8
Training set	1,2,3,4,5,8,9,10,13	1,2,5,6,7,9,10,11,12
Optimum components	2	2
N	13	13
DOF	10	10
r^2	0.9269	0.9564
q^2	0.7732	0.8991
F-test	63.3898	109.7579
r^2se	0.0243	0.0195
q^2se	0.0428	0.0298
pred_r^2	0.2028	0.5467
pred_r^2se	0.1259	0.0946

Table 2.3: 2D QSAR model optimization by Principal component regression methods

Model No. / Parameters	117
Test set	11,12,6,7
Training set	1,2,3,4,5,8,9,10,13
Optimum components	2
N	13
DOF	10
r^2	0.8437
q^2	0.7295
F-test	26.9877
r^2se	0.0398
q^2se	0.0523
pred_r^2	0.5481
pred_r^2se	0.0837

2.3. Three dimensional (3-D) QSAR studies

In the kNN-MFA method three models were generated for the selected members of training and test sets, and the corresponding best two models are reported herein. VLife Molecular Design Suite 3.5 allows user to choose probe, grid size, and grid interval for the generation of descriptors. The variable selection methods along with the corresponding parameters are allowed to be chosen, and optimum models are generated by maximizing q^2. k-Nearest neighbor molecular field analysis (kNN-MFA) requires suitable alignment of given set of molecules. To derive the kNN-MFA descriptor fields, a 3D cubic lattice with grid spacing of 2 A˚ in x, y, and z dimensions were created to encompass the aligned molecules. kNN-MFA descriptors were calculated using sp^3 carbon probe atom with a van der Waals radius of 2A˚ and a charge of +1.0 with default cut off energy 30 kcal/mol to generate steric field, electrostatic and hydrophobic fields. The steric, and electrostatic energy values were truncated at a default value of ±30 kcal/mol. The kNN-MFA steric, and electrostatic fields thus generated were scaled by the standard method in the software. The 3D-QSAR studies were performed by kNN-MFA using stepwise forward backward, simulated annealing selection method and genetic algorithm method. The software produced more than 7568 descriptors and prior to model development descriptors having zero values or same values were removed which resulted in more than total 2500 descriptors for all the compounds in separate columns. This algorithm allows constructing training sets covering all descriptor space areas occupied by representative points. kNN-MFA with simulated annealing and stepwise variable selection was employed for selection of variables to obtain the QSAR model. The standard leave one out (LOO) procedure was implemented to calculate cross validated r^2 (q^2) value, that is a molecule in the training set was eliminated and its biological activity was predicted as the weighted average activity of the k most similar molecules.

2.4. Molecular alignment

Molecular alignment was used to visualize the structural diversity in the given set of molecules. This was followed by generation of common rectangular grid around the molecules. The Atom based alignment method was used for alignment by considering the common elements of the series. kNN-MFA method requires suitable alignment of given set of molecules after optimization, alignment was carried out by Atom based alignment method (Fig. 2).

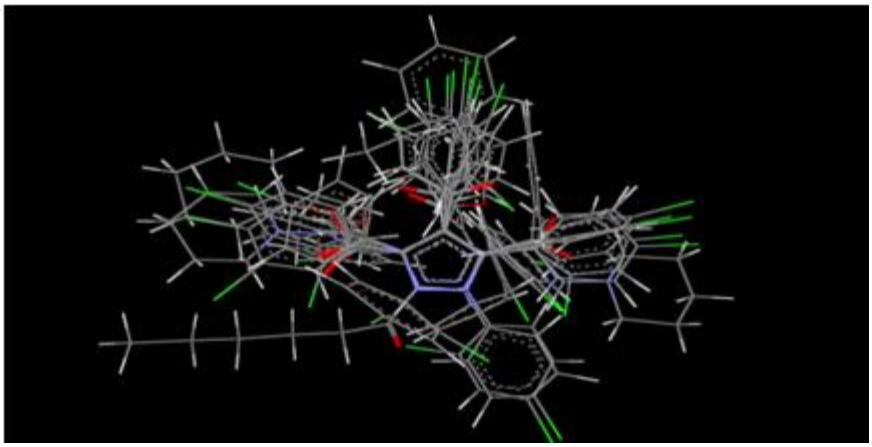


Figure 2: 3D view of aligned molecules by Atom based type of method of alignment

2.5. k-Nearest neighbor (KNN) method

The kNN methodology relies on a simple distance learning approach whereby an unknown member is classified according to the majority of its k-nearest neighbors in the training set. The nearness is measured by an appropriate distance metric (e.g., a molecular similarity measure calculated using field interactions of molecular structures). This method employs the kNN classification principle combined with the stepwise selection procedure for optimization of the number of nearest neighbors (k) used to estimate the activity of each compound and optimization of selection of variable from the original pool of all molecular descriptors (steric and electrostatic fields at the lattice points) that are used to calculate similarities between compounds). The descriptors that get selected in a given model are the field points either of steric or of electrostatic nature at particular locations in a common grid around reported set of molecules. For utilizing these descriptors for new ligand design, we consider the field values at different grid points of compounds cluster having most active compound. The extrema of field values of compounds in the cluster of most active compounds decide the range of field values which is preferred and recommended for new compound design.

2.5.1. kNN-MFA Stepwise (SW) forward variable selection

In stepwise (SW) forward backward variable selection algorithm, the search procedure begins with developing a trial model step by step with a single independent variable and to each step. Independent variables are added one at a time, examining the fit of the model by using the PLS cross-validation procedure. Thus, the model is repeatedly altered from the previous one by

adding or removing a predictor variable in accordance with the 'stepping criteria' (in this case F= 4 for inclusion; F=3.99 for exclusion for the forward–backward selection method). The method continues until there is no more significant variable remaining outside the model (Table 3).

Table 3: Best results of 3D QSAR model obtained by kNN-MFA method

Model No. / Parameters	20	36
kNN-MFA method	Stepwise Forward Backward (SWFB)	Genetic Algorithm (GA)
Test set	11,13,3,9	11,12,6,7
kNN	2	3
N	13	13
DOF	10	10
q^2	0.5374	0.6970
q^2se	0.0647	0.0442
pred_r^2	0.3548	0.0919
pred_r^2se	0.0948	0.1362
Descriptors	E_547 S_1176	S_469, E_937

2.6. Development and validation of QSAR models

Models were generated by using significant statistical methods, namely, Multiple linear regression (MLR). The multiple linear regression model and its estimation using ordinary least squares (OLS) is doubtless the most widely used tool. The multiple linear regression model assumes a linear (in parameters) relationship between a dependent variable y_i and a set of explanatory variables $x_i = (x_i0; x_i1; :::; x_iK)$. X_ik is also called an independent variable, a covariate or a regressor. The first regressor $x_i0 = 1$ is a constant unless otherwise specified. Multiple linear regression attempts to model the relationship between two or more explanatory variables and a response variable by fitting a linear equation to observed data. Every value of the independent variable *x* is associated with a value of the dependent variable *y*. Here all the calculated descriptors were considered as independent variable and biological activity as dependent variable.

The cross-validation analysis was performed using the leave-one-out method. The following statistical parameters were considered to compare the generated QSAR models: correlation coefficient (r), squared correlation coefficient (r^2), predicted r^2 (pred_r^2), and Fischer's value (F) [24]. To validate the generated QSAR models, the leave-one-out (LOO) method was used, indicated as the value of q2 (cross-validated explained variance), which is a measure of the internal predictive ability of the model. The cross-validated r2 (q2) value was calculated, where yi and $\hat{y}_i$ are the actual and predicted activities of the i^{th} molecule respectively, and y_{mean} is the average activity of all the molecules in the training set.

Both summations are over all molecules in the training set and hence the predictions were based on the current trial solution, the q^2 obtained indicates the predictive power of the current model[25].

$$q^2 = 1 - \frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - y_{mean})^2}$$

The predicted r^2 (pred_r^2) value was calculated, where yi and $\hat{y}_i$ are the actual and predicted activities of the i^{th} molecule in test set, respectively, and y_{mean} is the average activity of all molecules in the training set. Both summations are over all molecules in the test set. The pred_r^2 value is indicative of the predictive power of the current model for external test set[25].

$$pred\_r^2 = 1 - \frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - y_{mean})^2}$$

To evaluate the statistical significance of the QSAR model or an actual data set, we have employed a one-tail hypothesis testing. The robustness of the QSAR models for experimental training sets was examined by comparing these models to those derived for random data sets. Random sets were generated by rearranging biological activities of the training set molecules[25].

3. RESULTS AND DISCUSSION

The importance and utility of the new 2D and 3D QSAR method discussed has been established by applying it to known sets of molecules as described above. All the calculated descriptors were considered as independent variable and biological activity as dependent variable. In 2D QSAR analysis, significant methods like Multiple linear regression analysis, Partial Least Square (PLS)

and Principal Component Regression (PCR) were applied to generate the model having good q^2 and pred_r^2 values, one of which was selected having good internal and external predictivity. Selection of training and test set was by Manual data selection and random data selection method. Training and test set were selected if they follow the unicolumn statistics, i.e. maximum of the test is less than maximum of training set and minimum of the test set is greater than of training set, which is prerequisite for further QSAR analysis. This result shows that the test is interpolative i.e., derived from the min-max range of 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 of the two sets The QSAR models developed by kNN-MFA include both the electrostatic, steric descriptors along with their range to indicate their importance for interaction in molecular field. Models 20 and 36 are with 3D QSAR studies. QSAR investigations of the substituted 5-N-phenylsulfamoyl-2-(2-(nitrooxy) (acetoxy)benzoic acid derivatives series resulted in several QSAR equations. Some statistically significant 2D and 3D QSAR models were chosen for discussion.

3.1 2D-QSAR model:

Model 21:

By Random data selection method;

IC_{50}=--0.1362(SaascCount) + 0.0950 (SdssCcount) – 0.0556 ($SsCH_3$Count) – 0.0432 (T-C-S-6) + 1.6935

n = 13, Degree of freedom = 8, r^2 = 0.9236, q^2 = 0.8360, F test = 24.1907, r^2 se = 0.0290, q^2 se = 0.0424, pred_r^2 = 0.3738, pred_r^2 se = 0.1083

Among all the significant models the above is the best model generated for anti-inflammatory. The equation explains 92.36% (r^2 = 0.9236) of the total variance in the training set and has an internal (q^2) and external (pred_r^2) predictive ability of ~83.60% and ~37.38% respectively. The F-test = 24.1907 which is far greater than the F-tabulated value = 3.2850 (http://url.ie/fjj2). This shows the statistical significance of 99.99% of the model, which means that the probability of failure of the model is 1 in 10,000. All the standard error values are very low (less than 1) hence this model is highly acceptable.

In this QSAR model, the negative coefficient of SaascCount (it is descriptor defines the total number of CH group connected with three single bond) on the biological activity indicates that lower the value leads to the better activity, while higher the value indicates less biological activity. The negative coefficient of SdssCcount (This descriptor defines the total number of carbon connected with one double and two single bond.) on the biological activity indicates that higher the value leads to the better activity, while lower the value indicates less biological activity. The negative coefficient of $SsCH_3Count$ (It is descriptors defines the total number of CH_3 group connected with single bond. The negative coefficient of T-C-S-6 (It is the count of carbon atoms separated from sulphur atom by 6 bonds.

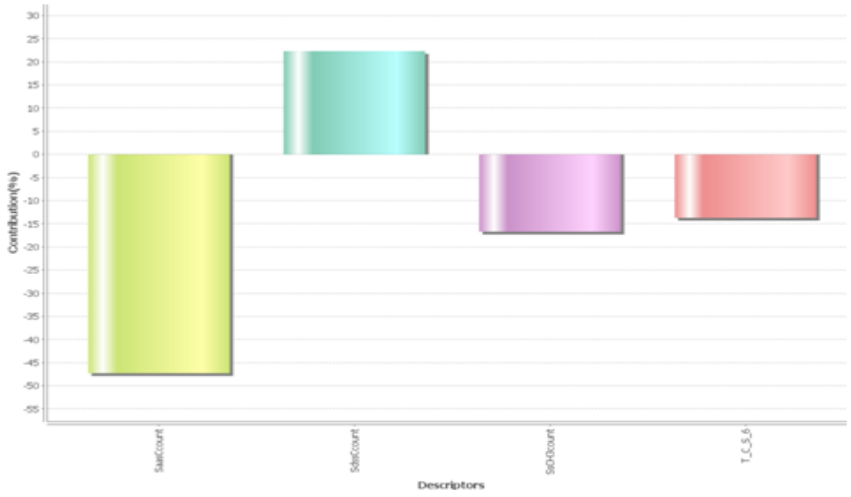


Figure 3a: Contribution plot of 2D-QSAR Model 21

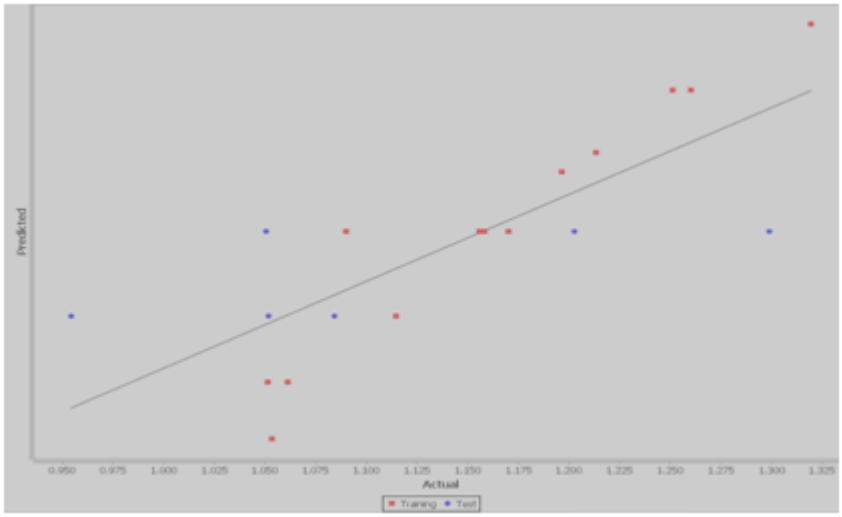


Figure 3b: Fitness plot showing actual activity versus predicted activity of Model 21

3.2 2D-QSAR model:

Model 22:

By Random data selection method;

IC_{50}=0.1401 (T-2-S-6) -0.0322 (T-C-O-7) + 0.0145 (T-C-C-5) -0.0861 (T-2-Cl-5) + 1.1475

n = 13, Degree of freedom = 10, r^2 = 0.9269, q^2 = 0.7732, F test = 63.3898, r^2 se = 0.0243, q^2 se = 0.0428, pred_r^2 = 0.2028, pred_r^2 se = 0.1259

Among all the significant models the above is the best model generated for anti-inflammatory. The equation explains 92.69% (r^2 = 0.9269) of the total variance in the training set and has an internal (q^2) and external (pred_r^2) predictive ability of ~77.32% and ~20.28% respectively. The F-test = 63.3898 which is far greater than the F-tabulated value = 3.2850 (http://url.ie/fjj2). This shows the statistical significance of 99.99% of the model, which means that the probability of failure of the model is 1 in 10,000. All the standard error values are very low (less than 1) hence this model is highly acceptable.

In this QSAR model, the positive coefficient of T-2-S-6 (that is any double bonded sulphur atom, separated from any other double bonded atom by 6 bond) on the biological activity indicates that higher the value leads to the better activity, while lower the value indicates less biological activity. The negative coefficient of T-C-O-7 (That is any carbon atom separated from oxygen atom by 7 bond) on the biological activity indicates that lower the value leads to the better activity, while higher the value indicates less biological activity. The positive coefficient of T-C-C-5 (that is any carbon atom separated from any other carbon atom by 5 bond) on the biological activity indicates that higher the value leads to the better activity, while lower the value indicates less biological activity. The negative coefficient of T-2-Cl-5 (that is any double bonded atom separated from chlorine atom by 5 bond).

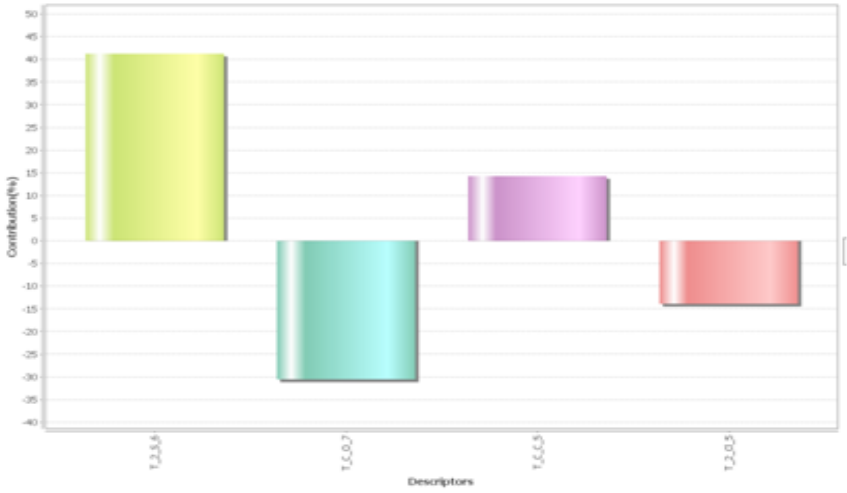


Figure 4a: Contribution plot of 2D-QSAR Model 25

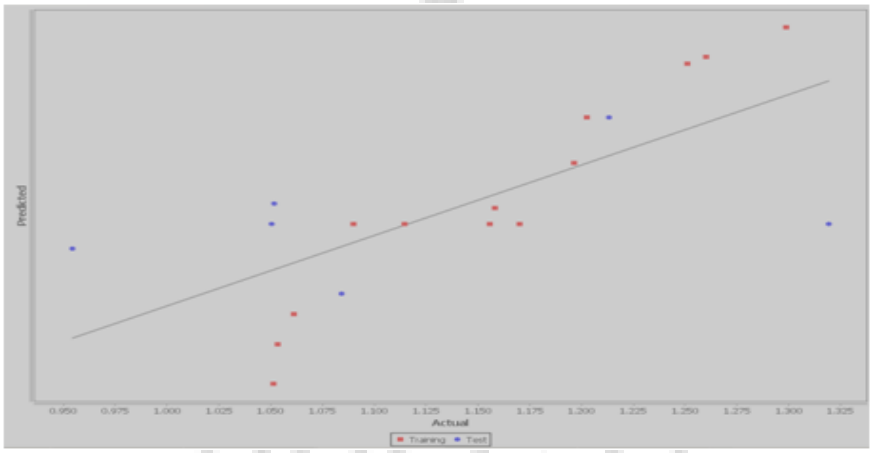


Figure 4b: Fitness plot showing actual activity versus predicted activity of Model 25

3.3 2D-QSAR model:

Model 44:

By Random data selection method;

$IC_{50=}$ -0.1119 (SaasCcount) – 0.0580 ($SsCH_3E$.index) +0.0606 (SdssCcount) +0.0221 (T-2-N-1) +1.5325

n = 13, Degree of freedom = 10, r^2 = 0.9564, q^2 = 0.8991, F test = 109.7579, r^2 se = 0.0195, q^2 se = 0.0298, pred_r^2 = 0.5467, pred_r^2 se = 0.0946

Among all the significant models the above is the best model generated for anti-inflammatory. The equation explains 95.64% (r^2 = 0.9564) of the total variance in the training set and has an

internal (q^2) and external (pred_r^2) predictive ability of ~89.91% and ~54.67% respectively. The F-test = 109.7579 which is far greater than the F-tabulated value = 3.2850 (http://url.ie/fjj2). This shows the statistical significance of 99.99% of the model, which means that the probability of failure of the model is 1 in 10,000. All the standard error values are very low (less than 1) hence this model is highly acceptable.

In this QSAR model, the negative coefficient of SaasCcount (it is descriptor defines the total number of CH group connected with three single bond) on the biological activity indicates that lower the value leads to the better activity, while higher the value indicates less biological activity. The negative coefficient of $SsCH_3E\_index$ (It is Electrotopological state indices for number of CH_3 group connected with one single bond)) on the biological activity indicates that lower the value leads to the better activity, while higher the value indicates less biological activity. The positive coefficient of SdssCcount (This descriptor defines the total number of carbon connected with one double and two single bond.) on the biological activity indicates that higher the value leads to the better activity, while lower the value indicates less biological activity. The positive coefficient T-2-N-1 (That is any double bonded atom separated from nitrogen atom by 1 bond).

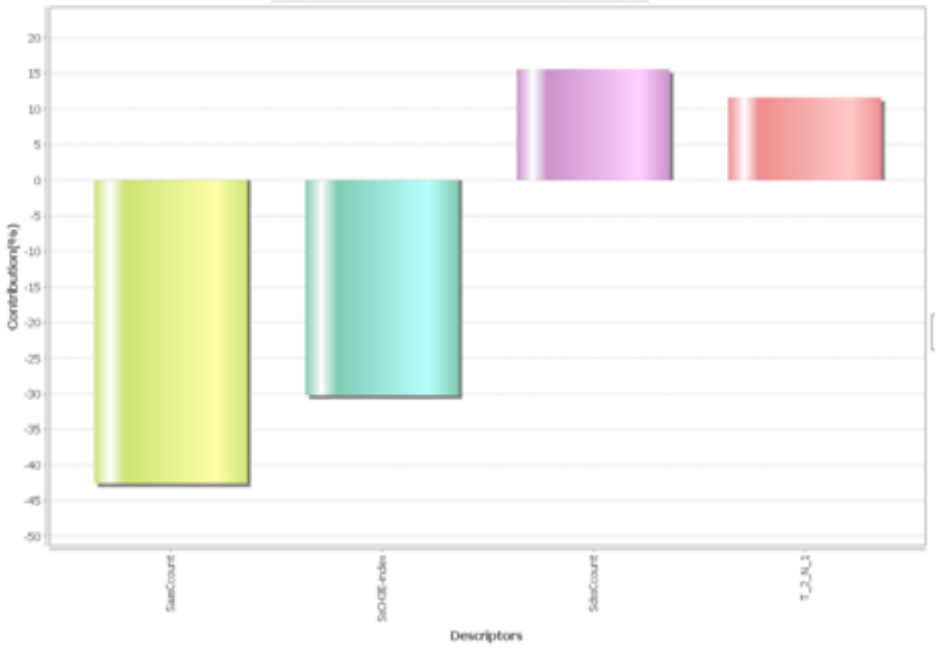


Figure 5a: Contribution plot of 2D-QSAR Model 44

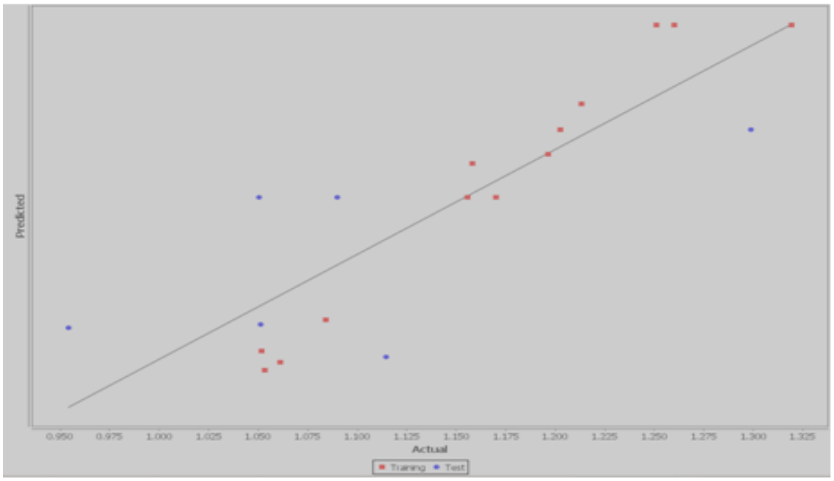


Figure 5b: Fitness plot showing actual activity versus predicted activity of Model 44

3.4 2D-QSAR model:

Model 117:

By Random data selection method;

IC_{50}= -0.1008 (SaasCcount) – 0.0608 ($SsCH_3E$-index) + 0.0526 (T-C-N-1) +1.6170

n = 13, Degree of freedom = 10, r^2 = 0.8437, q^2 = 0.7295, F test = 26.9877, r^2 se = 0.0398, q^2 se = 0.0523, pred_r^2 = 0.5481, pred_r^2 se = 0.0837

Among all the significant models the above is the best model generated for anti-inflammatory. The equation explains 84.37% (r^2 = 0.8437) of the total variance in the training set and has an internal (q^2) and external (pred_r^2) predictive ability of ~72.95% and ~54.81% respectively. The F-test = 26.9877 which is far greater than the F-tabulated value = 3.2850 (http://url.ie/fjj2). This shows the statistical significance of 99.99% of the model, which means that the probability of failure of the model is 1 in 10,000. All the standard error values are very low (less than 1) hence this model is highly acceptable.

In this QSAR model, the negative coefficient of SaasCcount (it is descriptor defines the total number of CH group connected with three single bond) on the biological activity indicates that lower the value leads to the better activity, while higher the value indicates less biological activity. The negative coefficient of $SsCH_3E$-index (It is Electrotopological state indices for number of CH_3 group connected with one single bond)) on the biological activity indicates that lower the value leads to the better activity , while higher the value indicates less biological

activity. The positive coefficient of T-C-N-1 (that is any carbon atom separated from nitrogen by 6 bond).

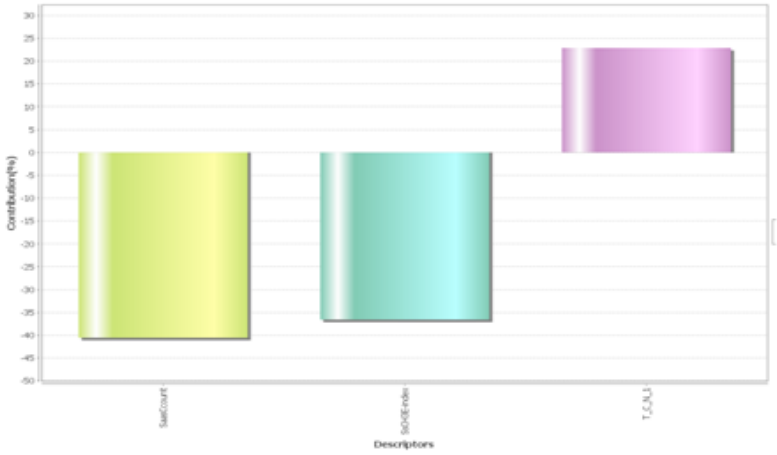


Figure 6a: Contribution plot of 2D-QSAR Model 117

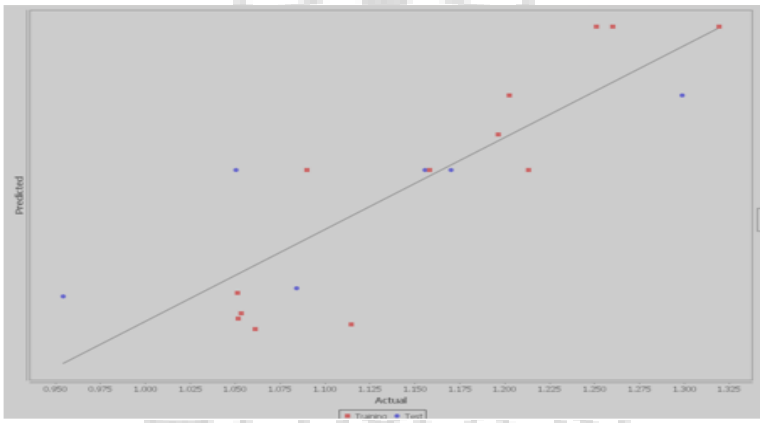


Figure 6b: Fitness plot showing actual activity versus predicted activity of Model 117

3.5 3D-QSAR model:

Model 20:

By stepwise forward backward method;

IC_{50}= E_547 (-6.2071,-5.5922) + S_1176 (30.00,30.00)

N= 13, Degree of freedom = 10, q^2 = 0.5374, q^2se = 0.0647, pred_r^2 =-0.3548, and pred_r^2 se = 0.0948

According to the **kNN-MFA methodology** Ic_{50} is a function of independent variables, steric and electrostatic fields. Values of it prove the equation statistically to be significant. It also shows the

predictive power of the model as 53.74%. It showed steric and electrostatic field energy of interactions between probe (CH) and compounds at their corresponding spatial grid points of 547, 1176. Numbers nearest neighbor's k of 2 were observed with this model i.e. two values are proved statistically significant. It is observed from the **Figure 6** that the negative coefficient of E_547 suggested that electronegative substituent may be favourable on the position of salicylic acid ring for better activity. Even the steric factor S_1176 is positive which indicates the favourability of bulky groups on the salicylic acid ring to increase the activity.

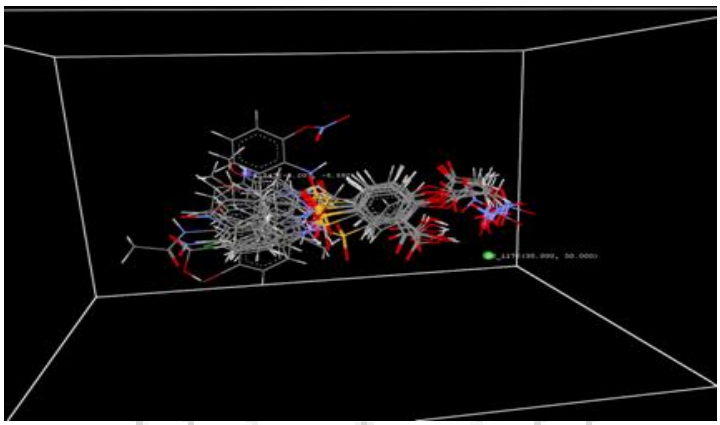


Figure 7a: Contribution plot of 3D-QSAR Model 20

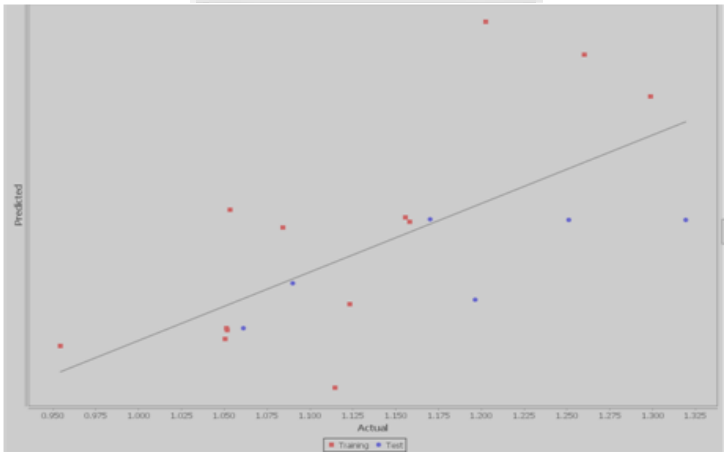


Figure 7b: Fitness plot showing actual activity versus predicted activity of Model 20

Model 36:

By stepwise forward backward method;

IC_{50}= E_937 (-2.4827,-0.4193) + S_469 (0.0643,0.1358)

N= 13, Degree of freedom = 10, q^2 = 0.6970, q^2se = 0.0442, pred_r^2 =-0.3548, and pred_r^2 se = 0.0948

According to the **kNN-MFA methodology** Ic_{50} is a function of independent variables, steric and electrostatic fields. Values of it prove the equation statistically to be significant. It also shows the predictive power of the model as 53.74%. It showed steric and electrostatic field energy of interactions between probe (CH) and compounds at their corresponding spatial grid points of 937, 469. Numbers nearest neighbor's k of 3 were observed with this model i.e. two values are proved statistically significant. It is observed from the **Figure 6** that the negative coefficient of E_937 suggested that electronegative substituent may be favourable on the position of salicylic acid ring for better activity. Even the steric factor S_469 is positive which indicates the favourability of bulky groups on the salicylic acid ring to increase the activity.

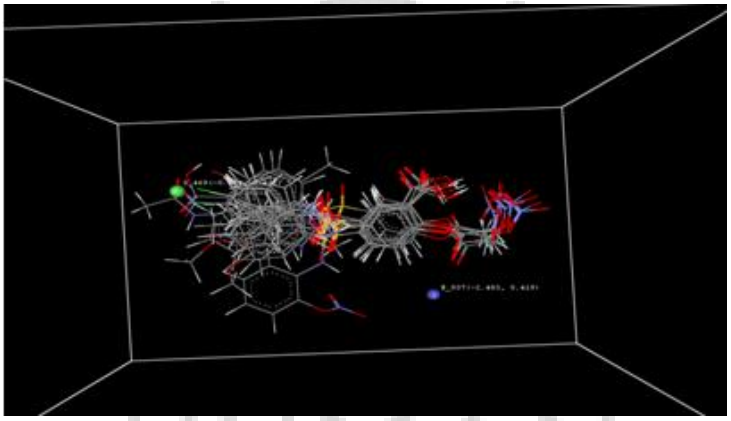


Figure 8a: Contribution plot of 3D-QSAR Model 36

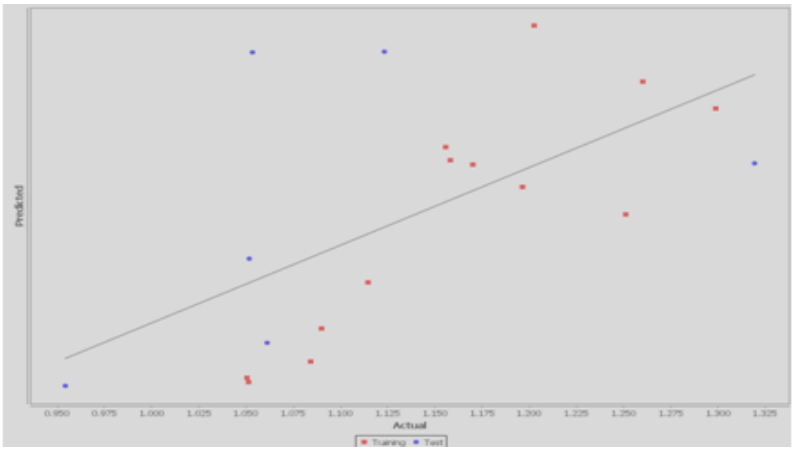


Figure 8b: Fitness plot showing actual activity versus predicted activity of Model 36

4. CONCLUSIONS

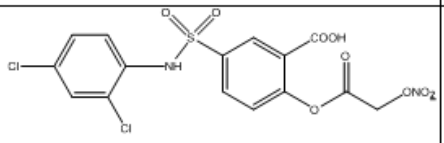
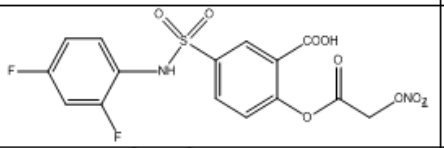
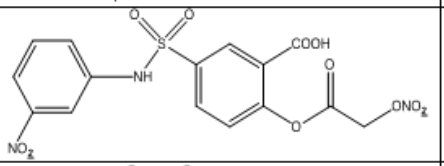
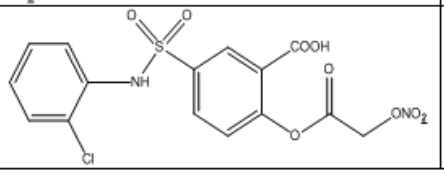
In the present investigation, all proposed QSAR models were statistically significant, thus, from above QSAR investigations it could be concluded that 2D/3D descriptors properties of substituted 5-phenylsulfamoyl-2-(2-(nitrooxy) (acetoxy)benzoic acid derivatives are mainly involved in treatment of drug abuse disorders. The good correlation between experimental and predicted biological activity for compounds in the test set further highlights the reliability of the constructed QSAR model. The requirements for the more potent biological activity are explored with 2D, 3D and group based QSAR studies. The 2D technique indicates the importance of XlogP, SssCH3-Eindex, SssCH3-count, and SlogP of the compounds on the activity. The 3D QSAR analysis makes it possible to relate chemical structures of ligands and their binding affinity with respect to different bio targets by using the kNN-MFA techniques. Thus it provides a direct view of factors expressed in terms of molecular fields (electrostatic, steric) affecting the binding affinity. This in turn could give the reasonably good prediction of binding affinity. The location and range of function values at the field points selected by the models provide clues for the design of new molecules. Hence, this method is expected to provide a good alternative for the drug design.

The 3D-QSAR model showed that electrostatic effects dominantly determine the binding affinities and these QSAR models developed in this study would be useful for the development of new drugs as a medicament for the drug abuse disorder. The 2D-QSAR studies revealed that alignment independent descriptors were the major contributing descriptors. The descriptor values obtained in this study helped in quantification of the structural features of 5-phenylsulfamoyl-2-(2-(nitrooxy) (acetoxy)benzoic acid derivative.

After successful QSAR studies, attempts were made to predict the activities of the newly designed analogues of these reported compounds. We have designed 32 compounds among which 11 compounds are showing higher activity than the reported analogues. In future, we can synthesize these compounds using the selected scheme and confirm their activity by carrying out *in vivo* evaluation.

Table 4: Newly designed salicylic acid derivative with their predicted biological activity

These compounds have more biological activity than the reported.

Sr. No.	Newly Designed molecules	Activity
1		1.15715
2		1.15714
3		1.5715
4		1.12219

5. ACKNOWLEDGMENT

The author wishes to express gratitude to V-life Science Technologies Pvt. Ltd. for providing the software for the study. Also the authors are thankful to the Trustee, Bhujbal Knowledge City for providing the necessary facilities to carry out the research work.

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