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Three Dimensional Quantitative Structure Activity Relationship (QSAR) Analysis on Arylbenzofuran Derivatives as Histamine H_3 Antagonists using k-Nearest Neighbor Molecular Field Analysis

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ABSTRACT

A three dimensional quantitative structure activity relationship (3D QSAR) using k nearest neighbor molecular field analysis (kNN MFA) method was performed on a series of arylbenzofuran derivatives as H_3-receptor antagonists. This study was performed with 29 compounds (data set) using sphere exclusion (SE) algorithm and random selection method for the division of the data set into training and test set. kNN-MFA methodology with stepwise (SW), simulated annealing (SA) and genetic algorithm (GA) was used for building the QSAR models. A predictive model was generated with SW-kNN MFA having internal predictivity 70.55% (q2 = 0.7055) and external predictivity 60.00 % (pred_r2 = 0.60). Model showed that steric (S_579), electrostatic (E_453) and hydrophobic (H_779) interactions play important role in determining H_3-receptor antagonistic activity. The kNN-MFA contour plots provided further understanding of the relationship between structural features of substituted arylbenzofuran derivatives and their activities which should be applicable to design newer potential H_3-receptor antagonists.

Keywords: 3D-QSAR, kNN-MFA, H_3-receptor antagonists, Arylbenzofuran derivatives.

INTRODUCTION

The histamine H_3 receptor is a G-protein-coupled receptor described earlier as central histamine modulating autoreceptors [1] and later as heteroreceptors regulating release of other neurotransmitters e.g., acetylcholine, dopamine, serotonin, glutamate etc. Activation of histamine H_3 receptor (H3R) by the endogenous ligand, histamine [1-5], reduces neurotransmitter release, while blockade of the H_3 receptors leads to enhanced neurotransmitter release [6-10]. This enhanced neurotransmitter release is thought to be responsible for enhance vigilance or alertness [11], wakefulness [12], nasal congestion [13-14], and in some cases an

anti-obesity effect [15-17] upon administration of H3R antagonists. Thus, H3 receptor antagonists enhance levels of histamine therefore they may be used as potential therapeutic agents for attention deficit/hyperactivity disorder, Alzheimer's disease, mild cognitive impairment or schizophrenia and obesity.

The thirst for discovery of new chemical entities of therapeutic interest has been continued since for many years to medicinal chemistry experts. In recent years, a substantial progress that has been made by computational chemistry led new challenges to drug discovery by rational process. Quantitative structure activity relationship (QSAR) which has become a popular tool for establishing quantitative relationship between biological activity and descriptors representing physico-chemical properties of the compounds in a series using statistical methods and it helps to predict the biological activities of newly designed analogues contributing to the drug discovery processes [18].

The main objective of the present study is the search for novel arylbenzofuran derivatives that would show a promise to become useful H_3-receptor antagonist. A series aryl benzofuran derivatives which were reported as H_3-receptor antagonists selected for QSAR study [19].

MATERIALS AND METHODS

Data Set: In the present study a data set of aryl benzofuran derivatives (29 molecules) as human H_3-receptor antagonistic activity [19] has been taken from the literature for QSAR studies (Table-1). The reported Ki values hH_3 binding affinity (μM), have been converted to the logarithmic scale [pKi (moles)], for QSAR study.

Molecular Modeling Study: Molecular modeling and kNN-MFA studies were performed on HCL computer having genuine Intel Pentium Dual Core Processor and Windows XP operating system using the software Molecular Design Suite (MDS) [20]. Structures were drawn using the 2D draw application and converted to 3D structures. Structures were optimized by energy minimization and geometry optimization was done using Merck Molecular Force Field (MMFF) method using Gasteiger Charge with 10000 as maximum number of cycles, 0.01 as convergence criteria (root mean square gradient) and 1.0 as constant (medium's dielectric constant which is 1 for in vacuo) in dielectric properties. The default values of 30.0 and 10.0 Kcal/mol were used for electrostatic and steric energy cutoff.

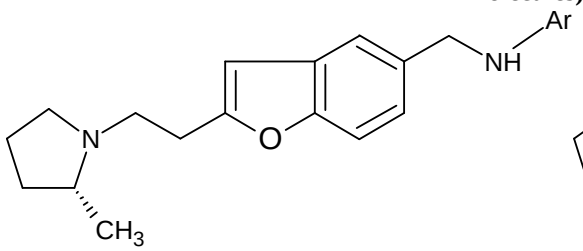
Molecular alignment: The selected dataset were aligned by using template based alignment method using most active molecule (7h) as a reference molecule (**2)** and structure (**1)** as a template (Figure-1). The alignment of all the molecules on the template is shown in **Figure-2**. In the template based alignment method, a template structure was defined and used as a basis for alignment of a set of molecules.

GRID Size: Once the molecules are aligned, a grid or lattice is established which surrounds the set of compounds in potential receptor surface. Current study uses grid resolution 2 A^o.

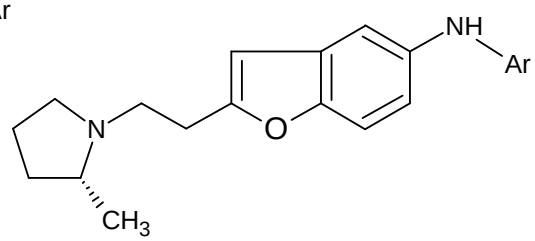
Descriptor calculation: Once the molecules are aligned, a molecular field is computed on a grid of points in space around the molecule. This field provides a description of how each molecule

will tend to bind in the active site. Descriptors representing the steric, electrostatic and hydrophobic interaction energies were computed at the lattice points of the grid using a methyl probe of charge +1.

Table 1: General structure of the Aryl benzofuran derivatives and their biological activities (data set of 29 molecules)



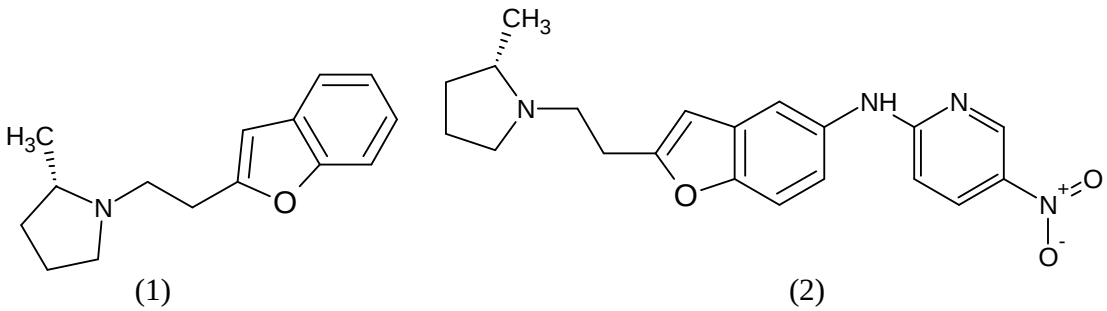
A (1-18)



B (19-29)

S. No.	Compound	Ar substituent	human H_3 pKi
1	6a	2-nitrophenyl	9.50
2	6b	4-nitrophenyl	9.13
3	6c	4-(2,2,2-trifluoroacetyl)phenyl	9.13
4	6d	3-cyanophenyl	9.40
5	6e	6-chloropyridazine-3-yl	9.51
6	6f	3-cyanopyrazin-2-yl	8.98
7	6g	Pyrazin-2-yl	9.53
8	6h	5-bromopyrimidin-2-yl	8.91
9	6i	Pyrimidin-5-yl	9.16
10	6j	5-ethylpyrimidin-2-yl	9.48
11	6k	Pyrimidin-2-yl	9.07
12	6l	5-nitrothiazol-2-yl	9.72
13	6m	3-nitropyridin-2-yl	9.36
14	6n	3,5-dinitropyridin-2-yl	9.15
15	6o	2,6-dicyanopyridin-4-yl	9.88
16	6p	3-cyanopyridin-2-yl	9.13
17	6q	5-cyanopyridin-2-yl	9.46
18	6r	5-nitropyridin-2-yl	9.52
19	7a	2-nitrophenyl	9.66
20	7b	4-cyanophenyl	9.57
21	7c	3-cyanophenyl	9.53
22	7d	5-ethyl-pyrimidin-2-yl	9.21
23	7e	Pyrimidin-2-yl	9.51
24	7f	Pyrimidin-5-yl	10.12
25	7g	Pyrazin-2-yl	9.98
26	7h	5-nitropyridin-2-yl	10.32
27	7i	3-nitropyridin-2-yl	9.87
28	7j	3-cyano-6-methylpyridin-2-yl	8.53
29	7k	5-trifluoromethyl-pyridin-2-yl	9.27

Generation of training and test set: In order to evaluate the QSAR model externally, data set was divided into training and test set using Random selection and Sphere Exclusion methods. Training set is used to develop the QSAR model for which biological activity data are known. Test set is used to challenge the QSAR model developed based on the training set to assess the predictive effectiveness of the model which is not included in model generation.



(1) (2)

Figure-1: Structure of template (1) and reference molecule (2) used in template based alignment.

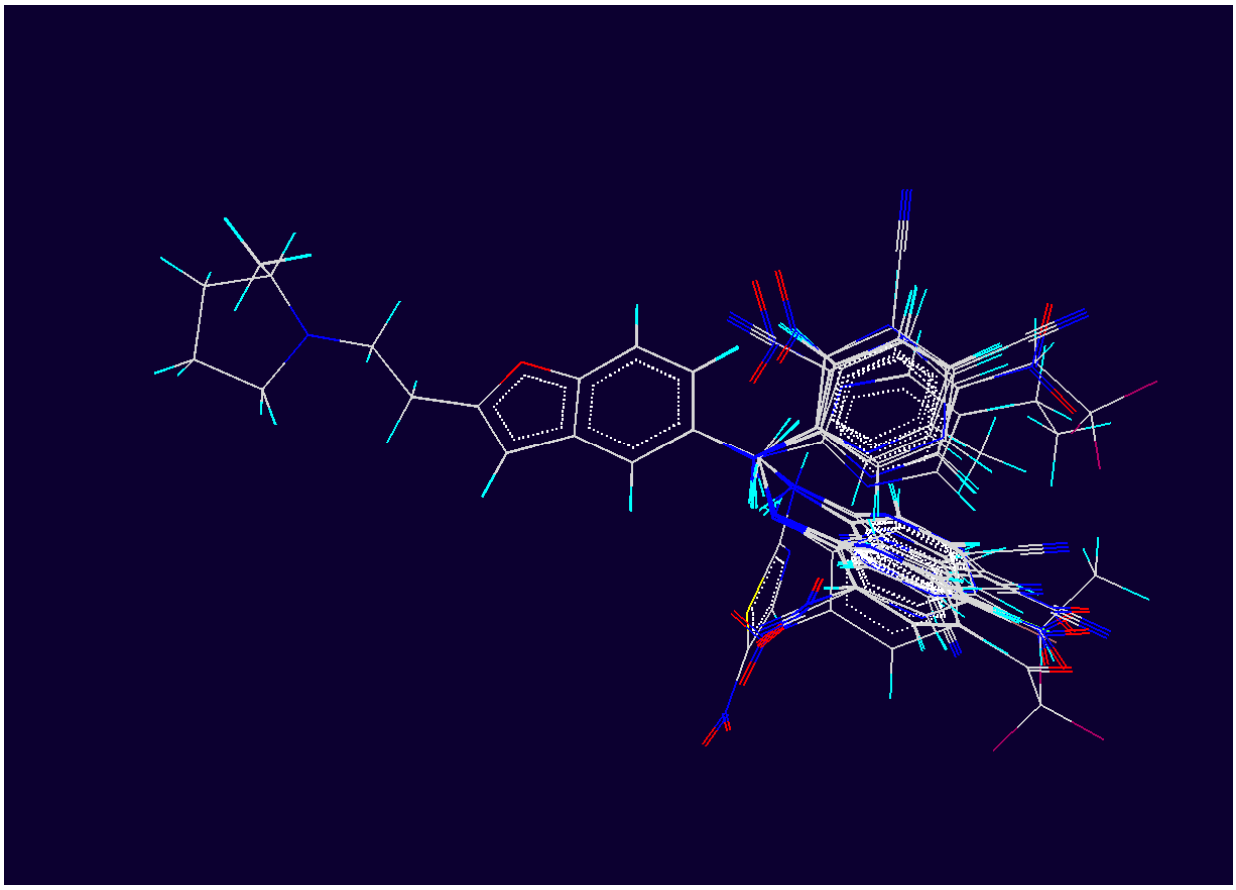


Figure-2: 3D-alignment of molecules (template based).

Sphere exclusion method: Sphere exclusion algorithm was used for generation of training and test sets. The whole data set was divided into training and test sets using sphere exclusion algorithm [21]. This algorithm allows constructing training sets covering all descriptor space areas occupied by representative points. The higher the dissimilarity level c is, the smaller the training set is and the larger the test set is and vice versa. It is expected that the predictive ability of QSAR models generally decrease when the dissimilarity level

increases. Once the training and test sets are generated, kNN methodology is applied to descriptors generated over grid.

Random selection method: In order to construct and validate the QSAR models, both internally and externally, the data sets were divided into training set (85% of total data set) and test sets [15%] in a random manner. 10 trials were run.

kNN-MFA methodology for building QSAR models: Models were generated by kNN-MFA [22] in conjunction with stepwise (SW) forward-backward, simulated annealing (SA) and genetic algorithm (GA) variable selection methods with pK_i activity field as dependent variable and descriptors as independent variable.

The kNN technique is a conceptually simple approach to pattern recognition problems. In this method, an unknown pattern is classified according to the majority of the class memberships 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). The standard kNN method is implemented simply as follows: (1) calculate distances between an unknown object (u) and all the objects in the training set; (2) select k objects from the training set most similar to object u, according to the calculated distances (k is usually an odd number); (3) classify object u with the group to which a majority of the k objects belongs. An optimal k value is selected by the optimization through the classification of a test set of samples or by the leave-one out cross-validation. The variables and optimal k values are chosen using different variable selection methods.

Stepwise forward-backward variable selection method (SW-kNN MFA): This method uses stepwise variable selection and k-NN principle [23] to build QSAR model. It involves a step-by-step search procedure that begins by addition of a single independent variable with optimal k value (optimizing k value from the given range of k values) and highest sum of weighted k-nearest neighbor cross validation (q^2) and external validation ($pred\_r^2$) value amongst all available descriptors to form a model. The parameter settings used for SW-kNN MFA are as follows:

Cross correlation limit as 0.5, maximum number of variable in final equation as n/5 (n is number of compounds in training set), term selection criteria as q^2, Ftest in as 4 and Ftest out as 3.99, variance cut-off as 0 and Scaling as Auto Scaling, number of maximum neighbors as 5, number of minimum neighbors as 2 and distance based weighted average as prediction method.

Simulated Annealing k-NN QSAR Algorithm (SA-kNN MFA): Simulated annealing is to simulate a physical process called annealing, in which a system is heated to a high temperature and then is gradually lowered to a preset temperature value (e.g. room temperature). During this process, the system samples possible configurations according to Boltzmann distribution. At equilibrium, low energy states will be mostly populated.

Steps involved in simulated annealing kNN-MFA method [22] can be summarized as follows-

(i) A subset of number of variables (*nvar)* descriptors is selected randomly (*nvar* is a number between 1 and the total number of available descriptors) as a hypothetical pharmacophore (HP), *nvar* is usually set to different values in several different runs.
(ii) HP is validated by weighted k-nearest neighbor cross validation procedure.
(iii) Steps 1 & 2 are repeated. The goal is to find the best pharmacophore that maximizes the q^2 value of the kNN-MFA model.

Development of SA k-NN QSAR method was done using algorithm as described in the literature. The parameter settings used for simulated annealing in the present study are as follows:

Maximum temperature as 100, minimum temperature as 0.01, cross correlation limit as 0.5, terms in model as n/5 (n is number of compounds in training set), iteration at given temperature as 5, decrease temperature by as 10, seed as 0, perturbation limit as 1, term selection criteria as q^2, variance cut-off as 0 and Scaling as Auto Scaling, number of maximum neighbors as 5, number of minimum neighbors as 2 and distance based weighted average as prediction method.

Genetic algorithm k-NN QSAR Algorithm (GA-kNN MFA): Genetic algorithms are derived from an analogy with the spread of mutations in a population. In this analogy, "individuals" are represented as a one-dimensional string of bits. An initial population of individuals is created, usually with random initial bits. A fitness function is used to estimate the "quality" of an individual, so that the "best" individuals receive the best fitness scores. Individuals with the best scores are more likely to propagate their genetic material to offspring through crossover, in which pieces of genetic material are taken from each parent and recombined to create the child. After many such mating steps, the average fitness of the individuals in the population increases, as good combinations of genes are discovered and spread through the population. Genetic algorithms are especially good at searching problem spaces having a large number of dimensions, since they conduct a very efficient, directed sampling of the large space of possibilities [24].

The parameter settings used for genetic algorithm in the present study are as follows:

Cross correlation limit as 0.5, chromosome length as n/5 (n is number of compounds in training set), cross over probability as 0.95, mutation probability as 0.05, population as 10, number of generations as 1000, convergence criteria as 0.01, seed as 0, term selection criteria as q^2, variance cut-off as 0 and Scaling as Auto Scaling, number of maximum neighbors as 5, number of minimum neighbors as 2 and distance based weighted average as prediction method.

Validation of the models: Models were validated internally and externally.
*a) Internal validation [Cross-Validation (q^2) **using** weighted k-Nearest Neighbor]:* In cross validation, a compound is eliminated in the training set and its biological activity is predicted on the basis of the k-NN principle, i.e., as the weighted average activity of k most similar molecules (k is set to 1 initially). The similarities are evaluated as Euclidean distances between compounds using only the subset of descriptors that corresponds to the

current model. This step is repeated until every compound in the training set has been eliminated and its activity is predicted once.

b) External Validation [(pred_r^2) using weighted k-Nearest Neighbor]: Following procedure was applied for external validation.
(1) Predict biological activity of a compound in the test set on the basis of the k-NN principle, i.e., as the weighted average activity of k (that correspond k value for highest q^2 value) most similar molecules in the training set. The similarities are evaluated as Euclidean distances between compounds using only the subset of descriptors that corresponds to the current model (for highest q^2 value).
(2) Repeat step 1 for every compound in the test set.
(3) Calculate the predicted r^2 (pred_r^2) value using following equation, where y_i and y_* are the actual and predicted activities of the i^{th} compound in test set, respectively, and y_{mean} is the average activity of all compounds in the training set. Both summations are over all compounds in the test set. The obtained pred_r^2 value is indicative of the predictive power of the current k-NN QSAR model for external test set.

$$pred\_r^2 = 1 - \sum (y_i - y_*)^2 / \sum (y_i - y_{mean})^2$$

RESULTS AND DISCUSSION

Different training and test set of arylbenzofuran derivatives were constructed using sphere exclusion (dissimilarity level 6.4 to 11.3) and random data selection methods. 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 (Table-2). 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. k-Nearest neighbor molecular field analysis (kNN-MFA) was applied using stepwise (SW), simulated annealing (SA) and genetic algorithm (GA) approaches for building QSAR models. Results of models developed by SW-kNN MFA, SA-kNN MFA and GA-kNN MFAusing sphere exclusion and random data selection methods are shown in Table-3 and 4 respectively. Significant QSAR model generated is shown in Table-5.

Following statistical measure was used to correlate biological activity and molecular descriptors: n = number of molecules, Vn = number of descriptors, k = number of nearest neighbor, df = degree of freedom, r2= squared correlation coefficient, q2 = cross validated correlation coefficient (by the leave-one out method), pred_r2 = predictive correlation coefficient for external test set, pred_r2se = coefficient of correlation of predicted data set, Z score = the Z score calculated by q2 in the randomization test, and α = the statistical significance parameter obtained by the randomization test. Data fitness plot for models 1 is shown in Figure-3.

Table-2: Uni-Column Statistics for Model 1 for training and test set activity

Column Name	Average	Max	Min	Std Dev	Sum
Training set (pK_i)	9.4225	10.3200	8.5300	0.3993	226.1400
Test set (pK_i)	9.4900	9.9800	9.1600	0.3160	47.4500

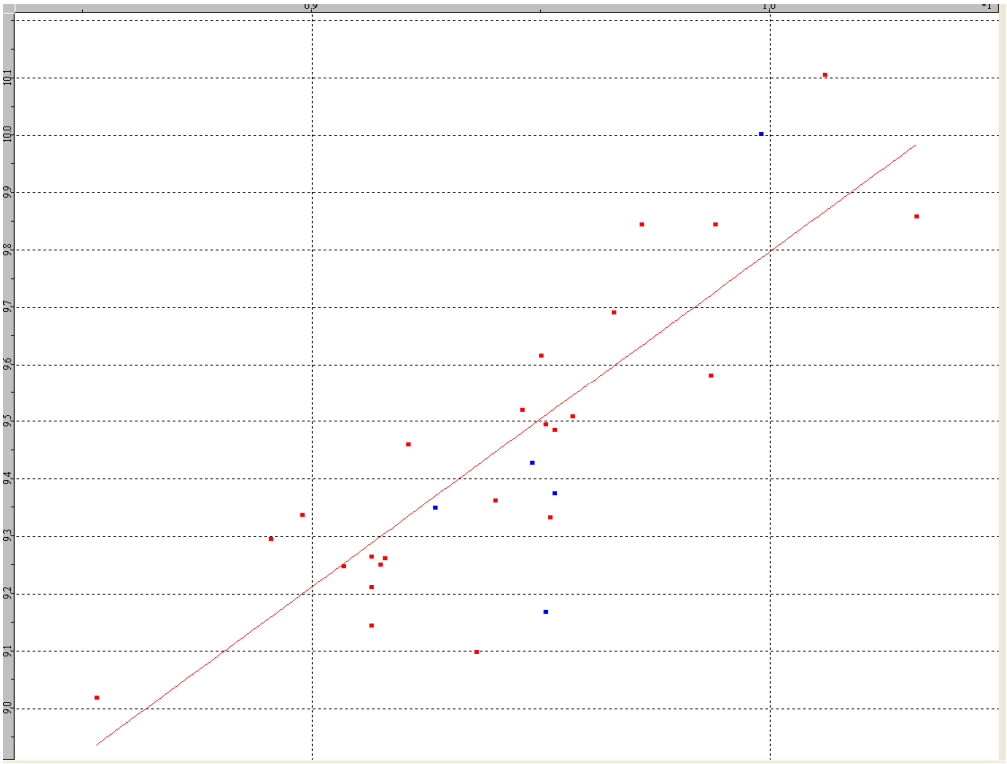


Figure-3: Data fitness plot for Models 1.

Table-3: Result of kNN-MFA study using sphere exclusion selection method

Dissimilarity Value	Test set mol	SW-KNN MFA		SA-KNN MFA		GA-KNN MFA	
		q^2	pred_r^2	q^2	pred_r^2	q^2	pred_r^2
6.4	7g,6k,6q	0.4953	-0.6001	0.2974	0.4077	-0.0837	-0.4209
8.6	7g,6k,6q,7i	0.4388	-0.7149	0.3773	0.7840	0.2014	-0.1216
9.1	7g,6g,6q,6e,7i	0.5226	-1.7112	0.3486	0.0053	0.1085	-0.7840
9.7	7g,6g,6q,6e,6k7i	0.4158	0.0605	0.2928	-0.8923	0.2513	-1.4970
9.9	7g,6g,6q,6e,6k7c,7i	0.4485	-1.3145	0.3971	0.5670	0.2190	-0.1691
10.0	7g,6g,6q,6e,6j,6f,7c,7i	0.6114	-1.4136	0.4273	0.3077	0.4386	0.3507
10.5	7g,6g,6q,6h,6f,6j,7c,6n,7i	0.4933	-1.7903	0.3259	-0.0169	-0.1580	0.0030
10.8	6i,7g,7c,6b,6q,6f,6k,6h,6n,7i	0.5925	-0.5398	0.2912	-0.3838	-0.0195	-0.4324
11.2	6i,7g,7c,6b,6q,6f,6k,6h,7d,6m,7j	0.6696	-0.3037	0.5613	-0.0503	0.4509	-0.1551
11.3	6i,7g,7c,6b,6q,6f,6j,6k,6d,6e,6n,7d,7j	0.6429	-0.7624	0.4403	0.0707	0.2088	0.0465

Table-4: Result of kNN-MFA study using random selection method (85%)

Trial	Test set mol	SW-KNN MFA		SA-KNN MFA		GA-KNN MFA	
		q^2	pred_r^2	q^2	pred_r^2	q^2	pred_r^2
1	6f,6g,6h,6l,7k	0.5480	0.0040	0.5157	-0.8560	0.1239	0.0050
2	6k,6r,7d,7j,7k	0.7026	-0.3651	0.6144	-0.5943	0.1252	-0.7451
3	6m,6q,7c,7g,7k	0.6034	-1.0143	0.3326	-1.0604	-0.0097	0.4758
4	6b,6d,7e,7g,7k	0.6601	0.1925	0.4010	0.0309	0.3037	0.2905
5	6e,6k,7d,7h,7k	0.4552	-0.7532	0.4855	0.7057	0.2879	0.1505
6	6a,6b,7h,7j,7k	0.4913	0.4131	0.4710	-0.2702	0.1480	-0.0275
7	6j,6m,7a,7d,7k	0.7024	-2.7085	0.4431	-6.6419	0.2849	-1.2646
8	6e,6j,7c,7g,7k	**0.7055**	**0.6000**	0.4979	-1.7788	0.0477	-0.4712
9	6f,6h,6l,6n,7k	0.6788	-0.2652	0.3889	0.5099	0.0375	0.3901
10	6e,6k,7b,7d,7k	0.4890	-0.0040	0.3966	-1.8837	0.4186	-0.6511

Table-5: Statistical significant model generated using kNN-MFA method

Parameters	**Model-1 Random (85%; Trial -8)**
Training Set Size (n)	24
Test set size	5
k nearest neighbour	2
Degree of freedom	20
q^2	0.7055
q^2se	0.2185
pred_r^2	0.6000
pred_r^2se	0.1936
Descriptors	E_453 -1.2715 -1.1427 H_779 0.4510 0.5717 S_579 -0.0447 -0.0439

Table-6: Actual and predicted biological activity for Training set

S. No.	**Training Set Mol**	**Actual**	**Predicted**
1	6a	9.5	9.61634
2	6b	9.13	9.14518
3	6c	9.13	9.26526
4	6d	9.4	9.36219
6	6f	8.98	9.33695
7	6g	9.53	9.48493
8	6h	8.91	9.29628
9	6i	9.16	9.26287
11	6k	9.07	9.24823
12	6l	9.72	9.845
13	6m	9.36	9.09912
14	6n	9.15	9.25061
15	6o	9.88	9.84422
16	6p	9.13	9.21145
17	6q	9.46	9.52007
18	6r	9.52	9.33374
19	7a	9.66	9.69165
20	7b	9.57	9.50919
22	7d	9.21	9.45954
23	7e	9.51	9.49514
24	7f	10.12	10.1049
26	7h	10.32	9.85901
27	7i	9.87	9.58165
28	7j	8.53	9.02027

Table-7: Actual and predicted biological activity for Test Set

S. No.	**Test Set Mol**	**Actual**	**Predicted**
5	6e	9.51	9.16894
10	6j	9.48	9.42818
21	7c	9.53	9.3747
25	7g	9.98	10.0027
29	7k	9.27	9.34964

Result of the observed and predicted biological activity for the training and test compounds in the Model 1 is shown in Table-6 and 7 respectively. The plot of observed vs. predicted activity of training and test sets for model 1 is shown in Figure-4**.** From the plot it can be seen that kNN-

MFA model is able to predict the activity of training set quite well (all points are close to regression line) as well as external.

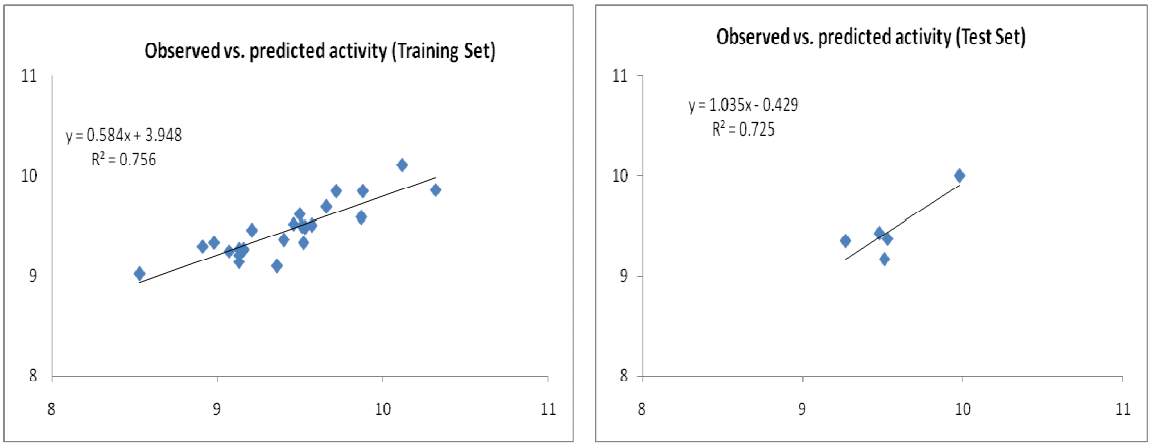


Figure-4: Graph between actual and predicted activity for training and test set (Model 1).

Sphere exclusion (SE) algorithm and random selection methods were used for constructing training and test sets. kNN-MFA methodology with stepwise (SW), simulated annealing (SA) and genetic algorithm (GA) was used for building the QSAR models. For the selected data set sphere exclusion (SE) data selection method does not result in any predictive model. A predictive model was generated with SW-kNN MFA method with random data selection method (pred_r2 = 0.60). The kNN-MFA contour plot (Figure-5) provided further understanding of the relationship between structural features of arylbenzofuran derivatives and their activities which should be applicable to design newer potential H_3 receptor antagonistic activity.

Interpretation of Model: It is produced by using stepwise kNN-MFA method having internal predictivity 70.55% (q2 = 0.7055) and external predictivity 60.00 % (pred_r2 = 0.60). kNN-MFA plot is shown in Figure-5. This model showed that steric (S_579), electrostatic (E_453) and hydrophobic (H_779) interactions play important role in determining H_3-receptor antagonistic activity. Steric field descriptor (S_579) has negative range (-0.0447 to -0.0439) indicates that negative steric potential is favorable for increase in activity and hence less bulky substituent group is preferred in that region.

Compounds having more bulky substituent group is not favorable for biological activity. Electrostatic field descriptor (E_453) has negative range (-1.2715 to -1.1427) indicates that negative electrostatic potential is favorable for increase in activity and hence less electronegative substituent group is preferred in that region. Compounds having more electronegative substituent group is not favorable for biological activity. Hydrophobic field descriptor (H_779) has positive range (0.4510 to 0.5717) indicates that positive hydrophobic potential is favorable for increase in activity and hence more hydrophobic substituent group is preferred in that region. Compounds having less hydrophobic substituent group is not favorable for biological activity.

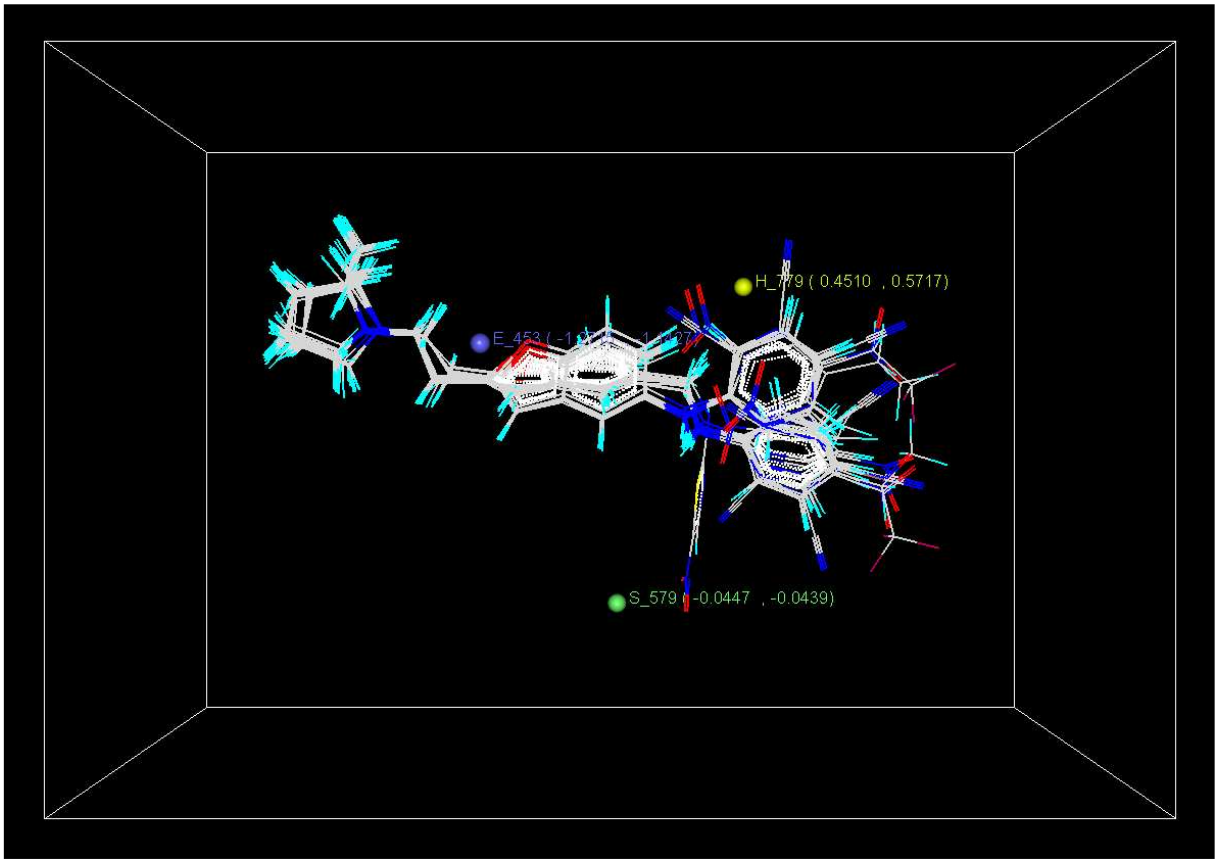


Figure-5: kNN-MFA result plot; 3D-alignment of molecules with the important steric, electrostatic and hydrophobic points contributing with ranges of values shown in parenthesis for model 1.

CONCLUSION

No significant model was generated in sphere exclusion data selection method. For the selected data set predictive model was generated with random data selection method. Model developed to predict the structural features of arylbenzofuran derivatives to inhibit H_3-receptor reveals useful information about the structural features requirement for the molecule. The master grid obtained for the various kNN-MFA models show that negative value in electrostatic field descriptors indicates the negative electrostatic potential is required to increase activity and hence less electronegative substituents group is preferred in that position. Compounds having more electronegative substituents group is not favorable for biological activity. Negative range in steric descriptors indicates less bulky substituents group is preferred in that region. Positive range in hydrophobic field descriptor indicates that positive hydrophobic potential is favorable for increase in activity and hence more hydrophobic substituent group is preferred in that region. On the basis of hydrophobic, electrostatic and steric potential contributions to the developed model in this work is useful in describing QSAR of arylbenzofuran derivatives as H_3-receptor antagonistic activity and can be employed to design new derivatives with potent inhibitory activity.

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REFERENCES

[1] S. J. Hill, C. R. Ganellin, H. Timmerman, J.-C. Schwartz, N. P. Shankley, J. M. Young, W. Schunack, R. Levi, H. L. Haas, *Pharmacol. Rev.*, **1997**, 49, 253.
[2] J.-M. Arrang, M. Garbarg, , J.-C. Schwartz, *Nature*, **1983**, 302, 832.
[3] R. Leurs, P. Blandina, C. Tedford, H. Timmerman, *Trends Pharmacol. Sci.*, **1998**, 19, 177.
[4] T. W. Lovenberg, B. L. Roland, S. J. Wilson, X. Jiang, J. Pyati, A. Huvar, M. R. Jackson, M. G. Erlander, *Mol. Pharmacol.*, **1999**, 55, 1101.
[5] R. Leurs, R. A. Bakker, H. Timmerman, I. J. P. de Esch, *Nat. Rev. Drug Disc.*, **2005**, 4, 107.
[6] S. Morisset, A. Rouleau, X. Ligneau, F. Gbahou, J. Tardivel-Lacombe, H. Stark, W. Schunack, C. R. Ganellin, J.-C. Schwartz, J.-M. Arrang, *Nature*, **2000**, 408, 860.
[7] A. Rouleau, X. Ligneau, J. Tarvidel-Lacombe, S. Morisset, F. Gbahou, J.-C. Schwartz, J.-M. Arrang, *Br. J. Pharmacol.*, **2002**, 135, 383.
[8] P. Ma, R. Zemmel, *Nat. Rev. Drug Disc.*, **2002**, 1, 571-2.
[9] L. B. Hough, *Mol. Pharmacol.*, **2001**, 59, 415-419.
[10] R. Leurs, M. Hoffmann, K. Wieland, H. Timmerman, *Trends Pharmacol. Sci.*, **2000**, 21, 11-12.
[11] A. A. Hancock, G. B. Fox, In Cognition Enhancing Drugs; Buccafusco, J. J., Ed.; Birkha¨user Verlag: Basel, Switzerland, **2004**, p 97.
[12] A. J. Barbier, C. Berridge, C. Dugovic, A. D. Laposky, S. J. Wilson, J. Boggs, L. Aluisio, B. Lord, C. Mazur, C. M. Pudiak, X. Langlois, W. Xiao, R. Apodaca, N. I. Carruthers, T. W. Lovenberg, *Br. J. Pharmacol.*, **2004**, 143, 649.
[13] R. L. McLeod, C. A. Rizzo, R. E., Jr. West, R. Aslanian, K. McCormick, M. Bryant, Y. Hsieh, W. Korfmacher, G. G. Mingo, L. Varty, S. M. Williams, N.-Y. Shih, R. W. Egan, J. A. Hey, *J. Pharmacol. Exp. Ther.*, **2003**, 305, 1037.
[14] R. Aslanian, M. W. Mutaki, N.-Y. Shih, K. D. McCormick, J. J. Piwinski, P. C. Ting, M. M. Albanese, M. Y. Berlin, X. Zhu, S. C. Wong, Y. J. Rosenblum, R. West, S. She, S. M. Williams, M. Bryant, J. A. Hey, *Bioorg. Med. Chem. Lett.*, **2002**, 12, 937.
[15] A. A. Hancock, *Curr. Opin. Investig. Drugs*, **2003**, 4, 1190.
[16] A. A. Hancock, Y. L. Bennani, E. N. Bush, T. A. Esbenshade, R. Faghih, G. B. Fox, P. Jacobson, V. Knourek-Segel, K. M. Krueger, M. E. Nuss, J. B. Pan, R. Shapiro, D. G. Witte, B. B. Yao, *Eur. J. Pharmacol.*, **2004**, 487, 183.
[17] K. Malmolof, F. Zaragoza, V. Golozoubova, H. H. F. Refsgaard, T. Cremers, K. Raun, B. S. Wulff, P. B. Johansen, B. Westerink, K. Rimvall, *Int. J. Obesity*, **2005**, 29, 1402.
[18] M.M.C.,Ferreira, *J Braz. Chem. Soc.*, **2002**, 13, 742.
[19] M. Cowart, R. Faghih, M.P. Curtis, G.A. Gfesser, Y.L. Bennani, L.A. Black, L. Pan, K.C. Marsh, J.P. Sullivan, T.A. Esbenshade, G.B. Fox, A.A. Hancock, *J. Med. Chem.*, **2005**, 48, 38-55.
[20] VLife Sciences Technology Pvt. Ltd. Pune-411045, Web: www.vlifesciences.com.
[21] M. Shen, A. LeTiran, Y. Xiao, A. Golbraikh, H. Kohn, A. Tropsha, *J. Med. Chem.*, **2002**, 45, 2811-2823.

[22] W. Zheng, A. Tropsha, *J. Chem. Inf. Comput. Sci.*, **2000**, 40, 185-194.
[23] S. Ajmani, J. Kamaiakar, S.A. Kulkarni, *J. Chem. Inf.* Model., **2006**, 46, .
[24] D. Rogers and A. J. Hopfinger, *J. Chem. Inf. Comp. Sci.*, **1994**, 34, 854-866.