ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

QSAR Rationales for the 5-HT_{2A} Receptor Antagonistic Activity of 2-Alkyl-4-aryl-Pyrimidine Fused Heterocycles.

Manju Choudhary, Pradeep Pilania and Brij Kishore Sharma*.

Department of Chemistry, Government PG College Bundi-323 001, Rajasthan, India.

ABSTRACT

The 5-HT_{2A} receptor binding affinities of the 2-alkyl-4-aryl-pyrimidine fused heterocycles have been quantitatively expressed in terms of topological and molecular features. The analysis revealed that less number of rotatable bonds (descriptor RBN), a more hydrophobic nature (descriptor MLOGP) and less polar surface area (descriptor PSA) in a molecular structure will be favorable to the binding affinity. A lower positive values of descriptors PW4 (path/walk 4 - Randic shape index) and MATS2m (Moran autocorrelation –lag 2/weighted by atomic masses) and higher value of descriptor MATS1v (Moran autocorrelation –lag 1/weighted by atomic van der Waals volumes) will augment the activity. Additionally, a lower value of descriptor BEHm1 (highest eigenvalue n. 1 of Burden matrix/weighted by atomic masses), higher value of descriptor BEHp1 (highest eigenvalue n. 1 of Burden matrix / weighted by atomic polarizabilities) and a higher value of 7^{th} order charge index (GGI7) will be beneficiary to the activity. The derived models and participating descriptors in them have suggested that the substituents of 2-alkyl-4-aryl-pyrimidine fused heterocycles have sufficient scope for further modification.

Keywords: QSAR, 2-Alkyl-4-aryl-pyrimidine fused heterocycles, 5-HT_2 antagonists, binding affinity, combinatorial protocol in multiple linear regression (CP-MLR).

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The neurotransmitter serotonin was first isolated and identified in as 5-hydroxytryptamine (5-HT) [1,2]. The existence of at least 14 different subtypes of 5-HT receptors each of which is encoded by distinct genes is confirmed by molecular cloning studies [1]. Based on pharmacology, amino acid sequence, gene organization and second messenger coupling pathways, 5-HT receptors have been divided into seven families [1-7] and designated as 5-HT[1-7].

All of the 5-HT receptor subtypes are coupled to G-proteins except 5-HT_3 receptor which is a ligand gated ion-channel [4]. It had been shown in perceptive operational studies that the monoamine elicits a complex array of pharmacological and physiological responses by acting at a diversity of 5-HT receptors. The action on at least one of the 5-HT receptor subtypes is the basis of the therapeutic value of many widely prescribed CNS drugs [5]. The 5-HT_{2A} receptor subtype has implications in a variety of behavioral processes and neuropsychiatric disorders [6,7]. This receptor subtype also appears to be the site of action of many hallucinogenic compounds. LSD, mescaline and bufotenin act as agonists where as atypical antipsychotics such as risperdal, olanzapine, and clozapine act as high affinity antagonists of the 5-HT_{2A} receptor [5]. The antidepressant mirtazepine has affinity for presynaptic □$_2$ receptors and also possesses pharmacology which includes the antagonism of the 5-HT_2 receptor subtypes [5]. The lack of selectivity of these and other drugs may result side effects. The designing of selective 5-HT receptor agonists or antagonists may be helpful to discover better tolerated medicines.

The role of 5-HT_{2A} antagonists in the treatment of certain sleep disorders has been suggested in pharmacological studies [8]. Both, the selective and non-selective 5-HT_{2A} antagonists increase the amount of time humans spend in slow wave sleep which is the most restorative stage of the sleep cycle [9]. The representative reported 5-HT_{2A} antagonists are ritanserin [7-9], eplivanserin [10], MDL-100907 [11] and □-fluorosulfone, structurally related to MDL-100907 [12]. The □-fluorosulfone exhibited an improved cardiovascular profile.

More than 70% homology in the transmembrane domain of the 5-HT_{2A-2C} receptor subtypes [5] is the main challenge in the discovery of subtype selective 5-HT_{2A} antagonists. Determination of the therapeutic value of a selective 5-HT_{2A} antagonist is dependent upon the discovery of such molecules having favorable pharmacokinetics.

In a high-throughput screen of internal compound collection Sanfilippo et al. had identified aminopyrimidine as a high affinity ligand for the 5-HT_{2A} receptor which have also shown significant affinity for the 5-HT_{2B} and 5-HT_{2C} receptor subtypes.

In an attempt to find a molecule with increased selectivity over the 5-HT_{2B} and 5-HT_{2C} receptors Shireman et al. [14] have reported a series of 2-alkylpyrimidines. In view of the importance of 5-HT_{2A} selective antagonists in the clinical management of sleep disorders, a quantitative structure–activity relationship is attempted on the binding affinities of these alkylpyrimidine derivatives. The present study is aimed at rationalizing the substituent variations of these analogues to provide insight for the future endeavours.

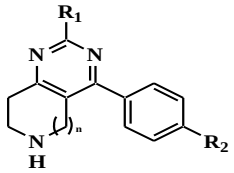
MATERIALS AND METHODS

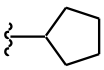
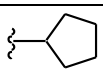
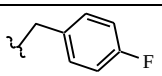
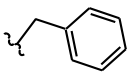
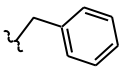
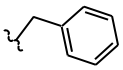
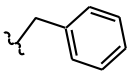
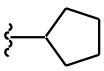
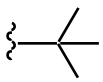
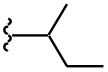
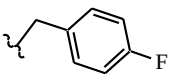
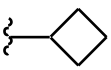
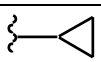
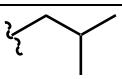
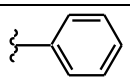
Chemical structure database and biological activity

This study comprises a chemical structure database of twenty 2-alkylpyrimidine derivatives, reported by Shireman et al. [14]. The receptor binding was performed using the human recombinant 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. The affinity of these derivatives for the three different human 5-HT_2 subtypes was evaluated by competitive radioligand binding assays using [^{3}H]ketanserin (h5-HT_{2A}) or [^{3}H]mesulergine (h5-HT_{2B} and h5-HT_{2C}). The assays were performed on membranes prepared from NIH3T3 stably transfected with h5-HT_{2A} or CHO stably transfected with h5-HT_{2B} and h5-HT_{2C}.[15] The structural variations and the binding affinities of titled compounds have been given in Table 1. The reported activity data on molar basis has been used for subsequent QSAR analyses as the response variables. For the purpose of modeling all 20 analogues have been divided into training and test sets. Out of the 20 analogues, one fourth compounds (5) have been placed in the test set for the validation of derived models. The training and test set compounds are also listed in Table 1.

ISSN: 0975-8585

Table 1: Structures and observed and modeled 5-HT binding affinity of 2-Alkyl-4-aryl-pyrimidine fused heterocycles.



Cpd.	n	R_1	R_2	$pK_i(5\text{-}HT_{2A})$[a]				
				Obs.	Eq. 6	Eq. 7	Eq. 8	Eq. 9
1[b]	1	$-NEt_2$	CH_3	7.70	7.89	7.73	7.62	8.05
2	2		Cl	5.92	6.21	6.16	6.05	6.16
3	2		F	8.40	8.00	8.37	7.95	8.04
4	2		F	7.89	8.50	8.26	8.18	8.27
5	2		CH_3	8.60	9.00	9.13	8.79	8.72
6	2		OCH_3	7.96	7.99	7.81	7.77	8.12
7	2		CN	7.47	7.30	7.53	7.93	7.77
8	2		F	9.15	8.72	8.56	8.51	8.59
9[b]	2		F	9.22	8.68	8.70	8.68	8.68
10	2		CH_3	9.40	9.08	9.43	9.20	9.02
11	2		CH_3	9.10	9.18	9.00	9.29	9.42
12[b]	1		F	7.70	8.16	8.15	8.38	8.29
13	1		F	8.66	7.92	7.98	8.11	8.20
14	1		F	8.30	8.40	7.74	8.13	8.03
15	1		F	7.96	7.84	7.93	7.90	7.67
16	1		F	7.89	8.30	8.01	8.28	8.30
17[b]	1		F	7.96	7.90	7.95	7.88	7.92
18	1		F	7.40	7.31	7.61	7.26	7.08
19	1		F	7.54	7.89	8.12	8.29	8.23
20[b]	1		F	7.30	7.87	7.70	7.48	7.57

[a]On molar basis, taken from reference[14]; [b]Compounds included in test set.

ISSN: 0975-8585

Theoretical molecular descriptors

Table 2: Descriptor classes[a] used along with their definition and scope for modeling the 5-HT_{2A} binding affinity of 2-alkylpyrimidine derivatives.

Descriptor class (acronyms)	Definition and scope
Constitutional (CONST)	Dimensionless or 0D descriptors; independent from molecular connectivity and conformations
Topological (TOPO)	2D-descriptor from molecular graphs and independent conformations
Molecular walk counts (MWC)	2D-descriptors representing self-returning walks counts of different lengths
Modified Burden eigenvalues (BCUT)	2D-descriptors representing positive and negative eigenvalues of the adjacency matrix, weights the diagonal elements and atoms
Galvez topological charge indices (GALVEZ)	2D-descriptors representing the first 10 eigenvalues of corrected adjacency matrix
2D-autocorrelations (2D-AUTO)	Molecular descriptors calculated from the molecular graphs by summing the products of atom weights of the terminal atoms of all the paths of the considered path length (the lag)
Functional groups (FUNC)	Molecular descriptors based on the counting of the chemical functional groups
Atom centered fragments (ACF)	Molecular descriptors based on the counting of 120 atom centered fragments, as defined by Ghose-Crippen
Empirical (EMP)	1D-descriptors represent the counts of non-single bonds, hydrophilic groups and ratio of the number of aromatic bonds and total bonds in an H-depleted molecule
Properties (PROP)	1D-descriptors representing molecular properties of a molecule

[a]Reference [17]

The structures of the compounds under study have been drawn in 2D ChemDraw [16]. The drawn structures were then converted into 3D modules using the default conversion procedure implemented in the CS Chem3D Ultra. The energy of these 3D-structures was minimized in the MOPAC module using the AM1 procedure for closed shell systems. This will ensure a well defined conformer relationship among the compounds of the study. All these energy minimized structures of respective compounds have been ported to DRAGON software [17] for the computation of descriptors for the titled compounds (Table 1). This software offers several hundreds of descriptors from different perspectives corresponding to 0D-, 1D-, and 2D-descriptor modules. The outlined modules comprised of ten different classes, namely, the constitutional (CONST), the topological (TOPO), the molecular walk counts (MWC), the BCUT descriptors (BCUT), the Galvez topological charge indices (GALVEZ), the 2D autocorrelations (2D-AUTO), the functional groups (FUNC), the atom-centered fragments (ACF), the empirical descriptors (EMP), and the properties describing descriptors (PROP). For each of these classes the DRAGON software computes a large number of descriptors which are characteristic to the molecules under multi-descriptor environment. The definition and scope of these descriptor's classes is given in Table 2. The combinatorial protocol in multiple linear regression (CP-MLR) [18] procedure has been used in the present work for developing QSAR models. Before the application of CP-MLR procedure, all those descriptors which are intercorrelated beyond 0.90 and showing a correlation of less than 0.1 with the biological endpoints (descriptor vs. activity, $r < 0.1$) were excluded. This has reduced the total dataset of the compounds from 464 to 89 descriptors as relevant ones for the 5-HT_{2A} binding activity. A brief description of the computational procedure is given below.

Model development

The CP-MLR is a 'filter' based variable selection procedure for model development in QSAR studies [18]. Its procedural aspects and implementation are discussed in some of our recent publications [19-23]. It involves selected subset regressions. In this procedure a combinatorial strategy with appropriately placed 'filters' has been interfaced with MLR to result in the extraction of diverse structure-activity models, each having unique combination of descriptors from the dataset under study. In this, the contents and number of variables to be evaluated are mixed according to the predefined confines. Here the 'filters' are significance evaluators of the variables in regression at different stages of model development. Of these, filter-1 is set in terms of inter-parameter correlation cutoff criteria for variables to stay as a subset (filter-1, default value 0.3 and upper limit ≤ 0.79). In this, if two variables are correlated higher than a predefined cutoff value the respective variable combination is forbidden and will be rejected. The second filter is in terms of t-values of regression coefficients of variables associated with a subset (filter-2, default value 2.0). Here, if the ratio of regression coefficient and associated standard error of any variable is less than a predefined cutoff value then the variable combination will be rejected. Since successive additions of variables to multiple regression equation will increase successive multiple correlation coefficient (r) values, square-root of adjusted multiple correlation coefficient of regression equation, r-bar, has been used to compare the internal explanatory power of models with different number of variables. Accordingly, a filter has been set in terms of predefined threshold level of r-bar (filter-3, default value 0.71) to decide the variables' 'merit' in the model formation. Finally, to exclude false or artificial correlations, the external consistency of the variables of the model have been addressed in terms of cross-validated R^2 or Q^2 criteria from the leave-one-out (LOO) cross-validation procedure as default option (filter-4, default threshold value $0.3 \leq Q^2 \leq 1.0$). All these filters make the variable selection process efficient and lead to unique solution. In order to collect the descriptors with higher information content and explanatory power, the threshold of filter-3 was successively incremented with increasing number of descriptors (per equation) by considering the r-bar value of the preceding optimum model as the new threshold for next generation.

Model validation

In this study, the data set is divided into training set for model development and test set for external prediction. Goodness of fit of the models was assessed by examining the multiple correlation coefficient (r), the standard deviation (s), the F-ratio between the variances of calculated and observed activities (F). A number of additional statistical parameters such as the Akaike's information criterion, AIC [24,25], the Kubinyi function, FIT [26,27], and the Friedman's lack of fit, LOF [28], (Eqs. 1-3) have also been derived to evaluate the best model.

$$AIC = \frac{RSS \times (n + p')}{(n - p')^2} \quad (1)$$

$$FIT = \frac{r^2 \times (n - k - 1)}{(n + k^2) \times (1 - r^2)} \quad (2)$$

$$LOF = \frac{RSS/n}{\left[1 - \frac{k(d+1)}{n}\right]^2} \quad (3)$$

where, RSS is the sum of the squared differences between the observed and the estimated activity values, k is the number of variables in the model, p' is the number of adjustable parameters in the model, and d is the smoothing parameter. The AIC takes into account the statistical goodness of fit and the number of parameters that have to be estimated to achieve that degree of fit. The FIT, closely related to the F-value (Fisher ratio), was proved to be a useful parameter for assessing the quality of the models. The main disadvantage of the F-value is its sensitivity to changes in k (the number of variables in the equation, which describe the model), if k is small, and its lower sensitivity if k is large. The FIT criterion has a low sensitivity toward changes in k-values, as long as they are small numbers, and a substantially increasing sensitivity for large k-values. The model that produces the minimum value of AIC and the highest value of FIT is considered

potentially the most useful and the best. The LOF takes into account the number of terms used in the equation and is not biased, as are other indicators, toward large numbers of parameters. A minimum LOF value infers that the derived model is statistically sound.

The internal validation of derived model was ascertained through the cross-validated index, Q^2, from leave-one-out and leave-five-out procedures. The LOO method creates a number of modified data sets by taking away one compound from the parent data set in such a way that each observation has been removed once only. Then one model is developed for each reduced data set, and the response values of the deleted observations are predicted from these models. The squared differences between predicted and actual values are added to give the predictive residual sum of squares, PRESS. In this way, PRESS will contain one contribution from each observation. The cross-validated Q^2_{LOO} value may further be calculated as

$$Q^2_{LOO} = 1 - PRESS/SSY \qquad (4)$$

where, SSY represents the variance of the observed activities of molecules around the mean value. In leave-five-out procedure, a group of five compounds is randomly kept outside the analysis each time in such a way that all the compounds, for once, become the part of the predictive groups. A value greater than 0.5 of Q^2-index hints toward a reasonable robust model.

The external validation or predictive power of derived model is based on test set compounds. The squared correlation coefficient between the observed and predicted values of compounds from test set, r^2_{Test}, has been calculated as

$$r^2_{Test} = 1 - \frac{\sum (Y_{Pred(Test)} - Y_{(Test)})^2}{\sum (Y_{(Test)} - \bar{Y}_{(Training)})^2} \qquad (5)$$

where, $Y_{Pred(Test)}$ and $Y_{(Test)}$ indicate predicted and observed activity values, respectively of the test-set compounds, and $\bar{Y}_{(Training)}$) indicate mean activity value of the training set. r^2_{Test} is the squared correlation coefficient between the observed and predicted data of the test-set. A value greater than 0.5 of r^2_{Test} suggests that the model obtained from training set has a reliable predictive power.

Y-randomization

Chance correlations, if any, associated with the CP-MLR models were recognized in randomization test [29,30] by repeated scrambling of the biological response. The data sets with scrambled response vector have been reassessed by multiple regression analysis (MRA). The resulting regression equations, if any, with correlation coefficients better than or equal to the one corresponding to the unscrambled response data were counted. Every model has been subjected to 100 such simulation runs. This has been used as a measure to express the percent chance correlation of the model under scrutiny.

RESULTS AND DISCUSSION

In multi-descriptor class environment, exploring for best model equation(s) along the descriptor class provides an opportunity to unravel the phenomenon under investigation. In other words, the concepts embedded in the descriptor classes relate the biological actions revealed by the compounds. For the purpose of modeling study, 5 compounds have been included in the test set for the validation of the models derived from 15 training set compounds. A total number of 89 significant descriptors from 0D-, 1D- and 2D-classes have been subjected to CP-MLR analysis with default 'filters' set in it. Statistical models in two and three descriptor(s) have been derived successively to achieve the best relationship correlating 5-HT_{2A} binding affinity. These models (with 89 descriptors) were identified in CP-MLR by successively incrementing the filter-3 with increasing number of descriptors (per equation). For this the optimum r-bar value of the preceding level model has been used as the new threshold of filter-3 for the next generation. A total number of 11 models in three descriptors were obtained. These models shared 19 descriptors. These descriptors along with their physical meaning, average regression coefficients and total incidences are listed in Table 3.

ISSN: 0975-8585

The selected models in three descriptors are given below.

$$pK_i(5\text{-}HT_{2A}) = -1.165(0.461)PW4 - 2.056(0.465)MATS2m + 1.819(0.366)MLOGP + 9.231$$
$$n = 15,\ r = 0.901,\ s = 0.424,\ F = 15.901,\ FIT = 1.987,\ LOF = 0.366,\ AIC = 0.310,$$
$$Q^2_{LOO} = 0.654,\ Q^2_{L5O} = 0.528,\ r^2_{randY}(sd) = 0.235(0.141),\ r^2_{Test} = 0.614 \quad (6)$$

$$pK_i(5\text{-}HT_{2A}) = -2.395(0.483)BEHm1 + 2.895(0.489)BEHp1 - 1.459(0.456)PSA + 7.990$$
$$n = 15,\ r = 0.899,\ s = 0.428,\ F = 15.481,\ FIT = 1.935,\ LOF = 0.374,\ AIC = 0.317,$$
$$Q^2_{LOO} = 0.668,\ Q^2_{L5O} = 0.528,\ r^2_{randY}(sd) = 0.237(0.179),\ r^2_{Test} = 0.717 \quad (7)$$

$$pK_i(5\text{-}HT_{2A}) = 2.398(0.422)GGI7 - 1.904(0.439)MATS2m + 1.716(0.684)MATS1v + 6.923$$
$$n = 15,\ r = 0.893,\ s = 0.440,\ F = 14.458,\ FIT = 1.807,\ LOF = 0.395,\ AIC = 0.335,$$
$$Q^2_{LOO} = 0.670,\ Q^2_{L5O} = 0.715,\ r^2_{randY}(sd) = 0.167(0.128),\ r^2_{Test} = 0.646 \quad (8)$$

$$pK_i(5\text{-}HT_{2A}) = -1.729(0.692)RBN + 3.416(0.601)GGI7 - 2.218(0.453)MATS2m + 8.665$$
$$n = 15,\ r = 0.892,\ s = 0.441,\ F = 14.409,\ FIT = 1.801,\ LOF = 0.396,\ AIC = 0.336,$$
$$Q^2_{LOO} = 0.626,\ Q^2_{L5O} = 0.564,\ r^2_{randY}(sd) = 0.178(0.153),\ r^2_{Test} = 0.624 \quad (9)$$

Table 3: Descriptors[a] identified for modeling the 5-HT_{2A} binding affinity of 2-alkylpyrimidine derivatives along with the average regression coefficient[b], standard deviation and the total incidence.

Descriptor	Avg reg coeff (sd) total incidence	Descriptor	Avg reg coeff (sd) total incidence
RBN	-1.922(0.273)2	Ram	1.764(0.000)1
BLI	1.490(0.000)1	PW4	-1.165(0.000)1
BEHm1	-2.395(0.000)1	BEHe1	2.143(0.000)1
BEHp1	2.895(0.000)1	BEHp3	1.771(0.384)2
BELp4	2.459(0.000)1	GGI3	1.849(0.000)1
GGI7	2.696(0.670)5	MATS2m	-2.059(0.157)3
MATS6m	-2.002(0.346)3	MATS1v	1.759(0.060)2
MATS8e	-1.967(0.000)1	GATS5p	1.574(0.000)1
Hy	-7.119(0.000)1	PSA	-1.306(0.266)3
MLOGP	1.760(0.082)2		

[a]The descriptors are identified from the three parameter models emerged from CP-MLR protocol with filter-1 as 0.79; filter-2 as 2.0; filter-3 as 0.5; filter-4 as $0.3 \le Q^2 \le 1.0$; number of compounds in the study are 15; CONST: RBN, number of rotatable bonds constitutional descriptors; TOPO: Ram, ramification index; BLI, Kier benzene-likeliness index; PW4, path/walk 4 - Randic shape index; BCUT: BEHm1, highest eigenvalue n. 1 of Burden matrix / weighted by atomic masses; BEHe1, highest eigenvalue n. 1 of Burden matrix / weighted by atomic Sanderson electronegativities; BEHp1, highest eigenvalue n. 1 of Burden matrix / weighted by atomic polarizabilities; BEHp3, highest eigenvalue n. 3 of Burden matrix / weighted by atomic polarizabilities, BELp4, lowest eigenvalue n. 4 of Burden matrix / weighted by atomic polarizabilities, GALVEZ: GGI3, topological charge index of order 3; GGI7, topological charge index of order 7; 2D-AUTO: MATS2m, Moran autocorrelation - lag 2 / weighted by atomic masses; MATS6m, Moran autocorrelation - lag 6 / weighted by atomic masses; MATS1v, Moran autocorrelation - lag 1 / weighted by atomic van der Waals volumes; MATS8e, Moran autocorrelation - lag 8 / weighted by atomic Sanderson electronegativities; GATS5p, Geary autocorrelation – lag 5 / weighted by atomic polarizabilities; PROP: Hy, hydrophilic factor; PSA, fragment-based polar surface area; MLOGP, Moriguchi octanol-water partition coeff. (logP).

In above regression equations, the values given in the parentheses are the standard errors of the regression coefficients. The $r^2_{randY}(sd)$ is the mean random squared multiple correlation coefficient of the regressions in the activity (Y) randomization study with its standard deviation from 100 simulations. In the randomization study (100 simulations per model), none of the identified models has shown any chance correlation. The signs of the regression coefficients suggest the direction of influence of explanatory variables in the models.

The descriptor RBN belongs to CONST class of Dragon descriptors. The constitutional class descriptors are based on simple constitutional facts and are independent from molecular connectivity and conformations.

The descriptor RBN represents number of rotatable bonds in a molecular structure and its correlation to the activity is negative which suggests that lesser number of rotatable bonds in a molecular structure will be favorable to the binding affinity. The descriptors MLOGP and PSA are PROP class descriptors. Descriptor MLOGP is Moriguchi octanol-water partition coefficient (logP) and PSA is fragment-based polar surface area. These descriptors reflect upon the hydrophobic property of a molecule and polar surface area of a fragment, respectively. The positive and negative contribution of descriptors MLOGP and PSA respectively, to the activity advocate a more hydrophobic nature and less polar surface area in a molecule for augmented activity.

The participated descriptor PW4 is from the TOPO class of Dragon descriptors. The TOPO class descriptors are based on a graph representation of the molecule and are numerical quantifiers of molecular topology obtained by the application of algebraic operators to matrices representing molecular graphs and whose values are independent of vertex numbering or labeling. They can be sensitive to one or more structural features of the molecule such as size, shape, symmetry, branching and cyclicity and can also encode chemical information concerning atom type and bond multiplicity. The descriptor PW4 is the path/walk 4 - Randic shape index. This descriptor contributed negatively to the activity suggesting that a lower positive value of it would be beneficiary to the activity.

The descriptors MATS2m and MATS1v, in above models, are representatives of 2D-AUTO class of Dragon descriptors. The 2D-AUTO descriptors, MATSke and GATSke have their origin in autocorrelation of topological structure of Moran and of Geary [31,32], respectively. The computation of these descriptors involves the summation of different autocorrelation functions corresponding to the different fragment lengths and lead to different autocorrelation vectors corresponding to the lengths of the structural fragments [33]. Also a weighting component in terms of a physicochemical property has been embedded in these descriptors. As a result, these descriptors address the topology of the structure or parts thereof in association with a selected physicochemical property. In these descriptors' nomenclature, the penultimate character, a number, indicates the number of consecutively connected edges considered in its computation and is called as the autocorrelation vector of lag k (corresponding to the number of edges in the unit fragment). The very last character of the descriptor's nomenclature indicates the physicochemical property considered in the weighting component for its computation. The participated descriptor MATS2m (Moran autocorrelation –lag 2/weighted by atomic masses) correlate negatively to the activity suggesting the unfavorable conditions associated with lag 2 weighted by atomic masses. The positive correlation of other descriptor MATS1v (Moran autocorrelation –lag 1/weighted by atomic van der Waals volumes) suggest the favorable conditions associated with lag 1 weighted by atomic van der Waals volumes.

The descriptors BEHm1 and BEHp1 are from the BCUT class of Dragon descriptors. The BCUT descriptors are the first 8 highest and the lowest absolute eigenvalues, BEHwk and BELwk, respectively, for the modified Burden adjacency matrix. Here w refers to the atomic property and k to the eigenvalue rank. The ordered sequence of the highest and the lowest eigenvalues reflect upon the relevant aspects of molecular structure, useful for similarity searching. The negative and positive contribution of descriptors BEHm1 and BEHp1 to the activity respectively, suggest a lower value of descriptor BEHm1 and a higher value of descriptor BEHp1 to enhance the activity.

Descriptor GGI7 is the lone representative of the GALVEZ class of descriptors. The GALVEZ descriptors are the Galvez topological charge indices and have their origin in the first ten eigenvalues of the polynomial of corrected adjacency matrix of the compounds. All the GALVEZ class descriptors belong to two categories. Of this one category corresponds to the topological charge index of order n (GGIn) and the other to the mean topological charge index of order n (JGIn), where 'n' represents the order of eigen value. The positive influence of descriptor GGI7 (topological charge index of 7th order) from this class to the activity suggested that a higher value of 7th order charge index would be beneficiary to the activity.

These models have accounted for up to 81.18 percent variance in the observed activities. The values greater than 0.5 of Q^2-index is in accordance to a reasonable robust QSAR model. The pK_i values of training set compounds calculated using Equations (6) to (9) have been included in Table 1. These models are validated with an external test set of five compounds listed in Table 1. The predictions of the test set compounds based on external validation are found to be satisfactory as reflected in the test set r^2 (r^2_{Test}) values and the predicted activity values are also reported in Table 1. The plot showing goodness of fit between observed and calculated activities for the training and test set compounds is given in Figure 1.

ISSN: 0975-8585

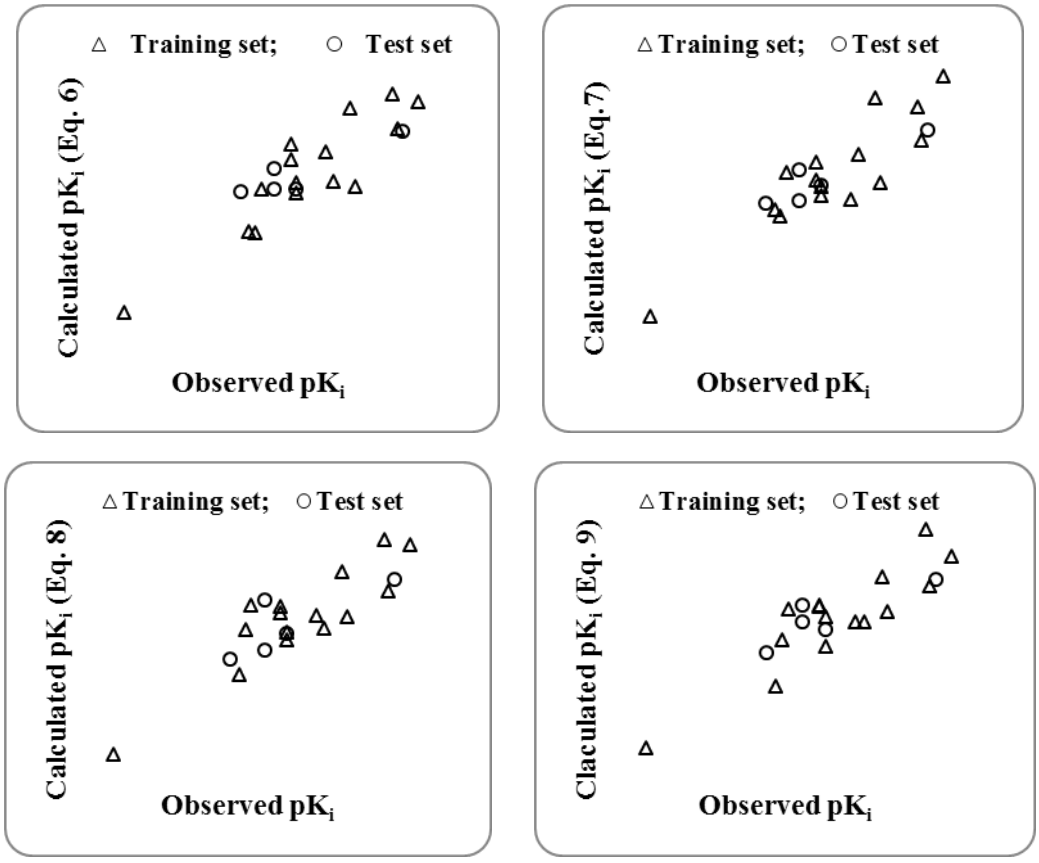


Figure 1: Plots of observed versus calculated pK$_i$ values for the 2-alkylpyrimidine derivatives

The SAR study of Shireman et al. [14] was aimed at to obtain molecules with increased selectivity versus the 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors. An attempt has been made in the present study to explain the selectivity for 5-HT$_2$ receptor subtypes in the form of selectivity ratio (SR) in terms of quantifying parameters (descriptors). For a given receptor the binding affinity has been reported as K$_i$ values. The selectivity ratio (SR) was calculated as the ratio of K$_i$ values for 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors and the same was then expressed on negative logarithmic scale as –log (SR). The ratios K$_i$(5-HT$_{2A}$)/K$_i$(5-HT$_{2B}$) and K$_i$(5-HT$_{2A}$)/K$_i$(5-HT$_{2C}$) has been represented by SR1 and SR2, respectively. Two different pools of relevant descriptors were used to explore significant QSARs through CP-MLR. The relevant descriptors were obtained by exclusion (descriptors which are intercorrelated beyond 0.90 and showing a correlation of less than 0.1 with the biological endpoints were excluded). A total number of 80 and 84 descriptors emerged as relevant ones for SR1 and SR2, respectively. These descriptors were then subjected to CP-MLR and 8 models for SR1 and 5 models for SR2 were obtained. The selected models for the SR1 and SR2 are presented below.

$$-\log SR1 = 2.574(0.385)nR06 + 1.496(0.509)IVDE + 1.767(0.498)GATS2p - 1.971$$
$$n = 15,\ r = 0.934,\ s = 0.421,\ F = 25.250,\ FIT = 3.156,\ LOF = 0.362,\ AIC = 0.307,$$
$$Q^2_{LOO} = 0.771,\ Q^2_{L5O} = 0.855,\ r^2_{randY}(sd) = 0.242(0.152),\ r^2_{Test} = 0.566 \quad (10)$$

$$-\log SR1 = 1.355(0.369)MAXDP - 1.848(0.783)X1A + 2.039(0.627)nCs + 0.555$$
$$n = 15,\ r = 0.899,\ s = 0.517,\ F = 15.560,\ FIT = 1.945,\ LOF = 0.544,\ AIC = 0.461,$$
$$Q^2_{LOO} = 0.618,\ Q^2_{L5O} = 0.643,\ r^2_{randY}(sd) = 0.197(0.146),\ r^2_{Test} = 0.912 \quad (11)$$

$$-\log SR1 = -0.883(0.332)nR07 + 1.451(0.399)MAXDP - 1.295(0.571)JGI5 + 1.014$$
$$n = 15,\ r = 0.898,\ s = 0.518,\ F = 15.428,\ FIT = 1.928,\ LOF = 0.548,\ AIC = 0.464,$$
$$Q^2_{LOO} = 0.669,\ Q^2_{L5O} = 0.755,\ r^2_{randY}(sd) = 0.199(0.124),\ r^2_{Test} = 0.517 \quad (12)$$

$$-\log SR1 = 2.273(0.641)BEHe1 - 2.011(0.426)GATS5e + 2.231(0.569)nCs - 0.117$$
$$n = 15,\ r = 0.894,\ s = 0.530,\ F = 14.613,\ FIT = 1.826,\ LOF = 0.572,\ AIC = 0.485,$$

Q^2_{LOO} = 0.713, Q^2_{L5O} = 0.724, r^2_{randY}(sd) = 0.241(0.159), r^2_{Test} = 0.727 (13)

-log SR2 = 1.242(0.255)RBN – 1.472(0.247)BELv7 + 1.994(0.302)BEHe1 + 0.545
n = 15, r = 0.909, s = 0.198, F = 17.457, FIT = 2.182, LOF = 0.079, AIC = 0.067,
Q^2_{LOO} = 0.641, Q^2_{L5O} = 0.687, r^2_{randY}(sd) = 0.217(0.149), r^2_{Test} = 0.525 (14)

-log SR2 = -0.722(0.186)X2Av + 0.929(0.234)IC2 + 1.131(0.272)BEHe1 + 0.772
n = 15, r = 0.903, s = 0.203, F = 16.347, FIT = 2.043, LOF = 0.084, AIC = 0.071,
Q^2_{LOO} = 0.582, Q^2_{L5O} = 0.707, r^2_{randY}(sd) = 0.223(0.133), r^2_{Test} = 0.572 (15)

-log SR2 = -0.972(0.202)X2Av +1.016(0.292)BEHe1 + 0.533(0.163)nCt + 1.333
n = 15, r = 0.880, s = 0.225, F = 12.601, FIT = 1.575, LOF = 0.103, AIC = 0.087,
Q^2_{LOO} = 0.603, Q^2_{L5O} = 0.698, r^2_{randY}(sd) = 0.209(0.147), r^2_{Test} = 0.575 (16)

-log SR2 = -1.147(0.234)BLI + 0.771(0.278)LP1 + 0.416(0.165)nCt + 1.609
n = 15, r = 0.857, s = 0.244, F = 10.142, FIT = 1.267, LOF = 0.122, AIC = 0.103,
Q^2_{LOO} = 0.510, Q^2_{L5O} = 0.522, r^2_{randY}(sd) = 0.187(0.109), r^2_{Test} = 0.674 (17)

From the correlation of the participated descriptors to the activity, in above models, the presence of more number of six membered rings (nR06) and higher number of sp^3 hybridized secondary carbon atoms (nCs) in a molecular structure and higher values of mean information content vertex degree equality (IVDE)), atomic polarizabilities weighted Geary autocorrelation of lag-2 (GATS2p), maximal electrotopological positive variation (MAXDP) and the highest eigenvalue n. 1 of Burden matrix / weighted by atomic Sanderson electronegativities (BEHe1) would be in favor of selectivity toward 5-HT_{2A} receptor. On the other hand higher values of atomic Sanderson electronegativities weighted Geary autocorrelation of lag 5 (GATS5e), average connectivity index, chi-1 (X1A), mean topological charge index of order 5 (JGI5) and presence of more number of seven membered rings in a molecular structure would be beneficiary to the 5-HT_{2B} activity.

The contribution of participated descriptors in regression models for the SR2 activity suggest that a higher value of information content index of 2-order neighbourhood symmetry (IC2), highest eigenvalue n. 1 of Burden matrix / weighted by atomic Sanderson electronegativities (BEHe1), Lovasz-Pelikan index which is related to leading eigen value (LP1) and presence of more rotatable bonds (RBN) and sp^3 hybridized tertiary carbon atoms in a molecular structure are in favor of SR2. Additionally, lower values of lowest eigenvalue n. 7 of Burden matrix / weighted by atomic van der Waals volumes (BELv7), average valence connectivity index, chi-2 (X2Av) and Kier benzene-likeliness index (BLI) are advantageous to the 5-HT_{2A} as compared to 5-HT_{2C} activity.

These models have accounted for up to 87.23 and 82.62 percent variances in the observed activities (SR1 and SR2, respectively). The values greater than 0.5 of Q^2-index and r^2_{Test} hints that these models are reasonable robust QSAR models and the predictions of the test set compounds based on external validation are satisfactory. The predicted activity values of test set compounds are mentioned in Table 4.

The regression analysis for the SR1 and SR2 activities has also been performed through CP-MLR using the 89 descriptors which were employed for 5-HT_{2A} binding activity. QSARs have also been obtained for the SR1 activity in the descriptor pool of SR2 activity and vice versa. The results are represented in Table 5. It is evident from this table that the most of the descriptors emerged in these models are part of the model equations discussed above and hold scope to quantify the biological endpoints.

ISSN: 0975-8585

Table 4: observed and modeled 5-HT selectivity ratio of 2-Alkyl-4-aryl-pyrimidine fused heterocycles.

Cpd.	K_i(nM)[a] 5-HT			-logSR1[b]					-logSR2[c]				
	2A	2B	2C	Obs.	Eq.10	Eq.11	Eq.12	Eq.13	Obs.	Eq.14	Eq.15	Eq.16	Eq.17
1[d]	20	70	300	0.544	0.915	0.768	0.310	1.244	1.176	1.437	1.105	1.290	1.017
2	1200	270	5000	-0.648	-0.698	-0.646	-0.192	-0.598	0.620	0.753	0.894	0.677	0.809
3	4	20	80	0.699	0.551	0.554	0.625	0.532	1.301	1.400	1.391	1.552	1.555
4	13	30	200	0.363	0.526	1.155	1.039	0.833	1.187	1.397	1.078	1.255	1.185
5	2.5	1	23	-0.398	0.161	-0.097	-0.301	-0.483	0.964	0.960	1.188	1.098	0.908
6	11	4.8	120	-0.360	-0.151	-0.472	-0.348	0.673	1.038	0.863	1.006	1.299	1.123
7	34	20	460	-0.230	-0.481	-0.093	0.059	-0.294	1.131	1.240	1.327	1.361	1.246
8	0.7	40	50	1.757	1.667	2.006	1.421	2.095	1.854	1.536	1.611	1.667	1.656
9[d]	0.6	30	20	1.699	1.275	1.821	1.461	1.759	1.523	1.536	1.545	1.536	1.487
10	0.4	2.5	15	0.796	0.886	0.519	0.067	0.602	1.574	1.628	1.488	1.404	1.232
11	0.8	22	38	1.439	1.373	1.939	1.048	1.885	1.677	1.808	1.937	1.974	1.836
12[d]	20	2500	560	2.097	2.297	1.979	2.438	1.914	1.447	1.674	1.769	1.737	1.737
13	2.2	220	110	2.000	1.704	1.406	2.015	0.968	1.699	1.535	1.501	1.431	1.413
14	5	70	100	1.146	0.950	1.291	1.101	1.177	1.301	1.607	1.107	1.010	1.702
15	11	2250	1020	2.311	1.838	1.384	1.625	1.774	1.967	1.882	1.839	1.824	1.650
16	13	2200	1000	2.228	2.709	2.165	2.398	2.236	1.886	1.674	1.813	1.843	1.907
17[d]	11	210	485	1.281	1.785	1.570	1.993	1.424	1.644	1.870	1.868	1.667	1.657
18	40	9000	5000	2.352	1.723	1.826	2.011	2.118	2.097	2.154	2.244	2.009	2.012
19	29	400	1000	1.140	1.838	1.657	2.026	1.079	1.538	1.397	1.411	1.430	1.601
20[d]	50	1000	4590	1.301	1.877	1.136	1.862	1.514	1.963	1.879	1.923	2.211	2.069

[a]Reference [14], [b]SR1= K_i(5-HT_{2A})/K_i(5-HT_{2B}), [c]SR2= K_i(5-HT_{2A})/K_i(5-HT_{2C}) and [d]Test set compounds.

Table 5: Models obtained for SR activities in various pools of descriptors through CP-MLR.

S. No.	Analysis for the	Models	
1	SR1 (in descriptors used for the analysis of 5-HT_{2A})	-log SR1= 1.121(0.309)MAXDP + 3.136(0.649)D/Dr06 - 2.077(0.680)BELp3 + 0.327 n = 15, r = 0.936, s = 0.414, F = 26.213, Q^2_{LOO} = 0.801, Q^2_{LSO} = 0.796, r^2_{Test} = 0.552	(18)
		-log SR1= -1.885(0.491)X0Av + 2.407(0.475)GATS5p +2.189(0.602)nCs + 0.585 n = 15, r = 0.915, s = 0.477, F = 18.861, Q^2_{LOO} = 0.697, Q^2_{LSO} = 0.748, r^2_{Test} = 0.580	(19)
		-log SR1= 1.844(0.333)MAXDP + 1.519(0.605)BEHm1 + 2.071(0.614)nCs -1.316 n = 15, r = 0.904, s = 0.505, F = 16.417, Q^2_{LOO} = 0.633, Q^2_{LSO} = 0.699, r^2_{Test} = 0.593	(20)
		-log SR1= 3.750(0.792)BEHp3 + 2.996(0.596)nCs -4.077(0.727)MR -0.247 n = 15, r = 0.900, s = 0.513, F = 15.810, Q^2_{LOO} = 0.648, Q^2_{LSO} = 0.645, r^2_{Test} = 0.693	(21)
2	SR1 (in descriptors used for the analysis of SR2)	–log SR1 = 2.574(0.385)nR06 + 1.496(0.509)IVDE + 1.767(0.498)GATS2p –1.971 n = 15, r = 0.934, s = 0.421, F = 25.250, Q^2_{LOO} = 0.771, Q^2_{LSO} = 0.855, r^2_{Test} = 0.566	(10)
		-log SR1= -2.411(0.501)X0Av -2.196(0.426)BEHv5 + 1.655(0.569)nCs + 3.070 n = 15, r = 0.916, s = 0.472, F = 19.349, Q^2_{LOO} = 0.716, Q^2_{LSO} = 0.657, r^2_{Test} = 0.613	(22)
		–log SR1 = 1.355(0.369)MAXDP –1.848(0.783)X1A + 2.039(0.627)nCs + 0.555 n = 15, r = 0.899, s = 0.517, F = 15.560, Q^2_{LOO} = 0.618, Q^2_{LSO} = 0.643, r^2_{Test} = 0.912	(11)
		–log SR1 = –0.883(0.332)nR07 + 1.451(0.399)MAXDP –1.295(0.571)JGI5 + 1.014 n = 15, r = 0.898, s = 0.518, F = 15.428, Q^2_{LOO} = 0.669, Q^2_{LSO} = 0.755, r^2_{Test} = 0.517	(12)
3	SR2 (in descriptors used for the analysis of 5-HT_{2A})	-logSR2= 1.242 (0.255)RBN – 1.472(0.247)BELv7 + 1.994(0.302)BEHe1 + 0.545 n = 15, r = 0.909, s = 0.198, F = 17.457, Q^2_{LOO} = 0.641, Q^2_{LSO} = 0.545, r^2_{Test} = 0.525	(23)
		-logSR2= 0.522(0.246)Ram + 1.516(0.276)GATS2p – 1.133(0.218)C-002 + 0.858 n = 15, r = 0.899, s = 0.207, F = 15.484, Q^2_{LOO} = 0.704, Q^2_{LSO} = 0.522, r^2_{Test} = 0.546	(24)
		-logSR2= -1.036(0.252)BELv7 + 1.559(0.299)BEHe1 + 0.727(0.175)JGI6 + 0.664 n = 15, r = 0.886, s = 0.219, F = 13.471, Q^2_{LOO} = 0.526, Q^2_{LSO} = 0.559, r^2_{Test} = 0.590	(25)
4	SR2 (in descriptors used for the analysis of SR1)	-log SR2 = 1.242(0.255)RBN – 1.472(0.247)BELv7 + 1.994(0.302)BEHe1 + 0.545 n = 15, r = 0.909, s = 0.198, F = 17.457, Q^2_{LOO} = 0.641, Q^2_{LSO} = 0.687, r^2_{Test} = 0.525	(14)
		-log SR2 = -0.722(0.186)X2Av + 0.929(0.234)IC2 + 1.131(0.272)BEHe1 + 0.772 n = 15, r = 0.903, s = 0.203, F = 16.347, Q^2_{LOO} = 0.582, Q^2_{LSO} = 0.707, r^2_{Test} = 0.572	(15)
		-log SR2 = -0.972(0.202)X2Av +1.016(0.292)BEHe1 + 0.533(0.163)nCt + 1.333 n = 15, r = 0.880, s = 0.225, F = 12.601, Q^2_{LOO} = 0.603, Q^2_{LSO} = 0.698, r^2_{Test} = 0.575	(16)
		-log SR2 = -1.147(0.234)BLI + 0.771(0.278)LP1 + 0.416(0.165)nCt + 1.609 n = 15, r = 0.857, s = 0.244, F = 10.142, Q^2_{LOO} = 0.510, Q^2_{LSO} = 0.522, r^2_{Test} = 0.674	(17)

CONCLUSIONS

In conclusion, the present study has provided structure–activity relationships of the binding affinities of 2-Alkyl-4-aryl-pyrimidine fused heterocycles to 5-HT_2 receptor in terms of structural requirements. The binding affinity has, therefore become the function of the cumulative effect of different structural features which were identified in terms of individual descriptors. In order to improve the 5-HT_6 receptor binding affinity of a compound, less number of rotatable bonds (descriptor RBN), a more hydrophobic nature (descriptor MLOGP) and less polar surface area (descriptor PSA) in a molecular structure will be favorable to the binding affinity. A lower positive values of descriptors PW4 (path/walk 4 - Randic shape index) and MATS2m (Moran autocorrelation –lag 2/weighted by atomic masses) and higher value of descriptor MATS1v (Moran autocorrelation –lag 1/weighted by atomic van der Waals volumes) will augment the activity. Additionally, a lower value of descriptor BEHm1 (highest eigenvalue n. 1 of Burden matrix/weighted by atomic masses), higher value of descriptor BEHp1 (highest eigenvalue n. 1 of Burden matrix / weighted by atomic polarizabilities) and a higher value of 7^{th} order charge index (GGI7) will be beneficiary to the activity.

ACKNOWLEDGEMENTS

Authors are thankful to their Institution for providing necessary facilities to complete this study. MC thanks CSIR, New Delhi, India for financial support in the form of SRF.

REFERENCES

[1] Hoyer D, Hannon JP, Martin GR. Pharmacol Biochem Behav 2002; 71:533-554.
[2] Hoyer D, Martin GR. Behav Brain Res 1996; 73:263-268.
[3] Raymond JR, Mukhin YV, Gelasco A, Turner J, Collinsworth G, Gettys TW, Grewal J S, Garnovskayam MN. Pharmacol Ther 2001; 92:179-212.
[4] Thompson AJ, Lummis SCR. Curr Pharm Des 2006; 12:3615-3630.
[5] Roth BL, Lopez E, Patil S, Kroeze WK. Neuroscientist 2000; 6:252-262.
[6] Leyson JE. Curr Drug Targets CNS Neurol Disord 2004; 3:11-26.
[7] Stefanski R, Goldberg SR. CNS Drugs 1997; 7:388-409.
[8] Dugovic C. J Sleep Res 1992; 1:163-168.
[9] Sharpley AL, Elliott JM, Attenburrow M-J, Cowen PJ. Neuropharmacology 1994; 33:467-471.
[10] Rinaldi-Carmona M, Congy C, Santucci V, Simiano J, Gautret B, Neliat G, Labbeeuw B, Le Fur G, Soubrie P, Breliere JC. J Pharmacol Exp Ther 1992; 262:759-768.
[11] Kehne JH, Baron BM, Carr AA, Chaney SF, Elands J, Feldman DJ, Frank RA, Van Giersbergen PLM, McCloskey TC, Johnson MP, McCarthy DR, Poirot M, Senyah Y, Siegel BW, Widmaier C. J Pharmacol Exp Ther 1996; 277:968-981.
[12] (a) Fish LR, Gilligan MT, Humphries AC, Ivarsson M, Ladduwahetty T, Merchant KJ, O'Connor D, Patel S, Philipps E, Vargas HM, Hutson PH, MacLeod AM. Bioorg Med Chem Lett 2005; 15:3665-3669. (b) Fletcher SR, Burkamp F, Blurton P, Cheng SKF, Clarkson R, O'Connor D, Spinks D, Tudge M, van Niel MB, Patel S, Chapman K, Marwood R, Shepheard S, Bentley G, Cook GP, Bristow LJ, Castro JL, Hutson PH, MacLeod AM. J Med Chem 2002; 45:492-503.
[13] Sanfilippo PJ, Urbanski M, Williams L, Press JB, Katz LB, Shriver DA, Fernandez JA, Shatynski D, Offord SJ. Eur J Med Chem 1992; 27:655-661.
[14] Shireman BT, Dvorak CA, Rudolph DA, Bonaventure P, Nepomuceno D, Dvorak L, Miller KL, Lovenberg TW, Carruthers NI. Bioorg Med Chem Lett 2008; 18:2103–2108.
[15] Bonaventure P, Nepomuceno D, Miller K, Chen J, Kuei C, Kamme F, Tran D, Lovenberg TW, Liu C. Eur J Pharmacol 2005; 513:181.
[16] ChemDraw Ultra 6.0 and Chem3D Ultra, Cambridge Soft Corporation, Cambridge, USA. http://www.cambridgesoft.com
[17] Dragon software (version 1.11-2001) by R. Todeschini, V. Consonni, Milano, Italy. http://www.talete.mi.it/dragon.htm
[18] Prabhakar YS. QSAR Comb Sci 2003; 22:583.
[19] Sharma S, Prabhakar YS, Singh P, Sharma BK. Eur J Med Chem 2008; 43:2354.
[20] Sharma BK, Pilania P, Singh P. J Enzy Inhibn Med Chem 2009; 24:607.
[21] Sharma S, Sharma BK, Pilania P, Singh P, Prabhakar YS. J Enzy Inhibn Med Chem 2009; 24:1024.
[22] Sharma BK, Pilania P, Singh P, Prabhakar YS. SAR QSAR Environ Res 2010; 21: 169-.

[23] Sharma BK, Pilania P, Sarbhai K, Singh P, Prabhakar YS. Mol Divers 2010; 14:371.
[24] Akaike H. Information theory and an extension of the minimum likelihood principle. In: Petrov BN,Csaki F. (eds) Second international symposium on information theory. Akademiai Kiado, Budapest, 1973; 267.
[25] Akaike H. IEEE Trans Automat Contr 1974; AC-19:716-723.
[26] Kubinyi H. Quant Struct-Act Relat 1994; 13:285.
[27] Kubinyi H. Quant Struct-Act Relat 1994; 13:393.
[28] Friedman J. In: Technical report no. 102. Laboratory for computational statistics. Stanford University, Stanford, 1990.
[29] So S-S, Karplus M. J Med Chem 1997; 40:4347.
[30] Prabhakar YS, Solomon VR, Rawal RK, Gupta MK, Katti SB. QSAR Comb Sci 2004; 23:234.
[31] Moran PAP. Biometrika 1950; 37:17.
[32] Geary RC. Incorp Statist 1954; 5:115.
[33] Broto P, Moreau G, Vandycke C. Eur J Med Chem 1984; 19:66.