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Original article

A novel range based QSAR study of human neuropeptide Y (NPY) Y5 receptor inhibitors

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Abstract

A conventional QSAR study has been carried out using thermodynamic and other descriptors, on a set of arylsulfonamidomethylcyclohexyl derivatives as antagonists of potential obesity drug target human neuropeptide Y Y5 receptor. In addition, a novel range based method was applied to obtain a QSAR model so that the information contained in the compounds for which an approximate value instead of exact value of inhibitory activity was available could be included in the model. Analysis of models suggests that range based model is better in screening biologically active compounds from chemical library. The conventional model is able to predict activity accurately only for active compounds whereas the range based method is better in discriminating active and inactive compounds.

Keywords: Obesity; Neuropeptide Y; QSAR; GFA

1. Introduction

Obesity has emerged as an epidemic which requires effective strategies for its management [1]. In addition to the lifestyle habits, deregulation of various hypothalamic mechanisms which control the food intake and its expenditure for energy production has been implicated in the development and progression of this disease [2].

The role of neuropeptide Y, a 36 amino acid peptide is well recognized in the regulation of feeding behavior [3–6]. Alterations in the NPY level are associated with various diseases like cardiovascular disorders [7], psychological disorders [8], immune system disorders [9], inflammatory diseases [10], alterations in alcohol consumption behavior [11] etc. The appetite stimulation action is mainly mediated through the activation of Y1 and Y5 receptors [12]. Thus development of a cheap and potent antagonist of these receptors might be a good approach for antiobesity drug discovery.

Here we describe the quantitative structure activity relationship study of a set of arylsulfonamidomethylcyclohexyl derivatives which act as inhibitors of human neuropeptide Y Y5 receptor [13]. In addition to developing a good QSAR model by conventional method in which only those compounds are considered for model generation for which exact value of activity is given in literature, we also developed a QSAR model by incorporating those compounds for which, only some range of activity was given in place of the exact activity value. The idea behind the range based method is that in many cases exact value of the activity is not given in the literature for a large number of molecules and these molecules are not considered for QSAR model generation. However, these molecules contain some structural information which makes them inactive and this information should be utilized to understand the QSAR of that dataset. Moreover a QSAR model can predict biological activity accurately only for those compounds which are within the range of training set compounds. Thus the range of training set compounds should be as wide as possible. The model developed by the range based approach in this study is better in classifying the compounds as active and inactive. While the conventional method predicted the

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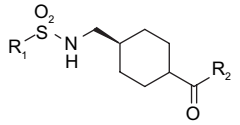
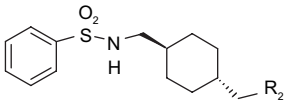
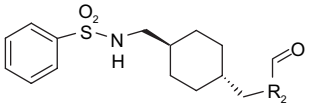
Table 1a
Structure and experimental activity of the amide, amine and *N*-formyl derivatives used in the study



Compound a1-43



Compound b1-4



Compound c1-3

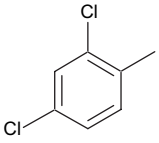
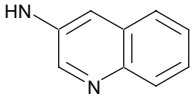
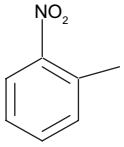
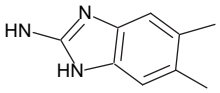
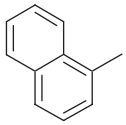
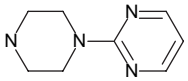
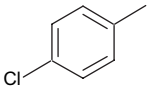
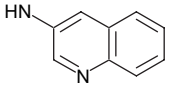
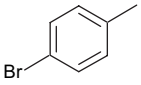
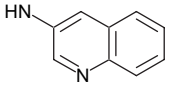
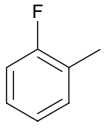
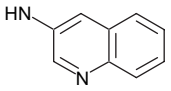
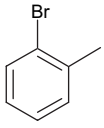
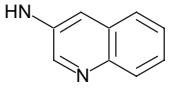
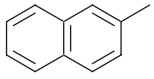
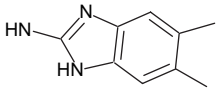
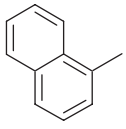
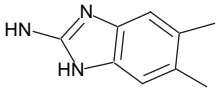
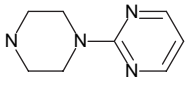
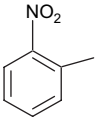
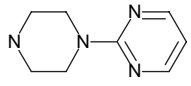
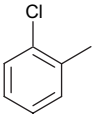
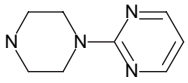
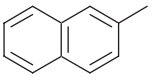
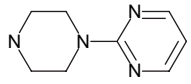
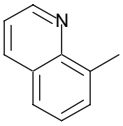
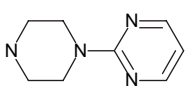
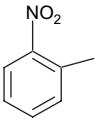
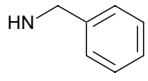
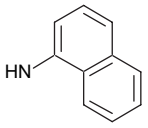
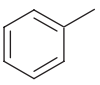
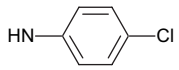
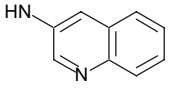
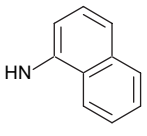
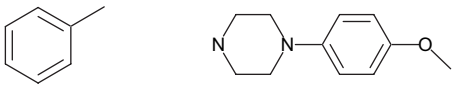
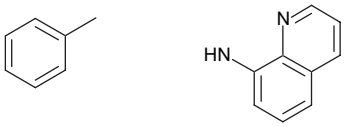
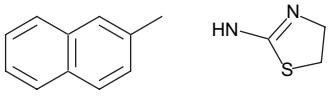
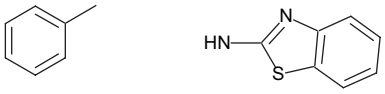
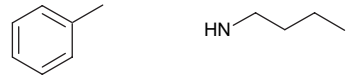
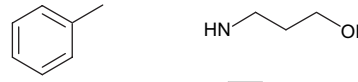
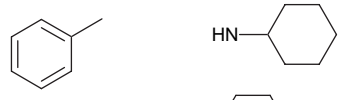
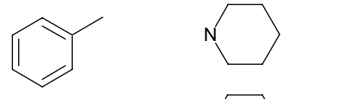
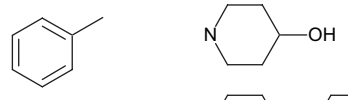
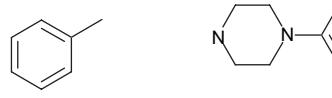
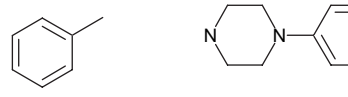
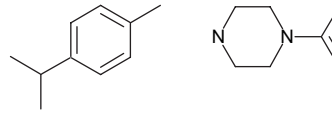
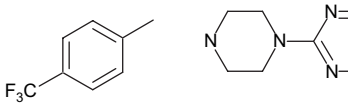
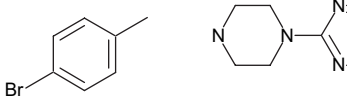
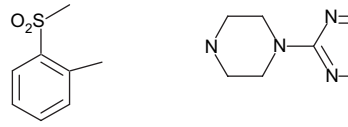
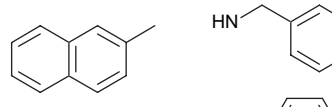
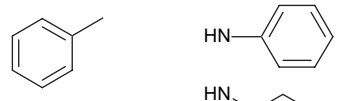
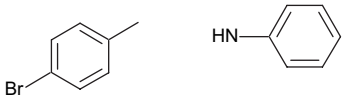
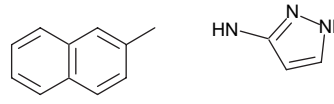
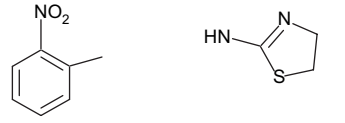
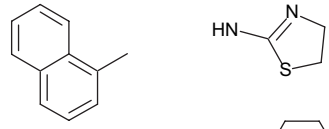
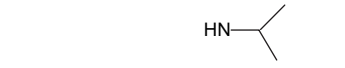
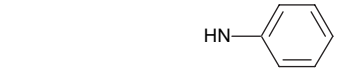
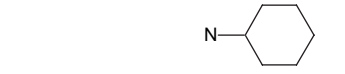
Mol id	R_1	R_2	IC_{50}	Mol id	R_1	R_2	IC_{50}
Compounds with IC_{50} between 0 and 10 nM (pIC_{50} between 2 and 3)							
a1			2.12	**a2**			4.16
Compounds with IC_{50} between 10 and 100 nM (pIC_{50} between 1 and 2)							
a3			19.2	**a4**			38.9
a5			34.6	**a6**			30.4
a7			17.3	**a8**			79.9
a9			79.9				
Compounds with IC_{50} between 100 and 1000 nM (pIC_{50} between 0 and 1)							
a10			748	**a11**			425
a12			138	**a13**			416
a14			179	**a15**			335
a16			573	**a17**			885
a18			106	**b1**			113

Table 1a (*continued*)

Compounds with IC_{50} between 1000 and 10,000 nM (pIC_{50} between −1 and 0)

a_{19}		9240	**a_{20}**		1910
a_{21}		4220	**a_{22}**		2840

Compounds with IC_{50} between 10,000 and 100,000 nM (pIC_{50} between −2 and −1)

a_{23}		$>10^4$	**a_{24}**		$>10^4$
a_{25}		$>10^4$	**a_{26}**		$>10^4$
a_{27}		$>10^4$	**a_{28}**		$>10_4$
a_{29}		$>10^4$	**a_{30}**		$>10_4$
a_{31}		$>10^4$	**a_{32}**		$>10^4$
a_{33}		$>10^4$	**a_{34}**		$>10^4$
a_{35}		$>10^4$	**a_{36}**		$>10^4$
a_{37}		$>10^4$	**a_{38}**		$>10^4$
a_{39}		$>10^4$	**a_{40}**		$>10^4$
a_{41}		$>10^4$	**a_{42}**		$>10^4$
a_{43}		$>10^4$	**b_2**		$>10^4$
b_3		$>10^4$	**b_4**		$>10^4$
c_1		$>10^4$	**c_2**		$>10^4$
c_3		$>10^4$			

Structure and experimental activity (in terms of IC_{50} in nM unit) of the amide, amine and *N*-formyl derivatives used in the study are described in this table. As the structures for thio(urea) derivatives are little different, they are given in a separate table.

activity accurately only for active compounds, the range based method worked well for both active and inactive compounds. Thus the model developed using range based method should be used for better screening of compounds from chemical database for their Neuropeptide Y Y5 inhibitory activity.

2. Methods

2.1. Dataset

The inhibitory activity of the arylsulfonamidomethylcyclohexyl derivatives for neuropeptide Y Y5 receptor was taken from literature [13] in terms of IC_{50} values. The structures and experimental values of activity for the compounds used in this study are shown in Tables 1a and 1b. The IC_{50} values were converted to pIC_{50} to get the linear relationship in the equation using the following formula:

$$pIC_{50} = -\log IC_{50}$$

Before conversion to pIC_{50}, activity value in nM unit was converted to μM unit to clearly distinguish between active and inactive compounds. In the case of nM IC_{50}, all the values of pIC_{50} were in negative range, making the interpretation difficult.

2.2. Molecular modeling

All the compounds were built using build molecule module of Sybyl6.9 software package installed on a Silicon Graphics Fuel Work station [14]. The geometry optimization calculations for each compound were carried out using the semiempirical method AM1 included in MOPAC 6.0 [15]. The following specific software options were employed while performing AM1 studies: convergence = normal, optimization = full, state = singlet, net charge = 0 e.u., time limit = 3600 s, keyword = mmok.

2.3. Descriptor calculation

Topological, information content, spatial, structural, thermodynamic, electronic and E-state indices types of descriptors were calculated using the Cerius^2 4.9 software package [16]. Topological indices are 2D descriptors based on graph theory concepts. These help to differentiate the molecules according to their size, degree of branching, flexibility and overall shape. Information content descriptors view molecule graphs as a source of certain probability distributions to which Shannon's statistical information theory tools can be applied [17]. Spatial descriptors are 3D descriptors which determine the shape and size of compounds. E-state indices or electrotopological descriptors are numerical values computed for each atom in a molecule and encode information about both the topological environment of the atom and the electronic interactions due to all other atoms in the molecule [18]. Some of the descriptors included in the study are listed and described in Table 2.

2.4. Regression analysis

The total number of descriptors calculated was more than 150 but some of the descriptors were rejected because they contain a value of zero for all the compounds. Further, the inter correlation of descriptors was taken into account and the highly correlated descriptors were grouped together manually from the correlation table. Only 1 descriptor from a group of correlated descriptors was taken for further study. From the remaining descriptors, the selection of variables to obtain the QSAR models was carried out using genetic function approximation (GFA) method. GFA is a genetics based method of variable selection which combines Holland's genetic algorithm (GA) with Friedman's multivariate adaptive regression splines (MARS) [19,20]. The GFA method works in the following way: first of all a particular number of equations (set at 100 by default in the Cerius^2 software) are generated randomly, then pairs of ''parent'' equations are chosen randomly from this set of 100 equations and ''crossover'' operations are performed at random. The number of crossovers was set by default at 5000 and the equations were generated by using a fixed length of 5 so that the generated equation has 4 descriptors and 1 constant value. The goodness of each progeny equation is assessed by Friedman's lack of fit (LOF) score which is given by the following formula:

$$LOF = LSE/\{1 - (c + dp)/m\}^2$$

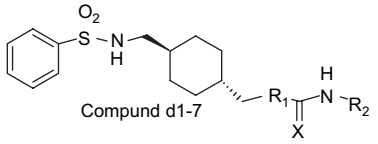
where LSE is the least-squares error, c is the number of basis functions in the model, d is the smoothing parameter, p is the number of descriptors and m is the number of observations in the training set. The smoothing parameter which controls the scoring bias between equations of different sizes was set at a default value of 1.0 and the new term was added with a probability of 50%. Only the linear equation terms were used for model building which is set by default in the software. The best equation out of the 100 equations was taken based on the statistical parameters such as regression coefficient, adjusted regression coefficient, regression coefficient cross validation and F-test values.

3. Results and discussion

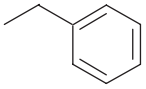
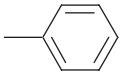
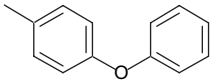
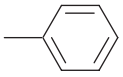
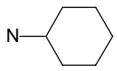
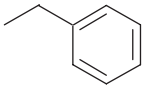
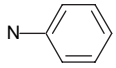
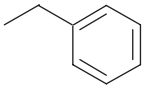
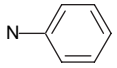
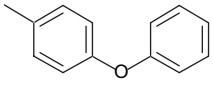
3.1. Conventional method

Statistically significant model was developed by taking only those compounds for which exact IC_{50} values were given. Out of the 24 compounds for which exact IC_{50} values were given (tested by same biological assay) 19 compounds were taken in the training set and 5 in the test set. The compounds in the training and test set were selected in such a way so as to maintain the maximum diversity of the structure and activity. Using genetic function approximation method to select the variables the following equation was developed:

Table 1b
Structure and experimental activity of the thio(urea) derivatives used in the study



Compund d1-7

Mol id	R_1	R_2	X	IC_{50}	Mol id	R_1	R_2	X	IC_{50}
Compounds with IC_{50} between 10,000 and 100,000 nM (pIC_{50} between −2 and −1)									
d_1			O	10,200					
Compounds with IC_{50} above 10^5 nM (pIC_{50} less than −2)									
d_2			O	$>10^5$	**d_3**			O	$>10^5$
d_4			S	$>10^5$	**d_5**			O	$>10^5$
d_6			O	$>10^5$	**d_7**			O	$>10^5$

Structure and experimental activity (in terms of IC_{50} in nM unit) of the thio(urea) derivatives used in the study are described in this table.

$$pIC_{50} = -7.65701 - 0.984981 * Atype\_N\_72 - 2.8118 * Atype\_O\_60 + 0.768097 * Atype\_C\_26 + 0.638227 * CHI - 2 \quad (1)$$

$N = 19$, LOF = 0.381, $r^2 = 0.874$, $r^2_{adj.} = 0.838$, F-test = 24.283, LSE = 0.128, $r = 0.935$, $q^2 = 0.772$, $r^2_{pred} = 0.505$

where N is the number of compounds in training set, LOF is lack of fit, r^2 is squared correlation coefficient, $r^2_{adj.}$ is the square of adjusted correlation coefficient, F-test is a statistical parameter which compares 2 models differing by 1 or more variables to see if the more complex model is more reliable than the less complex one. The model is supposed to be good if the F-test is above a threshold value. LSE is least-square error, r is correlation coefficient, q^2 is the square of the correlation coefficient of the cross validation and $r^2_{pred.}$ is the square of predicted correlation coefficient calculated from the predicted activity of test set compounds. Atype_N_72, Atype_O_60 and Atype_C_26 are atom-type-based AlogP descriptors. These descriptors developed by Ghose and Crippen [21] use the atomic contribution of individual atom types towards the overall hydrophobicity of molecules. Carbon, hydrogen, oxygen, nitrogen, sulfur and halogens are classified into 110 atom types. After several revisions the number of atom classifications has increased to 120 [22]. Each AlogP98 atom-type value represents the number of atoms of that type in the molecule. CHI-2 is the Kier and Hall connectivity index of order 2. Kier and Hall generalized and extended the branching index approach developed by Randic into a series of descriptors that came to be known as chi molecular indices. For calculation of these indices bond paths of

Table 2
Description of the parameters used in the study

Type	Descriptors
E-state indices	Electrotopological-state indices
Electronic	Sum of partial charges, sum of formal charges, dipole moment, energy of the highest occupied orbital, energy of the lowest unoccupied orbital
Information content	Information of atomic composition index, information indices based on the A-matrix, information indices based on the D-matrix, multigraph information content indices
Spatial	Radius of gyration, Jurs descriptors, shadow indices, area, density, PMI, V_m
Structural	Number of chiral centers, molecular weight, number of rotatable bonds, number of hydrogen-bond acceptors, number of hydrogen-bond donors
Thermodynamic	Log of the partition coefficient, log of the partition coefficient atom-type value, desolvation free energy of water, desolvation free energy of octanol, heat of formation, molar refractivity
Topological	Wiener index, Zagreb index, Hosoya index, Kier and Hall molecular connectivity index, Balaban indices

The descriptors used to build the QSAR models in this study are described in this table. They are divided into 6 broad categories which include E-state indices, electronic, information content, spatial, structural, thermodynamic, topological descriptors etc.

different lengths are considered. Thus for second order connectivity index only pairs of edges (bonds) are considered in the molecular graph. The predicted activity of training and test set compounds by this model is given in Tables 3a and 3b, respectively. This model explains more than 87% variation in the biological activity of these compounds and also suggests that atom-type-based AlogP98 descriptors are important in the QSAR study of these compounds.

3.2. Range based approach

In addition to the conventional method of developing QSAR model we also tried a range based approach by incorporating all compounds in the model. Out of the 57 neuropeptide Y Y5 receptor inhibitors exact values of IC_{50} were given only for 24 compounds. So by conventional method of QSAR, rest of the compounds could not be used for model generation and the information contained in those compounds could not be harnessed. In our range based approach, different ranges of biological activity were defined, each range being 1 log IC_{50} unit wide. Compounds were divided into different groups according to the range of their biological activity. The mid point of the range was assigned as the activity of all the compounds in that group or range in place of the exact value of pIC_{50}. For example, all those compounds having pIC_{50} value between 1 and 2 were given a value of 1.5, similarly compounds with pIC_{50} between 2 and 3 were given a value of 2.5 and so on. Compounds for which the mentioned activity (IC_{50}) value was $>10^4$ nM (10 μM) were assigned a value of pIC_{50} as −1.5 and for which the mentioned IC_{50} was $>10^5$ nM (100 μM) were given a value of pIC_{50} as −2.5. Compounds with $pIC_{50} > -1$ were considered as active and with $pIC_{50} < -1$ were considered as inactive. The activity values for the compounds are given in Tables 1a and 1b.

Table 3a
Actual and predicted (by model 1) activities of training set compounds

Compound	Actual pIC_{50}	Predicted pIC_{50}	Residual
a_1	2.674	2.704	−0.030
a_2	2.381	1.868	0.513
a_3	1.717	1.025	0.692
a_5	1.461	1.588	−0.127
a_6	1.517	1.531	−0.014
a_7	1.762	1.531	0.231
a_8	1.098	1.429	−0.331
a_{10}	0.126	−0.195	0.321
a_{12}	0.860	0.914	−0.053
a_{13}	0.381	1.069	−0.688
a_{14}	0.747	1.025	−0.278
a_{15}	0.475	0.312	0.163
a_{17}	0.053	0.324	−0.271
a_{18}	0.975	0.423	0.552
a_{19}	−0.966	−0.966	0
a_{20}	−0.281	0.369	−0.650
a_{22}	−0.453	−0.563	0.110
b_1	0.947	1.016	−0.069
d_1	−1.009	−0.940	−0.069

Actual activity and the activity predicted by model 1 for the training set of compounds are given in this table with the residual value as the difference of the two (actual activity−predicted activity).

Table 3b
Actual and predicted (by model 1) activities of test set compounds

Compound	Actual pIC_{50}	Predicted pIC_{50}	Residual
a_4	1.410	1.588	−0.178
a_9	1.098	1.385	−0.287
a_{11}	0.372	1.508	−1.136
a_{16}	0.242	0.369	−0.127
a_{21}	−0.625	−0.571	−0.054

Actual activity and the activity predicted by model 1 for the test set of compounds are given in this table with the residual value as the difference of the two (actual activity−predicted activity).

Out of 57, 10 compounds were taken in test set and rest in training set. The selection of test set compounds was in such a way so as to represent each class and higher the number of compounds in a class more the compounds taken in test set for that particular class. So 5 more compounds were added to the test set taken for model 1. The same set of descriptors was calculated again and correlation matrix was developed for the various descriptors along with pIC_{50} value. Based on the correlation matrix some of the descriptors were rejected from the highly correlated descriptors. The model generated shows 2 compounds as outliers. The reason for compound **d1** to be detected as outlier may be that it is the only urea derivative which shows activity (pIC_{50}) in the range of −1 to −2. For all other urea derivatives activity range is below −2. Similarly for compound **a17** activity range is 0−1 whereas for similar compounds, for example, compounds **a38** and **a39** activity range is −1 to −2. For rest of the compounds model was generated using genetic function approximation as:

$$\begin{aligned} pIC_{50} = {} & -3.55704 + 1.75378 * \text{Radius of gyration} - 1.52078 \\ & * \text{Atype\_S\_107} + 0.662542 * \text{S\_aaaC} - 0.055225 * \text{MR} \\ & - 0.932474 * \text{Atype\_N\_73} \end{aligned} \quad (2)$$

$N = 45$, LOF = 0.459, $r^2 = 0.850$, $r^2_{\text{adj.}} = 0.831$, F-test = 44.148, LSE = 0.278, $r = 0.922$, $q^2 = 0.793$, $r^2_{\text{pred}} = 0.879$

Radius of gyration is a 3D spatial descriptor which is calculated by considering the coordinates of various atoms relative to center of mass. Thus it is a measure of the size of the molecule. Atype_S_107 and Atype_N_73 are atom-type-based AlogP descriptors as described in model 1. S_aaaC is an electrotopological index and the symbol indicates sum descriptor for carbon with 3 aromatic bonds. MR is molar refractivity which depends on molecular weight and density of the compound. This model was used to predict the activity of the test set compounds and out of the 5 active compounds 4 were predicted to be active and out of 5 inactive compounds all 5 were predicted to be inactive. But when model 1 was used to predict the activity of this test set, it predicted all the 5 active compounds as active but out of 5 inactive compounds it predicted only 1 compound as inactive and rest 4 as active, total number of active compounds by model 1

Table 3c
Mid point of range and predicted pIC_{50} of test set compounds

Compound	Range of pIC_{50}	Mid point of range	Predicted pIC_{50} (model 1)	Predicted pIC_{50} (model 2)
a_4	2 to 1	1.5	1.588	1.228
a_9	2 to 1	1.5	1.385	2.271
a_{11}	1 to 0	0.5	1.508	0.014
a_{16}	1 to 0	0.5	0.369	1.136
a_{21}	0 to −1	−0.5	−0.571	*−1.382*
a_{25}	−1 to −2	−1.5	−2.628	−1.217
a_{34}	−1 to −2	−1.5	*1.452*	−1.058
a_{42}	−1 to −2	−1.5	*−0.132*	−1.904
d_2	−2 to −3	−2.5	*−0.389*	−2.453
d_6	−2 to −3	−2.5	*0.562*	−2.637

Mid point of range and predicted pIC_{50} by both the conventional and range based methods are given in this table for test set compounds.

is 9. Thus model 2 is better in classifying the compounds as active and inactive. The actual (range based) and predicted activities by model 1 and model 2 for the test set compounds are given in Table 3c. The inter correlation of various descriptors was checked and the correlation matrices for models 1 and 2 are given in Tables 4a and 4b, respectively.

The reasons behind better predictability of compounds as active and inactive by model 2 may be the broad range of activity and larger number of compounds taken in training set in this model, as it is a well known fact that no QSAR model is universally applicable. A QSAR model can predict accurately only for those compounds which fall in the applicable range of that QSAR model. The applicable range of model is determined by the descriptor space covered in the training set. Broader the range of compounds taken in training set, better is the predictability of the model. This assumption worked very well in the case of neuropeptide Y Y5 inhibitors. In future the same assumption may be tried on other inhibitors also and a better QSAR model can be developed compared to conventional method.

4. Conclusion

Based on the available data on the neuropeptide Y Y5 receptor inhibitors a statistically significant QSAR model was generated by using conventional QSAR approach. This model can be used to predict the NPY Y5 receptor antagonistic activity of unknown compounds of similar chemical structure. In addition to it a new approach based on the use of the range of activity values was proposed. Our work suggests that derived activity values based on the range in which the activity of a given compound lies can be used to develop QSAR model. We proposed that QSAR model generated using range based approach can be used to predict whether a given compound of this series is active or inactive. Statistical data as well as prediction of activity for test set compounds indicate that the model derived by this approach can be used as a filter for screening database of compounds with good predictability. In the present scenario of focused combinatorial library and HTS screening what is important for a biologist is to know how many of the compounds from a given database might be active and how many may be inactive, not the exact activity value of a compound, and our range based method classifies very accurately the active compounds as active and inactive compounds as inactive. Thus this approach could be useful in screening compounds from a large focused library.

Table 4a
Correlation matrix for the biological activity and the descriptors used in model 1

	Atype_N_72	Atype_O_60	Atype_C_26	CHI-2	pIC_{50}
Atype_N_72	1.000				
Atype_O_60	0.000	1.000			
Atype_C_26	−0.177	0.257	1.000		
CHI-2	0.182	0.057	−0.320	1.000	
pIC_{50}	−0.315	−0.404	0.374	0.374	1.000

The inter correlation of descriptors with each other and with the biological activity are given in the table. The table shows that descriptors are independent of each other.

Table 4b
Correlation matrix for the biological activity and the descriptors used in model 2

	MR	Atype_S_107	Atype_N_73	Radius of gyration	S_aaaC	pIC_{50}
MR	1.000					
Atype_S_107	−0.185	1.000				
Atype_N_73	−0.244	−0.055	1.000			
Radius of gyration	0.508	−0.090	0.121	1.000		
S_aaaC	−0.264	0.251	0.455	0.254	1.000	
pIC_{50}	−0.297	−0.057	0.277	0.432	0.614	1.000

The inter correlation of descriptors with each other and with the biological activity are given in the table. In this table, also it is clear that descriptors are not related to each other, so there is no redundancy in the model.

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