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QUANTITATIVE STRUCTURE ACTIVITY RELATIONSHIP (QSAR) STUDIES ON 5-CYANO, N1, 6-DISUBSTITUTED, 2-THIOURACIL DERIVATIVES AS CENTRAL NERVOUS SYSTEM DEPRESSANTS

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Quantitative structure activity relationship (QSAR) studies of twenty four 5-cyano, N1, 6-disubstituted, 2-thiouracil derivatives were performed for their central nervous system (CNS) depressant (locomotor) activity using VlifeMDS3.5 software. Partial least square (PLS) linear regression analysis coupled with stepwise variable selection method was applied to derive QSAR models which were further validated for statistical significance and predictive ability by internal and external validation. The best QSAR model was selected, having correlation coefficient $r^2 = 0.9014$ and cross validated squared correlation coefficient $q^2 = 0.8120$ with external predictive ability of pred_$r^2 = 0.6692$. The QSAR model indicated that the vdWSurfaceArea (van der Waals surface area of the molecule), dipole moment, YcompDipole (y component of the dipole moment) and T_2_F_1 (count of number of double bounded atoms (i.e. any double bonded atom, T_2) separated from fluorine atom by 1 bond in a molecule) were the important determinants for CNS depressant (locomotor) activity.

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Keywords: QSAR, thiouracil, locomotor activity, PLS, CNS depressant

1. Introduction

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. As an application of computational chemistry, nowadays, quantitative structure activity relationship (QSAR) has become more popular tool for the prediction of biological activities of molecules. The quantitative relations between the chemical properties of a molecule (physicochemical, structural and conformational) and the biological response assist to understand the driving forces for the drugs action and helps to predict the biological activities of newly designed analogues statistically thus, contributing to the drug discovery processes [1].

During past two decades, a significant attention has been focused on design and development of pyrimidine heterocyclic compounds of biological interest [2-7]. As pyrimidine constitutes the base for thiamine, uracil and cytosine nitrogen bases which are building blocks of the nucleic acids, there has been growing interest in this particular heterocycle. Many papers have reported QSAR investigations on the derivatives of pyrimidine [8-12].

In this context, a series of twenty four 5-cyano, N1, 6-disustituted, 2-thiouracil derivatives were synthesized by the tertiary condensation (Fig. 1) of aryl-substituted thiourea, appropriate aldehyde and ethylcyanoacetate catalyzed by potassium carbonate in presence of small amount of ethanol using microwave irradiation technique [13]. The structures of the compounds were

confirmed by FTIR and NMR spectroscopy studies. The synthesized thiouracil derivatives were further evaluated for CNS depressant (locomotor activity) in mice using actophotometer. The antimicrobial and antinociceptive activities of these compounds have been already reported [14, 15]. All thiouracil derivatives produced significant decrease (53.92 ± 1.90 – 89.50 ± 1.66) in the locomotor activity in mice. A log-dose response relationship was established and the pEC_{50} values for all compounds were determined. These values were further used as a data set (Table 1) to obtain various QSAR models.

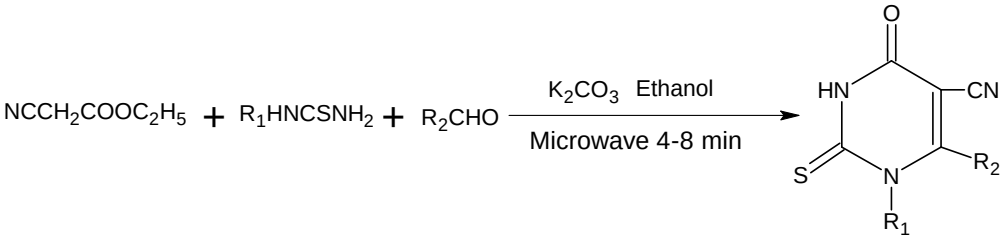
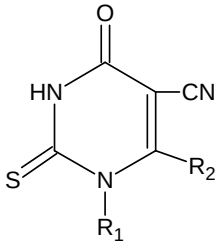


Fig. 1 Scheme of synthesis of thiouracil derivatives

Table 1 Nature of R group and pEC_{50} values of thiouracil derivatives.



Compound Code	Nature of R1	Nature of R2	pEC_{50} (-log EC_{50} or 1/ EC_{50}) μmol
P1	Phenyl	Phenyl	0.5534
P2	Phenyl	4-hydroxy phenyl	0.7598
P3	Phenyl	4-methoxy phenyl	0.8260
P4	4-Chloro-phenyl	Phenyl	0.5678
P5	4-Chloro-phenyl	4-hydroxy phenyl	0.6683
P6	4-Chloro-phenyl	4-methoxy phenyl	0.7735
P7	4-Methyl-phenyl	Phenyl	0.7927
P8	4-Methyl-phenyl	4-hydroxy phenyl	0.7749
P9	4-Methyl –phenyl	4-methoxy phenyl	0.7761
P10	4-Methoxy-phenyl	Phenyl	0.7958
P11	4-Methoxy-phenyl	4-hydroxy phenyl	0.7657
P12	4-Methoxy-phenyl	4-methoxy phenyl	0.8535
P13	4-Fluoro-phenyl	Phenyl	0.5712
P14	4-Fluoro-phenyl	4-hydroxy phenyl	0.6928
P15	4-Fluoro-phenyl	4-methoxy phenyl	0.7814
P16	2, 4-dimethyl-phenyl	Phenyl	0.7975
P17	2, 4-dimethyl-phenyl	4-hydroxy phenyl	0.7601
P18	2, 4-dimethyl-phenyl	4-methoxy phenyl	0.8045
P19	3-Methyl-phenyl	Phenyl	0.7629
P20	3-Methyl-phenyl	4-hydroxy phenyl	0.7610
P21	3-Methyl-phenyl	4-methoxy phenyl	0.7667
P22	4-Nitro-phenyl	Phenyl	0.5581
P23	4-Nitro-phenyl	4-hydroxy phenyl	0.6638
P24	4-Nitro-phenyl	4-methoxy phenyl	0.7899

As a part of ongoing research to design novel compounds with potent locomotor activity, this work was aimed to establish quantitative relationship between CNS depressant (locomotor) activity and 2D (two dimensional) descriptors of 5-cyano, N1, 6-disubstituted, 2-thiouracil derivatives. Partial least square (PLS) linear regression method was used to generate various QSAR models. Three best models were selected and validated for their regression coefficient, internal and external predictive ability and statistical significance. All models were further interpreted to investigate the contribution of various descriptors in CNS depressant (locomotor) activity.

2. Experimental

2.1. Biological data

A data set of pEC_{50} values (locomotor activity) of twenty four compounds was used for 2D QSAR studies (Table 1). The micromolar concentrations of thiouracils required to produce fifty percent response (EC_{50}) in animals were converted to negative logarithmic values (pEC_{50}) for undertaking the QSAR study.

2.2. Modeling software

All twenty four compounds were built on workspace of molecular modeling software VLifeMDS (Version 3.5 VLife Sciences Technologies Pvt Ltd., Pune, India). The QSAR models were built on the same software.

2.3. Optimization of molecules

All molecules were batch optimized for the minimization of energies using MMFF (Merck Molecular Force Field) in MOPAC module of VLifeMDS software until the root mean square (rms) gradient reached value 0.001 kcal/mol A° before they were undertaken for 2D QSAR studies.

2.4. Selected descriptors

Most stable structure for each compound was generated after energy minimization and used for calculating various physico-chemical descriptors like thermodynamic, steric and electronic. The various descriptors selected for 2D QSAR were vdWSurfaceArea (van der Waals surface area of the molecule), –vePotentialSurfaceArea (total van der Waals surface area with negative electrostatic potential of the molecule), +vePotentialSurfaceArea (total van der Waals surface area with positive electrostatic potential of the molecule) dipole moment, YcompDipole (y component of the dipole moment), element count, slogP, path count, cluster, distance based topological indices, connectivity index, hydrophobic and hydrophilic areas like SAMostHydrophilic (Most hydrophilic value on the vdW surface by Audry Method using Slogp), SAMostHydrophobicHydrophilic Distance (distance between most hydrophobic and hydrophilic point on the vdW surface by Audry Method using Slogp), SAHydrophilicArea (vdW surface descriptor showing hydrophilic surface area by Audry Method using SlogP) and SKMostHydrophilic (Most hydrophilic value on the vdW surface by Kellog Method using Slogp), radius of gyration, Wiener's index, moment of inertia, semi- empirical descriptors, HOMO (Highest occupied molecular orbital), LUMO (lowest unoccupied molecular orbital), heat of formation and ionization potential. Besides these all alignment independent descriptors were also calculated. The hydrophobic descriptors govern the movement of a drug molecule across the biological membranes in order to interact with the receptor by van der Waals binding forces whereas both electronic and steric descriptors influence the affinity of a drug molecule necessary for proper drug- receptor interaction.

2.5. Generation of training and test set of compounds

The optimal training and test sets were generated by either random selection method or the sphere exclusion algorithm. A commonly used ratio of training to validation objects (test set), which was also adopted in this work, is 80%: 20% [16]. However, rational splitting was accomplished by applying a sphere-exclusion type algorithm [17-20]. In classical sphere-exclusion algorithm the molecules are selected whose similarities with each of the other selected molecules are not higher than a defined threshold. Each selected molecule generates a hyper-sphere around itself, so that any molecule inside the sphere is excluded from the selection in the train set and driven toward the test set. The number of compounds selected and the diversity among them can be determined by adjusting the radius of the sphere (R) [17].

2.6. Statistical computation

All the calculated descriptors were considered as independent variable and biological activity as dependent variable. VLife Molecular Design Suite (VLifeMDS software was used to generate QSAR models by Partial Least Squares Regression (PLSR) method analysis. Statistical measures used were the number of compounds in regression *n*, the regression coefficient r^2, the *F*–test (Fischer's value) for statistical significance *F*, the cross–validated correlation coefficient q^2 and the standard error of estimation r^2 and q^2. The regression coefficient r^2 is a relative measure of fit by the regression equation. It represents the part of the variation in the observed data that is explained by the regression. The correlation coefficient values closer to 1.0 represent the better fit of the regression. The *F*–test reflects the ratio of the variance explained by the model and the variance due to the error in the regression. High values of the *F*–test indicate that the model is statistically significant. The predictive ability of the generated correlations was evaluated by cross validation method employing a 'leave–one–out' scheme. Validation parameters considered were cross validated q^2. The predictive ability of the selected model was also confirmed by external validation of test set compounds which is also denoted with pred_r^2.

3. Results and discussion

3.1. Model generation

QSAR investigations of the 5-cyano, N1, 6-disustituted, 2-thiouracil analogues series resulted in several QSAR equations. Some statistically significant QSAR models were chosen for discussion.

pEC_{50} = + 0.0603 Dipole Moment
+ 0.0019 vdWSurfaceArea
– 0.1784 (MODEL 1)

n = 19, Test Set Size = 5 ((P6, P8, P10, P15 and P24), r^2 = 0.8523, q^2 = 0.8152, *F* = 98.1114, r^2 se = 0.0381, q^2 se = 0.0426, pred_r^2 = 0.4926, pred_r^2se = 0.0496, R^2 = 0.8109.

pEC_{50} = + 0.0021 vdWSurfaceArea
+ 0.0657 DipoleMoment
+ 0.0625 T_2_F_1
+ 0.0079 YcompDipole
–0.2770 (MODEL 2)

n = 19, Test Set Size = 5 ((P9, P13, P18, P23 and P24), r^2 = 0.9014, q^2 = 0.8120, *F* = 73.1247, r^2 se = 0.0295, q^2 se = 0.0407, pred_r^2 = 0.6692, pred_r^2se = 0.0588, R^2 = 0.8480.

pEC_{50} = + 0.0028 –vePotentialSurfaceArea
+ 0.0022 SAHydrophilicArea
+ 17.8277 SAMostHydrophilic
– 0.0351 T_N_O_5
+ 0.0487 DipoleMoment
– 0.0016 Quadrupole3
+ 0.0954 SAMostHydrophobicHydrophilicDistance
+ 0.0018 +vePotentialSurface Area
– 0.2488 SKMostHydrophilic
+ 2.1505 (MODEL 3)

n = 18, Test Set Size = 6 (P8, P10, P15, P18, P19 and P21), r^2 = 0.9676, q^2 = 0.8377, *F* = 139.5127, r^2 se = 0.0195, q^2 se = 0.0436, pred_r^2 = 0.4807, pred_r^2se = 0.0506, R^2 = 0.9008.

In these equations n is the number of molecules (Training set) used to derive the QSAR models, r^2 is the regression coefficient, q^2 is the cross-validated r^2 (by the leave-one out method), pred_r^2 is the predicted r^2 for the external test set, *F* is the Fisher ratio, q^2 se and r^2 se are the standard errors of cross-validated coefficient and regression coefficient respectively, pred_r^2se is the standard error of predicted r^2 for the external test set and R^2 is the correlation coefficient for observed vs. predicted biological activity.

The QSAR models 1 and 2 were obtained by random method of training and test data selection [16], where 80% (19) of the total molecules were selected for training set while remaining were selected as test set molecules (5). The QSAR models 3 was obtained by sphere exclusion method [17] of training and test data selection, where 18 of the total molecules were selected for training set while remaining were selected as test set molecules (6).

3.2. Validation of QSAR models

All three QSAR models have shown good correlation between their corresponding descriptors and biological activity. Also large values of *F* indicated that the model fit in all cases was not a chance occurrence and all models were statistically significant [21]. However, a QSAR model is considered to be predictive, if the following conditions are satisfied: pred_r^2 > 0.5 and q^2 > 0.6 [22-25]. Table 2 shows comparative predicted activities along with residuals by three models. Figures 2, 3 and 4 represents the fitness plots of observed vs. predicted biological activity for the models 1, 2 and 3 respectively. The model 3 has excellent goodness of fit (r^2 = 0.9676), good internal predictive ability (q^2 = 0.8377) and excellent fitness plot (R^2 = 0.9008) but poor ability to predict the activities of test set molecules (pred_r^2 = 0.4807) which have not been included to build the QSAR model. The model 1 has also good correlation coefficient (r^2 = 0.8523), good internal predictive ability (q^2 = 0.8152) but slightly poor external predictive ability (pred_r^2 = 0.4926) with fitness plot of (R^2 = 0.8109). However, model 2 shows excellent correlation coefficient (r^2 = 0.9014), good internal predictive ability (q^2 = 0.8120) and fitness plot (R^2 = 0.8480). Further, it has better external predictive ability for the test set molecules (pred_r^2 = 0.6692). From the above equations, it was also observed that 85.23 % (r^2 = 0.8523), 90.14 % (r^2 = 0.9014) and 96.76 % (r^2 = 0.9676) of the variation in the biological activity was accounted for by the parameters used in the equations 1, 2 and 3 respectively. This signifies that model 2 can be considered as a most predictive and best model among all the three QSAR models evaluated.

Table 2 Comparative observed and predicted activities (LOO) of thiouracil derivatives by QSAR models

Compound	Observed pEC_{50}	Predicted pEC_{50} *					
		Model 1	Residuals	Model 2	Residuals	Model 3	Residuals
P1	0.5534	0.6119	-0.0585	0.5829	-0.0295	0.5574	-0.004
P2	0.7598	0.7204	0.0394	0.7111	0.0487	0.7468	0.013
P3	0.8260	0.7849	0.0411	0.8236	0.0024	0.8406	-0.0146
P4	0.5678	0.6014	-0.0336	0.5792	-0.0114	0.5828	-0.015
P5	0.6683	0.6815	-0.0132	0.6738	-0.0055	0.6618	0.0065
P6	0.7735	0.7631 [a]	0.0104	0.7732	0.0003	0.7512	0.0223
P7	0.7927	0.7556	0.0371	0.7838	0.0089	0.7684	0.0243
P8	0.7749	0.7590 [a]	0.0159	0.7880	-0.0131	0.7578 [a]	0.0171
P9	0.7761	0.8179	-0.0418	0.8316 [a]	-0.0555	0.7576	0.0185
P10	0.7958	0.7601 [a]	0.0357	0.7886	0.0072	0.7643 [a]	0.0315
P11	0.7657	0.7560	0.0097	0.7847	-0.019	0.7512	0.0145
P12	0.8535	0.8831	-0.0296	0.8872	-0.0337	0.8757	-0.0222
P13	0.5712	0.5882	-0.017	0.6293 [a]	-0.0581	0.5767	-0.0055
P14	0.6928	0.6563	0.0365	0.7158	-0.023	0.6784	0.0144
P15	0.7814	0.7118 [a]	0.0696	0.7653	0.0161	0.7144 [a]	0.067
P16	0.7975	0.7448	0.0527	0.7367	0.0608	0.7911	0.0064
P17	0.7601	0.7779	-0.0178	0.7829	-0.0228	0.7985	-0.0384
P18	0.8045	0.8416	-0.0371	0.8565 [a]	-0.052	0.8439 [a]	-0.0394
P19	0.7629	0.7449	0.018	0.7498	0.0131	0.7584 [a]	0.0045
P20	0.7610	0.7138	0.0472	0.7127	0.0483	0.7536	0.0074
P21	0.7667	0.8037	-0.037	0.7975	-0.0308	0.8406 [a]	-0.0739
P22	0.5581	0.5914	-0.0333	0.5791	-0.021	0.5777	-0.0196
P23	0.6638	0.6307	0.0331	0.6114 [a]	0.0524	0.6642	-0.0004
P24	0.7899	0.7317 [a]	0.0582	0.7459 [a]	0.044	0.8018	-0.0119

* indicates predicted activity by leave one out cross validation; [a] indicates molecules of test set.

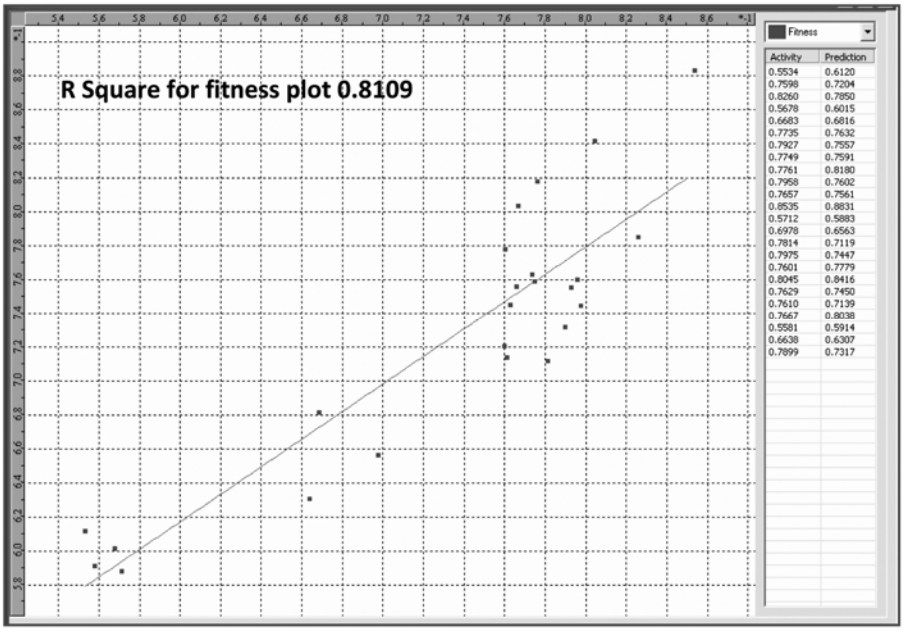


Fig. 2 The plot of observed versus predicted activity for model 1

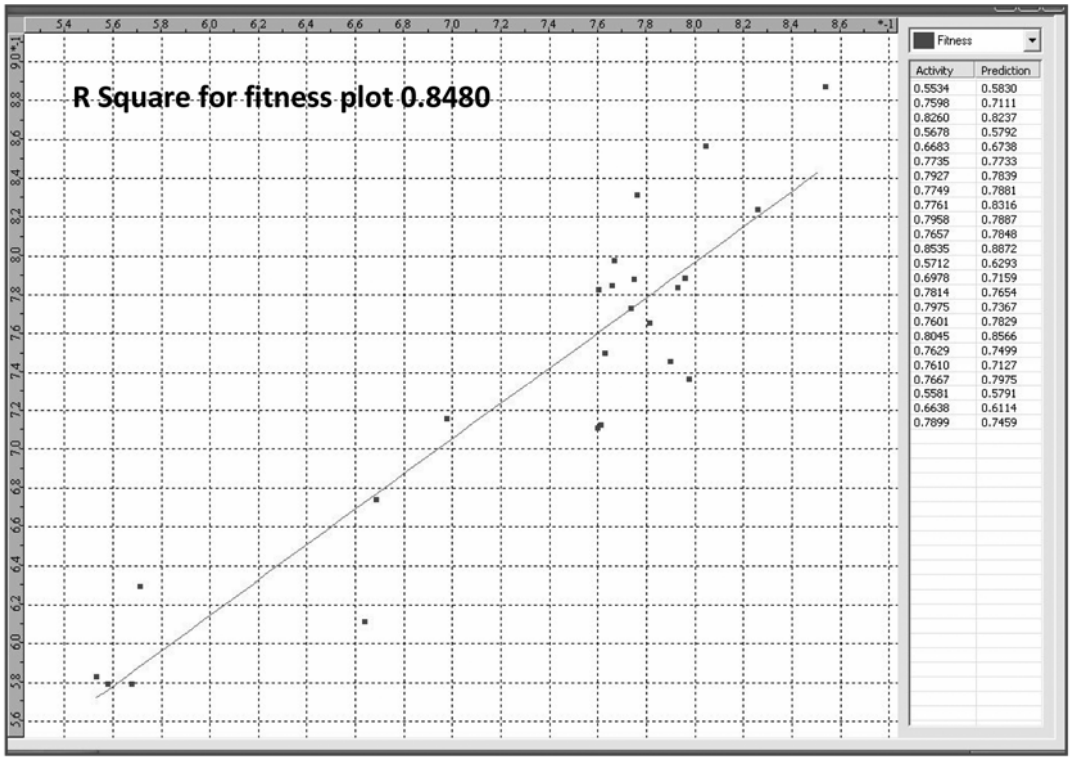


Fig. 3 The plot of observed versus predicted activity for model 2

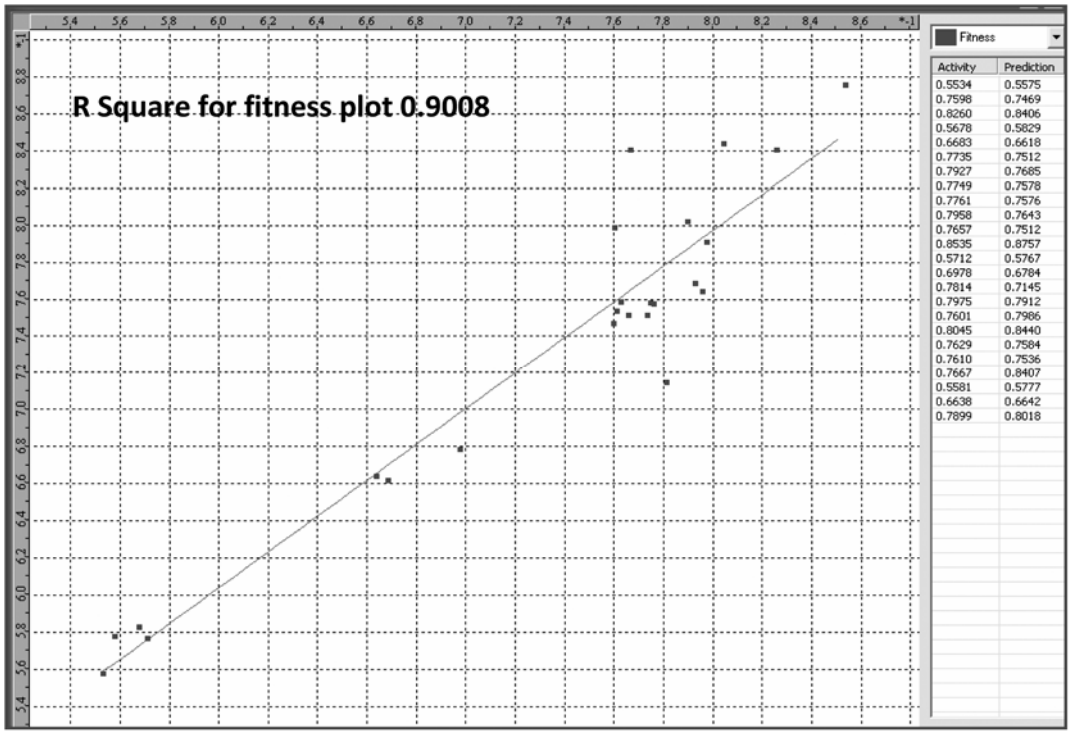


Fig. 4 The plot of observed versus predicted activity for model 3.

The descriptor data was further analyzed to check data spread by calculating the mean and standard deviation for all QSAR models. Table 3 shows uni-column statistics for the activity data of training and test set.

Table 3 Uni-column Statistics for QSAR models

Model 1 Uni-column Statistics for training set					
Column Name	Average	Max	Min	StdDev	Sum
Activity	0.7214	0.8535	0.5534	0.0964	13.7069
Model 1 Uni-column Statistics for test set					
Column Name	Average	Max	Min	StdDev	Sum
Activity	0.7831	0.7958	0.7735	0.0096	3.9155
Model 2 Uni-column Statistics for training set					
Column Name	Average	Max	Min	StdDev	Sum
Activity	0.7377	0.8535	0.5534	0.0885	14.0169
Model 2 Uni-column Statistics for test set					
Column Name	Average	Max	Min	StdDev	Sum
Activity	0.7211	0.8045	0.5712	0.1006	3.6055
Model 3 Uni-column Statistics for training set					
Column Name	Average	Max	Min	StdDev	Sum
Activity	0.7187	0.8535	0.5534	0.0982	12.9362
Model 3 Uni-column Statistics for test set					
Column Name	Average	Max	Min	StdDev	Sum
Activity	0.7810	0.8045	0.7629	0.0164	4.6862

The max of the test should be less than or equal to max of train set and the min of the test should be greater than or equal to min of train set. Table 3 indicated that in all cases the test set was interpolative i.e. derived within the min-max range of the train set. The mean and standard deviation of the train and test set provided insight to the relative difference of mean and point density distribution (along mean) of the two sets. In model 1 and model 3, the means of the test sets were higher than the train sets showed the presence of relatively more active molecules as compared to the inactive ones. Also in both the cases a relatively higher standard deviation in train sets indicated that training sets had widely distributed activity of the molecules as compared to the test sets [26].

In model 2 the mean of the test set was slightly lower than the train set showed the presence of relatively slightly less active molecules as compared to the inactive ones. Also a relatively slightly higher standard deviation in test set indicated that test set has widely distributed activity of the molecules as compared to the training set [26].

3.3. Interpretation of QSAR models

Model 1

In this QSAR equation, the positive contribution of dipole moment on the biological activity indicated that the increase in dipole moment of molecule leads to better locomotor activity. The positive coefficient of vdWSurfaceArea showed that increase in vdWSurfaceArea is beneficial for the activity [26].

Model 2

QSAR model 2 demonstrates that positive contribution of vdWSurfaceArea and dipole moment on the biological activity indicated that the increase in vdWSurfaceArea and dipole moment of molecule leads to better locomotor activity. The positive coefficient of YcompDipole showed that increase in YcompDipole is not detrimental for the activity. The next most important descriptor influencing activity variation is T_2_F_1 and is directly proportional to the activity. This descriptor indicates that increase in the count of number of double bounded atoms (i.e. any double bonded atom, T_2) separated from fluorine atom by 1 bond in a molecule will lead to positive effect on the activity [26].

Model 3

QSAR model 3 shows that positive contribution of –vePotentialSurfaceArea, +vePotentialSurfaceArea, SAMostHydrophilic, dipole moment, SAMostHydrophobicHydrophilic Distance and SAHydrophilicArea on the biological activity indicated that the increase in the values of these descriptors leads to better locomotor activity. The negative coefficient of Quadrupole3 and SKMostHydrophilic showed that increase in the values of these descriptors is detrimental for the activity. The next most important descriptor influencing activity variation is T_N_O_5 and is inversely proportional to the activity. The negative coefficient of this descriptor indicates that, the decrease in the count of number of nitrogen atoms (single double or triple bonded) separated from any oxygen atom by 5 bonds in a molecule will lead to positive effect on the activity [26].

4. Conclusions

In the present investigation, all proposed QSAR models were statistically significant. However, model 2 could be considered as best one in terms of its excellent internal and external predictive abilities. According to model 2, the CNS depressant (locomotor) activity of 5-cyano, N1, 6-disubstituted, 2-thiouracil derivatives was positively contributed with electrostatic parameter like van der Waals surface area of the molecule, electronic parameters like dipole moment and Y component of Dipole moment and alignment independent descriptor T_2_F_1. Thus, from above QSAR investigations it could be concluded that electrostatic and electronic properties of thiouracil derivatives are mainly involved in eliciting CNS depressant (locomotor) activity.

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