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QSAR studies of imidazo (1,5-*a*) quinoxalines amides, carbamates and ureas as potent GABA modulators

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Quantitative structure activity relationship (QSAR) studies have been performed on imidazo(1,5-*a*)quinoxalines amides, carbamates and ureas. The QSAR models have been developed by using multiple linear regression in order to identify descriptors, which are actually focusing towards the biological activity. Leave out-group of rows method, usually employed in cross validation analysis, are used to validate the developed model. The best predictive QSAR model derived, had r^2cv of 0.81, non-cross validated r^2 of 0.87, predictive r^2 for test set 0.61 and standard error of estimate 0.23. The model reveals that multidimensional steric [verloop B_5 (subs 4)], hydrophobic [Log P (whole molecule)], electro topological [Ipso atom E state index (subs 2) and sum of E state indices (subs 4)] and hydrogen bond donor [ADME H bond donor (subs 4)] descriptors have significant impact on GABA modulator activity of the compounds. The results clearly indicate the soundness and predictive ability of the model. Using this model novel anxiolytics and anticonvulsants can be obtained with improved potency and pharmacokinetic profile.

Keywords: QSAR, imidazo(1,5-*a*)quinoxalines derivatives, GABA modulators, tools for structure activity relationship (TSAR)

GABA, was first discovered in the brain by Roberts and Awapara in 1950, functions as an inhibitory neurotransmitter in the CNS[1]. Since then, the complicated mechanisms that contribute to GABA-mediated neurotransmission have been studied using electrophysiological, pharmacological, and molecular biological techniques[2]. Generally, GABA serves as an inhibitory neurotransmitter that decreases neuronal excitability by increasing the conductance of post-synaptic membranes to Cl^- ion[3-5]. Its inhibitory effect plays an important role in all central neurons, and it has been suggested that 30% or more of central neurons may use GABA as a neurotransmitter. GABA is essential for the overall balance between neuronal excitation and inhibition that is vital to normal brain function. Too much excitation, or too little inhibition, can result in a range of conditions like convulsions, anxiety, high blood pressure, restlessness and insomnia. Restoration of the balance between excitation and inhibition is a major aim of therapies that target GABA-mediated neuronal inhibition. GABA produces neuronal inhibition by acting on an amazing diversity of membrane-bound receptors; the three major classes of these receptors are $GABA_A$, $GABA_B$ and $GABA_C$ receptors[6,7]. $GABA_A$ and $GABA_C$ are ligand gated ion channels (LGICs) that mediate fast synaptic transmission *via* the movement of ion through the channels while $GABA_B$ receptor are G-protein coupled receptors. Anxiety is an adoptive response to stressful or threatening stimuli while definitive pathophysiological mechanisms of the various anxiety disorders have yet to be fully elucidated and epilepsy is a common chronic neurological disorder that is characterized by recurrent unprovoked seizures. Abnormal neuronal excitability, a major characteristic of epilepsy is thought to occur as a result of disruption of the depolarization and repolarization mechanisms of the cell (this is termed the "excitability of neuronal tissue"). GABA systems have been implicated in the pathogenesis of anxiety and epilepsy. $GABA_A$ receptor complex has emerged as the most promising target for the better understanding of their disorders. Design and synthesis of more selective and higher affinity ligands that display prominent pharmacological activities are required and which in turn serve to further elucidate the physiological and pharmacological functions of $GABA_A$/BzR, which may result in new subtype-

selective agents for the treatment of anxiety, convulsions or memory deficits as well as decreasing the potential for side-effects. $GABA_A$ receptors are the target for several important centrally acting drugs notably benzodiazepines, barbiturates and neurosteroids, out of which barbiturates were withdrawn because of their severe side-effects and toxicity[8].

Novel medicines are typically developed by using a trial and error approach which is time consuming and costly. The application of quantitative structure activity relationship (QSAR) methodologies to this problem has the potential to greatly decrease the time and effort required to discover new medicines or improve current ones. QSAR is extremely useful and rational approach in designing new chemical entities with improved therapeutic profile and less toxicity and this is generally considered to be prime function of QSAR. The main hypothesis in the QSAR approach is that all properties (physical, chemical and biological) of a chemical substance are statistically related to its molecular structure and the success of this approach can be explained by the possibility to estimate the properties of new chemical compounds without the early need to synthesize and test them[9]. In this paper we report a QSAR studies on a series of imidazo(1-5*a*)quinoxalines amides, carbamates and ureas with a purpose to elucidate the chemical features involved in drug receptor interaction.

Results and Discussion

The QSAR studies were carried out using the 45 compounds of imidazo(1,5-*a*)quinoxalines amides, carbamates and ureas (**Table I**). The statistically significant model was constructed from the training set by using five parameters. When the multiple regression analysis was performed without deleting any outlier, we retrieved the values of r = 0.87, r^2 = 0.72, r^2cv = 0.42. The low value of r^2cv suggested the low predictivity of the model. In order to improve the predictivity and reliability of the model the outliers were detected and it was found that two compounds 65 and 77 were having high residual values and they were too far away from the regression line.

Model developed (equation 1 and 2) after deleting the above said two compound showed a high value of r^2cv = 0.81 and also satisfied all the other statistical criterias (**Tables II** and **III**).

Original Data

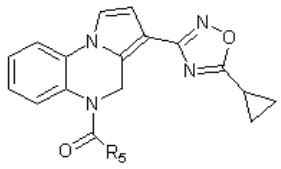
Y = -0.32667023*X1 [Log P (whole molecule)] +0.87090772*X2 [ipso atom E state index (subs 2)] -0.069402575*X3 [sum of E state indices (subs 4)] -0.68182576*X4 [ADME H bond donor (subs 4)] +0.27933711*X5 [verloop B_5 (subs 4)] + 0.78422374 (Eqn. 1)

Standardized Data

Y = -0.20606874*S1 [Log P (whole molecule)] +0.22911786*S2 [ipso atom E state index (subs2)] -0.37281889*S3 [sum of E state indices (subs 4)] -0.29356796*S4 [ADME H bond donor (subs 4)] +0.25286701*S5 [verloopB_5 (subs 4)] -0.3862353 (Eqn. 2)

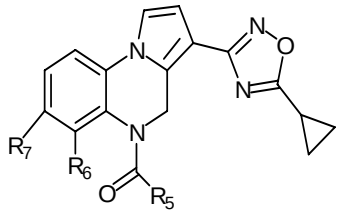
The regression equation 1 reveals that the parameters entered in the final model are not equally relevant. Out of five three parameters are negatively correlated and two parameters are positively

Table Ia — Imidazo(1,5-*a*)quinoxalines amides, carbamates, ureas and thiocarbamates



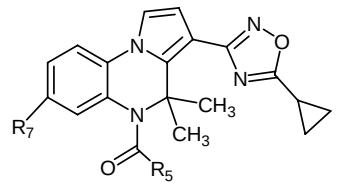
Compd	R^5	K_i(nM)	Log K_i (nM)
33	Me	1.5	-0.176
34	CF_3	143	-2.155
35	Ph	4.3	-0.633
36	2-pyridyl	1.1	-0.041
37	2-furan	0.43	0.366
38	2-pyrrole	1.1	-0.041
39	NH_2	7.2	-0.857
40	NHMe	2.8	-0.044
41	NMe_2	1.0	0
42	NHEt	3.5	-0.544
43	NEt_2	0.85	0.070
44	NHiPr	3.4	-0.531
45	Pyrrolidine	0.45	0.346
46	Morpholine	0.81	0.091
47	Aniline	16	-1.20
48	OtBu	1.9	-0.278
49	OMe	1.5	-0.176
50	OiPr	1.8	-0.255
51	SMe	1.3	-0.113
52	SEt	2.1	-0.322
53	SPh	11	-1.041

Table Ib — Imidazo(1,5-*a*)quinoxalines ureas with fluoro, chloro, or methyl A-ring substituents



Compd	R^5	R^6	R^7	K_i(nM)	Log K_i (nM)
54	NMe_2	F	H	0.89	0.050
55	NMe_2	H	F	0.61	0.214
56	NMe_2	Cl	H	0.71	0.148
57	NMe_2	H	Cl	3.7	-0.568
58	Pyrrolidine	F	H	0.64	0.193
59	Pyrrolidine	H	F	0.56	0.251
60	Pyrrolidine	Cl	H	0.73	0.136
61	Pyrrolidine	H	Cl	1.1	-0.041
62	Morpholine	F	H	0.48	0.318
63	Morpholine	H	F	0.68	0.167
64	Morpholine	H	Cl	2.3	-0.361
65	Morpholine	Me	H	4.6	-0.662

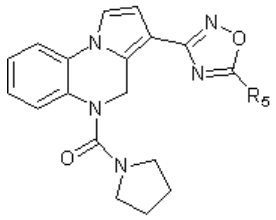
Table Ic — Imidazo (1,5-*a*) quinoxalines amides, carbamates, ureas and thiocarbamates with *gem*-4, 4 dimethyl substitutions



Compd	R^5	R^7	K_i(nM)	Log K_i (nM)
66	Me	H	4.9	-0.690
67	NH_2	H	86	-1.934
68	NHMe	H	21	-1.322
69	NMe_2	H	3.1	-0.491
70	Pyrrolidine	H	2.9	-0.462
71	OMe	H	6.8	-0.832
72	SMe	H	5.1	-0.707
73	NMe_2	H	3.1	-0.491
74	Pyrrolidine	Cl	3.3	-0.51

correlated to the biological activity. By observing the absolute values of the coefficient in the regression equation with the standardized data leads to the conclusion that log P belongs to the hydrophobic descriptor, Ipso atom E state index (subs 2) and sum

Table Id — Imidazo(1, 5-*a*)quinoxalines ureas with selected 5′-alkyloxadiazole substituents



Compd	$R^{5'}$	K_i(nM)	Log K_i (nM)
75	Et	0.65	0.187
76	iPr	1.1	-0.041
77	tBu	14.7	-1.167

Table II — Statistical parameters obtained for the best model

QSAR parameters	Value
No. of molecules in training set	34
No. of molecules in test set	9
Correlation coefficient (r)	0.93
r^2 (Test set)	0.61
r^2 (Training set)	0.87
r^2cv	0.81
F value	38.82
S value	0.23
Predictive sum of square	2.305
Residual sum of square	1.544

of E state indices (subs 4) are electro topological descriptors, verloop B_5 (subs 4) is multidimensional steric descriptor and H bond donor plays an important role in drug-receptor interaction, aqueous solubility and partitioning. Properties such as oral bio-availability or membrane permeability have often been correlated to the numbers of hydrogen bond donor and log P in a molecule thus log P is an important property in describing the affinity of the compounds in terms of their partitioning the biological membranes. By observing equation 1 it is clear that by decreasing Log P values the activity increases. Thus by decreasing the lipohilicity of the whole molecule by introducing hydrophilic groups such as –OH, NH_2, CO, NO_2 would surely lead to the development of newer agents with better pharma-cokinetic and pharmacodynamic profile. The electro topological parameter, ipso atom E state index (subs 2) insights the importance of steric conformations. Ipso atom E-state indices represents structural information in QSAR model, and describes the

Table III — Statistical test values of descriptors used in best model

	Abbreviation	Coefficient	Jacknife SE	Covariance SE	t-value
Log P (whole molecule)	X1	-0.3266	0.0439	0.0741	-4.4044
Ipso Atom E-State index (subst.2)	X2	0.8709	0.0180	0.1714	5.0801
Sum of E-State indices (subst.4)	X3	-0.0694	0.0085	0.0081	-8.5318
ADME H-bond donors (subst.4)	X4	-0.6818	0.1283	0.1086	-6.2746
Verloop B5 (subst.4)	X5	0.2793	0.0541	0.0502	5.5548

potential for non-covalent intermolecular interaction. The information encoded in the E-state value for an atom is the electron accessibility of that atom. The atom closest to a given atom has the greatest influence on its E-state value. The third important parameter is sum of E state indices (subs 4), the general concept of the E state indices is to characterize each atom of a molecule in terms of its potential for electronic interactions which is influenced by the neighboring atom. The result indicates that the use of the ipso atom E state index descriptor provides a structure space in which molecular structures are organized in chemically meaningful ways so that inhibitory properties associated with those structures can also be expected to be usefully organized. The Verloop parameters are a set of multidimensional steric descriptors that can be used to characterize the shape and volume of the substituent, which are very important in explaining the steric influence of substituents in the interaction of organic compounds with macromolecular drug receptors. Verloop B5 parameter describes the width of the substituent in the direction perpendicular to Length (L)[10]. As it correlates positively with the biological activity therefore the substitution of bulkier groups at R_5 position will enhance the biological activity. It can be exemplified with the compounds named 37, 45, and 62 as they have high biological activity due to the presence of bulkier groups like 2-furan, pyrrolidine, and morpholine respectively. Fujita *et al.* devised hydrogen bonding indicator variable which simply took the value of unity if a molecule or substituent was capable of forming a hydrogen bond and of zero if it was incapable of doing so[11]. The number of H-bonds donors affect the ability of a given molecule to pass physiological barriers such as blood brain barrier or its solubility and absorption into the haematic flux[12]. As it correlates negatively to the biological activity, it means that substitution at R_5 position by that atoms or groups which are not capable to behave like H-bond donor will enhance the binding interact-tions and biological activity. External validation of the above constructed QSAR model was carried out by using the test set compounds. And it was analyzed that the values obtained for actual and predictive activity were comparable to each other. **Tables IV** and **V** shows the comparison between actual and predicted values for training and test set. And the **Figures 1** and **2** show the plot between actual versus predictive values for training and test set respectively. The proposed model has high-predicted ability and could be useful aid to the costly and time consuming experiments for determining the activity of imidazo (1,5-*a*)quinoxalines amides, carbamates and ureas as GABA receptor modulators.

Materials and Methods

All the computational studies were performed using TSAR 3.3 software. TSAR is an integrated analysis package for the interactive investigation of structure activity relationships and is based on obtaining correlation between numerical description of molecular properties and biological activity. Series of imidazo(1,5-*a*)quinoxalines amides, carbamates and ureas which have high affinity for GABA reported by E. Jon Jacobsen *et al.*[13]. were taken from literature and are listed in **Table I**. The inhibitory values expressed in terms of nanomolar affinity k_i (nM)[14] were converted into inverse logarithmic scale (Log k_i). The compounds named U-78875 and diazepam were excluded from the model because of dissimilar structures to that of remaining series.

Structure preparation and descriptors calculations

All the chemical structures were sketched using TSAR 3.3 and were saved as *.sdf files. A correct alignment is of the utmost importance for creating useful and predictive models, therefore the three-dimensional structures of all the molecules were generated using CORINA 3D package. Partial charges were derived using the Charge-2 and their geometries were optimized using the cosmic module, which includes valence terms as bond potentials, bond

Table IV — Comparison between actual and predicted values of training set

Compd	Actual value	Predicted value
34	-2.155	-2.122
35	-0.633	-0.366
38	-0.041	-0.274
39	-0.857	-0.693
40	-0.447	-0.692
41	0	0.097
42	-0.544	-0.596
44	-0.531	-0.839
45	0.346	0.253
46	0.091	0.148
47	-1.2	-0.845
48	-0.278	-0.655
50	-0.255	-0.483
51	-0.113	-0.023
52	-0.322	0.072
53	-1.041	-0.926
55	0.214	0.088
56	0.148	-0.081
57	-0.568	-0.081
58	0.193	0.278
59	0.251	0.251
60	0.136	0.051
61	-0.041	0.070
62	0.318	0.211
63	0.167	0.149
66	-0.69	-0.554
67	-1.934	-1.508
68	-1.322	-1.425
70	-0.462	-0.482
72	-0.707	-0.684
73	-0.491	-0.844
74	-0.51	-0.656
75	0.187	0.072
76	-0.041	-0.037

angles, torsional potential and nonbonded terms as electrostatic interactions and Van der Waals interaction. The dataset was randomly divided into training set of 36 compounds and test set of 9 compounds, as described in previous literature[15,16]. The test set included compounds 33, 37, 36, 43, 49, 54, 64, 69, 71 and remaining all the other compounds were included in the training set. The training set was used for the building of QSAR model and the test set for examining the predictive capability of the model.

Table V — Comparison between actual and predicted values of test set

Compd	Actual value	Predicted value
36	-0.041	-0.387
43	0.07	-0.067
49	-0.176	-0.336
54	0.05	0.078
69	-0.491	-0.648
71	-0.832	-0.904
33	-0.176	0.145
37	0.366	0.184
64	-0.361	-0.036

More than 250 molecular descriptors including hydrophobic, steric and electronic were calculated for whole molecule and their substituents. The data reduction is very important in reducing data redundancy that could lead to low predictivity of the models. Descriptors with the same values for all the compounds were discarded. To further reduce the data, a correlation matrix was generated to study the data patterns. The correlation values involved in correlation matrix indicates the extent of co-linearity and on this basis the highly intercorrelated parameters can be identified. The data reduction was done by pairwise correlation[17]. Among the highly intercorrelated descriptors the one that had high correlation with biological activity was kept and other was discarded. This process was repeated number of times and finally five descriptors Log P (whole molecule), H bond donor (subs 4), verloop B_5 (subs 4), sum of E state indices (subs 4), and ipso atom E state index (subs 2) were retrieved that were highly correlated with biological activity and were not having intercorrelation among each other (**Table VI**).

Multivariate analysis

Multiple linear regression (MLR) is most widely used method in developing QSAR model. MLR is a simple technique and can produce equal or better models compared to partial least square or neural networks. The relationship between the selected parameters and biological activity was quantified by the multiple linear regression implemented in TSAR Version 3.3. The relationship between the independent variables (physico-chemical parameters) and dependent variable (biological activity) was analyzed and the best model was selected on the basis of various statistical parameters like correlation

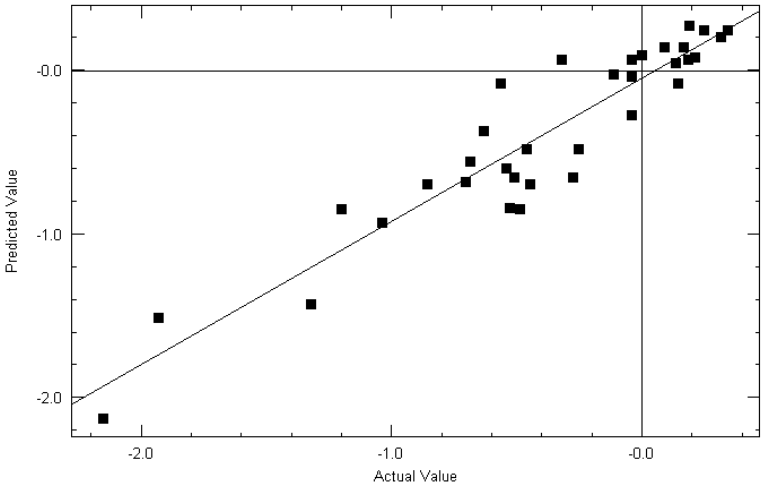


Figure 1 — Graph between actual and predicted value (training set)

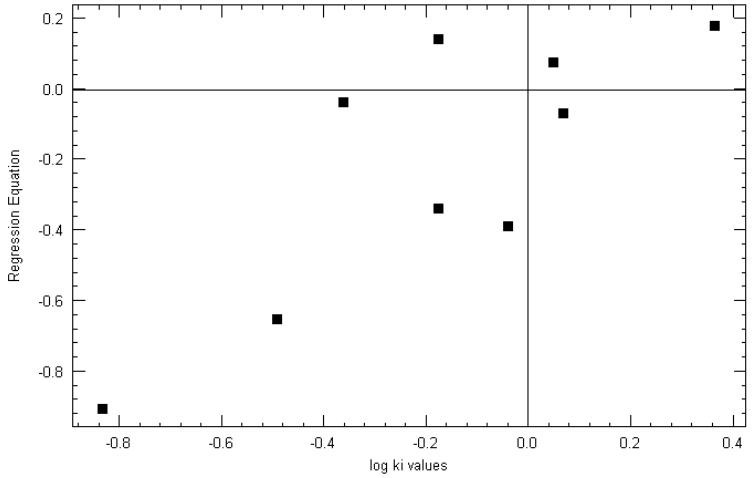


Figure 2 — Graph between actual and predicted value (test set)

coefficient (r), square of the correlation coefficient (r^2), Fischer test (f-value), and standard deviation (SD). The correlation coefficient (r) measures the quality of fit of the model where as square of the correlation coefficient (r^2) is used to describe the goodness of fit of the data. The Fischer test (f-value) is used to measure the levels of the statistical significance of the regression model. Standard deviation is the square root of the variance, and measure of the magnitude of the residuals, accounting for accuracy. It should be small but not lower than the standard deviation of experimental data. The study of outliers must include the explanation of the anomalous behavior in order to justify the removal of the compound[18]. The detection of outliers is necessary as they can cause problems. Due to the presence of outliers the real biological activity cannot be predicted well. An outlier can only be deleted from training set compounds and they can be detected by finding out the compound whose residual value is greater as in comparison with the other compounds or the compounds that are far away from the regression line can behave like outlier two compounds were identified as outliers and were deleted. The cross-validated correlation coefficient (r^2_{CV}) is used as a diagnostic tool to evaluate the predictive power or the goodness of prediction of an equation generated using a regression method. The value of r^2_{CV} was determined using the leave-out-groups of rows method in which the groups of some rows are removed from the data set and its activity is predicted using the model derived from rest of the data set. Then that group of row is returned to the training set and another group of row is removed. This procedure

Table VI — Correlation matrix

	Log P (whole molecule)	Ipso Atom E-State index (subst.2)	Sum of E-State indices (subst.4)	ADME H-bond donors (subst.4)	Verloop B5 (subst.4)	Log Ki values
Log P (whole molecule)	1					
Ipso Atom E-State index (subst.2)	-0.118079	1				
Sum of E-State indices (subst.4)	0.118209	0.258984	1			
ADME H-bond donors (subst.4)	-0.43446	-0.092115	-0.059640	1		
Verloop B5 (subst.4)	0.140756	0.323509	0.273573	-0.251352	1	
Log Ki values	-0.187188	0.43613	-0.412187	-0.437319	0.442754	1

is repeated until it accesses the predicted value of our model. The predictive capability of the QSAR model was also determined from a test set of nine compounds that were excluded during model development. The predictive r^2 for all nine test set compounds was also established.

Conclusion

A QSAR analysis using 45 compounds was succesfully carried out to build a statistically significant model possessing a good correlative and predictive ability. The detailed structural investigation revealed that the anxiolytic and antiepileptic activity exhibited by the series is predominantly explained by the steric factors of the substituents, hydrophobic character and presence of hydrogen bonding groups and hence modulation of the any of these properties could be used to optimize activity. The validation technique utilized in this work illustrates the accuracy and robustness of the constructed model by calculating its fitness on training set and test set.

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