
A Quantitative Structure-Activity Study of 3-Phenyl-substituted Imidazo[1,5-a]quinoxalin-4-ones and Imidazo[1,5-a]quinoxaline Ureas having Affinity at the Benzodiazepine Receptor Complex

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A series consisting of imidazo[1,5-a]quinoxalin-4-ones and imidazo[1,5-a]quinoxaline ureas, with a substituted phenyl ring in each class of these compounds, was reported to have high affinity for benzodiazepine receptor complex. In order to establish the exact relationship between activity and various quantifying parameters, these compounds were subjected to quantitative structure-activity relationship (QSAR) analysis. The study reveals that for *in vitro* radioligand displacement activities of substituted imidazo[1,5-a]quinoxalin-4-ones, a chloro group at R₆, a relatively smaller group such as fluoro at R₇, the less hydrophobic and more electron-donating groups, respectively, in *para*- and *meta*-positions of phenyl ring at R₃ are necessary. In a related class of imidazo[1,5-a]quinoxaline ureas, the bulky group such as a pyrrolidine ring at R₆, a less hydrophobic *para*-substituent on phenyl ring, a fluoro group at R₇, and the unsubstituted R₆-position are advantageous. In the piperazine urea sub-series, which was reported to possess biophasic efficacy, it emerged that a methoxy group at R₃, a 3,5-dimethyl piperazine moiety at R₆ and a fluoro group at R₇ are essential, whereas R₄ is required to be unsubstituted.

The discovery of benzodiazepines (BZs) and their receptors have opened a new era in the discovery and characterization of drugs acting on the central nervous system (CNS). These drugs have a wide range of therapeutic applications¹. They may be used in the pharmacotherapy of anxiety, and related emotional disorders, in the treatment of sleep disorders, status epileptic and other convulsive states. They are also used as centrally acting muscle relaxants and can be the drugs of choice in anesthesiology. However, the mechanism of action of these, unusually well tolerated and broadly effective drugs, was elucidated only after the discovery and characterization of benzodiazepine receptors (BZR) from the brain tissue^{2,3}. The BZR, in fact, is not an independent receptor, but is coupled to the γ -aminobutyric acid (GABA) receptor and is a part of the GABA_A-controlled chloride ion channel. Thus, the BZs and related compounds are not directly responsible for their observed biological actions

but act as modulators of GABA binding to its receptor, which then directly alters the gating of the GABA-dependent transmembrane chloride ion channel^{4,5}. GABA acts on at least two different receptor types⁶⁻¹⁰; the action of BZs seems to be restricted to synaptic effect of GABA that are mediated by the GABA_A-receptors. Drugs that interact with the BZR and allosterically modulate the action of GABA on neuronal chloride ion flux have a continuum of intrinsic activity¹¹⁻¹³, ranging from full agonists (anxiolytic, hypnotic and anticonvulsant agents) through antagonists to inverse agonists (proconvulsant and anxiogenic agents). Partial agonists exist within this continuum and may be devoid of BZ-mediated side effects such as physical dependence, ethanol potentiation, amnesia, oversedation and muscle relaxation^{14,15}. Furthermore, several different receptor subunits (α , β , γ , δ) combine to form the GABA_A-receptor complex^{16,17}. The drugs discriminating these subunits might be free from the side effects are thus preferred at the clinical level.

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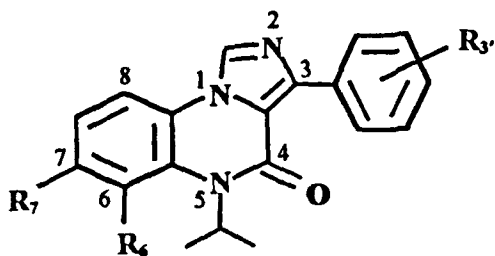


Fig. 1: Structures of imidazo[1,5-a]quinoxalin-4-ones

Jacobsen *et al.* have reported¹⁸ a series comprising of imidazo[1,5-a]quinoxalin-4-ones and imidazo[1,5-a]quinoxaline ureas. These compounds, exemplified in Figs. 1 and 2, contain a substituted phenyl ring at the 3-position and are reported to have high affinity for benzodiazepine receptor complex. More importantly, compounds from these classes displayed varying efficacy, ranging from antagonists to full agonists and several compounds also showed the *in vitro* partial agonist like properties. The binding affinities of these compounds at the benzodiazepine receptor in rat cortical membrane were determined by the competition experiments with radiolabelled [³H]flunitrazepam.

The present study aims at establishing quantitative relationship between the *in vitro* radioligand displacement data and various physicochemical and structural parameters of the title compounds. The importance of electronic, steric and hydrophobic parameters in the displacement of either [³H]flunitrazepam or [³H]diazepam from the receptor binding have already been established in our earlier publications¹⁹⁻²².

MATERIALS AND METHODS

For the present study, the electronic parameter, σ , the molar refraction parameter, MR, the hydrophobic constant, π and the van der Waals volume, V_w have emerged the best quantifying descriptors to explain the variations in the biological activities of aforementioned compounds.

The V_w , being a theoretical accessible parameter, stands to account for the lipophilicity and/or the bulk of substituents. For a given substituent, it was calculated assuming spherical shape of atoms²³. Care was taken to incorporate the corrections for the overlap of atomic orbitals and for branching in the chain wherever required. The importance of this parameter in the QSAR studies, have been documented in the past²⁴⁻³⁰. The σ , π and MR parameters were taken from the compilation of Hansch and Leo³¹. Some specific alteration in the chemical struc-

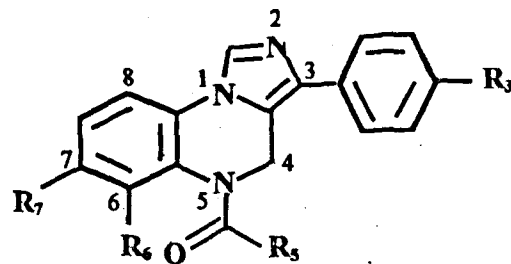


Fig. 2: Structures of imidazo[1,5-a]quinoxaline ureas

ture of the compounds was specified through the dummy parameters. All these explaining parameters and the activity values (expressed as pK_i on molar scale) for the compounds under study, are listed in the Tables 1, 2 and 3. In order to obtain the QSARs, multiple regression analysis (MRA) following the method of least squares was considered. A number of statistical parameters were obtained in conjunction with such calculations to assess the significance of the derived results. These are the multiple correlation coefficient, R (or correlation coefficient r), standard error of estimates, s and F -value representing the ratio of the variances of calculated to observed activities. The \pm data within parentheses, associated with coefficients of the descriptors in a regression equation, are the 95% confidence intervals.

RESULTS AND DISCUSSION

From the listed compounds (Fig. 1) of Table 1, the pK_i values of congeners 6 and 10 are uncertain (<5.00) and congener 3 is the lone compound which contains R_3 , = 4'-Me. On eliminating these three compounds from the data set, the MRS surfaced a highly significant correlation, shown in Eq. (1):

$$pK_i = 0.575(\pm 0.208)I_6 - 2.355(\pm 0.558)MR_7 - 2.260(\pm 0.395)\pi_4 - 1.962(\pm 0.549)\sigma_3 + 7.503$$

$$n = 15, R = 0.939, s = 0.267, F(4, 10) = 18.572 \quad (1)$$

The dummy variable, I_6 , for R_6 -substitution, was arbitrarily assigned a value 1 for chloro substituent and 0 otherwise. The other parameters, namely σ , MR, π , V_w , field F and resonance R , attempted for this position remained unsuccessful. The F -value obtained for the above equation is significant at 99% level [$F_{4,10}(0.01) = 5.99$] and R^2 accounts for 88% of the variance in the activity values. From Eq. (1), it appears that a substituent such as fluoro having smaller MR value at R_7 and chloro at R_6 are advantageous. In addition, a less hydrophobic substituent at *para*-position and a more electron-donating substituent at *meta*-position of 3-phenyl ring lead to the better

TABLE 1 : QSAR PARAMETERS AND *IN VITRO* BINDING AFFINITIES AT THE BENZODIAZEPINE RECEPTOR OF IMIDAZO[1,5-a]QUINOXALIN-4-ONES^a

S. No.	R ₃	R ₆	R ₇	I ₆	MR ₇	π ₄	σ ₃	pK _i (M)	
								Obsd ^b	Calcd ^c
1.	H	H	H	0	0.103	0.00	0.00	7.41	7.26
2.	4'-OMe	H	H	0	0.103	-0.02	0.00	7.50	7.31
3.	4'-Me	H	H	0	0.103	0.56	0.00	6.76	- ^d
4.	4'-Cl	H	H	0	0.103	0.71	0.00	5.54	5.66
5.	4'-F	H	H	0	0.103	0.14	0.00	6.94	6.94
6.	4'-CF ₃	H	H	0	0.103	0.88	0.00	<5.00	- ^d
7.	3'-OMe	H	H	0	0.103	0.00	0.12	6.68	7.03
8.	3'-F	H	H	0	0.103	0.00	0.34	6.32	6.59
9.	3'-CF ₃	H	H	0	0.103	0.00	0.43	6.73	6.42
10.	2'-OMe	H	H	0	0.103	0.00	0.00	<5.00	- ^d
11.	H	H	F	0	0.092	0.00	0.00	7.40	7.29
12.	4'-OMe	H	F	0	0.092	-0.02	0.00	7.09	7.33
13.	4'-F	H	F	0	0.092	0.14	0.00	7.23	6.97
14.	4'-F	H	Cl	0	0.603	0.14	0.00	5.77	5.77
15.	4'-OMe	Cl	H	1	0.103	-0.02	0.00	7.56	7.88
16.	4'-F	Cl	H	1	0.103	0.14	0.00	7.84	7.52
17.	4'-OMe	Me	H	0	0.103	-0.02	0.00	7.30	7.31
18.	4'-F	Me	H	0	0.103	0.14	0.00	6.90	6.94

^aFig. 1 for structures; ^btaken from reference 18; ^ccalculated using Eq. (1); ^doutlier compounds in deriving Eq. (1)

displacement potency of a compound. Based on these inferences, a simple compound having 4'-aminophenyl, chloro and fluoro substituents, respectively at 3, 6 and 7-positions in the imidazo[1,5-a]quinoxalin-4-one moiety is predicted to possess the theoretical pK_i value of 10.68. This predicted compound may, therefore elicit, displacement activity about 3 order of magnitude higher than that of the most active compound of the training set. The calculated pK_i values, using Eq. (1) for the compounds in Table 1, closely resemble the observed ones and all independent variables of this equation have no mutual correlations (*r*-value: I₆ vs MR₇ = 0.097, I₆ vs π₄ = 0.062, I₆ vs σ₃ = 0.176, MR₇ vs π₄ = 0.081, MR₇ vs σ₃ = 0.111 and π₄ vs, σ₃ = 0.221).

With regard to imidazol[1,5-a]quinoxaline ureas (Fig. 2) in Table 2, the van der Waals volume of group R₅, molar refraction of group R₇ and hydrophobicity of group R₃ were found to be the most appropriate variables while a dummy parameter, I₆ was suitable to account for the binary variation at R₆. The derived correlation is shown in Eq. (2):

$$pK_i = 0.704(\pm 0.153)I_6 - 2.270(\pm 0.495)MR_7 - 1.047(\pm 0.475)\pi_3 - 1.711(\pm 0.277)V_w(5) + 10.679$$

n = 16, *R* = 0.936, *s* = 0.234, *F*(4, 11) = 19.613 (2)

This equation is highly significant in statistical sense as its *R*²-value accounts for 88% of variance and the *F*-value stands significant at 99% level [*F*_(4,11)(0.01) = 5.67]. The calculated pK_i values obtained using this equation

TABLE 2 : QSAR PARAMETERS AND *IN VITRO* BINDING AFFINITIES AT THE BENZODIAZEPINE RECEPTOR OF IMIDAZO[1,5-a]QUINOXALINE UREAS^a

S. No.	R ₃	R ₅	R ₆	R ₇	π ₃	V _w (5)	I ₆	MR ₇	pK _i (M)	
									Obsd ^b	Cald ^c
1.	H	pyrrolidine	H	H	0.00	0.705	0	0.103	9.43	9.24
2.	F	pyrrolidine	H	H	0.14	0.705	0	0.103	9.01	9.09
3.	Me	pyrrolidine	H	H	0.56	0.705	0	0.103	8.81	8.65
4.	F	morpholine	H	H	0.14	0.786	0	0.103	8.79	8.95
5.	F	3,4-dimethyl piperazine	H	H	0.14	1.120	0	0.103	8.23	8.38
6.	F	3,4,5-trimethyl piperazine	H	H	0.14	1.282	0	0.103	7.88	8.11
7.	H	piperidine	H	H	0.00	0.859	0	0.103	8.78	8.98
8.	F	morpholine	H	F	0.14	0.786	0	0.092	8.67	8.98
9.	F	morpholine	H	Cl	0.14	0.786	0	0.603	7.82	7.82
10.	F	morpholine	Cl	H	0.14	0.786	1	0.103	8.53	8.25
11.	F	3,5-dimethyl piperazine	H	F	0.14	1.120	0	0.092	8.60	8.41
12.	F	3,4,5-trimethyl piperazine	H	F	0.14	1.282	0	0.092	8.30	8.13
13.	F	3,5-dimethyl piperazine	Cl	H	0.14	1.120	1	0.103	7.48	7.68
14.	F	3,4,5-trimethyl piperazine	Cl	H	0.14	1.282	1	0.103	7.32	7.40
15.	OMe	3,5-dimethyl piperazine	H	H	-0.02	1.120	0	0.103	8.86	8.55
16.	OMe	3,4,5-trimethyl piperazine	H	H	-0.02	1.282	0	0.103	8.38	8.27

^aFig. 2 for structures; ^bsee footnote under Table 1; ^ccalculated using Eq. (2)

and listed in Table 2 are in close agreement with the observed ones. All variables to the right hand side of Eq. (2) have shown their mutual independence (r -value : π₃ vs V_w(5) = 0.277, π₃ vs I₆ = 0.042, π₃ vs MR₇ = 0.021, V_w(5) vs I₆ = 0.168, V_w(5) vs MR₇ = 0.227 and I₆ vs MR₇ = 0.115). In order to have potent analogues, Eq. (2) reveals that a less bulky group at R₅, a less hydrophobic group at R₃, and a fluoro group having smaller MR parameter at R₇ are essential while the position R₆ is required to be unsubstituted.

Amongst the listed compounds of Table 2, the subclass of eight piperazine ureas (Nos. 5, 6 and 11-16) was reported to have biophasic efficacy¹⁸ and such analogues become more antagonistic with increase in their concentration. The biophasic effect displayed by these conge-

ners is most likely due to their ability to bind at two different sites, a high-affinity site and a low-affinity site³², on the GABA_A complex. The biophasic properties observed for this subclass are unique and have not been reported so far for other classes of BZR ligands. The variations of R₃, R₅, R₆ and R₇ were positions, in these compounds, may suitably be described by the dummy variables alone, as all these positions are having only binary substitutions. For example, the dummy variables I₃, I₆ and I₇ were each assigned a value 1 for the substitutions, -F at R₃, 3,4,5-trimethylpiperazine at R₅, -Cl at R₆ and -F at R₇ while, in the same order, a value 0 were taken for each of them for the substitutions, -OMe, 3,4-dimethylpiperazine, H and H at these substituted positions. Using this strategy of independent variables, MRA yielded a highly significant correlation Eq. (3):

TABLE 3 : QSAR PARAMETERS AND *IN VITRO* BINDING AFFINITIES AT THE BENZODIAZEPINE RECEPTOR OF IMIDAZO[1,5-a]QUINOXALINE UREAS^a

S. No.	R ₃	R ₅	π ₃	I ₅	V _w (5)	pK _i (M)	
						Obsd ^b	Cald ^c
1.	H	NMe ₂	0.00	1	0.501	8.47	7.62
2.	F	NMe ₂	0.14	1	0.501	8.53	7.63
3.	Me	NMe ₂	0.56	1	0.501	7.95	7.67
4.	F	NH ₂	0.14	0	0.177	6.67	7.29
5.	F	NHEt	0.14	0	0.439	7.31	7.57
6.	F	NH ^t Bu	0.14	0	0.701	6.55	- ^d
7.	F	OMe	0.14	0	0.304	7.33	7.43
8.	F	O ^t Pr	0.14	0	0.562	7.29	7.70
9.	F	p-F-Ph	0.14	0	0.831	7.69	7.98
10.	F	m-F-Ph	0.14	0	0.831	8.23	7.98
11.	F	o-F-Ph	0.14	0	0.831	7.37	7.98

^aFig. 2 for structures; where R₆ = R₇ = H; ^bsee footnote under Table 1; ^ccalculated using Eq. (5); ^doutlier compound in deriving Eq. (5)

$$pK_i = -0.565(\pm 0.094)I_3 - 0.322(\pm 0.066)I_5 - 0.655(\pm 0.094)I_6 + 0.395(\pm 0.094)I_7 + 8.781$$

$$n = 8, R = 0.993, s = 0.094, F(4, 3) = 56.314 \quad (3)$$

All variables to the right hand side of the above equation are mutually orthogonal. The *F*-value obtained is significant at 99% level [*F*_{4,3}(0.01) = 28.7] and *R*² accounts for 99% of the variance. From the binary substitution at a given position, this equation has shown the preference for one alternative substitution only that may lead to the better displacement ability of a compound. For this reason, the substituent like -OMe, 3,5-dimethylpiperazine and -F are required respectively at R₃-, R₅-, and R₇-positions, whereas the R₆-position better remains unsubstituted. Such guidelines regarding structural changes in BZR ligands, having biophasic efficacy may help in future synthetic efforts.

Listed data for the compounds in Table 3 have resulted into correlation Eq. (4):

$$pK_i = -1.033(\pm 1.108)\pi_3 + 1.087(\pm 0.671)I_5 + 1.199(\pm 0.331)V_w(5) + 6.814$$

$$n = 11, R = 0.818, s = 0.456, F(3, 7) = 4.736 \quad (4)$$

In above equation, the dummy variable I₅ was chosen for R₅-position so as to account for the presence or absence of certain structural feature thereon. Thus, I₅ = 1 or 0 stands to denote the presence or absence of -NMe₂ substituent at this position. The large positive coefficient of I₅ in the above equation hinted the preference for -NMe₂ substituent at R₅-position. Further, the calculated pK_i-value for compound 6 in Table 3 was largely deviating from its observed one. This is only the analogue in which the mono-substituted nitrogen has a bulky-^tBu group at R₅-position. The same was, therefore, ignored and the resulting significant correlation is given by Eq. (5):

$$pK_i = -1.033(\pm 0.728)\pi_3 + 1.349(\pm 0.449)I_5 + 1.090(\pm 0.220)V_w(5) + 6.791$$

$$n = 10, R = 0.913, s = 0.300, F(3, 6) = 10.048 \quad (5)$$

The *F*-value obtained in above equation, still significant at 99% level [*F*_{3,6}(0.01) = 8.45] has substantially been increased and the ± data associated with regression coefficient of π₃ is also lowered. Similarly, *R*² accounts for 83% of the variance. Further, the descriptors of Eq. (5) are poorly intercorrelated (*r*-value : π₃ vs I₅ = 0.045, π₃ vs V_w(5) = 0.312 and I₅ vs V_w(5) = 0.143). The calculated pK_i

values, obtained from this equation closely resemble the observed ones (Table 3). Further, it appears from Eq. (5) that a bulkier dialkyl substituted amino group at R₅-position and a less hydrophobic *para*-substituent on 3-aryl ring are advantageous in raising the *in vitro* displacement ability of a compound against the [³H]flunitrazepam.

Thus the present study provides a rationale for the *in vitro* ligand displacement affinity to the BZR, and opens up new vistas to the drug-receptor interaction at the molecular level. The study also provides the basis for the selection of substituents in analogue-design strategy.

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