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QSAR Studies of 6-aryl- 6*H*-pyrrolo [3, 4-d] pyridazine analogues as high-affinity ligands of the α2δ subunit of voltage-gated calcium channels

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ABSTRACT

The anticonvulsant activity of 6-aryl- 6H- pyrrolo (3, 4-d) pyridazine (PPZ) analogues was quantitatively analyzed in terms of physico chemical parameters by regression analysis. Structural requirements for maximal binding affinity to a novel site of $\alpha_2\delta$ subunit were derived. The leave –one –out cross validation method was used to judge the predictive power of model equations. The high binding affinity of molecule is expected to have more bulky and electron donating groups.

Keywords: 6-aryl- 6*H*-pyrrolo(3,4-d) pyridazine; Quantitative Structure Activity Relationship; Steric factor; lipophilicity; electronic parameter; cross validation molecular mechanics.

INTRODUCTION

All anticonvulsant drugs must exert their actions by modulating the activity of the basic mediators of neuronal excitability: voltage and neurotransmitter–gated ion channels. The $\alpha_2\delta$ Ca^{2+} channel subunit is responsible for chronic pain states and axiety. Gabapentin (Fig.1) is unique among Ca^{2+} channel ligands in that it acts at the $\alpha_2\delta$ subunit rather that at the α_1 subunit. Since the $\alpha_2\delta$ subunit appears to be common to all voltage- dependent Ca^{2+} channel [1,2], it is conceivable that gabapentin modulates the activity of more than one type of neuronal Ca^{2+} channel. It is possible that gabapentin exerts functional effects only with particular combinations of subunits. Moreover, these effects may be observed only under conditions that mimic closely the excessive repetitive discharges that characterize clinical epilepsy. Further studies on the cellular electrophysiological actions of gabentin, in a variety of systems, are required before the action of the drug at Ca^{2+}channels can be fully understood. The high affinity interaction of certain L–system substrates

(eg. L –leucine and L – Methionine [3]) with $\alpha_2\delta$ subunit is intriguing. The endogenous ligands such as these presumably compete with gabapentin in vivo for the binding site on the $\alpha_2\delta$ subunit.

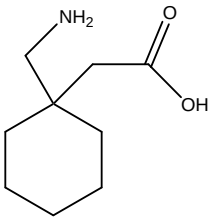


Figure 1.Gabapentin

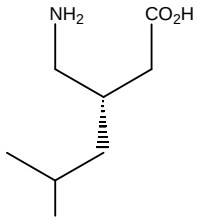


Figure 2. Pregabalin

This perhaps explains why the therapeutic concentration of gabapentin [4] is well above the K_D of the drug at the gabapentin binding site. Gabapentin is the first ligand describes which interaction with the $\alpha_2\delta$ subunit [5]. It is suggested that modulation of voltage-dependent neuronal Ca^{2+} channels are important in the antiepileptic action of ligands.Pregabalin (Fig.2) was also found to be therapeutic agent for the treatment of both neuropathic pain [6] and anxiety [7]. Recently, pyrrolo pyridazine (PPZ) [8] (Fig. 3) was identified as $\alpha_2\delta$ ligand through a high-throughput-binding assay in a program directed at the discovery of non amino acid gabapentin mimetics.

The close observation of analogues of PPZ reveals that electron-donating group present at ortho position in the phenyl ring enhances the binding affinity with the $\alpha_2\delta$. In order to quantify the binding affinity of analogues of PPZ to $\alpha_2\delta$ subunit, QSAR study was taken up.

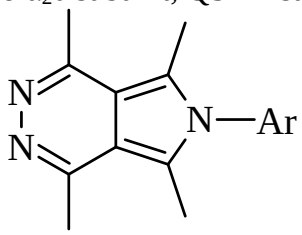


Figure 3. 6-aryl- 6*H*-pyrrolo (3,4-d) pyridazine

EXPERIMENTAL SECTION

A series of 18 compounds were subjected to molecular modeling using window Chem. SW Inc. [9] program. Molecules were built using molecular modeling pro. of Chem. SW followed by energy minimization. These were subjected to regression analysis using SSPS 10.0 software [10] to generate the best-fit correlation.

2.1.1 Data Set

The physicochemical parameters, like molar refractivity (MR), electronic parameter (σ_i) and lipophilicity (QlogP) of the substituents were calculated by molecular modeling pro.

2.2 Molecular Structure Building of QSAR Models

Regression analysis was applied to the analogues of PPZ. Appropriate descriptors or parameters like QlogP, MR and sigma i (σ_i) for the compound (Table-1) was correlated for the observed activity to know the explanatory variables. In the multiple regressions analysis different modeled equations were generated to predict and design a compound with best possible inhibitory property.

2.3 Chemical Descriptors

2.3.1 Steric Parameter

This parameter gives a measure of the steric factor and bulkiness of the given base molecule with various substituents. It is the molar volume corrected by the refractive index and represents size, and polarizability of a fragment or molecules. Molar refractive [11] is given by

$$MR = \frac{(n^2-1) \times (MW)}{(n^2+2) \times d} \quad (1)$$

Where, n is the refractive index, MW is the molecular weight, and d is the compound density.

$$\text{Log } (1/IC_{50}) = \sum a_i.x_i \quad (2)$$

Where, a_i is the correlation coefficient, x_i is the physico-chemical parameter.

The equations are labeled in the order in which they appear in the text with Arabic numbers in parentheses aligned to the left margin. In writing the equations use the following rules: vectors and matrices with bold, functions such as log, sin, cos normal, and all other mathematical symbols with italic.

2.3.2 Electronic Parameter

It is an electronic substituent descriptor reflecting the donating or accepting properties of a constituent.

2.3.3 Lipophilicity Parameter

The lipophilicity factor P is the most used property where P is defined by 1-octonol/water partition coefficient. All the QlogP values were calculated as per Bodor & Buchwald method in Chew SW [12].

2.4 Correlation analysis

Correlation between biological activity expressed as log $1/IC_{50}$ and physico- chemical parameters X_i (QlogP, MR and σ_i) (Table-1) were analyzed statistically by fitting the data to correlation equation (equation 2) consisting of various combinations of these parameters.

The statistical optimization is used to propose the best correlation model. The correlation coefficient, a_i for each term was determined by the least square method.

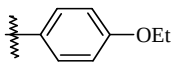
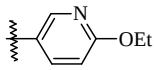
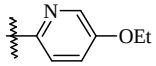
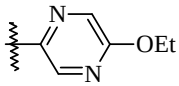
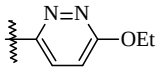
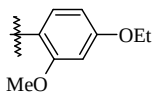
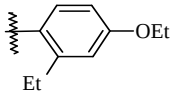
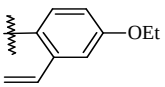
RESULTS AND DISCUSSION

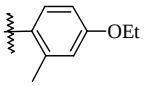
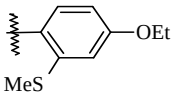
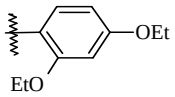
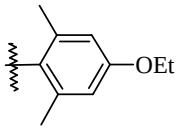
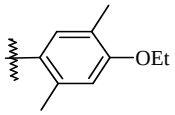
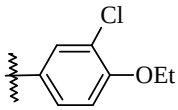
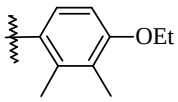
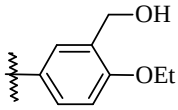
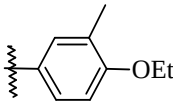
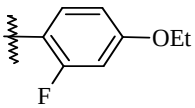
The set of 18 analogues of PPZ were first synthesized by Tao Hu group[13]the biological activity data presented in Table-1 were subjected to regression analysis. The correlation matrix was generated with 18 analogues. The correlation terms involved in the correlation matrix in Table-3 indicate the extent of co-linearity. The term close to unity indicates high co-linearity, while the value below 0.5 indicates that non co-linearity. The perusal of correlation matrix indicates QlogP (or) MR and σ_i are the indicative parameters. Large errors were noticed when QlogP was taken as an independent variable [14]. Hence, QlogP is not considered as a indicative parameter. MR and σ_i were considered only as indicative parameters.

Table 1.Physico-chemical parameters of 6-aryl –Pyrrolo [3,4-d] Pyridazine variants.

Compound	Activity	QlogP	MR	σ_i
PPZ 1	1.7404	4.2828	89.4986	0.0980
PPZ 2	0.6021	2.6918	87.6236	0.2250
PPZ 3	0.2553	2.6912	87.3378	0.1070
PPZ 4	0.2042	2.5468	85.5380	0.2340
PPZ 5	0.1140	2.5577	87.4711	0.2340
PPZ 6	3.3980	4.3053	96.0369	0.0083
PPZ 7	2.8860	5.2645	99.2159	0.0490
PPZ 8	2.6021	5.0757	99.2600	0.0086
PPZ 9	2.5683	4.7783	94.6149	0.0920
PPZ 10	2.5527	5.3356	102.4802	0.0800
PPZ 11	2.4815	4.7886	100.7800	0.1030
PPZ 12	2.2672	5.2737	99.7312	0.0860
PPZ 13	2.0254	5.2742	99.6561	0.1090
PPZ 14	2.0000	4.7267	94.3033	0.1150
PPZ 15	1.5441	5.2774	99.6561	0.1090
PPZ 16	1.5315	3.5740	96.3145	0.1150
PPZ 17	1.5186	4.7783	94.5398	0.1150
PPZ 18	1.4920	4.4434	89.7901	0.1620

Table 2. Activity Physicochemical properties of 6-aryl- 6H- pyrrolo (3, 4-d) pyridazine variants.

S.No.	Ar	Activity	σ_i	MR
1.		1.7404	0.098	89.4986
2.		0.6021	0.225	87.6236
3*.		0.2553	0.107	87.3378
4.		0.2042	0.234	85.5380
5.		0.1140	0.234	87.4711
6.		3.398	0.0083	96.0369
7.		2.8860	0.049	99.2159
8.		2.6021	0.0086	99.2600

9.		2.5683	0.092	94.6149
10.		2.5527	0.0083	102.4
11.		2.4815	0.103	100.784
12.		2.2672	0.086	99.7312
13.		2.0254	0.109	99.6561
14.		2.000	0.115	94.3033
15.		1.5441	0.109	99.6561
16.		1.5315	0.115	96.3145
17.		1.5186	0.115	94.5393
18.		1.4920	0.162	89.790

**Outlier*

Table 3. Correlation matrix of the 6-aryl- 6H- pyrrolo [3, 4-d] pyridazine variants.

	Activity	MR	σi	Ql ogP
Activity Pearson correlation	1.000	0.789	-0.855	0.817
Sig. (2-tailed)	0.000	0.000	0.000	0.000
MR Pearson correlation		1.000	-0.704	0.866
Sig.(2-tailed)		0.000	0.001	0.000
σ_i Pearson correlation			1.000	- 0.706

Sig.(2-tailed)	0.000	0.001
QlogP Pearson correlation		1.000
Sig.(2-tailed)		0.000

N=18

Table 4. Model equation generated for the 6-aryl- 6*H*- pyrrolo [3, 4-d] pyridazine variants.

1. A= 0.019(0.002) MR
N=18, R=0.903, %EV=81.5, SEE=0.885, F=74.785, Q=1.020, PRESS=13.3232, q^2_{cv}=0.1573.

2. A=8.823(3.039) σi
N=18, R=0.579, %EV =33.1, SEE=1.681, F=8.429, Q=0.344, PRESS=48.0882, q^2_{cv} = -2.0414.

3. A=0.03(0.002) MR-10.856 (1.605) σi
N=18, R=0.976, %EV=95.2, SEE=0.464, F=158.64, Q=2.1034, PRESS=3.9572, q^2_{cv} =0.7497.

4. A= 0.03(0.001) MR-10.662 (1.040) σi
N=17, R=0.989, %EV=97.9, SEE=0.318, F=347.30, Q=3.0786, PRESS=2.3408, q^2_{cv}=0.8252.

A=Activity, N=Number of data points, R=Regression constant, %EV=Percentage of explained variance, SEE =Standard error estimate, F=F ratio, Q=Quality factor, PRESS= Predictive sum of squares, q^2_{cv}=Cross-validation.

Table 5. Observed and Predicted activity values of 6-aryl- 6H- pyrrolo [3, 4-d] pyridazine variants (Model eq.3).

Compound	Observed activity	Predicted activity	Residual value
PPZ 1	1.7404	1.6211	0 .1193
PPZ 2	0.6021	0.1861	0 .4160
PPZ 3*	0.2553	1.4585	-1.2032
PPZ 4	0.2042	0.0258	0.1784
PPZ 5	0.1140	0.0838	0.0302
PPZ 6	3.3980	2.7910	0 .6070
PPZ 7	2.8860	2.4445	0.4415
PPZ 8	2.6021	2.8844	-0.2823
PPZ 9	2.5683	1.8397	0.7286
PPZ 10	2.5527	2.2059	0.3468
PPZ 11	2.4815	1.9052	0.5763
PPZ 12	2.2672	2.0583	0.2089
PPZ 13	2.0254	1.8064	0.2190
PPZ 14	2.0000	1.5807	0.4193

PPZ 15	1.5441	1.8064	-0.2623
PPZ 16	1.5315	1.6410	-0.1095
PPZ 17	1.5186	1.5878	-0.0692
PPZ 18	1.4920	0.9350	0.5570

**Outlier considered for model equation*

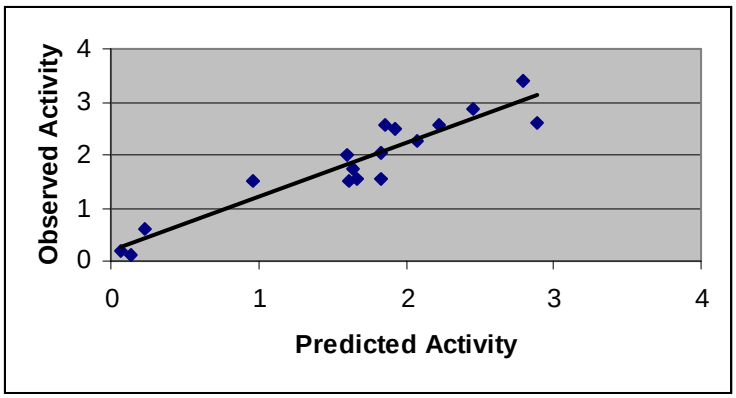


Figure 4. Plot of Observed Activity versus Predicted Activity.

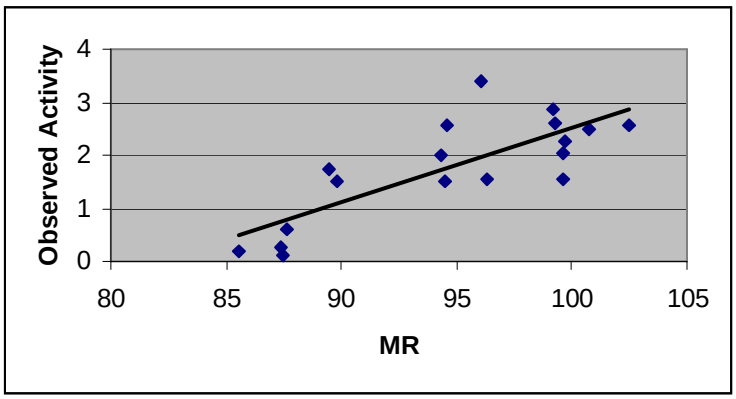


Figure 5. Plot of Observed Activity versus MR.

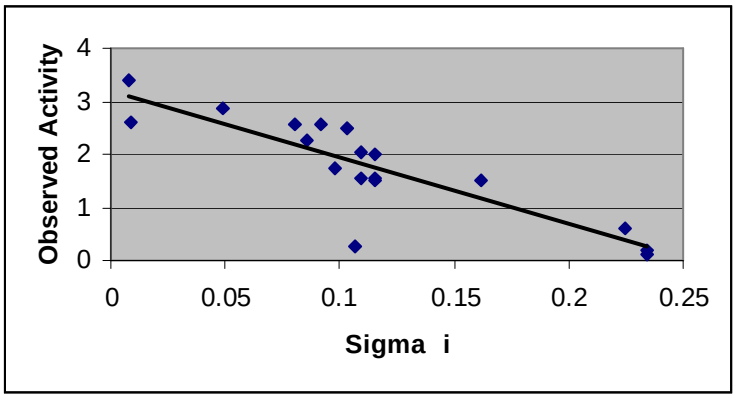


Figure 6. Plot of Observed Activity versus Sigma i (σ_i).

In the initial stage, mono parametric QSAR equations were generated with MR and σ_i separately. It is interesting to record that R_A^2 (adjusted) values take into account of the adjustment of R^2. Therefore, if a variable is added that does not contribute its fair share, the R^2_A value will actually decline. It is observed that by the addition of σ_i to the model (Equation-3, Table-4) R^2_A is increased. This supports bivariant dependence of biological activity. Hence, multiple regressions have been sought.

The regression technique was applied through the origin since there is no output (activity) without input (MR and σ_i). The resulted modeled equations explain the biological activity as a function of MR and σ_i.

$$\begin{aligned} &A=0.03(0.002)\ MR-10.856\ (1.605)\ \sigma_i \text{ ----------} \quad (3) \\ &N=18, R=0.976,\ \%EV=95.2, SEE=\ 0.464, F=158.64. \end{aligned} \tag{3}$$

Where N=Number of data points, R=Regression constant, %EV=Percentage of explained variance, SEE=Standard error estimation, F= F ratio.

Equation-3 shows that the value of % EV is 95.2 and to improve its value, outliers were sought and eliminated. In addition, the plot of the observed activity versus the predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. After the elimination of the outlier (PPZ 3), a second model was developed.

$$\begin{aligned} &A=0.03(0.002)\ MR - 10.779(1.100)\ \sigma_i \text{ ----------} \quad (4) \\ &N=17,\ R=\ 0.989,\ \%EV=\ 97.9,\ SEE=0.318,\ F=\ 347.30. \end{aligned} \tag{4}$$

There is an increase in R (0.976 to 0.989) and %EV (95.2 to97.9) values and decreases in SEE (0.464 to 0.318) and F value increases from (158.64 to 347.30). These trends support the statistical validity of the equation-4. In an attempt to investigate predictive potential of the proposed models, the cross validation parameters (q^2_{cv} and PRESS) were calculated and used. The predictive power of equations confirmed by leave-one-out (LOO) [15-16] cross validation method, where compounds are deleted one after another and prediction of the activity of the deleted compound is made based on the QSAR model. Cross-validation evaluates the validity of a model by how well it predicts the data rather than how well it fits the data. The cross-validation parameter, q^2_{cv} is mentioned at each respective equation (Table-4).

$$\mathbf{q^2_{cv}} = \frac{\mathbf{(SD - PRESS)}}{\mathbf{SD}} \tag{5}$$

Where the **PRESS** (predictive residual sum of squares) and **SD** (standard deviation) values are obtained as

$$\mathbf{PRESS} = \sum (\text{property}_{\text{observed}} - \text{property}_{\text{predicted}})^2 \tag{6}$$

$$\mathbf{SD} = \sum (\text{property}_{\text{observed}} - \text{property}_{\text{mean}})^2 \tag{7}$$

Equation-4 (Table-5) gives a good q^2_{cv} value. Its value should be always smaller than %EV. A modal is considered to be significant when $q^2_{cv} > 0.3$ [17].

Another cross-validation parameter, PRESS which is the sum of the squared differences between the actual and that predicted when the compound is omitted from the fitting process, also supports the predictive ability of equation–4. Its overall value decreases (Table-4). The quality factor, Q [18] is defined as the ratio of regression constant R to the standard error estimation (SEE) i.e. Q = R/SEE. This indicates that the higher the value of R and lower the value of SEE, higher is the magnitude of Q and the better will be the correlation. In present case, Q increases from 2.1034 to 3.0786 (Table-4). The predictive ability of the modeled equation-4 is seen in terms of the low residual values (Table-5). The excellent agreement between observed activities versus predicted activity is shown in the Figure-4. The linear relationship between observed activity and MR is shown in figure-5, indicates that as MR increases the activity increase which is also evident from the data (Table-1). As the bulkiness of substituent (Ar) increases, the activity increases. The inverse relationship is found between activity and σ_i (Table-2and Fig -6). The compound with high bulkiness and low σ_i is expected to have more anticonvulsant activity. So the structural requirements for maximal binding affinity to the $\alpha_2\delta$ subunit site are more bulky and electron-donating group present at ortho position in the phenyl ring. The activity site is expected to have electron accepting groups and large receptor site pit to accommodate the ligand effectively. The compounds PPZ 6 and 10 may be considered as effective anti convulsent agents.

The binding affinity of PPZ with $\alpha_2\delta$ subunit of voltage gated calcium channel depends on the chemical environment of phenyl ring system. The binding affinity diminishes when the phenyl ring is replaced by various heterocyclic such as pyridine, pyridazine or pyrimidine. Incorporating substituents on the phenyl ring revealed dramatic stereo-electronic effects in the binding affinity of pyrrolo pyridazine lead molecule to $\alpha_2\delta$ subunit.

The presence of the ortho-methoxy moiety most likely changes the conformation of the molecule by placing the two rings perpendicular to each other such that the phenyl group providing a comparable nonplanar conformation, resulting in improved binding affinity. However, incorporation additional methyl group, leading to dimethyl analogues either in the 3,4or5– position (PPZ-12,13 and 15) failed to significantly improve potency. Electron donating groups such as methoxy (PPZ 6), Ethyl (PPZ 7), vinyl (PPZ 8), thiomethyl (PPZ 10) and ethoxy (PPZ 11) improved the binding affinity significantly. However, optimum size of the ortho substituent (PPZ 10, 11) is also important in retaing the binding affinity. The optimum size is important in optimizing the binding affinity (PPZ 6). The ligand (PPZ 6) showed a 45 – fold increase in potency over the initial lead molecule (Fig .3). The electron withdrawing groups at ortho – position exemplified by (PPZ 18) lead to significant decrease in potency. In general analogues with electron donating groups are preferred and ortho- substituents are relatively more preferred then meta substituents.

$$pIC_{50} = -2.528\ (\pm 0.509) + 2.791\ (\pm 0.426)\ S_{av1} - 0.441\ (\pm 0.235)\ \pi_{pA}$$

$n = 18$ $R = 0.864$ $\%EV = 74.582$ $R^2_A = 0.712$ $F_{(2,15)} = 22.007$ $p<0.001$ $SEE = 0.259$ (5)

$PRESS = 1.328$ $SSY = 2.980$ $R^2_{CV} = 0.554$ $S_{PRESS} = 0.298$ $PSE = 0.272$

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