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Structure activity relationship studies on aminopyridine carboxamides as JNK-2 inhibitors

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Abstract

In this work we report QSAR studies on aminopyridine carboxamide inhibitors of JNK-2. QSAR models were constructed by multiple regression analysis using 46 compounds, validated by q^2, r^2_{cvext} and other procedures. The activity contributions of these compounds were determined from regression equation and the validation procedures that analyze the predictive ability of QSAR models were described. Among several descriptors that were considered, four variables resulted in a statistically significant model, based on FIT Kubinyi function, with r^2 = 0.685, q^2 = 0.750, $r^2_{cv,ext}$ = 0.994 and inter-correlation between descriptors being 0.38. Our results suggest that variables such as dipole moment, logP, Kier ChiV2 and shape flexibility play an important role in the inhibition of JNK-2 by aminopyridine carboxamide inhibitors.

Keywords: JNK-2; aminopyridine carboxamides; QSAR; regression.

INTRODUCTION

The c-Jun N-terminal kinases (JNKs) are members of the mitogen-activated protein (MAP) kinase family. Evidence indicated a crucial role of JNKs in mitochondrial dysfunctions (such as Alzheimer's, Parkinson's, or Huntington's disease) with subsequent initiation of neuronal apoptosis [1]. JNKs are involved in the mitochondrial pathology at different functional levels and finally JNKs trigger the expression of the pro-apoptotic proteins in the nucleus [2-6]. In contrast to the JNK-1 isoform, JNK-2 translocates to the nucleus and the mitochondria, where it acts downstream of MKK4 [7]. Hence, based on the importance of JNK-2 in mitochondrial dysfunctions, QSAR analysis was carried out to delineate the structural requirements [8, 9] of various aminopyridine carboxamides. From literature, various JNK-1[10] and JNK3 [11-13] QSAR studies are reported, whereas aminopyridine carboxamide inhibitors of JNK-2 were not

presented. To our knowledge no attempts have been made so far to build a QSAR model with these selected set of compounds. Statistically significant QSAR models were generated using multiple linear regression procedure.

RESULTS

Multivariate regression technique was employed using default parameters of Tsar Software with F to enter and F to leave being 4 to test the predictive power of the generated QSAR model. Cross-validation was carried out on complete data set which resulted in the following multivariate equation:

Log (1/C) = + 0.069*Dipole Moment X Component
- 0.381*logP
+ 0.293*Kier ChiV1
- 0.422*KAlpha2
+ 7.335
r = 0.760, r^2 = 0.577, q^2 = 0.696, F = 14.003, n = 46,
PRESS = 6.963, s = 0.412 (1)

Eq. 1 represents four significant descriptors and the data set was investigated for outliers by calculating the standard residuals shown in Table 4. Standardized residuals greater than 2 and less than -2 are usually considered large [14]. Compounds 31, 39 and 40 have standardized residuals -2.163, -3.374 and 2.465 and hence removed from the data set to obtain statistically validated best models [15].

After rejecting outliers from the data set, QSAR models were generated by dividing the set as 36 molecule training and a 7 molecule validation set (Table 4). The results obtained from the multiple linear regression procedure with varied number of variables are encouraging and the best models among many are shown below with their statistics.

Model-1:
Log (1/C) = - 0.385*logP
+ 0.229*Kier ChiV2
- 0.405*Shape Flexibility index
+ 7.406
r = 0.728, r^2 = 0.529, q^2 = 0.893, F =12.021, n = 36
PRESS = 4.88, s = 0.390 (2)

Model-2:
Log (1/C) = + 0.074*Dipole Moment X Component
- 0.384*logP
+ 0.238*Kier ChiV2
- 0.306*Shape Flexibility index
+ 6.713
r = 0.828, r^2 = 0.685, q^2 = 0.750, F =16.859, n = 36

PRESS = 3.268, s = 0.325 (3)

Model-3:

Log (1/C) = + 0.079*Dipole Moment X Component
- 0.384*logP
+ 0.549*Kier Chi3
- 0.179*KAlpha3 index
+ 0.004* Heat of Formation
+ 6.174

r = 0.827, r^2 = 0.683, q^2 = 0.634, F = 12.985, n = 36

PRESS = 3.28, s = 0.331 (4)

Table 1: Predictive ability of the three models with varying descriptors for validation sets

S. No	$R^2_{cv,ext}$	R^2	k	k'	X^a	Y^b
1	0.973	0.790	0.990	1.009	0.043	0.002
2	0.994	0.961	0.996	1.004	0	0.001
3	0.998	0.766	1.002	0.997	0.043	0.006

[a] $(R^2 - R_0^2) / R^2$; [b] $(R^2 - R_0'^2) / R^2$

Accordingly, all the three models passed the conditions for validation sets (Eqs. 6-9, Table 4). Different numbers of significant variables are obtained in each of the equations. However to select the most significant model, the number of variables entering the QSAR model are compared by FIT Kubinyi function (Eq. 5) [16].

$$FIT = R^2 (n - k - 1) / (n + k^2) (1 - R^2) \quad (5)$$

where n is the number of compounds in training set and k is the number of variables in the QSAR equation. The main disadvantage of the F value is its high sensitivity if k is small and lower sensitivity if k is large [16]. The best model should possess a high value of this function.

Table 2: Statistical results of the generated QSAR models

QSAR Model No.	N^a	r^2	PRESS	F test	FIT
Model 1	3	0.529	4.88	12.021	1.178
Model 2	4	0.685	3.27	16.859	1.287
Model 3	5	0.683	3.28	12.985	0.809

[a] *no. of variables in the model*

DISCUSSION

According to the statistical values of the models reported in table 2**,** we chose the model with four variables (Eq. 3) since this showed high FIT than others. This model accounts for the good internal predictive ability as shown by q^2 value of 0.750 and was able to explain 68.5% variance of inhibitory activities of aminopyridine carboxamides. The predictive residual sum of squares and the standard error of estimate are 3.268 and 0.325 respectively.

Further, inter-correlation between variables of the proposed QSAR model with the best FIT (Eq. 3) was checked to know about their independence. The results are presented in table 3, where, it is clear that the descriptors are not highly correlated. Figure 1 depicts the predictive ability of Eq. 3 when applied on validation set molecules. Observed versus predicted values of the validation set which illustrate the predictive ability of Eqs. 6-9 (Table 4) are depicted graphically in Figure 2 and 3.

Table 3: Inter-correlation between significant descriptors of Eq. 3

	X1 [a]	**X2**[b]	**X3**[c]	**X4**[d]
X1[a]	1.000			
X2[b]	0.050	1.000		
X3[c]	-0.028	0.388	1.000	
X4[d]	-0.321	-0.136	0.114	1.000

[a]*Dipole Moment X Component;* [b]*log P;* [c]*Kier ChiV2 index;* [d]*Shape Flexibility index*

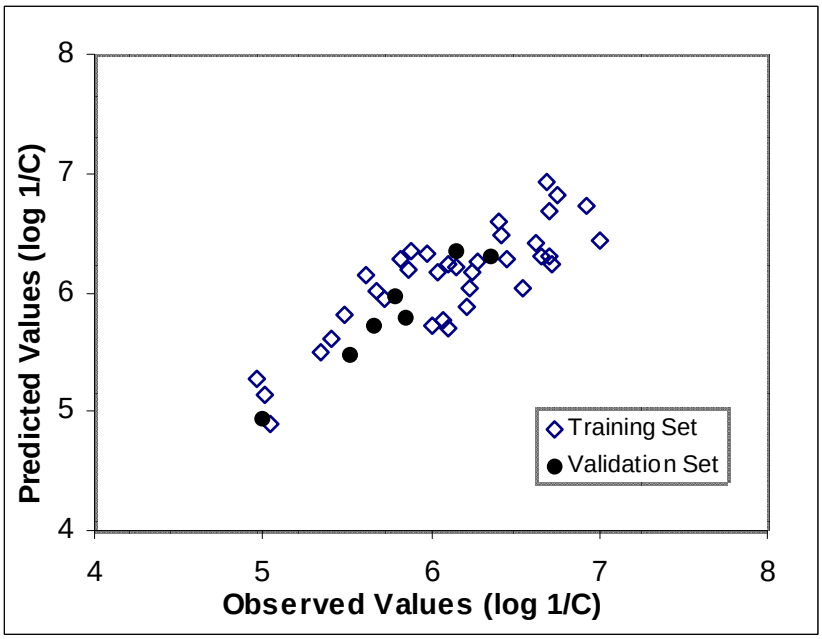


Figure 1: Observed and predicted values of molecules in training and validation set

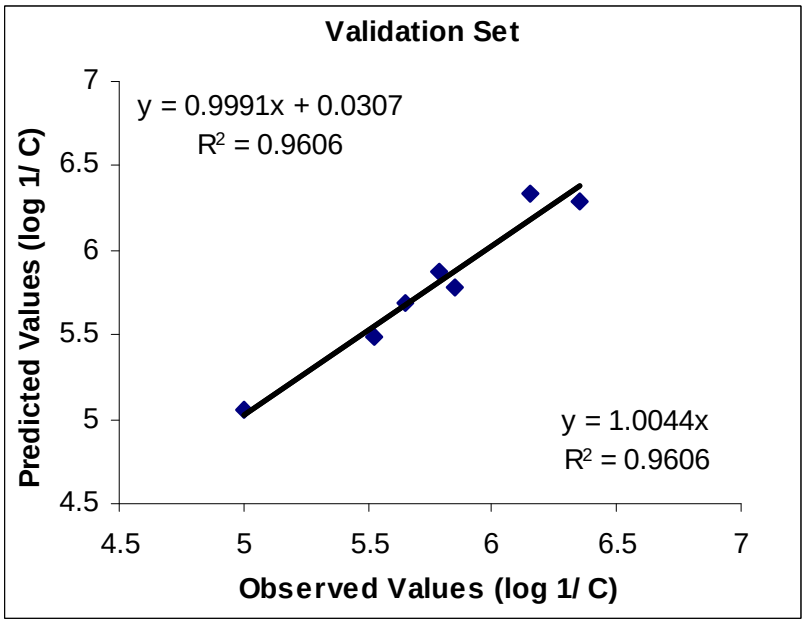


Figure 2: Regression plot between observed vs. predicted values of compounds from validation set justifying the predictive ability of QSAR model Eq. 3

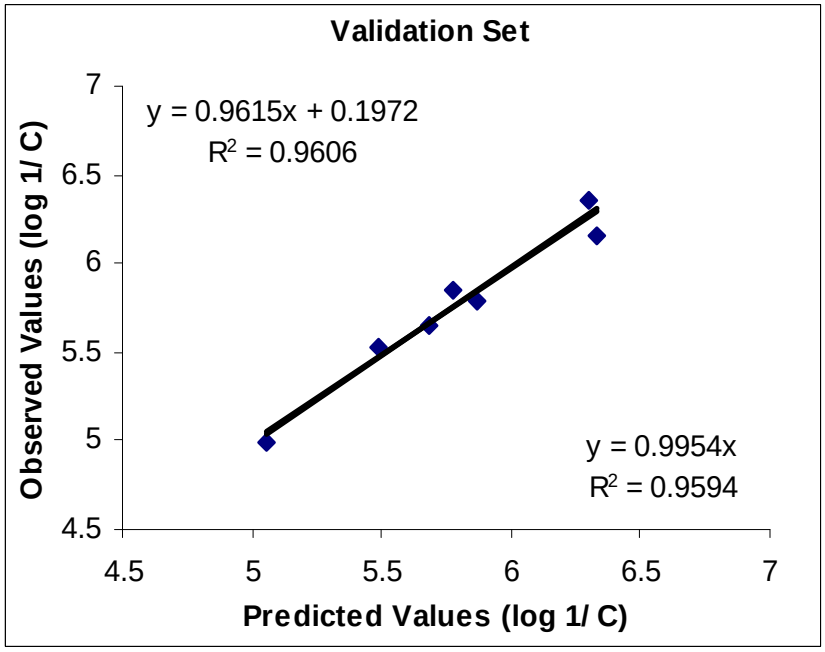


Figure 3: Regression plot between predicted vs. observed values of compounds from validation set justifying the predictive ability of QSAR model Eq. 3

A brief explanation of the descriptors utilized to generate the QSAR model:
Log P is a measure of hydrophobicity/lipophilicity and describes the distribution of a compound between organic and water phase. A value of log P > 0 indicates greater solubility in the organic phase whereas log P < 0 indicates greater solubility in the aqueous phase [17, 18].

Kier indices are most widely used in a variety of applications. The required information is embedded in the hydrogen suppressed framework and thus no experimental measurements are needed to define the molecular connectivity indices. The size, branching, unsaturation, cyclic and chemical nature of various chemical species that appear in a hydrogen–depleted molecular graph are determined by molecule connectivity [19].

The molecular flexibility index is a topological descriptor characterizing the conformational flexibility of a molecule. The estimation is entirely based on the structure with no dependence on physical measurement. It increases with homologation or the number of flexible bonds in the molecule and decreases with increased branching or cyclic nature [20].

Dipole moment is an electronic parameter and is due to the degree of charge separation in a molecule. It is important in case when dipole interactions are involved in ligand-receptor interactions. Dipole moment X component describes the moments using the substituent point of attachment as an origin with this bond placed along the X-axis. The components of μ along the X-axis (bond of attachment) are summed to give the bond dipole in Debyes [21].

The generated best QSAR model (Eq. 3) represents a negative contribution of Log P and shape flexibility to the activity. Majority of the compounds in the dataset have logP values greater than 1 and hence we can say there is less lipophilicity on the compounds. Since a decrease in logP increases lipophilicity and according to Eq. 3, an increased value of lipophilicity on molecules would favor cellular permeability. A negative contribution of Shape Flexibility index point towards conformational stability can be achieved by decreasing the rotational or flexible bonds with an increase in branching and/or cyclic nature would favor better binding and activity at the molecular level.

On the other hand, Dipole Moment X component and Kier chiV2 index contributes positively to the activity. Therefore, new variables with electron rich groups and increased partial charge on substituent along the X component enhances the interaction between the electron rich functional groups of the inhibitors and corresponding amino acid residues in the enzyme active site during enzyme inhibition.

MATERIALS AND METHODS

QSAR studies were carried out on aminopyridine carboxamide derivatives [22] whose inhibitory activities were reported in terms of IC_{50} in μM and were converted to negative logarithmic values of their molar concentration (C) in order to guarantee the linear distribution of data. The structures were sketched using ISIS (Integrated Scientific Information System) Draw 2.3 [23] software and the descriptors were calculated using Tsar (Tools for structure activity relationships) v3.3 software [24]. The three dimensional structures of all molecules were

generated using Corina 3D package. Charges were derived for every molecule and the geometries were optimized using cosmic module of Tsar.

The relationship between dependent variable log (1/C) and the independent variables (various physicochemical descriptors) was established by linear multiple regression technique using Tsar 3.3 software. The model is validated internally using leave-one-out (LOO) technique and externally by predicting the activities of validation set. Significant descriptors were chosen from the pool of descriptors based on the statistical data of analysis. The statistical quality of the generated QSAR model was evaluated [25, 26] based on the correlation coefficient (r), explained variance (r^2), standard error of estimate (s), F-value, cross-validation (q^2) and predictive residual sum of squares (PRESS).

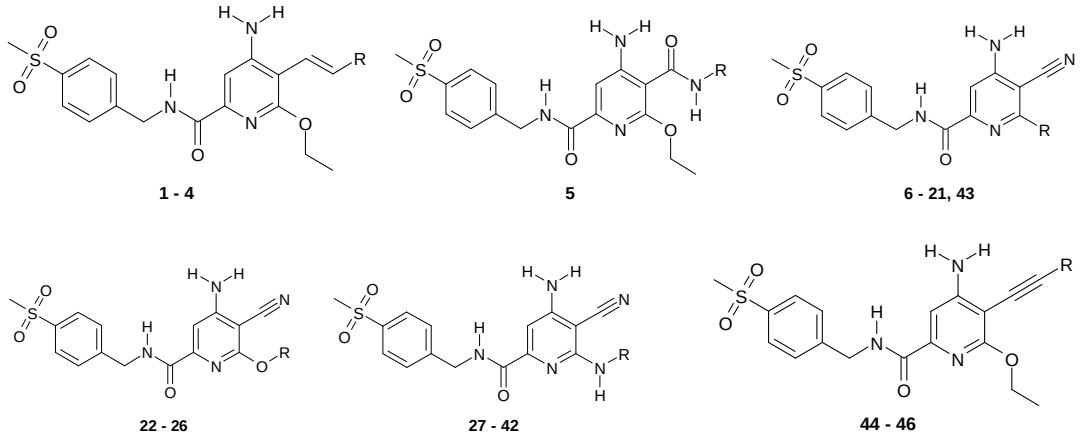
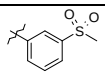
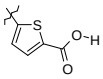
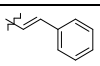
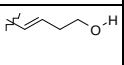
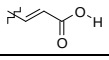
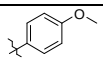
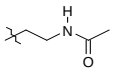
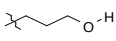
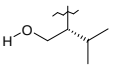


Table 4: Structures of aminopyridine carboxamide inhibitors and their biological data, calculated and standard residuals (Eq. 1), training and validation sets (Eq. 3).

Compound	R	Exp.[a] IC_{50}	Obs.[b] log (1/C)	Calc.[c] log (1/C)	Std.[d] Res.	Calc.[e] log (1/C)	Pred.[f] log (1/C)	Dipole Mom .X	LogP	Kier ChiV 2	Shape Flex.
1	H	1.46	5.410	5.749	-0.861	5.547		-2.760	1.789	7.731	6.719
2		4.13	5.039	4.803	0.600	4.846		-3.984	3.358	9.271	7.986
3		4.16	5.016	5.060	-0.112	5.046		-3.850	3.300	10.226	7.995
4	-CN	1.79	5.350	5.512	-0.411	5.480		-3.867	1.414	7.989	7.490
5	H	0.97	5.607	6.264	-1.669	6.058		-4.726	-0.026	7.852	6.852
6		0.84	5.72	5.872	-0.385	6.120		1.814	2.819	9.192	6.591

7		0.24	6.246	6.115	0.334	6.282		1.214	2.016	8.761	6.343
8		0.60	5.862	6.091	-0.581	6.314		3.310	2.048	8.942	6.882
9		0.097	6.699	6.670	0.074	6.626		-1.521	1.446	11.69	7.048
10		0.39	6.707	6.374	0.846	6.281		-0.299	1.828	9.066	6.060
11		0.53	5.874	6.335	-1.171	6.283		0.057	1.485	8.222	5.771
12		0.16	6.455	6.206	0.634	6.250		-3.892	1.055	9.851	6.789
13		0.68	5.813	6.252	-1.115	6.254		-3.517	1.057	9.708	6.806
14		0.34	6.079	5.741	0.860	5.844		-6.053	1.454	8.452	6.386
15*		0.66	5.791	5.840	-0.125	-	5.960	-4.600	1.454	8.446	6.386
16		0.86	5.677	5.945	-0.681	6.163		-4.695	0.810	8.337	6.641
17		0.23	6.207	5.961	0.624	5.861		-1.613	1.616	7.747	6.324
18		0.26	6.154	6.276	-0.310	6.230		2.971	1.616	7.747	6.324
19*		1.29	5.525	5.360	0.420	-	5.478	-1.751	2.833	8.991	7.032
20		0.24	6.222	5.984	0.605	6.021		0.039	0.865	8.162	7.490
21		0.43	5.969	6.274	-0.776	6.283		1.250	0.723	7.918	6.829
22		0.038	7.01	6.440	1.448	6.547		4.755	1.419	8.333	6.735
23		0.047	6.93	6.690	0.610	6.461		4.136	1.373	8.921	6.158
24		0.37	6.037	6.078	-0.104	6.275		4.174	1.877	8.872	7.328
25*		0.61	5.847	5.690	0.399	-	5.782	-2.332	2.166	9.628	7.266
26		0.45	6.002	5.573	1.091	5.809		-0.821	2.092	8.944	7.405
27		0.084	6.664	6.302	0.920	6.354		0.562	1.102	8.509	6.735
28		0.069	6.747	6.855	-0.275	6.517		0.731	0.743	8.629	5.629
29		0.16	6.397	6.583	-0.473	6.435		0.691	1.139	8.982	6.158
30*		0.29	6.154	6.297	-0.362	-	6.343	0.526	1.535	9.336	6.704
31#		4.7	4.967	5.818	-2.163	-	-	1.685	2.123	9.049	7.206

32*		1.0	5.653	5.496	0.398	-	5.724	0.393	2.375	9.373	7.779
33		0.087	6.68	6.820	-0.356	6.907		3.265	-0.795	8.353	7.612
34		0.081	6.726	6.135	1.502	6.351		-3.440	-0.617	8.620	8.227
35*		0.18	6.351	6.249	0.260	-	6.310	-0.517	-0.044	8.214	7.685
36		0.15	6.414	6.477	-0.159	6.496		0.378	-0.097	7.861	7.071
37		0.21	6.284	6.192	0.235	6.289		0.412	0.182	8.130	7.685
38		0.10	6.623	6.325	0.757	6.453		-1.071	-0.469	8.417	7.867
39#		9.2	4.656	5.983	-3.374	-	-	-2.874	0.326	8.940	7.902
40#		0.030	7.129	6.159	2.465	-	-	-2.545	0.317	8.438	7.294
41		0.32	6.101	6.199	-0.250	6.262		-1.961	0.317	8.438	7.294
42		1.4	5.489	5.718	-0.582	5.907		-2.635	1.188	9.388	8.122
43		0.12	6.539	6.018	1.325	6.188		-5.118	0.452	8.838	7.273
44	H	0.30	6.095	5.833	0.666	5.581		-4.194	1.322	7.632	6.566
45		4.61	4.957	5.271	-0.799	5.177		-5.458	1.160	8.263	8.380
46*		4.32	4.998	4.990	0.020	-	4.932	-5.480	1.440	8.342	8.781

** Validation set molecules; Outliers ; [a] Experimental values or activity IC_{50}(□M); [b] Logarithmic Molar concentration; [c] Calculated values from Eq. 1; [d] Standardized residuals from Eq. 1; [e] Calculated values from Eq. 3; [f] Predicted values from Eq. 3*

In the present study, thirty four descriptors were evaluated in terms of their efficacy to predict the activities of the investigated inhibitors. Various physicochemical, topological and electrostatic molecular descriptors considered for the analysis are: Total dipole, dipole components, LogP, Total lipole, Molecular refractivity, Connectivity indices (chi and chiV types) of atoms, bonds, path, and cluster, Shape indices, Molecular flexibility index, Topological descriptors, H-bond donors, H-bond acceptors. Parameters such as HOMO, LUMO, Ionization potential and heat of formation were calculated using AM1 Hamiltonian and BFGS optimization in vacuum.

Externally predicting the activities of validation set estimates predictive ability of the generated model. This criterion may not be sufficient for a QSAR model to be truly predictive therefore additional conditions such as external set cross-validation r^2 ($R^2_{cv,\ ext}$) and the regression of

observed activities against predicted activities and vice versa for validation set were employed according to the following equations [27, 14].

$$R^2_{cv,ext} > 0.5 \quad (6)$$

$$R^2 > 0.6 \quad (7)$$

$$(R^2 - R_0^2) / R^2 < 0.1 \text{ or } (R^2 - R_0'^2) / R^2 < 0.1 \quad (8)$$

$$0.85 \leq k \leq 1.15 \text{ or } 0.85 \leq k' \leq 1.15 \quad (9)$$

Calculations relating to R^2_{cvext}, R_0^2, R^2, slopes k of actual versus predicted and k' of predicted versus actual values are presented in detail in ref. 15.

CONCLUSION

Finally, considering the contributions of the variables of Eq. 3 on aminopyridine carboxamides would help in designing novel compounds with better inhibition. The positive correlation of Kier chiV2, Dipole Moment X component signifies the development of substituents with electron rich groups and dipole moment along the X component of the compound. The negative correlation of LogP and shape flexibility index indicates the development of new analogs with increased lipophilicity and a fractional decrease in the flexible bonds. The robustness of the QSAR model predictive ability (Eqs. 6-9) of Eq. 3 on validation set illustrated the reliability of the model. Thus the QSAR model generated demonstrates a promising method for designing better aminopyridine carboxamide analogs as inhibitors of JNK-2.

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