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FUJITA BAN ANALYSIS OF SOME SUBSTITUTED GLUTAMAMIDES

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ABSTRACT
Introduction: Like glucose, glutamine is a major substrate for the cancer cell. Glutamine supplies the major portions of nitrogen atoms in the synthesis of DNA and RNA. Numerous studies on glutamine metabolism in cancer show that glutamic acid and glutamine analogs may be developed as future anticancer agents. A QSAR study was performed on some previously synthesized glutamine analogs to understand the substitutional requirements for their anticancer activity. Twenty eight 1,5-N, N'-disubstituted-2-(substituted benzenesulphonyl) glutamamides were considered and the QSAR study was performed using the Fujita-Ban model, which does not require the use of physico-chemical parameters. A software Tanagra v1.4 was used to calculate the contribution of substituents to biological activity by PLSR analysis. A good QSAR model was obtained considering the biological activity of 25 analogs (3 compounds were not considered as they gave a poor r^2 value of 0.66) as evidenced by the statistical data(r=0.823, s=0.049067). The study helps to understand the relationship of the chemical structure of the glutamine analogs with the anticancer activity in a lucid manner and may be helpful in the synthesis of future glutamine analogs with better biological activity.

KEYWORDS: Anticancer activity, Fujita-Ban Model, Glutamamide analogues, QSAR (Quantitative structure activity relationship)

INTRODUCTION
The rate of success in drug discovery is exclusively dependent on the ability to identify, characterize novel, patentable newer 'target-drug-molecules' usually termed as New Chemical Entities (NCEs), which essentially possess the inherent capability and potential in the management and control of a specific disease or ailment; besides being efficacious and safer in character. Cancer chemotherapy is now of established value and a highly specialized field. With these objectives in mind, a plethora of glutamamide derivatives are being studied to develop them as future anticancer agents [1, 2].

In the synthesis of DNA and RNA, glutamine plays an important role. Glutamine supplies the 3rd and 9th nitrogen atoms of the purine ring, the 2nd amino group of guanine and the 3rd nitrogen atom and amino group of cytosine. Tumors require continuous supply of both

essential and non-essential amino acids as nitrogen source for increased biosynthesis of nucleic acid and protein. Glutamine, therefore, plays a key role in cancer cell growth.

The only circulatory sugar D-glucose and the non-essential amino acid L-glutamine are two major substrates for cancer. Also, glutamine is responsible for many physiological functions, involved in multiple metabolic pathways and cancer cases. It is an important component in the cell culture media for both carbon and nitrogen source. Patients of cancer often develop glutamine depletion in muscles due to uptake by tumors and chronic protein metabolism. On the basis of these, it can be assumed that structural variants of glutamine might possess possible antitumor activity [1].

In the present work, previously synthesized twenty eight analogs of 1,5-N,N'-Disubstituted-2-(Substituted Benzenesulphonyl) Glutamamides were selected (compounds no. 21,22 and 36 were later deselected since they gave a poor r^2 value). These compounds were considered in a QSAR study through Fujita-Ban model, which does not require the use of physico-chemical parameters. The calculation procedure is easier than that of the Free-Wilson model especially when the activity of the parent compound is known. It is also a less time consuming model than Hansch analysis. The Fujita-Ban analysis was performed to find out the effect of the substituents on glutamamide analogs and the antitumor activity.

MATERIALS AND METHODS

In the QSAR study using Fujita-Ban model [4], the structure of 1,5-N,N'-disubstituted-2-(substituted benzenesulphonyl) glutamamides was used. The general structure of these analogs is given in Fig. 1. Biological activities along with the substituent types of the glutamamide analogs are given in Table 1.

The method used in this study is a modification of the Free-Wilson technique. Here, the log of activity is considered to be a free energy related parameter which is additive in nature. Fujita and Ban in 1971 derived a linear equation for a set of substituents, in the form of eq. 1 as follows:

$$\text{Log BA} = \sum G_i X_i + \mu \qquad (1)$$

where, BA=biological activity, G_i is the log activity contribution or the log activity enhancement factor of the i^{th} substituent relative to that of H and X_i is a parameter which takes a value of 1 or 0 according to the presence or absence of the i^{th} substituent, and μ=log BA, calculated for the unsubstituted compound, i.e. parent compound.

A software named Tanagra v1.4 was used to calculate the contribution of various substituents to biological activity using partial least square regression analysis.

RESULTS AND DISCUSSION

Based on the parent structure of glutamamide analogs and the Fujita-Ban equation, 25 simultaneous linear equations, Eqs. 2-26, were generated with unknown variables. It is done to find out whether or not the addition of substituents (interaction terms) is of significance in improving the correlation between the structure of the 25 compounds with their biological activities. Some of these Eqs. 2-26 are shown below:

$\mu+R_2(Cl)=1.413$ (2)

$\mu+R_2(Cl)+R_4(CH_3)=1.331$ (3)

$\mu+R_2(Cl)+R_4(C_2H_5)=1.548$ (4)

$\mu+R_2(Cl)+R_4(n\text{-}C_3H_7)=1.632$ (5)

$\mu+R_2(Cl)+R_4(i\text{-}C_3H_7)=1.721$ (6)

…….. (..)

…….. (..)

$\mu+R_2(i\text{-}C_4H_9)+R_4(n\text{-}C_6H_{13})=1.626$ (24)

$\mu+R_2(t\text{-}C_4H_9)+R_4(CH_3)=1.516$ (25)

$\mu+R_2(t\text{-}C_4H_9)+R_4(C_2H_5)+R_5(C_2H_5)=1.532$ (26)

Solutions obtained by the method of least squares of these 25 equations, Eqs. 2-26, with the help of Tanagra v.1.4, gave individual contribution of each substituent group and that of the parent compound μ. These are given in Table 2. The Observed and Calculated biological activities of the 28 compounds are shown in Table 3. The Calculated activities of the compounds are obtained by summing up the values including that of the interaction term. The correlation obtained by plotting a graph between Observed and Calculated activities is poor, as judged from the regression coefficient, $r^2=0.660$.

Compounds no. 21, 22, and 36 are thus not considered so as to improve the regression coefficient value to $r^2=0.823$, as seen by again plotting a graph between Observed and Calculated biological activities.

In the following work, the statistical data validation (i.e. r^2, s values) was done [3]. For our data set the value of SS_{cal} and SS_{mean} was calculated and by using these values we derive the value of r^2.

$r^2=1-(SS_{cal}/SS_{mean})$ (27)

SS_{cal} is a measure of how much the Observed activity of a compound varies from calculated value. For each compound the difference between the Observed activity and the calculated activity is Residual activity. This is then squared and the values are added together to give the sum of squares (SS_{cal}).

SS_{mean} is a measure of how much the Observed activity varies from the mean of all the Observed activities. In the experimental analysis SS_{cal} should be less than SS_{mean}. For a perfect correlation the Calculated values for the activity would be the same as the Observed ones, and so SS_{cal} would be zero, i.e. $r^2=1$.

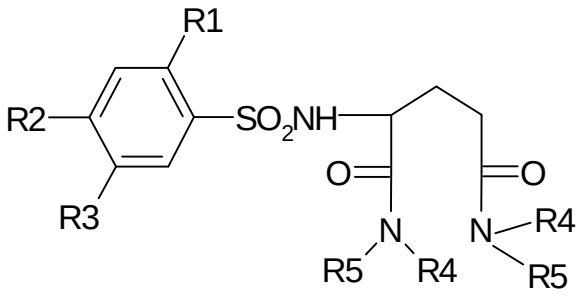
Table 4 carries out the statistical data validation. For the figures shown in the Table 4 and using Eqn. 27 the value of r^2 works out to be 0.820716, which is a good correlation.

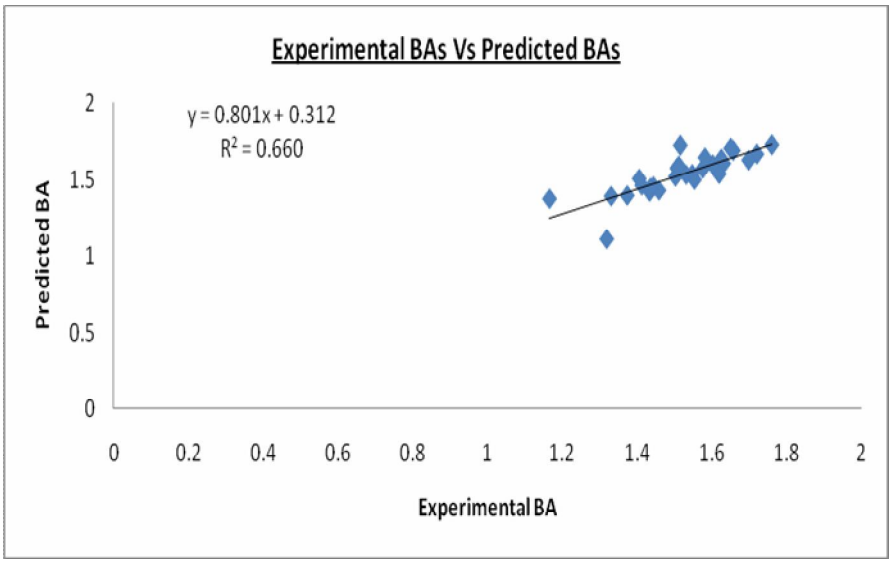
The Standard Deviation(s) for the data set is calculated by using Eqn. 28 and is dependent on the number of compounds (n) tested.

$$s^2 = SS_{cal}/(n-2) \quad (28)$$

The above eqn. gives a value of 0.002407 for the data provided in Table 4, i.e. s=0.04906.

Figure1: General structure of 1,5-N,N'-disubstituted-2-(substituted benzenesulphonyl) glutamamides[1].





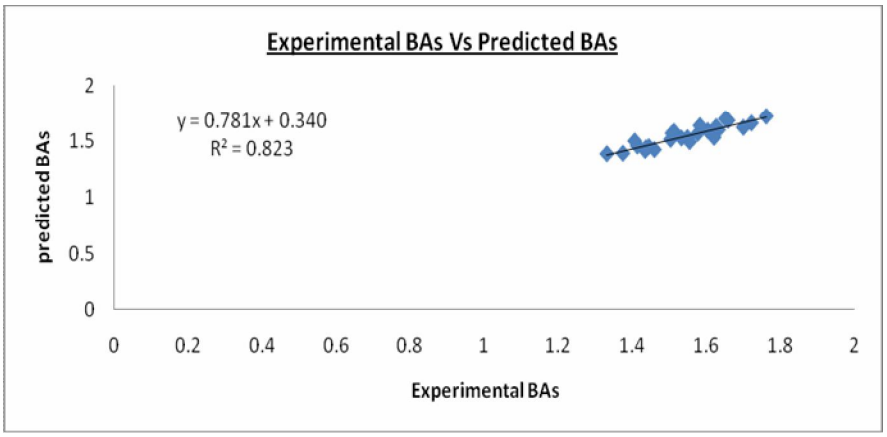


Table 1: Log biological activities of the compounds with their substituent groups

No	R_1	R_2	R_3	R_4	R_5	Log BA
10	H	Cl	H	H	H	1.413
11	H	Cl	H	CH_3	H	1.331
12	H	Cl	H	C_2H_5	H	1.548
13	H	Cl	H	n-C_3H_7	H	1.632
14	H	Cl	H	i-C_3H_7	H	1.721
15	H	Cl	H	n-C_4H_9	H	1.658
16	H	Cl	H	i-C_4H_9	H	1.512
17	H	Cl	H	CH_3	CH_3	1.620
18	H	Cl	H	C_6H_5	H	1.406
19	H	Cl	H	C_2H_5	C_2H_5	1.374
20	H	Cl	H	n-C_6H_{13}	H	1.603
21	Cl	H	Cl	H	H	1.166
22	Cl	H	Cl	CH_3	H	1.319
23	Cl	H	Cl	n-C_3H_7	H	1.434
24	Cl	H	Cl	c-C_6H_{11}	H	1.503
25	H	i-C_4H_9	H	H	H	1.554
26	H	i-C_4H_9	H	CH_3	H	1.459
27	H	i-C_4H_9	H	C_2H_5	H	1.576
28	H	i-C_4H_9	H	n-C_3H_7	H	1.582
29	H	i-C_4H_9	H	i-C_3H_7	H	1.651
30	H	i-C_4H_9	H	n-C_4H_9	H	1.761
31	H	i-C_4H_9	H	i-C_4H_9	H	1.699
32	H	i-C_4H_9	H	C_6H_5	H	1.444
33	H	i-C_4H_9	H	CH_3	CH_3	1.507
34	H	i-C_4H_9	H	c-C_6H_{11}	H	1.436
35	H	i-C_4H_9	H	n-C_6H_{13}	H	1.626
36	H	t-C_4H_9	H	CH_3	H	1.516
37	H	t-C_4H_9	H	C_2H_5	C_2H_5	1.532

Table 2: Contribution of each substituents to log biological activity using PLSR analysis.

Substituent & Constant	Log(BA)
R_1(Cl)	-0.097244
R_2(Cl)	-0.010941
R_2(i-C4H9)	0.026330
R_2(t-C4H9)	0.124187
R_3(Cl)	-0.097244
R_4(CH3)	-0.069622
R_4(C2H5)	0.073350
R_4(n-C3H7)	0.143730
R_4(i-C3H7)	0.203551
R_4(n-C4H9)	0.225585
R_4(i-C4H9)	0.127326
R_4(C6H5)	-0.043844
R_4(n-C6H13)	0.136249
R_4(c-C6H11)	0.241892
R_4(c-C6H13)	-0.052665
R_5(CH3)	0.145085
R_5(C2H5)	-0.139001
constant	1.469136

Table 3: Observed and Calculated biological activities of 28 analogs.

OBSERVED BA	PREDICTED BA
1.413	1.458309
1.331	1.388675
1.548	1.531971

1.632	1.602035
1.721	1.661975
1.658	1.684234
1.512	1.585725
1.62	1.533928
1.406	1.501864
1.374	1.392806
1.603	1.59428
1.166	1.371893
1.319	1.107755
1.434	1.418367
1.503	1.51616
1.554	1.495326
1.459	1.425692
1.576	1.568988
1.582	1.639052
1.651	1.698992
1.761	1.721251
1.699	1.622742
1.444	1.451771
1.507	1.570945
1.436	1.442279
1.626	1.631267
1.516	1.717865
1.532	1.528002

Table 4: Statistical validation of Biological Activity data

PreBA	Res BA	Sq. Res. BA	Obser BA-Obser mean	Sq of (Obs BA-Obs mean)
1.458309	-0.045309	0.00205	-0.13028	0.016972878
1.388675	-0.057675	0.00332	-0.21228	0.045062798
1.531971	0.016029	0.00025	0.00472	2.2278405
1.602035	0.029965	0.00089	0.08872	0.007871238
1.661975	0.059025	0.003483951	0.17772	0.031584398
1.684234	-0.026234	0.000688223	0.11472	0.013160678
1.585725	-0.073725	0.005435376	-0.03128	0.000978438
1.533928	0.086072	0.007408389	0.07672	0.005885958
1.501864	-0.095864	0.009189906	-0.13728	0.018845798
1.392806	-0.018806	0.000353666	-0.16928	0.028655718
1.59428	0.00872	7.60384E-05	0.05972	0.003566478
1.418367	0.015633	0.000244391	-0.10928	0.011942118
1.51616	-0.01316	0.000173186	-0.04028	0.001622478
1.495326	0.058674	0.003442638	0.01072	0.000114918
1.425692	0.033308	0.001109423	-0.08428	0.007103118

1.568988	0.007012	4.91681E-05	0.03272	0.001070598
1.639052	-0.057052	0.003254931	0.03872	0.001499238
1.698992	-0.047992	0.002303232	0.10772	0.011603598
1.721251	0.039749	0.001579983	0.21772	0.047401998
1.622742	0.076258	0.005815283	0.15572	0.024248718
1.451771	-0.007771	6.03884E-05	-0.09928	0.009856518
1.570945	-0.063945	0.004088963	-0.03628	0.001316238
1.442279	-0.006279	3.94258E-05	-0.10728	0.011508998
1.631267	-0.005267	2.77413E-05	0.08272	0.006842598
1.528002	0.003998	1.5984E-05	-0.01128	0.000127238

CONCLUSIONS

The present work upholds the additive nature of Fujita-Ban analysis and can be used as a good model by overcoming limitations and problems associated with Free-Wilson and Hansch analysis. The results illustrate successful applications of mathematical models on structure-activity studies. When modifications in the parent structure are made at various positions at the same time, the analysis with this model could be a powerful tool.

The results suggest that the glutamamide derivatives have excellent scope for further development as commercial anticancer agents. The study helps to substantiate that there is a definite correlation between the chemical structure of the glutamine analogs and the anticancer activity as evidenced by the statistical data.. The substitutions at various positions (R_1, R_2, R_3, R_4, R_5) at a time, shows a change in the biological activity of the compounds. The anticancer activity increased by substitution with chlorine and various alkyl groups so as to develop a useful 'lead'. These factors should be considered in designing future glutamine analogs. Since the analysis is based on the logarithmic activity data and the group contribution for an H substituent at any position is assigned a value of zero, the calculated group contribution values can be further analysed in terms of the linear free energy substituent effect parameters such as σ (Hammett substitution constant) and π (hydrophobic characteristics) so that their physicochemical meanings can be elucidated in certain cases. Further studies are necessary to develop better active glutamine analogs as the anticancer agents in near future.

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