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5-*N*-Substituted-2-(substituted benzenesulphonyl) glutamines as antitumor agents. Part II: Synthesis, biological activity and QSAR study

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Abstract—Cancer is a major killer disease throughout human history. Thus, cancer becomes a major point of interest in life science. It was proved that cancer is a nitrogen trap and tumor cells are avid glutamine consumers. The non-essential amino acid glutamine, which is a glutamic acid derivative, supplies its amide nitrogen to tumor cells in the biosynthesis of purine and pyrimidine bases of nucleic acids as well as takes part in protein synthesis. Based on these and in continuation of our composite programme of development of new potential anticancer agents through rational drug design, 17 new 5-*N*-Substituted-2-(substituted benzenesulphonyl) glutamines were selected for synthesis. These compounds as well as 36 earlier synthesized glutamine analogues were screened for antitumor activity using percentage inhibition of tumor cell count as the activity parameter. QSAR study was performed with 53 compounds in order to design leads with increased effectiveness for antitumor activity using both physicochemical and topological parameters. QSAR study showed that steric effect on the aromatic ring is conducive to the activity. *n*-butyl substitution on aliphatic side chain and atom no 12 is important for antitumor activity of glutamine analogues.

1. Introduction

Cancer is a disease of worldwide importance because it is a major killer throughout human history. Cancer has been described as a nitrogen trap.[1,2] The importance of non-essential amino acid glutamine in proliferation of human tumor cells was studied extensively.[3,4] All tumor cells studied were found to have a high activity of phosphate-dependent glutaminase utilizing glutamine from the medium during long-term culture.[3] Human hepatoma cells take up glutamine at rates several fold faster than the normal human hepatocytes.[4] L-glutamine is not only the precursor of the biosynthesis of purine and pyrimidine bases of DNA as well as used us a building block of proteins. Other than glucose it is one of the major substrates, if not the major substrate, for the energy metabolism of rapidly growing tumor cells.[5] Beside glucose, glutamine is assumed to be the main energy source in tumor cells.[5] Since no cells, whether cancerous or normal, cannot survive without the only circulatory sugar glucose whereas glutamine is a non-essential amino acid which is required by most of the cells and tissues, points out that it may be the major substrate for cancer. It also plays a central role in multiple metabolic pathways and considered to be the most essential component of tissue/cell culture media[6] for not only as the nitrogen source but also as the carbon source. Since most of the cells need non-essential amino acid glutamine for physiological functions and most of those normal cells are transformable to cancerous one, glutamine may play a significant role in cancer. After a definite time interval, all cells start mutation in cell culture medium, which is also indicative for the role of glutamine in cancer.[7]

Aryl sulphatase C (ASC) family of transporters is involved in the mediation of glutamine uptake and glutamine, in

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the form of glutamate, and cysteine are supplied perhaps for glutathione synthesis.[8] Glutamine dependant syntheses of Interleukin-2 by lymphocytes and Interleukin-1 by macrophages are hampered in cancer. These resulted in part to immunosuppression, which is a common manifestation of most of the anticancer drugs.[9] Reintroduction of thalidomide, a glutamic acid derivative, in a clinical trial for the treatment of various malignant tumors also supported the role of glutamine in cancer.[10] Besides these, azaserine and acivicin are antiglutamine agents.[11] Thus, the structural variants of glutamine attracted our attention to develop possible anticancer agents, which may act through glutamine and/or folic acid antagonism.

We have previously reported synthesis, biological evaluation by percentage inhibition of tumor weight and Quantitative Structure-Activity Relationship (QSAR) studies on some glutamine and glutamamide analogues as possible anticancer agents[12–18] as a part of our composite programme of rational drug design.[12–24] Here in this article, 17 new 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines, as shown in Figure 1, were synthesized in continuation of our earlier work.[16] These compounds as well as 36 earlier synthesized glutamine analogues[16] were screened for antitumor activity using percentage inhibition of tumor cell count as the activity parameter. QSAR studies were done using all 53 biologically evaluated glutamine analogues to explore the substitutional requirement to optimize leads for the improved anticancer activity.[12–17]

2. Results

2.1. Synthesis

Synthesis of the 5-*N*-substituted-2-(substituted benzenesulfonyl) glutamines were carried out in accordance with the Scheme 1 and the chemistry behind the synthesis is explained in the Experimental section. Physical data of all of the intermediates and final compounds are furnished in Tables 1 and 2, respectively. The yields of the title compounds were found good as shown in Table 2.

2.2. Biological evaluation

These 17 final compounds as well as 36 earlier synthesized glutamine analogues were biologically evaluated by dissolving those in phosphate buffered saline (PBS) or by suspending in PBS or by suspending in PBS with 2% Tween 80 (whenever necessary) separately. The solution or suspension of the test compounds were administered at a dose level of 2 mmol/kg/day intraperitonially (ip) for 7 consecutive days. One day after the intraperitonial inoculation of each mouse with 2×10^6 Ehrlich Ascites Carcinoma (EAC) cells,% inhibitions of tumor cell count were calculated by comparing the cell count of the test (T) with that of the control (C) using the expression $(1-T/C)\times100$.% Inhibition of tumor cell count was considered as the biological activity data for QSAR study. Biological activity data of these 53 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines are shown in Table 3. Some of the compounds possess promising anticancer activity and draw attention for future research on those.

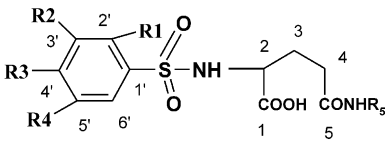
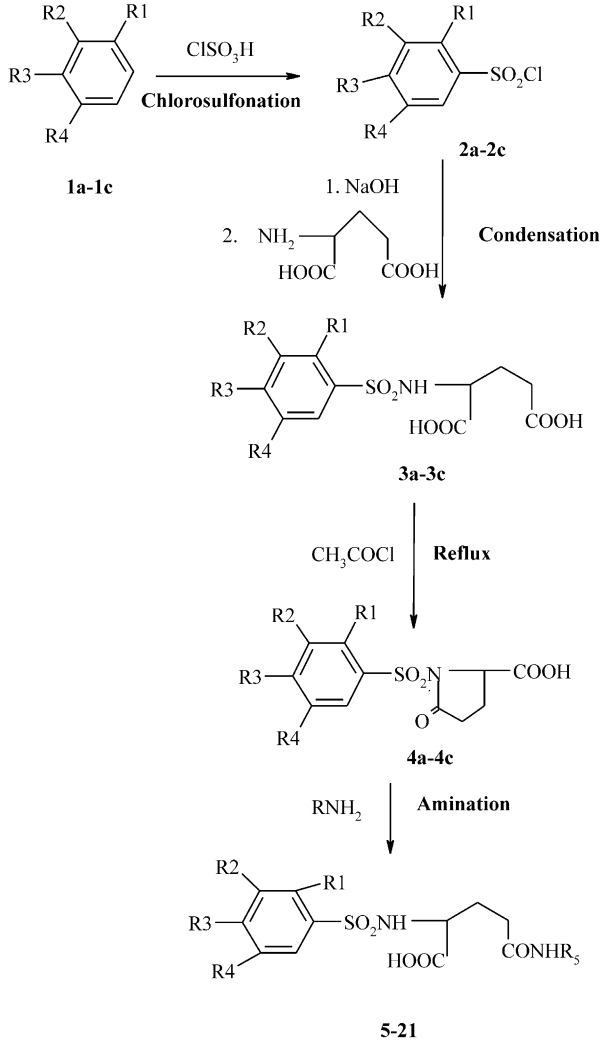


Figure 1. General structure of 5-*N*-substituted-2-(substituted benzenesulphonyl)-glutamines.

2.3. QSAR

In order to identify the chemical structural features required and/or responsible for antitumor activity of 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines attempts have been made to perform QSAR studies using physicochemical parameters as well as E-state index.[22–30] E-state value of each atom contains electronic and topological structural informations from all other atoms within the structure.

The congener series possesses the substitution on aromatic ring system at 1′, 2′, 3′, 4′ positions denoted as R_1, R_2, R_3, R_4 respectively and at 5-N position of the aliphatic side



Scheme 1. General synthetic route for the preparation of 5- *N*-substituted-2-(substituted benzenesulphonyl)-glutamines.

Table 1. Physical data of the intermediate compounds

Compd[a]	R_1	R_2	R_3	R_4	M.P. (°C)	%Yield	Molecular formula	MW	Elemental analysis (%)					
									Found			calculated		
									C	H	N	C	H	N
2a	H	NO_2	H	H	60–62	82.23	$C_6H_4N_1O_4S_1Cl$	221.62	32.31	1.73	6.21	32.48	1.80	6.32
2b	Cl	H	H	CH_3	17–19	80.31	$C_7H_6O_2S_1Cl_2$	225.09	36.89	2.58	—	37.32	2.66	—
2c	H	H	Br	H	73–75	77.90	$C_6H_4O_2BrS_1Cl$	255.52	28.11	1.48	—	28.18	1.56	—
3a	H	NO_2	H	H	107–108	74.30	$C_{11}H_{12}N_2O_8S_1$	332.29	39.45	3.53	8.39	39.72	3.61	8.43
3b	Cl	H	H	CH_3	131–133	60.32	$C_{12}H_{14}N_1O_6S_1Cl$	335.76	42.66	4.21	4.07	42.89	4.17	4.16
3c	H	H	Br	H	148–150	80.84	$C_{11}H_{12}N_1O_6S_1Br$	366.19	35.88	3.17	3.74	36.05	3.28	3.82
4a	H	NO_2	H	H	80–81	79.42	$C_{11}H_{10}N_2O_7S_1$	314.28	41.92	3.14	8.78	42.00	3.18	8.91
4b	Cl	H	H	CH_3	165–167	88.00	$C_{12}H_{12}N_1O_5S_1Cl$	317.76	45.12	3.68	4.29	45.32	3.78	4.41
4c	H	H	Br	H	95–97	64.00	$C_{11}H_{10}N_1O_5S_1Br$	348.18	37.84	3.39	3.95	37.91	3.45	4.02

[a] Compd = Compound.

Table 2. Physical data of different substituted benzenesulphonyl glutamines **5–21**

Compd[a]	R1	R2	R3	R4	R5	M.P. (°C)	% Yield	Molecular formula	M.W
5	H	NO_2	H	H	CH_3	148–150	58.02	$C_{12}H_{15}N_3O_7S_1$	345.33
6	H	NO_2	H	H	C_2H_5	169–171	63.70	$C_{13}H_{17}N_3O_7S_1$	359.36
7	H	NO_2	H	H	*n*-C_3H_7	173–175	65.30	$C_{14}H_{19}N_3O_7S_1$	373.39
8	H	NO_2	H	H	*i*-C_3H_7	171–173	70.00	$C_{14}H_{19}N_3O_7S_1$	373.39
9	H	NO_2	H	H	C_6H_5	194–196	59.51	$C_{17}H_{17}N_3O_7S_1$	407.40
10	H	NO_2	H	H	*n*-C_4H_9	154–156	64.80	$C_{15}H_{21}N_3O_7S_1$	387.41
11	H	NO_2	H	H	*n*-C_6H_{13}	139–141	70.00	$C_{17}H_{25}N_3O_7S_1$	415.47
12	Cl	H	H	CH_3	*i*-C_3H_7	149–151	53.80	$C_{15}H_{21}N_2O_5S_1Cl$	376.87
13	Cl	H	H	CH_3	*n*-C_3H_7	115–117	60.20	$C_{15}H_{21}N_2O_5S_1Cl$	376.87
14	Cl	H	H	CH_3	*n*-C_4H_9	84–86	57.80	$C_{16}H_{23}N_2O_5S_1Cl$	390.89
15	Cl	H	H	CH_3	*i*-C_4H_9	123–125	59.37	$C_{16}H_{23}N_2O_5S_1Cl$	390.89
16	Cl	H	H	CH_3	$C_6H_5CH_2$	119–121	55.70	$C_{19}H_{21}N_2O_5S_1Cl$	424.90
17	H	H	Br	H	*n*-C_4H_9	201–202	88.16	$C_{15}H_{21}N_2O_5S_1Br$	421.31
18	H	H	Br	H	*n*-C_6H_{13}	145–147	77.50	$C_{17}H_{25}N_2O_5S_1Br$	449.37
19	H	H	Br	H	*n*-C_3H_7	197–199	85.50	$C_{14}H_{19}N_2O_5S_1Br$	407.29
20	H	H	Br	H	*i*-C_4H_9	204–205	88.16	$C_{15}H_{21}N_2O_5S_1Br$	421.31
21	H	H	Br	H	C_6H_5	228–230	78.90	$C_{17}H_{17}N_2O_5S_1Br$	441.30

[a] Compd = Compound.

chain, which is designated as R_5 as shown in Figure 1. Physicochemical parameters were considered as the sum of the substitutents on the benzene ring for each compound. Among the various physicochemical parameters only MR value was considered. The E-state (ETSA) index is an atom/sub-molecular descriptor encoding both electronic and topological information. ETSA indices of the common atoms were calculated by using a computer programme 'Mouse' written in C^{++} language and developed in our laboratory.[31] The software can run in windows operating system and can calculate ETSA indices only. For calculation of E-state index, arbitrary numbering was used and these are shown in Figure 2. The physicochemical parameter ΣMR (sum of MR values at R_1, R_2, R_3, R_4 positions of the benzene ring), E-state index S_{12} and indicator parameter I, which were used in developing QSAR equations, are shown in Table 3. Correlation analysis of various physicochemical and topological parameters used in developing QSAR equations was performed. The intercorrelated parameters were discarded depending on their individual correlation with the biological activity (log BA). All possible combinations of parameters were considered. The resultant parameters were subjected to multiple regression analysis using log BA as the biological activity parameter with the help of a software 'Multiregress' developed in our laboratory.[32] The best possible combination of parameters, which is best fitted with the biological activity data found, was used for developing QSAR equations.

Stepwise development of the regression equation with various physicochemical parameters and deleting intercorrelated parameters, various combinations were tried and the best uni-parametric equation was

$$\text{Log BA} = 1.133\ (\pm 0.094) + 0.458\ (\pm 0.084)\Sigma\text{MR} \quad (1)$$

$N = 53$; R = 0.606; %EV = 36.68; F = 29.549;
$p < 0.0000$; S.E.E = 0.167; SSY = 2.241;
PRESS = 1.5198; $q^2 = 0.322$; $S_{PRESS} = 0.173$;
PSE = 0.169

where ΣMR is sum of the molar refractivities of the substituents in the benzene ring, N is the number of data points, R, %EV, F, p, S.E.E., SSY, PRESS, q^2, S_{PRESS}, P.S.E are correlation coefficient, percentage of explained variance, ratio between the variances of observed and calculated activities, probability factor related to the F-ratio, standard error of estimate, sum

Table 3. Antitumor activities and QSAR parameters of fifty three 5- *N*-substituted-2-(substituted benzenesulphonyl)-glutamines **5–57**

Compd[a]	R_1	R_2	R_3	R_4	R_5	S_{12}	I	ΣMR	BA (%TCI)	Log BA
5	H	NO_2	H	H	CH_3	11.117	0.000	0.740	21.74	1.337
6	H	NO_2	H	H	C_2H_5	11.186	0.000	0.740	31.34	1.496
7	H	NO_2	H	H	*n*-C_3H_7	11.243	0.000	0.740	10.47	1.020
8	H	NO_2	H	H	*i*-C_3H_7	11.251	0.000	0.740	41.38	1.617
9	H	NO_2	H	H	C_6H_5	11.387	0.000	0.740	32.75	1.515
10	H	NO_2	H	H	*n*-C_4H_9	11.289	1.000	0.740	45.45	1.658
11	H	NO_2	H	H	*n*-C_6H_{13}	11.361	0.000	0.740	48.86	1.689
12	Cl	H	H	CH_3	*i*-C_3H_7	11.303	0.000	1.160	31.69	1.501
13	Cl	H	H	CH_3	*n*-C_3H_7	11.295	0.000	1.160	83.098	1.920
14	Cl	H	H	CH_3	*n*-C_4H_9	11.341	1.000	1.160	61.97	1.792
15	Cl	H	H	CH_3	*i*-C_4H_9	11.347	0.000	1.160	40.00	1.602
16	Cl	H	H	CH_3	$C_6H_5CH_2$	11.460	0.000	1.160	56.42	1.751
17	H	H	Br	H	*n*-C_4H_9	11.269	1.000	0.890	41.76	1.621
18	H	H	Br	H	*n*-C_6H_{13}	11.341	0.000	0.890	48.71	1.688
19	H	H	Br	H	*n*-C_3H_7	11.222	0.000	0.890	20.88	1.320
20	H	H	Br	H	*i*-C_4H_9	11.269	0.000	0.890	30.76	1.488
21	H	H	Br	H	C_6H_5	11.367	0.000	0.890	39.56	1.597
22	H	H	H	H	*i*-C_4H_9	11.228	0.000	0.000	12.68	1.103
23	H	H	CH_3	H	*i*-C_3H_7	11.238	0.000	0.560	19.13	1.282
24	H	H	CH_3	H	*i*-C_4H_9	11.282	0.000	0.560	20.80	1.318
25	CH_3	H	H	NO_2	H	11.093	0.000	1.300	19.35	1.287
26	CH_3	H	H	NO_2	CH_3	11.202	0.000	1.300	46.77	1.670
27	CH_3	H	H	NO_2	C_2H_5	11.271	0.000	1.300	66.19	1.821
28	CH_3	H	H	NO_2	*n*-C_3H_7	11.328	0.000	1.300	45.00	1.653
29	CH_3	H	H	NO_2	*n*-C_4H_9	11.374	1.000	1.300	89.36	1.951
30	CH_3	H	H	NO_2	*i*-C_3H_7	11.336	0.000	1.300	42.20	1.625
31	CH_3	H	H	NO_2	*i*-C_4H_9	11.380	0.000	1.300	76.29	1.882
32	CH_3	H	H	NO_2	C_6H_{11}	11.504	0.000	1.300	71.80	1.856
33	CH_3	H	H	NO_2	C_6H_5	11.472	0.000	1.300	33.3	1.522
34	CH_3	H	H	NO_2	$C_6H_5CH_2$	11.493	0.000	1.300	70.15	1.846
35	CH_3	H	H	NO_2	*n*-C_5H_{11}	11.413	0.000	1.300	78.89	1.897
36	CH_3	H	H	NO_2	*n*-C_6H_{13}	11.446	0.000	1.300	67.38	1.828
37	H	NO_2	CH_3	H	H	11.062	0.000	1.300	40.32	1.605
38	H	NO_2	CH_3	H	CH_3	11.171	0.000	1.300	56.06	1.749
39	H	NO_2	CH_3	H	C_2H_5	11.241	0.000	1.300	31.29	1.495
40	H	NO_2	CH_3	H	*n*-C_3H_7	11.297	0.000	1.300	32.28	1.509
41	H	NO_2	CH_3	H	*n*-C_4H_9	11.343	1.000	1.300	80.74	1.907
42	H	NO_2	CH_3	H	*n*-C_5H_{11}	11.382	0.000	1.300	61.8	1.791
43	H	NO_2	CH_3	H	*n*-C_6H_{13}	11.415	0.000	1.300	65.14	1.814
44	H	NO_2	CH_3	H	*i*-C_3H_7	11.305	0.000	1.300	57.97	1.763
45	H	NO_2	CH_3	H	*i*-C_4H_9	11.349	0.000	1.300	62.85	1.798
46	H	NO_2	CH_3	H	C_6H_{11}	11.473	0.000	1.300	38.75	1.588
47	H	NO_2	CH_3	H	$C_6H_5CH_2$	11.462	0.000	1.300	28.22	1.450
48	H	NO_2	CH_3	H	C_6H_5	11.441	0.000	1.300	47.88	1.680
49	H	H	C_2H_5	H	CH_3	11.150	0.000	1.030	34.65	1.540
50	H	H	C_2H_5	H	C_2H_5	11.220	0.000	1.030	41.20	1.615
51	H	H	C_2H_5	H	*n*-C_3H_7	11.276	0.000	1.030	24.73	1.393
52	H	H	C_2H_5	H	*n*-C_4H_9	11.322	1.000	1.030	50.68	1.705
53	H	H	C_2H_5	H	*n*-C_5H_{11}	11.361	0.000	1.030	34.06	1.532
54	H	H	C_2H_5	H	*n*-C_6H_{13}	11.394	0.000	1.030	70.47	1.848
55	H	H	C_2H_5	H	*i*-C_3H_7	11.284	0.000	1.030	54.07	1.733
56	H	H	C_2H_5	H	$C_6H_5CH_2$	11.441	0.000	1.030	65.23	1.814
57	H	H	C_2H_5	H	C_6H_5	11.420	0.000	1.030	52.36	1.719
58	Mitomycin C								100.00	2.000
59	Azaserine								100.00	2.000
60	DON								100.00	2.000

[a] Compd = Compound.

of square of regression values, predicted residual sum of square, cross validated R^2, uncertainty factor, predictive standard error respectively. ΣMR explains 36.68% of the variances in the activity data. Positive coefficient of ΣMR indicated that the steric effect at benzene ring of these compounds is conducive to the anticancer activity. It may be hypothesized that the benzene ring of the substituted benzenesulphonyl glutamines has a tendency to interact with the receptor via dispersion forces. Combining E-state index along with ΣMR slightly improve the quality of the relation as follows:

$$\text{LogBA} = -6.305\ (\pm 2.434) + 0.380\ (\pm 0.082)\ \Sigma\text{MR} + 0.665\ (\pm 0.217)\ S_{12} \quad (2)$$

$N = 53$; R = 0.683; %EV = 46.66; F = 21.869; $p < 0.0000$; S.E.E = 0.155; SSY = 2.241; PRESS = 1.342; $q^2 = 0.401$; $S_{PRESS} = 0.164$; PSE = 0.159

Eq 2 explains 46.66% of the variation in anticancer activity. The ETSA index of atom 12 (S_{12}) in the equation

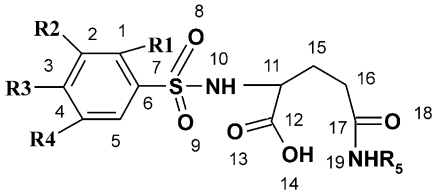


Figure 2. Structure of 5-*N*-substituted-2-(substituted benzenesulphonyl)-glutamines used for calculation of ETSA indices.

implies that carboxyl carbon of the glutamine portion has the major contribution to the activity of this type of compounds and higher value of S_{12} corresponds to the higher activity. Addition of indicator variable I for the presence of *n*-butyl at R_5 position (I takes value 1 for the presence of *n*-butyl, otherwise zero) increased explained variance of the equation (2) by about 7% and the correlation coefficient up to 0.728 in eq 3

$$\text{LogBA} = -6.227\ (\pm 2.307) + 0.382\ (\pm 0.0780)\ \Sigma\text{MR} + 0.656\ (\pm 0.206)\ S_{12} + 0.164\ (\pm 0.064)\ \text{I} \quad (3)$$

$N = 53$; R = 0.728; %EV = 53.06; F = 18.463; $p < 0.0000$; S.E.E = 0.147; SSY = 2.241; PRESS = 1.194; $q^2 = 0.467$; $S_{PRESS} = 0.156$; PSE = 0.150

Positive coefficient of I indicates that *n*-butyl substitution at R_5 position is advantageous to the anticancer activity.

Stepwise deletions of the compounds **13**, **33**, **47** and **46** which might be acting through a different mechanism of action help to improve the quality of the relation statistically as follows:

$$\text{LogBA} = -6.452\ (\pm 2.231) + 0.373\ (\pm 0.075)\ \Sigma\text{MR} + 0.676\ (\pm 0.199) S_{12} + 0.171 (\pm 0.061)\ \text{I} \quad (4)$$

DC = **13**, $N = 52$; R = 0.744; %EV = 55.32; F = 19.807; $p < 0.0000$; S.E.E = 0.142; $SSY = 2.154$; PRESS = 1.10; $q^2 = 0.489$; $S_{PRESS} = 0.151$; PSE = 0.145

$$\text{LogBA} = -7.260\ (\pm 2.201) + 0.381\ (\pm 0.073)\ \Sigma\text{MR} + 0.747\ (\pm 0.196)\ S_{12} + 0.164\ (\pm 0.060)\ \text{I} \quad (5)$$

DC = **13**, **33**; $N = 51$; R = 0.766; %EV = 58.65; F = 22.225; $p < 0.0000$; S.E.E = 0.137; SSY = 2.144; PRESS = 1.015; $q^2 = 0.526$; $S_{PRESS} = 0.147$; PSE = 0.141

$$\text{LogBA} = -8.310\ (\pm 2.087) + 0.393\ (\pm 0.069)\ \Sigma\text{MR} + 0.840\ (\pm 0.186)\ S_{12} + 0.155\ (\pm 0.056)\ \text{I} \quad (6)$$

DC = **13**, **33**, **47**; $N = 50$; R = 0.802; %EV = 64.26; F = 27.565; $p < 0.0000$; S.E.E = 0.128; SSY = 2.113; PRESS = 0.867; $q^2 = 0.590$; $S_{PRESS} = 0.137$; PSE = 0.132

$$\text{LogBA} = -9.177\ (\pm 2.061) + 0.402\ (\pm 0.066)\ \Sigma\text{MR} + 0.916\ (\pm 0.184)\ S_{12} + 0.148\ (\pm 0.054)\ \text{I}$$

DC = **13**, **33**, **47**, **46**; $N = 49$; R = 0.821; %EV = 67.32; F = 30.906; $p < 0.0000$; S.E.E = 0.123; SSY = 2.111; PRESS = 0.793; $q^2 = 0.625$; $S_{PRESS} = 0.133$; PSE = 0.127

where DC is the deleted compounds behave as outlier. The lower value of S_{PRESS}, P.S.E. in eq 7 suggest that the optimum numbers of variables are taken in the QSAR model. Deletions of compounds not only increase the correlation coefficient but also the variance ratio F and the squared cross-validated correlation coefficient. Thus, the eq 7 was statistically the best equation. This equation explains 67.32% of the variances in the activity data. The correlation matrices of eqs 3 and 7 are presented in Tables 4 and 5 respectively. The observed, calculated, residual and predicted residual (Press) values of eq 7 are shown in Table 6.

3. Discussion

The QSAR study of these 53 glutamine analogues showed that bulky substitutions at benzene ring might possesses positive contributions to the anticancer activity. Atom number 12, that is, carboxyl carbon of glutamine may play as the pharmacophoric atom for the biological activity. Amongst the different alkyl substitutions at 5-*N*-position of glutamine, *n*-butyl group contributes more positively towards the activity. The fact behind this points out the possibility of one of the major interactions may be due to optimum lipophilicity to bind with the glutamine receptor(s) site(s) of the cancer cells.

4. Conclusion

Our previous studies encourage us to develop structural variants of glutamine as possible anticancer agents. Thus, 17 new compounds were synthesized and characterized by chemical and instrumental methods. All compounds were obtained in good yields. These compounds alongwith 36 previously synthesized compounds were biologically evaluated for antitumor activity considering %

Table 4. Correlation matrix of eq 3

	S_{12}	I	ΣMR	LogBA
S_{12}	1.0000	0.0132	0.3126	0.4893
I		1.0000	−0.0077	0.2535
ΣMR			1.0000	0.6057
LogBA				1.0000

Table 5. Correlation matrix of eq 7

	S_{12}	I	ΣMR	LogBA
S_{12}	1.0000	0.0467	0.2644	0.5933
I		1.0000	0.0130	0.2616
ΣMR			1.0000	0.6548
LogBA				1.0000

Table 6. Observed (obs), calculated (calc), residual (res) and predicted residual (Pres) values of 5-*N*-Substituted-2- (substituted benzenesulphonyl)-glutamines on the basis of eq 7

Compd[a]	Obs	Calc	Res	Pres	Compd	Obs	Calc	Res	Pres
5	1.337	1.303	0.034	0.038	**31**	1.882	1.769	0.113	0.118
6	1.496	1.366	0.130	0.140	**32**	1.856	1.882	−0.026	−0.029
7	1.020	1.418	−0.398	−0.421	**34**	1.846	1.872	−0.026	−0.029
8	1.617	1.425	0.192	0.202	**35**	1.897	1.799	0.098	0.104
9	1.515	1.550	−0.035	−0.038	**36**	1.828	1.829	−0.001	−0.001
10	1.658	1.609	0.049	0.062	**37**	1.605	1.478	0.127	0.159
11	1.689	1.526	0.163	0.174	**38**	1.749	1.578	0.172	0.190
12	1.501	1.642	−0.141	−0.145	**39**	1.495	1.641	−0.146	−0.155
14	1.792	1.825	−0.033	−0.040	**40**	1.509	1.693	−0.184	−0.192
15	1.602	1.682	−0.080	−0.083	**41**	1.907	1.883	0.024	0.029
16	1.751	1.786	−0.035	−0.037	**42**	1.791	1.771	0.020	0.021
17	1.621	1.651	−0.030	−0.036	**43**	1.814	1.801	0.013	0.014
18	1.688	1.568	0.120	0.124	**44**	1.763	1.700	0.063	0.066
19	1.320	1.459	−0.139	−0.145	**45**	1.798	1.740	0.058	0.060
20	1.488	1.502	−0.014	−0.015	**48**	1.680	1.825	−0.145	−0.155
21	1.597	1.592	0.005	0.005	**49**	1.540	1.450	0.090	0.098
22	1.103	1.107	−0.004	−0.006	**50**	1.615	1.514	0.101	0.106
23	1.282	1.341	−0.059	−0.065	**51**	1.393	1.565	−0.172	−0.176
24	1.318	1.382	−0.064	−0.070	**52**	1.705	1.755	−0.050	−0.060
25	1.287	1.506	−0.219	−0.262	**53**	1.532	1.643	−0.111	−0.114
26	1.670	1.606	0.064	0.070	**54**	1.848	1.673	0.175	0.182
27	1.821	1.669	0.152	0.160	**55**	1.733	1.572	0.161	0.165
28	1.653	1.721	−0.068	−0.071	**56**	1.814	1.716	0.098	0.105
29	1.951	1.912	0.039	0.048	**57**	1.719	1.697	0.022	0.023
30	1.625	1.728	−0.103	−0.108					

[a] Compd = Compound.

inhibition of tumor cell count as the activity parameter. All compounds showed the anticancer activity. In order to identify the chemical structural features required and/or responsible for antitumor activity of 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamine, Quantitative Structure–Activity Relationship (QSAR) studies were performed using both physicochemical and topological parameters. The QSAR study throws some light on the further synthesis of better active 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines (QSAR analogues). In this regard, the most important consideration is the steric influence of benzene ring, which is conducive to anticancer activity. Increased lipophilicity, volume and steric bulk of R_1, R_2, R_3 and R_4 substituents correspond to higher value of ΣMR and hence result in increase in activity. The required substitution pattern at the aliphatic moiety is another important feature to consider for the synthesis of better active compounds. In aliphatic portion, atom 12 of Figure 2 is the most important one for the antitumor activity. The R_5 substituents should increase the value of S_{12} (ETSA index of atom 12). However, if *n*-butyl is present at R_5 position, steric factor at the benzene ring is only to be considered for further synthesis of this type of compounds. *n*-Butyl substitution at aliphatic side chain is important for antitumor activity may be due to its optimum lipophilicity.

5. Experimental

5.1. Synthesis

Seventeen new 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines were synthesized and chemically characterized by IR-, NMR- and Mass spectroscopy as well as C, H, N analysis.There were two type of substitutions each in R_1, R_2, R_3 and R_4 at aromatic ring whereas the aliphatic side chain contains nine substitutions at R_5 position.

5.1.1. Chemistry. Synthesis was started with chlorosulphonation of substitutedbenzenes **1**, to get corresponding sulphonyl chlorides **2**. This sulphonyl halide proved to be versatile synthon in the subsequent step in the preparation of substituted benzenesulphonyl glutamic acids. With the application of Schotton-Bauman reaction, 2-(substituted benzenesulphonyl) glutamic acids **3** were prepared by one-step condensation of **2** with L-glutamic acid. In this reaction alkaline medium was maintained to remove the hydrochloric acid which was formed during condensation. Reaction of the resulting intermediates **3** with acetyl chloride afforded cyclized acid intermediates 1-(substituted benzenesulphonyl)-5-oxopyrrolidine-2-carboxylic acids **4**. Aminolysis of the intermediates **4** with various amines afforded the corresponding glutamines **5**.

5.1.2. General. Melting points were measured on a capillary melting point apparatus and were uncorrected. All the compounds were characterized qualitatively and quantitatively by performing both analytical and spectrophotometric analysis. These compounds, which contained free carboxyl groups, were also characterized by matching neutralization equivalents.

The infrared spectra were recorded on BUCK M500 quick scanning Infrared spectrophotometer using KBr pellets. Running the spectrum of 0.05 mm polystyrene film did the finer calibration of the machine. The fre-

quencies were expressed in cm^{-1}. Proton Nuclear Magnetic Resonance (^1H NMR) spectra were collected at 25°C in the pulsed Fourier Transformation mode on Bruker DRX 500 or Bruker DRX 300 MHz spectrophotometers using the solvents described and was consistent with the proposed structures. Chemical shifts are reported in δ ppm (parts per million) relative to Tetramethyl Silane for deuteriorated dimethylsulfoxide (DMSO-d_6). Signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The mass spectra (FAB) were recorded on JEOL-JMS-SX-PNBA (p- nitrobenzyl alcohol) was used as matrix (M^+) which showed M + 1 peak at 154, 2M + 1 peak at 307. Elemental or microanalysis (C, H, N) of the compounds was performed on 2400 series II CHN analyzer of Perkin–Elmer.

5.1.3. General synthetic procedure. Method 1. Substituted benzenesulphonyl chloride (2a–c). To a mixture of substituted benzene (0.1 mol) in chloroform (50 mL), in a 500 mL flask equipped with dropping funnel, thermometer, reflux condenser, chlorosulphonic acid (0.25 mol) was added dropwise over a period of 45–60 min. The reaction mixture was magnetically stirred at 0°C in a bath containing freezing mixture of ice and salt. Chlorosulphonic acid was added in such a rate that the temperature of the reaction mixture does not exceed 5°C. After the complete addition of chlorosulphonic acid, the reaction mixture was stirred for another 45 min at room temperature and the mixture was poured on to crushed ice. The product was extracted with three 50-mL portions of chloroform, dried overnight over anhydrous sodium sulphate. Chloroform was distilled off. The product was sufficiently pure which was not attempted for further purification. It had been taken for the next step.

5.1.4. Method 2. 2-(Substituted benzenesulphonyl) glutamic acid (3a–3c). L-glutamic acid (14.7 g: 0.1 mol) was taken in a 250-mL conical flask and sodium hydroxide solution (2N) was added slowly till all glutamic acid dissolved and the mixture become distinctly alkaline to phenolphthalein. The reaction mixture was stirred on a magnetic stirrer and the temperature was maintained at 70°C using hot water-bath. Substituted benzenesulphonyl chloride (2: 0.15 moles) was added in small portions with constant stirring and sodium hydroxide (2N) was added time-to-time to keep the reaction mixture alkaline. The reaction was continued until a clear homogeneous solution resulted and thin layer chromatography showed the reaction was complete. After the reaction was over, it was allowed to cool to room temperature and filtered to separate undissolved solid matter, if any. The filtrate was acidified with concentrated hydrochloric acid and saturated with sodium chloride. The product was extracted with three 50 mL portions of ethyl acetate. Ethyl acetate layer was washed with brine solution (15 mL) and dried overnight over anhydrous sodium sulphate. The solvent was distilled off to get the desired diacid (3a–c).

5.1.5. Method 3. 1-(Substituted benzenesulphonyl)-5-oxopyrrolidine-2-carboxylic acid (4a–4c). 2-(Substituted benzenesulphonyl) glutamic acid (3: 0.01 mol) was taken in 100 mL round bottomed flask, fitted with reflux condenser and calcium chloride guard tube. Acetyl chloride (0.025 mol) was added to it and refluxed for 2 h on boiling waterbath. The completion of the reaction was tested by thin layer chromatography. After the reaction was completed, the reaction mixture was cooled to room temperature and poured onto crushed ice with continuous stirring. The precipitated product was filtered and recrystallized from water with charcoal treatment. Physical data of all intermediates are recorded in Table 1.

5.1.6. Method 4. 5-*N*-Substituted 2-(substituted benzenesulphonyl) glutamines 5–21. In a 50 mL of loosely stoppered conical flask, 1-(substituted benzene sulphonyl)-5-oxopyrrolidine-2- carboxylic acid (4: 0.1 mole) was suspended in 20 mL of water. To this, excess of amines (0.025 mole) were added and allowed to stand overnight. The reaction mixture was concentrated over steambath to remove excess of amines. It was cooled to room temperature and chilled in an icebath. The mixture was acidified with 6N hydrochloric acid. The precipitate was filtered and the residue was washed with cold water and finally recrystallized from dilute ethanol with charcoal treatment. Physical data of title compounds were recorded in Table 2.

5.1.7. 5-*N*-Methyl-2-(3′-nitro benzenesulphonyl) glutamine (5). MS (FAB): $M+H^+$ peak at *m*/*z* 346. ^{1}HNMR (300 MHz, DMSO-d_6): δ 12.70 (s, 1H, COOH), 8.50 (d, 1H, SO_2NH), 8.34 (d, 1H, H-2″), 7.98 (dd, 1H, H-6′), 7.88 (m, 2H, H-4″, H-5″), 7.76 (m, 1H, CONH), 3.78 (m, 1H, H-2), 3.05 (m, 2H, N-CH_3-1″), 2.02 (m, 2H, H_2-4), 1.84 (m, 1H, H_A-3), 1.63 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3318, 3114 (N–H str of CONH), 3032 (Ar–C–H str), 2882 (ali C–H str), 1698 (C=O str), 1564, 1522 (N=O str of Ar–NO_2), 1446 (ali C–H def), 1336 and 1165 (S=O str of SO_2NH), 975, 882 (C–N str of Ar–NO_2), 795 and 750 (Ar–C-H def). Anal. $C_{12}H_{15}N_3O_7S_1$ (C, H, N) calcd: 41.74, 4.38, 12.17; found: 41.45, 4.30, 12.26.

5.1.8. 5-*N*-Ethyl-2-(3′-nitro benzenesulphonyl) glutamine (6). MS (FAB): $M+H^+$ peak at *m*/*z* 360. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.72 (s, 1H, COOH), 8.48 (d, 1H, SO_2NH), 8.32 (d, 1H, H-2′), 7.94 (dd, 1H, H-6′), 7.85 (m, 2H, H-4′, H-5′), 7.76 (m, 1H, CONH), 3.81 (m, 1H, H-2), 3.02 (m, 2H, N-CH_2-1″), 2.08 (m, 2H, H_2-4), 1.88 (m, 1H, H_A-3), 1.65 (m, 1H, H_B-3), 0.99 (m, 3H, CH_3-2″). IR (KBr, cm^{-1}): 3320, 3110 (N–H str of CONH), 3028 (Ar–C–H str), 2885 (ali C–H str), 1702 (C=O str), 1563, 1520 (N=O str of Ar–NO_2), 1442 (ali C–H def), 1334 and 1164 (S=O str of SO_2NH), 973, 880 (C–N str of Ar–NO_2), 798 and 754 (Ar–C–H def). Anal. $C_{13}H_{17}N_3O_7S_1$ (C, H, N) calcd: 43.45, 4.77, 11.69; found: 43.38, 4.62, 11.52.

5.1.9. 5-*N*-*n*-Propyl-2-(3′-nitro benzenesulphonyl) glutamine (7). MS (FAB): $M+H^+$ peak at *m*/*z* 374.^{1}H NMR (300 MHz, DMSO-d_6): δ 12.69 (s, 1H, COOH), 8.45 (d, 1H, SO_2NH), 8.37 (d, 1H, H-2′), 7.88 (dd, 1H, H-6′), 7.80 (m, 2H, H-4′, H-5′), 7.72 (m, 1H, CONH), 3.78 (m, 1H, H-2), 3.06 (m, 2H, N-CH_2-1″), 2.10 (m,

2H, H_2-4), 1.92 (m, 1H, H_A-3), 1.69 (m, 1H, H_B-3), 1.28 (m, 2H, CH_2-2″), 0.97 (m, 3H, CH_3-3″). IR (KBr, cm^{-1}): 3322, 3105 (N–H str of CONH), 3032 (Ar–C–H str), 2890 (ali C–H str), 1698 (C=O str), 1564, 1525 (N=O str of Ar–NO_2), 1446 (ali C–H def), 1336 and 1166 (S=O str of SO_2NH), 973, 882 (C–N str of Ar–NO_2), 796 and 755 (Ar–C–H def). Anal. $C_{14}H_{19}N_3O_7S_1$ (C, H, N) calcd: 45.03, 5.13, 11.25; found: 45.25, 5.03, 11.09.

5.1.10. 5-*N*-*i*-Propyl-2-(3′-nitro benzenesulphonyl) glutamine (8). MS (FAB): $M+H^+$ peak at *m*/*z* 374. ^1H NMR (300 MHz, DMSO-d_6): δ 12.66 (s, 1H, COOH), 8.50 (d, 1H, SO_2NH), 8.35 (d, 1H, H-2′), 7.96 (dd, 1H, H-6′), 7.89 (m, 2H, H-4′, H-5′), 7.72 (m, 1H, CONH), 3.84 (m, 1H, H-2), 3.00 (m, 1H, N-CH-1″), 2.10 (m, 2H, H_2-4), 1.90 (m, 1H, H_A-3), 1.67 (m, 1H, H_B-3), 1.10–0.90 (m, 6H, CH_3-2″, CH_3-3″). IR (KBr, cm^{-1}): 3325, 3112 (N–H str of CONH), 3030 (Ar–C–H str), 2882 (ali C–H str), 1700 (C=O str), 1565, 1523 (N=O str of Ar–NO_2), 1445 (ali C–H def), 1332 and 1162 (S=O str of SO_2NH), 970, 882 (C–N str of Ar–NO_2), 794 and 750 (Ar–C–H def). Anal. $C_{14}H_{19}N_3O_7S_1$ (C, H, N) calcd: 45.03, 5.13, 11.25; found: 45.15, 5.20, 11.14.

5.1.11. 5-*N*-Phenyl-2-(3′-nitro benzenesulphonyl) glutamine (9). MS (FAB): $M+H^+$ peak at *m*/*z* 408. ^{1}HNMR (300 MHz, DMSO-d_6): δ 12.74 (s, 1H, COOH), 8.54 (d, 1H, SO_2NH), 8.37 (d, 1H, H-2′), 7.98 (dd, 1H, H-6′), 7.88 (m, 2H, H-4′, H-5′), 7.78 (m, 5H, ph. protons), 7.64 (m, 1H, CONH), 3.85 (m, 1H, H-2), 2.12 (m, 2H, H_2-4), 1.92 (m, 1H, H_A-3), 1.69 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3316, 3106 (N–H str of CONH), 3032 (Ar–C–H str), 2882 (ali C–H str), 1695 (C=O str), 1565, 1524 (N=O str of Ar–NO_2), 1444 (ali C–H def), 1336 and 1165 (S=O str of SO_2NH), 977, 884 (C–N str of Ar–NO_2), 800 and 756 (Ar–C–H def). Anal. $C_{17}H_{17}N_3O_7S_1$ (C, H, N) calcd: 50.12, 4.21, 10.31; found: 49.95, 4.11, 10.22.

5.1.12. 5-*N*-*n*-Butyl-2-(3′-nitro benzenesulphonyl) glutamine (10). MS (FAB): $M+H^+$ peak at *m*/*z* 388. ^1H NMR (300 MHz, DMSO-d_6): δ 12.68 (s, 1H, COOH), 8.42 (d, 1H, SO_2NH), 8.26 (d, 1H, H-2′), 7.90 (dd, 1H, H-6′), 7.80 (m, 2H, H-4′, H-5′), 7.74 (m, 1H, CONH), 3.76 (m, 1H, H-2), 3.00 (m, 2H, N-CH_2-1″), 2.12 (m, 2H, H_2-4), 1.92 (m, 1H, H_A-3), 1.70 (m, 1H, H_B-3), 1.38 (m, 2H, CH_2-2″), 1.20 (m, 2H, CH_2-3″), 0.88 (m, 3H, CH_3-4″). IR (KBr, cm^{-1}): 3322, 3114 (N–H str of CONH), 3034 (Ar–C–H str), 2888 (ali C–H str), 1705 (C≐O str), 1565, 1524 (N=O str of Ar–NO_2), 1446 (ali C–H def), 1335 and 1165 (S=O str of SO_2NH), 977, 884 (C–N str of Ar–NO_2), 794 and 752 (Ar–C–H def). Anal. $C_{15}H_{21}N_3O_7S_1$ (C, H, N) calcd: 46.50, 5.46, 10.85; found: 46.32, 5.50, 10.76.

5.1.13. 5-*N*-*n*-Hexyl-2-(3′-nitro benzenesulphonyl) glutamine (11). MS (FAB): $M+H^+$ peak at *m*/*z* 416. ‘p^{1}HNMR (300 MHz, DMSO-d_6): δ 12.66 (s, 1H, COOH), 8.54 (d, 1H, SO_2NH), 8.38 (d, 1H, H-2′), 7.98 (dd, 1H, H-6′), 7.88 (m, 2H, H-4′, H-5′), 7.79 (m, 1H, CONH), 3.85 (m, 1H, H-2), 3.08 (m, 2H, N–CH_2–1″), 2.10 (m, 2H, H_2-4), 1.90 (m, 1H, H_A-3), 1.68 (m, 1H, H_B-3), 1.55–1.10 (m, 8H, CH_2-2″, CH_2-3″, CH_2-4″, CH_2-5″), 0.85 (m, 3H, CH_3-6″). IR (KBr, cm^{-1}): 3325, 3114 (N–H str of CONH), 3032 (Ar–C–H str), 2887 (ali C–H str), 1708 (C=O str), 1566, 1522 (N=O str of Ar–NO_2), 1446 (ali C–H def), 1337 and 1166 (S=O str of SO_2NH), 975, 882 (C–N str of Ar–NO_2), 795 and 750 (Ar–C–H def). Anal. $C_{17}H_{25}N_3O_7S_1$ (C, H, N) calcd: 49.15, 6.07, 10.11; found: 49.03, 5.96, 10.26.

5.1.14. 5-*N*-*i*-Propyl-2-(2′-chloro-5′-methyl benzenesulphonyl) glutamine (12). MS (FAB): $M+H^+$ peak at *m*/*z* 377. ‘p^{1}HNMR (300 MHz, DMSO-d_6): δ 12.74 (s, 1H, COOH), 8.50 (d, 1H, SO_2NH), 8.20–8.06 (m, 2H, H-3′, H-6′), 7.82 (m, 1H, H-4′), 7.72 (m, 1H, CONH), 3.78m, 1H, H-2), 3.00 (m, 1H, N–CH–1″), 2.62 (s, 3H, Ar–CH_3), 2.08 (m, 2H, H_2-4), 1.86 (m, 1H, H_A-3), 1.67 (m, 1H, H_B-3), 0.95 (m, 6H, CH_3-2″, CH_3-3″). IR (KBr, cm^{-1}): 3316, 3105 (N–H str of CONH), 3024 (Ar–C–H str), 2882 (ali C–H str), 1698 (C=O str), 1560, 1516 (N=O str of Ar–NO_2), 1438 (ali C–H def), 1332 and 1160 (S=O str of SO_2NH), 975, 882 (C–N str of Ar–NO_2), 796 and 750 (Ar–C–H def). Anal. $C_{15}H_{21}N_2O_5S_1Cl$ (C, H, N) calcd: 47.81, 5.62, 7.43; found: 47.56, 5.37, 7.37.

5.1.15. 5-*N*-*n*-Propyl-2-(2′-chloro-5′-methyl benzenesulphonyl) glutamine (13). MS (FAB): $M+H^+$ peak at *m*/*z* 377. ‘p^{1}HNMR (300 MHz, DMSO-d_6): δ 12.70 (s, 1H, COOH), 8.44 (d, 1H, SO_2NH), 8.16–8.00 (m, 2H, H-3′, H-6′), 7.78 (m, 1H, H-4′), 7.64 (m, 1H, CONH), 3.81 (m, 1H, H-2), 3.05 (m, 2H, N–CH_2–1″), 2.66 (s, 3H, Ar–CH_3), 2.10 (m, 2H, H_2-4), 1.82 (m, 1H, H_A-3), 1.65 (m, 1H, H_B-3), 1.28 (m, 2H, CH_2-2″), 0.92 (m, 3H, CH_3-3″). IR (KBr, cm^{-1}): 3322, 3110 (N–H str of CONH), 3018 (Ar–C–H str), 2878 (ali C–H str), 1702 (C=O str), 1556, 1512 (N=O str of Ar–NO_2), 1444 (ali C–H def), 1336 and 1165 (S=O str of SO_2NH), 977, 885 (C–N str of Ar–NO_2), 798 and 754 (Ar–C–H def). Anal. $C_{15}H_{21}N_2O_5S_1Cl$ (C, H, N) calcd: 47.81, 5.62, 7.43; found: 47.68, 5.49, 7.39.

5.1.16. 5-*N*-*n*-Butyl-2-(2′-chloro-5′-methyl benzenesulphonyl) glutamine (14). MS (FAB): $M+H^+$ peak at *m*/*z* 391. ^1H NMR (300 MHz, DMSO-d_6): δ 12.72 (s, 1H, COOH), 8.46 (d, 1H, SO_2NH), 8.22–8.04 (m, 2H, H-3′, H-6′), 7.84 (m, 1H, H-4′), 7.68 (m, 1H, CONH), 3.76 (m, 1H, H-2), 3.02 (m, 2H, N-CH_2-1″), 2.70 (s, 3H, Ar–CH_3), 2.12 (m, 2H, H_2-4), 1.86 (m, 1H, H_A-3), 1.69 (m, 1H, H_B-3), 1.45–1.25 (m, 4H, CH_2-2″, CH_2-3″), 0.95 (m, 3H, CH_3-4″). IR (KBr, cm^{-1}): 3318, 3107 (N–H str of CONH), 3020 (Ar–C–H str), 2884 (ali C–H str), 1708 (C=O str), 1560, 1516 (N=O str of Ar–NO_2), 1442 (ali C–H def), 1334 and 1164 (S=O str of SO_2NH), 975, 882 (C–N str of Ar–NO_2), 796 and 752 (Ar–C–H def). Anal. $C_{16}H_{23}N_2O_5S_1Cl$ (C, H, N) calcd: 49.16, 5.93, 7.17; found: 48.97, 5.82, 7.07.

5.1.17. 5-*N*-*i*-Butyl-2-(2′-chloro-5′-methyl benzenesulphonyl) glutamine (15). MS (FAB): $M+H^+$ peak at *m*/*z* 391. ^{1}HNMR (300 MHz, DMSO-d_6): δ12.68 (s, 1H, COOH), 8.50 (d, 1H, SO_2NH), 8.25–8.10 (m, 2H, H-3′, H-6′), 7.88 (m, 1H, H-4′), 7.72 (m, 1H, CONH), 3.82 (m, 1H, H-2), 3.06 (m, 2H, N-CH_2-1″), 2.72 (s, 3H, Ar–CH_3), 2.16 (m, 2H, H_2-4), 1.90 (m, 1H, H_A-3), 1.70 (m, 1H, H_B-3), 1.45 (m, 1H, CH-2″), 1.15-0.95 (m, 6H, CH_3-

3″, CH_3-4″). IR (KBr, cm^{-1}): 3322, 3110 (N–H str of CONH), 3015 (Ar–C–H str), 2876 (ali C–H str), 1700 (C=O str), 1558, 1514 (N=O str of Ar–NO_2), 1446 (ali C–H def), 1336 and 1164 (S=O str of SO_2NH), 973, 880 (C–N str of Ar–NO_2), 794 and 748 (Ar–C–H def). Anal. $C_{16}H_{23}N_2O_5S_1Cl$ (C, H, N) calcd: 49.16, 5.93, 7.17; found: 48.88, 5.78, 7.12.

5.1.18. 5-*N*-Benzyl-2-(2′-chloro-5′-methyl benzenesulphonyl) glutamine (16). MS (FAB): $M+H^+$ peak at *m/z* 425. ^{1}HNMR (300 MHz, DMSO-d_6): δ 12.74 (s, 1H, COOH), 8.52 (d, 1H, SO_2NH), 8.22–8.12 (m, 2H, H-3′, H-6′), 7.90–7.80 (m, 6H, H-4′, ph.-protons), 7.74 (m, 1H, CONH), 4.20 (m, 2H, CH_2-ph), 3.80 (m, 1H, H-2), 2.72 (s, 3H, Ar-CH_3), 2.16 (m, 2H, H_2-4), 1.90 (m, 1H, H_A-3), 1.70 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3320, 3110 (N–H str of CONH), 3018 (Ar–C–H str), 2880 (ali C–H str), 1705 (C=O str), 1556, 1510 (N=O str of Ar–NO_2), 1442 (ali C–H def), 1334 and 1162 (S=O str of SO_2NH), 977, 884 (C–N str of Ar–NO_2), 798 and 752 (Ar–C–H def). Anal. $C_{19}H_{21}N_2O_5S_1Cl$ (C, H, N) calcd: 53.71, 4.98, 6.59; found: 53.45, 4.79, 6.42.

5.1.19. 5-*N*-*n*-Butyl-2-(4′-bromo benzenesulphonyl) glutamine (17). MS (FAB): $M+H^+$ peak at *m/z* 422. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.70 (s, 1H, COOH), 8.50 (d, 1H, SO_2NH), 8.26–7.94 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.74 (m, 1H, CONH), 3.78 (m, 1H, H-2), 3.05 (m, 2H, N–CH_2-1″), 2.10 (m, 2H, H_2-4), 1.90 (m, 1H, H_A-3), 1.68 (m, 1H, H_B-3), 1.35-1.15 (m, 4H, CH_2-2″, CH_2-3″), 0.95 (m, 3H, CH_3-4″). IR (KBr, cm^{-1}): 3322, 3114 (N–H str of CONH), 3032 (Ar–C–H str), 2880 (ali C–H str), 1705 (C=O str), 1565, 1522 (N=O str of Ar–NO_2), 1444 (ali C–H def), 1335 and 1164 (S=O str of SO_2NH), 975, 882 (C–N str of Ar–NO_2), 796 and 750 (Ar–C–H def). Anal. $C_{15}H_{21}N_2O_5S_1Br$ (C, H, N) calcd: 42.76, 5.02, 6.65; found: 42.58, 4.89, 6.51.

5.1.20. 5-*N*-*n*-Hexyl-2-(4′-bromo benzenesulphonyl) glutamine (18). MS (FAB): $M+H^+$ peak at *m/z* 450. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.74 (s, 1H, COOH), 8.53 (d, 1H, SO_2NH), 8.28–7.98 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.77 (m, 1H, CONH), 3.81 (m, 1H, H-2), 3.00 (m, 2H, N–CH_2-1″), 2.06 (m, 2H, H_2-4), 1.88 (m, 1H, H_A-3), 1.70 (m, 1H, H_B-3), 1.38–1.20 (m, 8H, CH_2-2″, CH_2-3″, CH_2-4″, CH_2-5″), 0.99 (m, 3H, CH_3-6″). IR (KBr, cm^{-1}): 3318, 3110 (N–H str of CONH), 3028 (Ar–C–H str), 2878 (ali C–H str), 1698 (C=O str), 1560, 1518 (N=O str of Ar–NO_2), 1440 (ali C–H def), 1336 and 1162 (S=O str of SO_2NH), 973, 878 (C–N str of Ar–NO_2), 795 and 752 (Ar–C–H def). Anal. $C_{17}H_{25}N_2O_5S_1Br$ (C, H, N) calcd: 95.44, 5.61, 6.23; found: 95.16, 5.33, 6.26.

5.1.21. 5-*N*-*n*-Propyl-2-(4′-bromo benzenesulphonyl) glutamine (19). MS (FAB): $M+H^+$ peak at *m/z* 408. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.68 (s, 1H, COOH), 8.54 (d, 1H, SO_2NH), 8.24–7.94 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.76 (m, 1H, CONH), 3.77 (m, 1H, H-2), 3.02 (m, 2H, N-CH_2-1″), 2.08 (m, 2H, H_2-4), 1.92 (m, 1H, H_A-3), 1.66 (m, 1H, H_B-3), 1.32 (m, 2H, CH_2-2″), 0.96 (m, 3H, CH_3-3″). IR (KBr, cm^{-1}): 3320, 3114 (N–H str of CONH), 3034 (Ar–C–H str), 2832 (ali C–H str), 1700 (C=O str), 1567, 1524 (N=O str of Ar–NO_2), 1446 (ali C–H def), 1332 and 1160 (S=O str of SO_2NH), 977, 884 (C–N str of Ar–NO_2), 798 and 754 (Ar–C–H def). Anal. $C_{14}H_{19}N_2O_5S_1Br$ (C, H, N) calcd: 41.29, 4.70, 6.88; found: 40.96, 4.65, 6.76.

5.1.22. 5-*N*-*i*-Butyl-2-(4′-bromo benzenesulphonyl) glutamine (20). MS (FAB): $M+H^+$ peak at *m/z* 422. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.72 (s, 1H, COOH), 8.52 (d, 1H, SO_2NH), 8.32–7.96 (m, 4H, H-2, H-3′, H-5′, H-6′), 7.78 (m, 1H, CONH), 3.72 (m, 1H, H-2), 2.98 (m, 1H, N-CH-1″), 2.02 (m, 2H, H_2-4), 1.86 (m, 1H, H_A-3), 1.64 (m, 1H, H_B-3), 1.15–0.99 (m, 6H, CH_3-2″, CH_3-3″). IR (KBr, cm^{-1}): 3324, 3116 (N–H str of CONH), 3028 (Ar–C–H str), 2876 (ali C–H str), 1700 (C=O str), 1564, 1520 (N=O str of Ar–NO_2), 1442 (ali C–H def), 1334 and 1162 (S=O str of SO_2NH), 973, 881 (C–N str of Ar–NO_2), 794 and 748 (Ar–C–H def). Anal. $C_{15}H_{21}N_2O_5S_1Br$ (C, H, N) calcd: 42.76, 5.02, 6.65; found: 42.25, 5.11, 6.48.

5.1.23. 5-*N*-Phenyl-2-(4′-bromo benzenesulphonyl) glutamine (21). MS (FAB): $M+H^+$ peak at *m/z* 442. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.68 (s, 1H, COOH), 8.48 (d, 1H, SO_2NH), 8.22–7.92 (m, 4H, H-2′, H-3′, H-5′, H-6′), 7.76 (m, 5H, ph.-protons), 7.64 (m, 1H, CONH), 3.76 (m, 1H, H-2), 2.10 (m, 2H, H_2-4), 1.92 (m, 1H, H_A-3), 1.65 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3320, 3110 (N–H str of CONH), 3034 (Ar–C–H str), 2878 (ali C–H str), 1702 (C=O str), 1564, 1520 (N=O str of Ar–NO_2), 1446 (ali C–H def), 1336 and 1164 (S=O str of SO_2NH), 977, 884 (C–N str of Ar–NO_2), 798 and 752 (Ar–C–H def). Anal. $C_{17}H_{17}N_2O_5S_1Br$ (C, H, N) calcd: 46.27, 3.88, 6.35; found: 46.08, 3.75, 6.26.

5.2. Pharmacology

Fifty-three 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines (**5**–**57**) were biologically evaluated for their possible antitumor activity in search of potential anticancer agents.

5.2.1. Tumor cells. EAC (Ehrlich Ascites Carcinoma) cells originated from human breast carcinoma by spontaneous passaging. It is an undifferentiated tumor, which has lost its epithelial character. On subcutaneous inoculation it grows in the form of solid nodes and upon intraperitoneal inoculation ascites rich tumor cells will be produced. EAC were maintained in vivo in Swiss Albino mice by passaging every 10 days. EAC cells of 9 days old were used for the screening of the entire final compounds **5**–**57**.

5.2.2. Animals. Female Swiss albino mice of 10 weeks old with an average body weight of 18–20 grams were used. All mice were kept on basal metabolic diet with water *ad libitum*.

5.2.3. Screening procedure. Two groups of Swiss Albino Mice, each containing 5 healthy mice of the same sex (female in this case), approximately of the same age and body weight (18–20 g), were selected at random and kept in two different cages under identical conditions.

One of these two groups served as control while the other as test. Ehrlich Ascites Carcinoma (EAC) cells were collected from the donor mice and were suspended in sterile isotonic solution (0.9% w/v NaCl). The numbers of tumor cells per mL of this suspension were counted under microscope with the help of haemocytometer. A definite number (about 2×10^6 cells/0.2 mL) of these living viable cells was injected or implanted into the peritoneal cavity of each mouse. In this instance, the tumor cells multiplied relatively freely within the peritoneal cavity and ascites developed. A day of incubation was allowed to establish the disease in the body before starting the drug administration. From the second day of transplantation up to the eighth day a suitable challenge dose (0.2 mmole/kg body weight) of the drug solution/suspension in sterile phosphate buffer (pH 7.2) was injected intraperitoneally to each mouse in the test group at 24 hr interval. Thus, seven doses of the drug were administered to each mouse in the test group. On the ninth day food and water was withheld 18 hr before the starting of the testing operation. The weights of all the animals were recorded before they were sacrificed. The peritoneal cavity was dissected and by a syringe the ascitic fluid was withdrawn to a suitable volume, collected in sterile ice-cold saline and preserved in ice bath. The total number of living cells/mL in the peritoneal fluid of the 5 mice in a group was calculated. The fluid was sucked by adsorbent cotton. The weight of the 5 mice after sacrifice was recorded.

The evaluation of the test drug was made by comparing the cell count of the test with that of the control.

The percentage inhibition of cell count was obtained by the following expression:

$$\text{Percentage inhibition of Ascitic cells (TCI)} = (1 - T/C) \times 100$$

where T is the average number of Ascitic cells/mL in test animals, C is the average number of Ascitic cells/mL in control animals. Mitomycin C (1 mg/kg body weight) was used as the universal antitumor standard, Azeserine and DON (10 mg/kg body weight) in sterile phosphate buffer (pH 7.2) were used as standard glutamine antagonist. Pharmacological results are shown in Table 3.

5.3. QSAR Methodology

5.3.1. Data set and parameters. Physicochemical parameters like molar refractivity (MR), hydrophobicity (π), steric parameter (Es) etc. are collected from literature. Among the various physicochemical parameter, only MR value taken as the sum of the substitution of the benzene ring for each compound. Other physicochemical parameters were discarded after statistical analysis. As E-state index[22–30] contain electronic and topological structural information E-state index is considered as the important parameter for the development of the QSAR equations. E-state index was calculated using program 'Mouse' developed in our laboratory.[31] Along with MR value, and E-state index and indicator parameter I for the presence of *n*-butyl group at R_5 position are recorded in Table 3.

5.3.2. Statistical analysis. Correlation analysis and multiple regression analysis[33–37] were carried out using software 'Multiregress' developed in our laboratory.[32] By the correlation analysis intercorrelated parameters were eliminated stepwise depending on their individual correlation with the biological activity. Correlation matrices are given in Tables 4 and 5 respectively.

Antitumor activity of the compounds was subjected to multiple regression analysis and the statistical quality of the equations was justified[38] by parameter like correlation coffiecient (r or R), standard error of the estimate (S.E.E), variance ratio (F) at specified degree of freedom (df).

5.3.3. QSAR validation. For the validation of the QSAR equation[39,40] PRESS, q^2, S_{PRESS}, PSE were used to predicting the activity of the compounds in the test set.

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