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Synthesis, Screening and Quantitative Structure–Activity Relationship (QSAR) Studies of Some Glutamine Analogues for Possible Anticancer Activity

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Abstract—We described the syntheses, biological activities and QSAR studies of 36 new 5-*n*-substituted-2-(substituted benzenesulphonyl) glutamines **6**–**41** with different substitutions. These compounds were designed as structural analogues of most reactive amino acid, 'glutamine' (GLN), especially in the tumor cells. They present the new basic lateral chains at R_5 position as well as different substitutions at 2′, 3′, 4′, and 5′ positions on the benzene ring. The synthesized compounds have been tested for antitumor activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice using percentage inhibition of tumor weight as inhibitory parameter. In order to elucidate the structural requirements for antitumor activity, quantitative structure–activity relationship (QSAR) studies have been performed using extra thermodynamic model of Hansch. QSAR equations showed that the electronic parameter (σ) on the aromatic ring system, steric parameter (Es) and to some extent Sterimol length of the substituent (L) on the aliphatic side chain correlate significantly with the antitumor activity. Resonance factor occupies the major electronic contribution on the aromatic ring system to the activity.

Introduction

Cancer has been described as nitrogen trap.[1] Glutamine (GLN), a non essential amino acid, plays a key role in tumor cell growth by supplying its amide nitrogen atoms in the biosyntheses of other amino acids, purine, pyrimidine bases,[2] aminosugars and coenzymes, via a family comprised of 16 amido transferases[3] with diversified mechanisms.[4] It also plays the central role in multiple metabolic pathways and considered to be the most essential component of tissue culture media for not only as nitrogen source but also as carbon source.[5] Rates of cell growth, DNA and protein synthesis and thymidine transport correlated with the GLN concentration in the culture media.[6,7] GLN uptake reported[6] to be mediated by the system of ASC (aryl sulphatase C) family of transporters. In all cells, regardless of the tissue origin, sodium-dependent GLN transport is mediated almost exclusively by a single carrier. Cell growth is a function of GLN influx and it was suggested that GLN supply glutamate and cystine perhaps for glutathione synthesis. The major products of GLN utilization by lymphocytes and macrophages in culture are glutamate, aspertate, lactate and ammonia (25% of the GLN used is completely oxidized). The high rate of utilization of GLN by the cells of immune system serves to maintain a high intracellular concentration of intermediates of biosynthetic pathways. GLN is synthesized in skeletal muscles. The synthesis of interleukin-2 by lymphocytes[8] and of interleukin-1 of macrophages is GLN dependent. Lowered plasma GLN concentration contributes at least in part to the immunosuppression,[9] which is common to most of the anticancer drugs. Overall, the amino acid GLN is main substrate in many biosynthetic pathways especially during cell division.

Hence, structural variants of GLN were synthesized which may supposedly show antitumor activities by GLN[10] and/or Folic acid antagonism. In order to identify the chemical structural features important for the antitumor activity, quantitative structure–activity relationship (QSAR) studies have been performed.

Results and Discussion

Syntheses of 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines is carried out according to Scheme 1.

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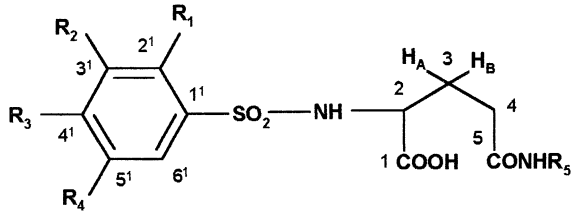


Figure 1. General structure for 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines.

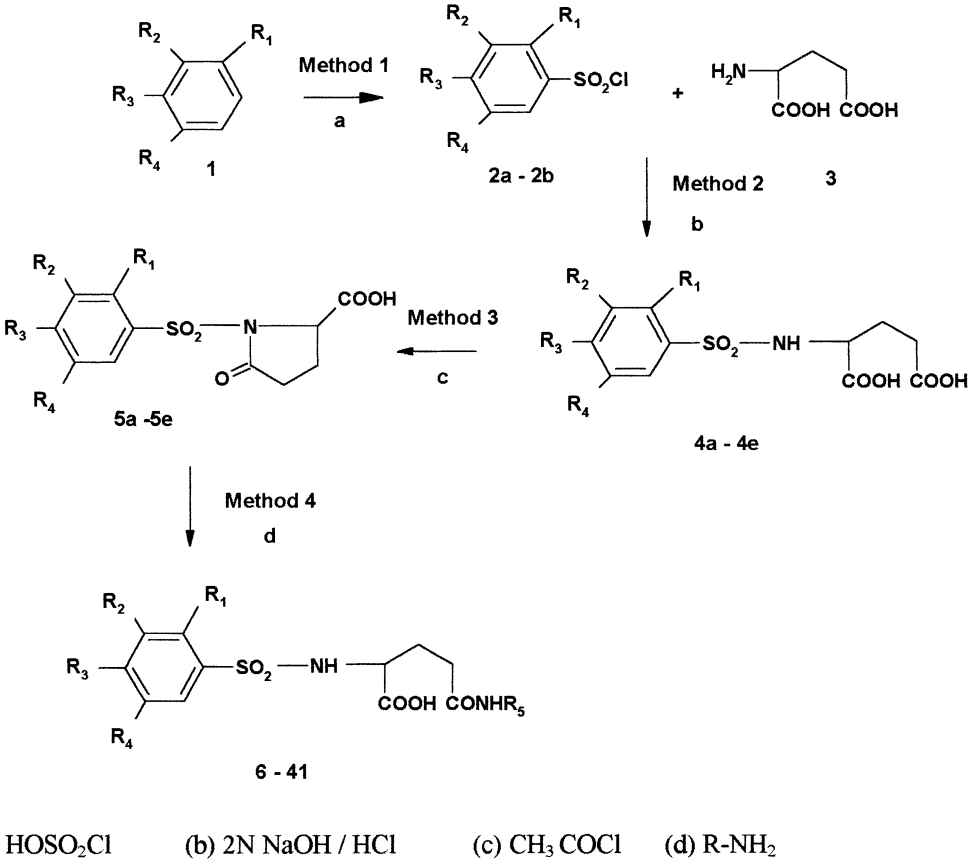
We started with chlorosulphonation[11] of substituted benzene **1**, to get corresponding sulphonyl chloride **2a**–**2b**, except in the case of *p*-tosyl chloride and benzenesulphonyl chloride, which were purchased. This halide proved to be versatile synthon[10] in the subsequent steps in the preparation of substituted benzenesulphonyl glutamic acids **4**. With the application of Schotten–Bauman reaction,[12] 2-(substituted benzenesulphonyl) glutamic acids **4a**–**4e** were prepared by one step condensation of **2** with L-glutamic acid **3**. In this reaction, alkaline medium was maintained to remove the hydrochloric acid which is formed during condensation. Reaction of resultant intermediate **4** with acetyl chloride afforded cyclized acid intermediate, 1-(substituted benzenesulphonyl)-5-oxopyrrolidine-2-carboxylicacids **5a**–**5e**. Nucleophilic displacement of intermediate **5** with amines[13] (amino-dehydroxylation) produced the corresponding amides **6**–**41** as crystalline solids with varying yields ranging from 48 to 98%. Physical data of the intermediates and final compounds are summarized in Tables 1 and 2, respectively.

Antitumor activity of the final compounds were evaluated by dissolving them in phosphate buffered saline (PBS) or by suspending in PBS with 2% Tween 80 (where and when necessary), 2 m mol/kg/day for 7 consecutive days, after intraperitoneal inoculation of mice with 2×10^6 EAC cells. For the tumor growth inhibition, antitumor activity was assessed on the basis of the percentage tumor inhibition (%TI), calculated from mean tumor weight treated (T) and control (C) mice on the day of evaluation. The% TI was calculated as $(1-T/C)\times100$. The biological activity results are presented in Table 3.

Development of QSAR equations

In order to identify the substituent effects on the antitumor activity, QSAR studies of 5-*N*-substituted 2-(substituted benzenesulphonyl) glutamines (Fig. 1) against Ehrlich ascites carcinoma (EAC) were performed by using the Hansch approach.[14] Biological activity (BA) data was calibrated to their logarithmic values (log BA) and are listed in Table 3.

The congener series possesses the substitution on the aromatic ring system at 2′, 3′, 4′, 5′ positions and at the 5-N position on the aliphatic side chain, as shown in Figure 1. Physicochemical parameters were taken as sum of all the substituents on the benzene ring for each compound.



Scheme 1. General synthetic protocol for 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines: (a) $HOSO_2Cl$; (b) 2 N NaOH/HCl; (c) CH_3COCl; (d) R–NH_2.

Table 1. Physical data of intermediate compounds

Compd	R_1	R_2	R_3	R_4	Mp (°C)	% Yield	Molecular formula	MW
2a	CH_3	H	H	NO_2	42–44	76.39	$C_7H_6N_1O_4S_1Cl$	235.50
2b	H	NO_2	CH_3	H	Liquid	83.54	$C_7H_6N_1O_4S_1Cl$	235.50
4a	H	H	CH_3	H	120–122	69.30	$C_{12}H_{15}N_1O_6S_1$	301.32
4b	CH_3	H	H	NO_2	138–140	73.20	$C_{12}H_{14}N_2O_8S_1$	346.32
4c	H	NO_2	CH_3	H	130–132	71.40	$C_{12}H_{14}N_2O_8S_1$	346.32
4d	H	H	C_2H_5	H	89–90	59.29	$C_{13}H_{17}N_1O_6S_1$	315.35
4e	H	H	H	H	145–147	56.66	$C_{11}H_{13}N_1O_6S_1$	287.29
5a	H	H	CH_3	H	75–77	74.46	$C_{12}H_{13}N_1O_5S_1$	283.30
5b	CH_3	H	H	NO_2	94–96	92.37	$C_{12}H_{12}N_2O_7S_1$	328.30
5c	H	NO_2	CH_3	H	38–39	93.64	$C_{12}H_{12}N_2O_7S_1$	328.30
5d	H	H	C_2H_5	H	40–41	92.55	$C_{13}H_{15}N_1O_5S_1$	297.30
5e	H	H	H	H	155–157	69.78	$C_{11}H_{11}N_1O_5S_1$	269.28

Table 2. Physical data of different substituted benzenesulphonyl glutamines **6–41**

Compd	R_1	R_2	R_3	R_4	R_5	Mp (°C)	% Yield	Molecular formula	MW
6	H	H	H	H	*i*-Butyl	160–162	98.04	$C_{15}H_{22}N_2O_5S_1$	342.42
7	H	H	CH_3	H	*i*-Propyl	176–178	53.70	$C_{15}H_{22}N_2O_5S_1$	342.42
8	H	H	CH_3	H	*i*-Butyl	179–181	75.90	$C_{16}H_{24}N_2O_5S_1$	356.44
9	CH_3	H	H	NO_2	H	178–180	70.00	$C_{12}H_{15}N_3O_7S_1$	345.33
10	CH_3	H	H	NO_2	CH_3	176–180	88.20	$C_{13}H_{17}N_3O_7S_1$	359.36
11	CH_3	H	H	NO_2	C_2H_5	192–194	70.00	$C_{14}H_{19}N_3O_7S_1$	373.39
12	CH_3	H	H	NO_2	*n*-C_3H_7	195	70.00	$C_{15}H_{21}N_3O_7S_1$	387.41
13	CH_3	H	H	NO_2	*n*-C_4H_9	218–220	86.60	$C_{16}H_{23}N_3O_7S_1$	401.44
14	CH_3	H	H	NO_2	*i*-Propyl	215–218	85.10	$C_{15}H_{21}N_3O_7S_1$	387.41
15	CH_3	H	H	NO_2	*i*-Butyl	212	90.00	$C_{16}H_{23}N_3O_7S_1$	401.44
16	CH_3	H	H	NO_2	C_6H_{11}	230	80.00	$C_{18}H_{25}N_3O_7S_1$	427.48
17	CH_3	H	H	NO_2	C_6H_5	210–213	79.00	$C_{18}H_{19}N_3O_7S_1$	421.43
18	CH_3	H	H	NO_2	$C_6H_5CH_2$	180–182	86.23	$C_{19}H_{21}N_3O_7S_1$	435.46
19	CH_3	H	H	NO_2	*n*-C_5H_{11}	146	82.67	$C_{17}H_{25}N_3O_7S_1$	415.46
20	CH_3	H	H	NO_2	*n*-C_6H_{13}	138–140	79.41	$C_{18}H_{27}N_3O_7S_1$	429.49
21	H	NO_2	CH_3	H	H	175–176	95.23	$C_{12}H_{15}N_3O_7S_1$	345.33
22	H	NO_2	CH_3	H	CH_3	184–185	70.81	$C_{13}H_{17}N_3O_7S_1$	359.36
23	H	NO_2	CH_3	H	C_2H_5	196–197	70.36	$C_{14}H_{19}N_3O_7S_1$	373.39
24	H	NO_2	CH_3	H	*n*-C_3H_7	193–194	56.81	$C_{15}H_{21}N_3O_7S_1$	387.41
25	H	NO_2	CH_3	H	*n*-C_4H_9	185–186	81.81	$C_{16}H_{23}N_3O_7S_1$	401.44
26	H	NO_2	CH_3	H	*n*-C_5H_{11}	179–180	65.88	$C_{17}H_{25}N_3O_7S_1$	415.47
27	H	NO_2	CH_3	H	*n*-C_6H_{13}	186–188	48.14	$C_{18}H_{27}N_3O_7S_1$	429.49
28	H	NO_2	CH_3	H	*i*-Propyl	192–194	85.10	$C_{15}H_{21}N_3O_7S_1$	387.41
29	H	NO_2	CH_3	H	*i*-Butyl	189–191	82.26	$C_{16}H_{23}N_3O_7S_1$	401.44
30	H	NO_2	CH_3	H	C_6H_{11}	195–197	65.90	$C_{18}H_{25}N_3O_7S_1$	427.48
31	H	NO_2	CH_3	H	$C_6H_5CH_2$	195–196	85.00	$C_{19}H_{21}N_3O_7S_1$	435.46
32	H	NO_2	CH_3	H	C_6H_5	219–221	80.00	$C_{18}H_{19}N_3O_7S_1$	421.43
33	H	H	C_2H_5	H	CH_3	162–164	74.45	$C_{14}H_{20}N_2O_5S_1$	328.39
34	H	H	C_2H_5	H	C_2H_5	150–152	68.78	$C_{15}H_{22}N_2O_5S_1$	342.42
35	H	H	C_2H_5	H	*n*-C_3H_7	145–147	67.75	$C_{16}H_{24}N_2O_5S_1$	356.44
36	H	H	C_2H_5	H	*n*-C_4H_9	155–157	78.45	$C_{17}H_{26}N_2O_5S_1$	370.47
37	H	H	C_2H_5	H	*n*-C_5H_{11}	162–164	46.69	$C_{18}H_{28}N_2O_5S_1$	384.50
38	H	H	C_2H_5	H	*n*-C_6H_{13}	168–170	83.56	$C_{19}H_{30}N_2O_5S_1$	398.52
39	H	H	C_2H_5	H	–$CH(CH_3)_2$	171–173	66.43	$C_{16}H_{24}N_2O_5S_1$	356.44
40	H	H	C_2H_5	H	$C_6H_5CH_2$	155–157	85.54	$C_{20}H_{24}N_2O_5S_1$	404.49
41	H	H	C_2H_5	H	C_6H_5	174–175	54.65	$C_{19}H_{22}N_2O_5S_1$	390.46

QSAR studies were performed against antitumor activities of the compounds in training set by multiple regression analysis. Physicochemical parameters used in developing QSAR models are compiled[15,16] in Tables 4 and 5. Correlation analysis was performed on all the descriptors and the resultant correlation matrix is given in Table 6. Depending on the intercorrelation among the independent descriptors and also the their individual correlation with BA, different possible combinations of parameters were subjected to multiple regression analysis.

Antineoplastic activity was found to have good correlation with Hammett's electronic constant ($\Sigma\sigma$) on the phenyl ring, steric factor (EsR_5) and to some extent on length (LR_5) of the substituents at the R_5 position, as shown in eq 1.

$$\begin{aligned}&\text{Log (BA)} = 1.5789\ (\pm 0.0929) - 0.3180\ (\pm 0.1267)\\&\Sigma\sigma + 0.3353\ (\pm 0.0504)\ \text{Es}\ R_5\ + 0.1113(\pm 0.0152)\\&\text{L}\ R_5 + 0.2460\ (\pm 0.715)I_1 + 0.2742\ (\pm 0.0576)I_2 \qquad (1)\\&n = 30\ R = 0.8731,\quad R^2 = 0.7623\quad F_{5,24} = 15.40,\\&\text{SEE} = 0.1014\quad p = 0.0000\end{aligned}$$

where, 'n' represents the number of data points, 'R' is correlation coefficient, 'F' is the F-ratio between variances

Table 3. Antitumor activities of 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines and ClogP and CMR values for the whole molecules

Compd	R_1	R_2	R_3	R_4	R_5	% Inhibition of			
						Tumor weight (BA)	Log (BA)	ClogP	CMR
6	H	H	H	H	*i*-Butyl	52.73	1.7220	0.9000	8.7000
7	H	H	CH_3	H	*i*-Propyl	50.00	1.6990	0.7800	8.7000
8	H	H	CH_3	H	*i*-Butyl	25.00	1.3980	1.4000	9.1600
9	CH_3	H	H	NO_2	H	37.50	1.5740	0.3000	7.9200
10	CH_3	H	H	NO_2	CH_3	68.75	1.8370	0.3400	8.3800
11	CH_3	H	H	NO_2	C_2H_5	25.00	1.3980	0.8600	8.8400
12	CH_3	H	H	NO_2	*n*-C_3H_7	50.00	1.6990	1.3900	9.3100
13	CH_3	H	H	NO_2	*n*-C_4H_9	62.50	1.7960	1.9200	9.7700
14	CH_3	H	H	NO_2	*i*-Propyl	62.50	1.7960	1.1700	9.3100
15	CH_3	H	H	NO_2	*i*-Butyl	12.00	1.0790	1.7900	9.7700
16	CH_3	H	H	NO_2	C_6H_{11}	33.00	1.5190	2.3700	10.5200
17	CH_3	H	H	NO_2	C_6H_5	33.00	1.5190	2.5700	10.4300
18	CH_3	H	H	NO_2	$C_6H_5CH_2$	60.17	1.7790	2.3600	10.8900
19	CH_3	H	H	NO_2	*n*-C_5H_{11}	60.83	1.7840	2.4500	10.2400
20	CH_3	H	H	NO_2	*n*-C_6H_{13}	67.37	1.8280	2.9800	10.7000
21	H	NO_2	CH_3	H	H	49.53	1.6950	0.2200	7.9200
22	H	NO_2	CH_3	H	CH_3	40.86	1.6110	0.2600	8.3800
23	H	NO_2	CH_3	H	C_2H_5	27.05	1.4320	0.7800	8.8400
24	H	NO_2	CH_3	H	*n*-C_3H_7	26.95	1.4310	1.3100	9.3100
25	H	NO_2	CH_3	H	*n*-C_4H_9	41.37	1.6170	1.8400	9.7700
26	H	NO_2	CH_3	H	*n*-C_5H_{11}	24.88	1.3960	2.3700	10.2400
27	H	NO_2	CH_3	H	*n*-C_6H_{13}	59.45	1.7740	2.9000	10.7000
28	H	NO_2	CH_3	H	*i*-Propyl	37.64	1.5760	1.0900	9.3100
29	H	NO_2	CH_3	H	*i*-Butyl	45.95	1.6620	1.7100	9.7700
30	H	NO_2	CH_3	H	C_6H_{11}	35.33	1.5480	2.2900	10.5200
31	H	NO_2	CH_3	H	$C_6H_5CH_2$	22.35	1.3490	2.2800	10.8900
32	H	NO_2	CH_3	H	C_6H_5	59.60	1.7750	2.4900	10.4300
33	H	H	C_2H_5	H	CH_3	90.45	1.9560	0.4700	8.2300
34	H	H	C_2H_5	H	C_2H_5	38.46	1.5850	1.0000	8.7000
35	H	H	C_2H_5	H	*n*-C_3H_7	65.64	1.8170	1.5300	9.1600
36	H	H	C_2H_5	H	*n*-C_4H_9	55.64	1.7450	2.0600	9.6200
37	H	H	C_2H_5	H	*n*-C_5H_{11}	56.36	1.7510	2.5900	10.0900
38	H	H	C_2H_5	H	*n*-C_6H_{13}	65.37	1.8150	3.1200	10.5500
39	H	H	C_2H_5	H	$-CH(CH_3)_2$	41.53	1.6180	1.3100	9.1600
40	H	H	C_2H_5	H	$C_6H_5CH_2$	37.50	1.5740	2.5000	10.7400
41	H	H	C_2H_5	H	C_6H_5	70.76	1.8500	2.7100	10.2800
42			Mitomycin-C			100.00	2.0000	—	–

ClogP, calculated log P; CMR, calculated molar refractivity.

Table 4. Aromatic substituent constants used in deriving QSAR equations[15,16]

Substituent	π	σ	MR	Es	$\mathcal{J}$	$\mathcal{R}$
H	0.00	0.00 (σ–*p*, σ–*m*) −0.06 (σ–*o*)	0.103	0.00	0.00	0.00
CH_3	0.56	−0.17 (σ–*p*) −0.36 (σ–*o*)	0.565	−1.24	−1.24	−0.13
C_2H_5	1.02	−0.15 (σ–*p*)	1.03	−1.31	−0.05	−0.10
NO_2	−0.28	0.71 (σ–*m*)	0.736	−2.52	0.67	0.16

MR values are scaled by factor of 0.1 as usual.

of the predicted and observed activities, and 'SEE' is standard error of the estimate. It explains 76.23% of variances in the activity.

The negative Σσ term in eq 1 explains the necessity of electron donating groups on the phenyl ring for better anticancer activity. This may be due to possible nucleophilic reactions of the ligand with the electrophilic functions of the receptor surface. The positive coefficient of EsR_5 indicates steric bulk at the R_5 position may not be useful for the activity or may be detrimental to the activity. The length of the R_5 substituent is also marginally contributing to the activity.

Indicator parameters, I_1 and I_2 were used for methyl and *i*-propyl groups at the R_5 position, as they were sharing larger residuals. Both I_1 and I_2 were found to be significantly contributing to the activity whereas, when an indicator parameter was used for the *i*-butyl group, there was no improvement in the QSAR model. This implies that smaller groups at the R_5 position may give the better ligand fit into the active site. Field ($\Sigma\mathcal{J}$) and Resonance ($\Sigma\mathcal{R}$) constants of the phenyl ring also yielded equally appreciable QSAR equations (eqs 2 and 3).

$$\begin{aligned}&\text{Log (BA)} = 1.5409\ (\pm 0.0811) - 0.1614(\pm 0.0563)\\&\Sigma\mathcal{J} + 0.3153\ (\pm 0.0475)\text{Es}R_5 + 0.1054\ (\pm 0.0147)\\&\text{L}\ R_5 + 0.2294\ (\pm 0.0687)I_1 + 0.2767\ (\pm 0.0555)I_2\\&n = 30,\quad R = 0.8812,\quad R^2 = 0.7765,\quad F_{5,24} = 16.68,\\&\text{SEE} = 0.0983,\quad p = 0.0000\end{aligned} \tag{2}$$

Table 5. Aliphatic substituent constants used in deriving QSAR equations[15,16]

Substituent	π	MR	Es	L	B_1	B_5
H	0.00	0.103	0.00	2.06	1.00	1.00
CH_3	0.04	0.357	−1.24	2.87	1.52	2.04
C_2H_5	0.56	0.817	−1.31	4.11	1.52	3.17
n-C_3H_7	1.09	1.29	−1.43	4.92	1.52	3.49
n-C_4H_9	1.62	1.747	−1.63	6.17	1.52	4.54
n-C_5H_{11}	2.15	2.217	−1.64	6.97	1.52	4.94
n-C_6H_{13}	2.68	2.677	−1.54	8.22	1.52	5.96
C_6H_{11}	2.07	2.497	−1.81	6.17	1.91	3.49
i-Propyl	0.87	1.287	−1.71	4.11	1.90	3.17
i-Butyl	1.49	1.747	−2.17	4.92	1.52	4.45
C_6H_5	2.27	2.277	−1.01	6.28	1.71	3.11
$C_6H_5CH_2$	2.06	2.867	−1.51	4.62	1.52	6.02

MR values are scaled by factor of 0.1 as usual.

Table 6. Correlation matrix for the descriptors used in QSAR studies

	Σπ	Σσ	ΣMR	ΣEs	ΣF	ΣR	πR_5	EsR_5	MRR_5	LR_5	Log (BA)	I_2	I_1
Σπ	1	−0.62	−0.49	0.94	−0.95	−0.91	0.19	−0.08	0.12	0.11	0.35	0.07	0.006
Σσ	−0.62	1	0.93	−0.82	0.81	0.86	−0.06	0.29	−0.03	0.00	−0.15	−0.23	0.055
ΣMR	−0.49	0.93	1	−0.76	0.74	0.81	−0.04	0.29	−0.03	0.02	−0.06	−0.21	0.075
ΣEs	0.942	−0.82	−0.76	1.00	−1.00	−1.00	0.16	−0.17	0.10	0.08	0.29	0.14	−0.02
Σ$\mathcal{F}$	−0.95	0.81	0.74	−1.00	1.00	0.994	−0.16	0.17	−0.10	−0.08	−0.30	−0.13	0.022
Σ$\mathcal{R}$	−0.91	0.86	0.81	−1.00	0.99	1.00	−0.15	0.20	−0.09	−0.06	−0.27	−0.15	0.032
πR_5	0.196	−0.06	−0.04	0.16	−0.16	−0.15	1.00	−0.42	0.96	0.90	0.10	−0.39	−0.49
EsR_5	−0.08	0.29	0.29	−0.17	0.17	0.197	−0.42	1.00	−0.51	−0.47	0.27	−0.13	0.137
MRR_5	0.12	−0.03	−0.04	0.1	−0.1	−0.1	0.97	−0.51	1.00	0.84	−0.01	−0.35	−0.48
LR_5	0.11	0.00	0.02	0.08	−0.08	−0.06	0.90	−0.47	0.84	1.00	0.20	−0.4	−0.43
Log (BA)	0.36	−0.15	−0.06	0.29	−0.30	−0.27	0.10	0.27	−0.01	0.21	1.00	0.29	0.293
I_2	0.08	−0.23	−0.21	0.14	−0.14	−0.15	−0.39	−0.13	−0.35	−0.4	0.29	1.00	0.389
I_1	0.01	0.06	0.07	−0.02	0.02	0.032	−0.49	0.14	−0.48	−0.43	0.29	0.38	1.00

Table 7. Observed and calculated activities of 5-*N*-substituted-2-(substituted benzenesulphonyl) glutamines

Compd	Observed	Eq 1		Eq 2		Eq 3		Eq 4	
		Pred	LOO	Pred	LOO	Pred	LOO	Pred	LOO
7	1.699	1.772	1.658	1.718	1.817	1.735	1.725	1.668	1.749
8	1.398	1.434	1.313	1.382	1.460	1.398	1.376	1.331	1.399
9	1.574	1.697	1.699	1.656	1.779	1.659	1.709	1.650	1.714
10	1.837	1.892	1.871	1.857	1.924	1.859	1.869	1.858	1.872
11	1.398	1.486	1.471	1.460	1.493	1.459	1.465	1.465	1.465
12	1.699	1.536	1.499	1.507	1.527	1.507	1.494	1.512	1.493
14	1.796	1.626	1.570	1.610	1.570	1.607	1.544	1.625	1.538
15	1.079	1.288	1.349	1.274	1.349	1.270	1.334	1.289	1.331
16	1.519	1.548	1.525	1.519	1.550	1.519	1.519	1.525	1.519
19	1.784	1.694	1.643	1.657	1.686	1.659	1.642	1.658	1.644
20	1.828	1.867	1.812	1.820	1.876	1.824	1.818	1.816	1.823
21	1.695	1.656	1.621	1.656	1.630	1.659	1.632	1.650	1.637
22	1.611	1.576	1.546	1.580	1.544	1.582	1.552	1.577	1.556
23	1.432	1.445	1.468	1.460	1.446	1.459	1.462	1.465	1.461
24	1.431	1.495	1.517	1.507	1.501	1.507	1.512	1.512	1.512
25	1.617	1.567	1.576	1.576	1.562	1.576	1.572	1.579	1.573
27	1.774	1.825	1.828	1.820	1.839	1.824	1.833	1.816	1.838
28	1.576	1.585	1.641	1.610	1.589	1.607	1.622	1.625	1.618
30	1.548	1.506	1.523	1.519	1.501	1.519	1.516	1.525	1.516
31	1.349	1.434	1.466	1.450	1.445	1.449	1.459	1.457	1.458
32	1.775	1.787	1.778	1.783	1.789	1.787	1.784	1.777	1.788
33	1.956	1.936	1.978	1.967	1.924	1.963	1.975	1.969	1.968
34	1.585	1.531	1.574	1.569	1.525	1.563	1.566	1.576	1.559
35	1.817	1.581	1.592	1.617	1.562	1.611	1.590	1.623	1.586
36	1.745	1.653	1.683	1.686	1.646	1.681	1.678	1.690	1.673
37	1.751	1.738	1.772	1.767	1.737	1.763	1.769	1.769	1.765
38	1.815	1.911	1.963	1.930	1.938	1.928	1.967	1.927	1.964
39	1.618	1.671	1.782	1.720	1.685	1.711	1.753	1.737	1.741
40	1.574	1.520	1.567	1.560	1.515	1.554	1.558	1.568	1.551
41	1.85	1.873	1.897	1.893	1.877	1.891	1.903	1.888	1.901

Pred, predicted activities; Observed, observed activities.

Table 8. Observed and calculated activities of compounds in test set as per QSAR models (eqs 1–4)

Compd	Observed	Calcd (eq 1)	Calcd (eq 2)	Calcd (eq 3)	Calcd (eq 4)
6	1.2950	1.2469	1.4183	1.3754	1.2939
13	1.5820	1.5793	1.6082	1.5758	1.5763
17	1.7160	1.7772	1.8284	1.7829	1.7865
18	1.4530	1.4571	1.4758	1.4502	1.4492
26	1.6940	1.6581	1.6526	1.6570	1.6585
29	1.3240	1.2890	1.2466	1.2737	1.2698

Calcd, calculated activities.

$$\text{Log (BA)} = 1.4635\ (\pm 0.0745) - 0.8034(\pm 0.2828)\ \Sigma\mathcal{R} + 0.3202(\pm 0.0478)\text{Es}R_5 + 0.1067\ (\pm 0.01477)\ \text{L}\ R_5 + 0.2336\ (+0.0690)I_1 + 0.2761\ (\pm 0.0557)\ I_2$$
$$n = 30\quad R = 0.8806,\quad R^2 = 0.7755,\quad F_{5,24} = 16.58,\quad \text{SEE} = 0.0985,\quad p = 0.0000 \qquad (3)$$

Both eqs 2 and 3 are significant at the 99% level. Also, they explain 77.60% of variances in activity. From these equations it is clear that resonance effect ($\Sigma\mathcal{R}$) significantly influences the biological activity when compared with field effect ($\Sigma\mathcal{J}$); possibly electron donating groups on the phenyl ring favor biological activity through resonance.

Hydrophobic interactions of the aromatic ring system were also found to be playing a role in ligand–receptor interactions (eq 4).

$$\text{Log (BA)} = 1.3975\ (\pm 0.0771) + 0.1501(\pm 0.0553)\ \Sigma\pi + 0.3011\ (\pm 0.0480)\text{Es}\ R_5 + 0.1021\ (\pm 0.0151)\ \text{L}\ R_5 + 0.2175\ (0.0697)\text{I}_1 + 0.2807\ (\pm 0.561)\text{I}_2$$
$$n = 30,\quad R = 0.8777,\quad R^2 = 0.7704,\quad F_{5,24} = 16.11,\quad \text{SEE} = 0.0996,\quad p = 0.0000 \qquad (4)$$

The data within the parentheses are significant at 99% level. The hydrophobic constant at the R_5 position was not found to contribute to the activity. The robustness of all these QSAR models was validated by the leave-one-out (LOO) method and the calculated activities are given in Table 7. All the models are equally robust and valid, which predicts the compounds in test set almost consistently. These calculated activities of the test compounds are given in Table 8.

Conclusion

All the final compounds have shown the range of anticancer activities depending on the nature of substitution at different parts of the structure. Standard drug Mitomycin-C at a dose level of 1 mg/kg body wt has shown 100% tumor inhibition. In order to identify the essential chemical structural features responsible for antitumor activity, QSAR studies were performed using the Hansch approach. From the QSAR study, it is evident that electron-donating groups[20] on the phenyl ring are essential for possible reactions of the ligand with electron deficient receptor surface. Resonance also plays a significant part in these interactions. Indicator parameters I_1 and I_2 and a positive steric parameter at the R_5 position explain why the smaller groups in that position may give better fit with the receptor site. Some possible hydrophobic interactions of the aromatic ring system with the receptor surface are not ruled out but a smaller coefficient of $\Sigma\pi$ and a lack of hydrophobic parameter in the aliphatic portion necessitates the substitution elsewhere on the basic nucleus for proper entry of the compound into the hydrophobic pocket of the receptor site.

Experimental

Chemistry

All the melting points were determined on Mel-Temp II melting point apparatus and are uncorrected. Elemental or micro analyses (C, H, N) of the compounds were performed on 2400 Series II CHN analyser of Perkin–Elmer. Analytical results indicated by the symbols of the elements are within ±0.4% of the theoretical values. Infrared spectra were performed on M-500 Model of Buck Scientific, using KBr discs. The frequencies are expressed in cm^{-1}. ^1H NMR spectra were recorded on Bruker (300 MHz), Varian gemini (200 MHz) and Bruker RDX (200 MHz) using Tetramethyl Silane (Me_4 Si) as an internal standard for solutions in $CDCl_3$ and DMSO-d_6. Chemical shifts are expressed in δ ppm (parts per million) down field from Me_4 Si and the coupling constant J in Hz. Splitting patterns have been designated as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublet), m (multiplet). Position of hydrogens described in ^1H NMR interpretation are as per general structure (Fig. 1) and substitutions at R_5 position has taken the superscript "″" (double dash). The mass spectra (FAB) were recorded on JEOL-JMS-SX-102 mass spectrometer. PNBA (*p*-nitro benzyl alcohol) was used as matrix (M^+) which showed the M + 1 peak at 154, 2M + 1 peak at 307.

Reactions were monitored by analytical thin-layer chromatography performed on silica gel G plates. The spots were located keeping the TLC plates in iodine chamber. *p-Toluene sulphonyl chloride,* o-*nitro toluene,* m-*nitro toluene, benzenesulphonyl chloride, chlorosulphonic acid,* L-*glutamic acid, chloroform, ethyl acetate, acetyl chloride and ethyl alcohol* were the commercial products.

General synthetic procedures

Method 1: substituted benzenesulphonyl chloride 2. To a mixture of substituted benzene (0.1 mol) in chloroform (50 mL), in a 500-mL flask equipped with dropping funnel, thermometer, reflux condensor, is added chlorosulphonic acid (0.25 mol) drop wise over a period of 45–60 min. The reaction mixture was magnetically stirred at 0 °C in a bath containing a freezing mixture of ice and salt. Chlorosulphonic acid was added at such a rate that the temperature of the reaction mixture did 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 and dried overnight over anhydrous sodium sulphate. Chloroform was distilled off. The product was sufficiently pure for no further purification. It has been taken for the next step.

Method 2. 2-(Substituted benzenesulphonyl) glutamic acid. 4a–4e. 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 until all the glutamic acid dissolved and the mixture became distinctly alkaline to phenolphthalein. The reaction mixture was stirred on the magnetic stirrer with temperature maintained at 70 °C using a hot water bath. Substituted benzenesulphonyl chloride (0.15 mol) was added in small portions with constant stirring and from time to time addition of sodium hydroxide (2 N) to keep the reaction mixture alkaline. The reaction was continued until a clear homogeneous solution resulted or the thin-layer chromatography showed the reaction to be 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 product.

2-(4′-Methyl benzenesulphonyl) glutamic acid 4a. IR (KBr, cm^{-1}): 3253, 3031 (Ar–C–H str), 2871 (ali C–H str), 1698 (C=O str), 1409, 1334 and 1150 (S=O str of SO_2NH), 985, 816 (Ar–C–H def), 658. Anal. $C_{12}H_{15}N_1O_6S_1$ (C, H, N) calcd: 47.84, 4.98, 4.04; found: 47.64, 4.78, 4.02.

2-(2′-Methyl-5′-nitro benzenesulphonyl) glutamic acid 4b. IR (KBr, cm^{-1}): 3531, 3366, 3031 (Ar–C–H str), 2871 (ali C–H str), 1679 (C=O str), 1515 (N=O str of Ar–NO_2), 1337, 1191 (S=O str of SO_2NH), 914, 792 and 733 (Ar–C–H def), 626. Anal. $C_{12}H_{14}N_2O_8S_1$ (C, H, N) calcd: 41.62, 4.05, 8.09; found: 41.72, 4.18, 8.21.

2-(4′-Methyl-3′-nitro benzenesulphonyl) glutamic acid 4c. ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.42 (d, 1H, H-2′), 8.05 (m, 1H, SO_2NH), 7.96 (dd, 1H, H-6′), 7.53 (d, 1H, H-5′), 3.88 (m, 1H, H-2), 2.58 (s, 3H, Ar–CH_3), 2.30 (m, 2H, H_2-4), 2.02 (m, 2H, H_A-3), 1.82 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3227, 3031 (Ar–C–H str), 2861 (ali C–H str), 1694 (C=O str), 1512 (N=O str of Ar–NO_2), 1404, 1336 and 1165 (S=O str of SO_2NH), 1104, 894, 713 (Ar–C–H def), 661. Anal. $C_{12}H_{14}N_2O_8S_1$ (C, H, N) calcd: 41.62, 4.05, 8.09; found: 41.59, 3.95, 8.12.

2-(4′-Ethyl benzenesulphonyl) glutamic acid 4d. IR (KBr, cm^{-1}): 3253, 3031 (Ar–C–H str), 2876 (ali C–H str), 1702 (C=O str), 1580, 1409, 1335 and 1148 (S=O str of SO_2NH), 979, 897, 783 (Ar–C–H def), 651. Anal. $C_{13}H_{17}N_1O_6S_1$ (C, H, N) calcd: 49.52, 5.40, 4.44; found: 49.83, 5.19, 4.32.

2-(Benzenesulphonyl) glutamic acid 4e. IR (KBr, cm^{-1}): 3191, 3031 (Ar–C–H str), 2881 (ali C–H str), 1726 (C=O str), 1695, 1428, 1375, 1303 and 1155 (S=O str of SO_2NH), 970, 888, 765 and 724 (Ar–C–H def). Anal. $C_{11}H_{13}N_1O_6S_1$ (C, H, N) calcd: 45.99, 4.53, 4.88; found: 45.96, 4.52, 4.62.

Method 3. 1-(Substituted benzenesulphonyl)-5-oxo pyrrolidine-2-carboxilic acids 5a–5e. 2-(Substituted benzenesulphonyl) glutamic acid (0.01 mol) was taken in 100-mL round-bottomed flask, fitted with reflux condensor and calcium chloride guard tube. Acetyl chloride (0.025 mol) was added to it and refluxed for 2 h in a boiling water bath. 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 then poured on to crushed ice with continuous stirring. The precipitated product was filtered and recrystallized from water with charcoal treatment.

1-(4′-Methyl benzenesulphonyl)-5-oxopyrrolidine-2-carboxilic acid 5a. IR (KBr, cm^{-1}): 3480, 3392, 2876 (ali C–H str), 1715 (C=O str), 1691, 1437, 1339 and 1154 (S=O str of SO_2NH), 944, 870, 789 and 736 (Ar–C–H def), 671. Anal. $C_{12}H_{13}N_1O_5S_1$ (C, H, N) calcd: 50.88, 4.59, 4.95; found: 50.68, 4.49, 5.21.

1-(2′-Methyl 5′-nitro benzenesulphonyl)-5-oxopyrrolidine-2-carboxilic acid 5b. IR (KBr, cm^{-1}): 3498, 3033 (Ar–C–H str), 2878 (ali C–H str), 1725 (C=O str), 1586, 1512 (N=O str of Ar–NO_2), 1338 and 1161 (S=O str of SO_2NH), 951, 887 (C–N str of Ar–NO_2), 792 and 736 (Ar–C–H def). Anal. $C_{12}H_{12}N_2O_7S_1$ (C, H, N) calcd: 43.90, 3.66, 8.54; found: 43.54, 3.56, 8.21.

1-(4′-Methyl 3′-nitro benzenesulphonyl)-5-oxopyrrolidine-2-carboxilic acid 5c. ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.58 (d, 1H, H-2′), 8.28 (dd, 1H, H-6′), 7.55 (d, 1H, H-5′), 4.92 (m, 1H, H-5), 2.65 (s, 3H, Ar–CH_3), 2.50 (m, 2H, H_2-4), 2.18 (m, 2H, H_A-3,), 1.25 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3191, 3031 (Ar–C–H str), 2866 (ali C–H str), 1743, 1694 (C=O str), 1517 (N=O str of Ar–NO_2), 1341 and 1165 (S=O str of SO_2NH), 965, 891, 750 and 708 (Ar–C–H def), 659. Anal. $C_{12}H_{12}N_2O_7S_1$ (C, H, N) calcd: 43.90, 3.66, 8.54; found: 43.43, 3.72, 8.56.

1-(Benzenesulphonyl)-5-oxo pyrrolidine-2-carboxilic acid 5e. IR (KBr, cm^{-1}): 3031 (Ar–C–H str), 2897 (ali C–H

str.), 1736 (C=O str), 1681, 1355, 1330 and 1167 (S=O str of SO_2NH), 1117, 952, 870, 750 and 721 (Ar–C–H def). Anal. $C_{11}H_{11}N_1O_5S_1$ (C, H, N) calcd: 49.07, 4.09, 5.20; found: 49.12, 4.21, 5.41.

Method 4. 5-*N*-Substituted-2-(substituted benzenesulphonyl) glutamines 6–41. In a 50-mL of loosely stoppered conical flask, 1-(substituted benzenesulphonyl)-5-oxopyrrolidine-2-carboxylic acid (0.1 mol) was suspended in 20 mL of water. To this excess of amines (0.025 mol) were added and allowed to stand overnight with continuous stirring. The reaction mixture was concentrated over a steam bath to remove excess of amines. Then it was cooled to room temperature and chilled in an ice bath. The mixture was then acidified with 6 N hydrochloric acid to eliminate any traces of unreacted amine. It was then filtered and the residue was washed many times with cold water and finally recrystallized with dilute ethanol.

5-*N*-*i*-Butyl-2-(benzenesulphonyl) glutamine 6. MS (FAB): $M+H^+$ peak at m/z 343. ^1H NMR (200 MHz, $CDCl_3$): δ 7.82 (m, 2H, H-2′, H-6′), 7.51 (m, 3H, H-3′, H-4′, H-5′), 7.24 (m, 1H, SO_2NH), 5.90 (m, 1H, CONH), 3.90 (m, 1H, H-2), 3.11 (m, 2H, N–CH_2-1″), 1.95–1.65 (m, 4H, H_2–4, H_2-3), 1.20 (m, 1H, CH-2″), 0.93 (m, 6H, CH_3-3″, CH_3-4″). IR (KBr, cm^{-1}): 3295, 3171 (N–H str of CONH), 2903, 2824 (ali C–H str), 1682, 1585, 1555, 1444 (ali C–H def), 1321 and 1155 (S=O str of SO_2NH), 887, 752 and 728 (Ar C–H def), 683. Anal. $C_{15}H_{22}N_2O_5S_1$ (C, H, N) calcd: 52.63, 6.43, 8.18; found: 52.54, 6.42, 8.53.

5-*N*-*i*-Propyl-2-(4′-methyl benzenesulphonyl) glutamine 7. MS (FAB): $M+H^+$ peak at m/z 343. ^1H NMR (200 MHz, $CDCl_3$): δ 7.71 (m, 2H, H-2′, H-6′), 7.30 (m, 2H, H-3′, H-5′), 7.14–6.87 (m, 2H, SO_2NH, CONH), 3.78 (m, 1H, H-2), 2.40 (s, 3H, Ar–CH_3), 2.23 (m, 2H, H_2-4), 2.38–1.72 (m, 3H, H_A-3, H_B-3, CH-1″), 0.92 (m, 6H, CH_3-2″, CH_3-3″). IR (KBr, cm^{-1}): 3298, 3186 (N–H str of CONH), 2921, 2875 (ali C–H str), 1677 (C=O str), 1583, 1540, 1443 (ali C–H def), 1420, 1321 and 1153 (S=O str of SO_2NH), 811, 687, 661. Anal. $C_{13}H_{18}N_2O_5S_1$ (C, H, N) calcd: 49.68, 5.73, 8.92; found: 49.72, 5.78, 9.52.

5-*N*-*i*-Butyl-2-(4′-methyl benzenesulphonyl) glutamine 8. MS (FAB): $M+H^+$ peak at m/z 357. ^1H NMR (200 MHz, $CDCl_3$): δ 7.71 (m, 2H, H-2′, H-6′), 7.27 (m, 2H, H-3′, H-5′), 7.14–6.87 (m, 2H, SO_2NH, CONH), 3.80 (m, 1H, H-2), 2.98 (m, 2H, N–CH_2-1″), 2.40 (s, 3H, Ar–CH_3), 2.25 (m, 2H, H_2-4), 2.21–1.68 (m, 3H, H_A-3, H_B-3, CH-2″), 0.89 (m, 6H, CH_3-3″, CH_3-4″). IR (KBr, cm^{-1}): 3304, 3197 (N–H str of CONH), 2895, 2868 (ali C–H str), 1702 (C=O str), 1588, 1555, 1442 (ali C–H def), 1327 and 1154 (S=O str of SO_2NH), 973, 808, 691. Anal. $C_{15}H_{22}N_2O_5S_1$ (C, H, N) calcd: 52.63, 6.43, 8.18; found: 52.52, 6.26, 8.08.

2-(2′-Methyl-5′-nitro benzenesulphonyl) glutamine 9. MS (FAB): $M+H^+$ peak at m/z 346. ^1H NMR (200 MHz, $CDCl_3$): δ 8.74 (d, 1H, $J=2.3$, H-6′), 8.27 (dd, 1H, $J_1=2.34$, $J_2=8.34$, H-4′), 7.67 (d, 1H, $J=8.58$, SO_2NH), 7.49 (d, 1H, $J=8.34$, H-3′), 6.54 (m, 2H, $CONH_2$), 3.86 (m, 1H, H-2), 2.80 (s, 3H, Ar–CH_3), 2.39 (m, 2H, H_2-4), 2.10 (m, 2H, H_A-3, H_B-3). IR (KBr, cm^{-1}): 3356, 3150 (N–H str of CONH), 3057 (Ar–C–H str), 1673 (C=O str), 1624, 1523 (N=O str of Ar–NO_2), 1435 (ali C–H def), 1340 and 1155 (S=O str of SO_2NH), 1113, 916, 865 (C–N str of Ar–NO_2), 795 and 733 (Ar–C–H def). Anal. $C_{16}H_{24}N_2O_5S_1$ (C, H, N) calcd: 53.93, 6.74, 7.86; found: 53.62, 6.71, 7.41.

5-*N*-Methyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 10. MS (FAB): $M+H^+$ peak at m/z 360. ^1H NMR (200 MHz, $CDCl_3$): δ 8.78 (d, 1H, $J=2.4$, H-6′), 8.24 (dd, 1H, $J_1=2.4$, $J_2=8.30$, H-4′), 7.66 (d, 1H, $J=8.58$, SO_2NH), 7.50 (d, 1H, $J=8.30$, H-3′), 6.83 (m, 1H, CONH), 3.86 (m, 1H, H-2), 2.80 (s, 3H, Ar–CH_3), 2.28 (m, 2H, H_2-4), 1.94 (m, 2H, H_A-3, H_B-3), 1.43 (m, 3H, N-CH_3). IR (KBr, cm^{-1}): 3301 (N–H str of CONH), 3044 (Ar–C–H str), 2920, 2888 (ali C–H str), 1691 (C=O str), 1634, 1511 (N=O str of Ar–NO_2), 1437 (ali C–H def), 1339 and 1154 (S=O str of SO_2NH), 1116, 792 and 737 (Ar–C–H def). Anal. $C_{12}H_{15}N_3O_7S_1$ (C, H, N) calcd: 41.74, 4.35, 12.17; found: 41.73, 4.36, 12.21.

5-*N*-Ethyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 11. MS (FAB): $M+H^+$ peak at m/z 374. ^1H NMR (200 MHz, $CDCl_3$): δ 8.76 (d, 1H, $J=2.4$, H-6′), 8.25 (dd, 1H, $J_1=2.46$, $J_2=8.34$, H-4′), 7.68 (d, 1H, $J=8.58$, SO_2NH), 7.49 (d, 1H, $J=8.34$, H-3′), 6.84 (m, 1H, CONH), 3.88 (m, 1H, H-2), 3.23 (m, 2H, N–CH_2-1″), 2.80 (s, 3H, Ar–CH_3), 2.34 (m, 2H, H_2-4), 2.08 (m, 2H, H_A-3, H_B-3), 1.13 (m, 3H, CH_3-2″). IR (KBr, cm^{-1}): 3315, 3160 (N–H str of CONH), 3067 (Ar–C–H str), 2887, 2825 (ali C–H str.), 1681 (C=O str), 1594, 1542, 1518 (N=O str of Ar–NO_2), 1422 (ali C–H def.), 1342 and 1152 (S=O str of SO_2NH), 967, 891 (C–N str of Ar–NO_2), 796 and 735 (Ar–C–H def), 633. Anal. $C_{13}H_{17}N_3O_7S_1$ (C, H, N) calcd: 43.45, 4.73, 11.70; found: 43.39, 4.68, 11.71.

5-*N*-*n*-Propyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 12. MS (FAB): $M+H^+$ peak at m/z 388. ^1H NMR (200 MHz, $CDCl_3$): δ 8.77 (d, 1H, $J=2.4$, H-6′), 8.25 (dd, 1H, $J_1=2.45$, $J_2=8.33$, H-4′), 7.48 (d, 1H, $J=8.38$, H-3′), 7.37 (m, 1H, SO_2NH), 6.58 (m, 1H, CONH), 3.89 (m, 1H, H-2), 3.17 (m, 2H, N–CH_2-1″), 2.81 (s, 3H, Ar–CH_3), 2.36 (m, 2H, H_2-4), 2.08 (m, 2H, H_A-3, H_B-3), 1.52 (m, 2H, CH_2-2″), 0.92 (m, 3H, CH_3-3″). Signal for SO_2NH appears to have merged in the solvent signal at 7.36. IR (KBr, cm^{-1}): 3316, 3147 (N–H str of CONH), 3055 (Ar–C–H str), 2910, 2886, 2827 (ali C–H str), 1674 (C=O str), 1586, 1567, 1549, 1509 (N=O str of Ar–NO_2), 1452 (ali C–H def), 1342 and 1160 (S=O str of SO_2NH), 1114, 913, 887 (C–N str of Ar–NO_2), 795 and 735 (Ar–C–H def). Anal. $C_{14}H_{19}N_3O_7S_1$ (C, H, N) calcd: 45.04, 5.09, 11.26; found: 45.21, 5.17, 11.34.

5-*N*-*n*-Butyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 13. MS (FAB):$M+H^+$ peak at m/z 402. ^1H NMR (200 MHz, $CDCl_3$): δ 8.74 (d, 1H, $J=2.34$, H-6′), 8.21 (dd, 1H, $J_1=2.38$, $J_2=8.33$, H-4′), 7.42 (d, 1H, $J=8.33$, H-3′), 7.34 (m, 1H, SO_2NH), 6.54 (m, 1H, CONH), 3.90 (m, 1H, H-2), 3.15 (m, 2H, N-CH_2-1″),

2.81 (s, 3H, Ar–CH_3), 2.36 (m, 2H, H_2-4), 1.98 (m, 2H, H_A-3, H_B-3), 1.52 (m, 2H, CH_2-2″), 0.92–0.81 (m, 6H, CH_3-3″, CH_3-4″). IR (KBr, cm^{-1}): 3315, 3221 (N–H str of CONH), 3033 (Ar–C–H str), 2905 (ali C–H str), 1683 (C=O str), 1548, 1507 (N=O str of Ar–NO_2), 1445 (ali C–H def), 1340 and 1160 (S=O str of SO_2NH), 1116, 792 and 736 (Ar–C–H def). Anal. $C_{15}H_{21}N_3O_7S_1$ (C, H, N) calcd: 46.51, 5.43, 10.85; found: 46.52, 5.44, 10.96.

5-*N*-*i*-Propyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 14. MS (FAB): $M+H^+$ peak at m/z 388. ^1H NMR (200 MHz, $CDCl_3$): δ 8.76 (d, 1H, $J=2.34$, H-6′), 8.24 (dd, 1H, $J_1=2.30$, $J_2=8.30$, H-4′), 7.83 (d, 1H, $J=7.9$, SO_2NH), 7.51 (m, 1H, H-3′ merged with $CHCl_3$ peak of $CDCl_3$), 6.69 (d, 1H, CONH), 4.01–3.86 (m, 2H, H-2, CH-1″), 2.80 (s, 3H, Ar–CH_3), 2.31 (m, 2H, H_2-4), 2.11–1.99 (m, 2H, H_A-3, H_B-3), 1.17–0.97 (m, 6H, CH_3-2″, CH_3-3″). IR (KBr, cm^{-1}): 3311, 3230 (N–H str of CONH), 2919, 2877 (ali C–H str.), 1674, 1630, 1538, 1511 (N=O str of Ar–NO_2), 1450 (ali C–H def.), 1339 and 1157 (S=O str of SO_2NH), 1117, 911, 796 and 737(Ar–C–H def). Anal. $C_{16}H_{23}N_3O_7S_1$ (C, H, N) calcd: 47.88, 5.73, 10.47; found: 47.42, 5.43, 10.76.

5-*N*-*i*-Butyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 15. MS (FAB): $M+H^+$ peak at m/z 402. ^1H NMR (300 MHz, $CDCl_3$): δ 8.75 (d, 1H, $J=2.2$, H-6′), 8.29 (dd, 1H, $J_1=2.2$, $J_2=8.32$, H-4′), 7.51 (d, 1H, $J=8.36$, H-3′), 6.07 (m, 1H, CONH), 5.99 (m, 1H, SO_2NH), 3.81 (m, 1H, H-2), 3.13 (m, 2H, N–CH_2-1″), 2.79 (s, 3H, Ar–CH_3), 2.50 (m, 2H, H_2-4), 2.13 (m, 2H, H_A-3, H_B-3), 1.21 (m, 1H, CH-2″), 0.94 (m, 6H, CH_3-3″, CH_3-4″). IR (KBr, cm^{-1}): 3316, 3160 (N–H str of CONH), 3033 (Ar–C–H str), 2909, 2878, 2824 (ali C–H str), 1675 (C=O str), 1584, 1566, 1509 (N=O str of Ar–NO_2), 1452 (ali C–H def), 1339 and 1158 (S=O str of SO_2NH), 1114, 886 (C–N str. of Ar–NO_2), 796 and 737 (Ar–C–H def), 633. Anal. $C_{15}H_{21}N_3O_7S_1$ (C, H, N) calcd: 46.51, 5.43, 10.85; found: 46.62, 5.76, 10.58.

5-*N*-Cyclohexyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 16. MS (FAB): $M+H^+$ peak at m/z 328. ^1H NMR (200 MHz, $CDCl_3$): δ 8.70 (m, 1H, H-6′), 8.28–8.23 (m, 2H, H-4′, H-3′), 7.53 (m, 1H, SO_2NH), 7.21 (m, 1H, CONH), 3.79 (m, 1H, H-2), 2.79 (s, 3H, Ar–CH_3), 2.57 (m, 2H, H_2-4), 2.23 (m, 2H, H_2-3), 1.94–1.67 and 1.34–1.11 (m, 11H, cyclohexyl protons). IR (KBr, cm^{-1}): 3320, 3176 (N–H str of CONH), 3046 (Ar–C–H str), 2882, 2809 (ali C–H str.), 1678, 1620, 1588, 1540, 1511 (N=O str of Ar–NO_2), 1441 (ali C–H def), 1340 and 1157 (S=O str of SO_2NH), 1115, 914, 887 (C–N str of Ar–NO_2), 795, 736 (Ar–C–H def). Anal. $C_{16}H_{23}N_3O_7S_1$ (C, H, N) calcd: 47.88, 5.73, 10.47; found: 48.32, 5.50, 10.73.

5-*N*-Phenyl 2-(2′-methyl 5′-nitro benzenesulphonyl) glutamine 17. MS (FAB): $M+H^+$ peak at m/z 422. ^1H NMR (300 MHz, DMSO-d_6): δ 8.60 (s, 1H, SO_2NH), 8.53 (d, 1H, H-6′), 8.32 (dd, 1H, H-4′), 8.27 (m, 1H, CONH), 7.62 (d, 1H, H-3′), 7.36–7.25 (m, 5H, H_5-ph), 3.82 (m, 1H, H-2), 2.72 (s, 3H, Ar–CH_3), 2.20 (m, 2H, H_2-4), 1.90 (m, 1H, H_A-3), 1.71 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3295, 3206 (N–H str of CONH), 3025 (Ar–C–H str), 1687 (C=O str), 1590, 1531 (N=O str of Ar–NO_2), 1431 (ali C–H def.), 1339 and 1158 (S=O str of SO_2NH), 902, 754 and 739 (Ar–C–H def), 689. Anal. $C_{18}H_{25}N_3O_7S_1$ (C, H, N) calcd: 50.58, 5.85, 9.84; found: 50.56, 5.64, 9.83.

5-*N*-benzyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 18. MS (FAB): $M+H^+$ peak at m/z 436. ^1H NMR (300 MHz, DMSO-d_6): δ 8.63 (s, 1H, SO_2NH), 8.54 (d, 1H, $J=2.34$, H-6′), 8.34 (dd, 1H, $J_1=2.42$, $J_2=8.33$, H-4′), 8.30 (m, 1H, CONH), 7.67 (d, 1H, $J=8.42$, H-3′), 7.33–7.20 (m, 5H, H_5-ph), 4.21 (m, 2H, CH_2–Ph), 3.77 (m, 1H, H-2), 2.72 (s, 3H, Ar–CH_3), 2.20 (m, 2H, H_2–4), 1.94 (m, 1H, H_A-3), 1.74 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3314, 3222 (N–H str of CONH), 3050 (Ar–C–H str), 2874 (ali C–H str), 1682 (C=O str), 1587,1504 (N=O str of Ar–NO_2,), 1444 (ali C–H def), 1338 and 1159 (S=O str of SO_2NH), 1114, 887 (C–N str of Ar–NO_2), 794 and 736 (Ar–C–H def), 697. Anal. $C_{18}H_{19}N_3O_7S_1$ (C, H, N) calcd: 51.31, 4.51, 9.98; found: 51.21, 4.34, 9.78.

5-*N*-*n*-Pentyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 19. MS (FAB): $M+H^+$ peak at m/z 416. ^1H NMR (300 MHz, DMSO-d_6): δ 8.63 (d, 1H, $J=6.32$, SO_2NH), 8.52 (d, 1H, $J=2.38$, H-6′), 8.34 (dd, 1H, $J_1=2.39$, $J_2=8.34$, H-4′), 7.73 (m, 1H, CONH), 7.67 (d, 1H, $J=8.42$, H-3′), 3.73 (m, 1H, H-2), 2.95 (m, 2H, N-CH_2-1″), 2.71 (s, 3H, Ar–CH_3), 2.08 (m, 2H, H_2-4), 1.89 (m, 1H, H_A-3), 1.69 (m, 1H, H_B-3), 1.29 (m, 6H, CH_2-2″,CH_2-3″, CH_2-4″), 0.85 (m, 3H, CH_3-5″). IR (KBr, cm^{-1}): 3320, 3227 (N–H str of CONH), 3041 (Ar–C–H str), 2871 & 2809 (ali C–H str), 1684 (C=O str), 1549, 1505 (N=O str of Ar–NO_2), 1442 (ali C–H def), 1340 and 1157 (S=O str of SO_2NH), 1097, 1051, 914, 793 and 737 (Ar–C–H def), 661. Anal. $C_{19}H_{21}N_3O_7S_1$ (C, H, N) calcd: 52.41, 4.83, 9.65; found: 52.39, 4.98, 9.76.

5-N-*n*-Hexyl-2-(2′-methyl-5′-nitro benzenesulphonyl) glutamine 20. MS (FAB): $M+H^+$ peak at m/z 430. ^1H NMR (300 MHz, DMSO-d_6): δ 8.63 (m, 1H, SO_2NH), 8.52 (d, 1H, $J=2.44$, H-6′), 8.34 (dd, 1H, $J_1=2.49$, $J_2=8.35$, H-4′), 7.73 (m, 1H, CONH), 7.67 (d, 1H, $J=8.47$, H-3′), 3.74 (m, 1H, H-2), 2.96 (m, 2H, N–CH_2-1″), 2.71 (s, 3H, Ar–CH_3), 2.15–2.03 (m, 2H, H_2-4), 1.95–1.87 (m, 1H, H_A-3), 1.75–1.65 (m, 1H, H_B-3) 1.38–1.14 (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, 3227 (N–H str of CONH), 3052 (Ar–C–H str), 2907, 2879, 2813 (ali C–H str), 1687 (C=O str), 1583, 1567, 1548, 1509 (N=O str of Ar–NO_2), 1445 (ali C–H def), 1341 and 1161 (S=O str of SO_2NH), 884 (C–N str of Ar–NO_2), 794 and 736 (Ar–C–H def), 662. Anal. $C_{17}H_{25}N_3O_7S_1$ (C, H, N) calcd: 49.16, 6.02, 10.12; found: 59.34, 6.21, 10.32.

2-(4′-Methyl-3′-nitro benzenesulphonyl) glutamine 21. MS (FAB): $M+H^+$ peak at m/z 346. ^1H NMR (300 MHz, DMSO-d_6): δ 8.47 (d, 1H, $J=8.86$, SO_2NH), 8.28 (d, 1H, $J=2.11$, H-2′), 7.99 (dd, 1H, $J_1=2.09$, $J_2=8.07$, H-6′), 7.70 (d, 1H, $J=8.07$, H-5′), 7.44 (m, 2H, $CONH_2$), 3.76 (m, 1H, H-2), 2.59 (s, 3H, Ar–CH_3), 2.11 (m, 2H, H_2-4), 1.90 (m, 1H, H_A-3), 1.71 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3375, 3283, 3196 (N–H str of CONH), 3036 (Ar–

C–H str), 1705 (C=O str), 1658, 1580, 1511 (N=O str of Ar–NO_2), 1339 and 1162 (S=O str of SO_2NH), 1102, 969, 878 (C–N str of Ar–NO_2), 787, 757, 707 (Ar–C–H def), 672. Anal. $C_{18}H_{27}N_3O_7S_1$ (C, H, N) calcd: 50.35, 6.29, 9.79; found: 49.46, 6.00, 10.18.

5-*N*-Methyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 22. MS (FAB): $M+H^+$ peak at *m/z* 360. ^1H NMR (300 MHz, DMSO-d_6): δ 12.72 (s, 1H, COOH), 8.47 (d, 1H, *J* = 8.87, SO_2NH), 8.30 (d, 1H, *J* = 1.81, H-2′), 7.96 (dd, 1H, J_1 = 1.81, J_2 = 8.07, H-6′), 7.70 (d, 1H, *J* = 8.14, H-5′), 7.68 (m, 1H, CONH), 3.80 (m, 1H, H-2), 2.59 (s, 3H, Ar–CH_3), 2.51 (m, 3H, N-CH_3), 2.08 (m, 2H, H_2-4), 1.88 (m, 1H, H_A-3), 1.67 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3319, 3181 (N–H str of CONH), 3025 (Ar–C–H str), 2887, 2819 (ali C–H str), 1690 (C=O str), 1573, 1517 (N=O str of Ar–NO_2, asymmetric) 1438 (ali C–H def), 1394, 1333 and 1164 (S=O str of SO_2NH), 970, 904, 880 (C–N str of Ar-NO_2), 798 and 755 (Ar–C–H def). Anal. $C_{12}H_{15}N_3O_7S_1$ (C, H, N) calcd: 41.74, 4.85, 12.17; found: 41.56, 4.24, 12.24.

5-*N*-Ethyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 23. MS(FAB): $M+H^+$ peak at *m/z* 374. ^1H NMR (300 MHz, DMSO-d_6): δ 12.73 (s, 1H, COOH), 8.47 (d, 1H, *J* = 8.82, SO_2NH), 8.31 (d, 1H, *J* = 1.74, H-2′), 7.96 (dd, 1H, J_1 = 1.92, J_2 = 8.1, H-6′), 7.75 (m, 1H, CONH), 7.71 (d, 1H, *J* = 8.16, H-5′), 3.80 (m, 1H, H-2), 3.01 (m, 2H, N–CH_2-1″), 2.59 (s, 3H, Ar–CH_3), 2.09 (m, 2H, H_2-4), 1.89 (m, 1H, H_A-3), 1.67 (m, 1H, H_B-3), 0.97 (m, 3H, CH_3-2″). IR (KBr, cm^{-1}): 3310, 3188 (N–H str of CONH), 3033 (Ar–C–H str), 2885, 2831 (ali C–H str), 1692 (C=O str), 1565, 1518 (N=O str of Ar–NO_2) 1436 (ali C–H def), 1333 and 1163 (S=O str of SO_2NH), 973, 879 (C–N str of Ar–NO_2), 796 and 754 (Ar–C–H def), 662. Anal. $C_{13}H_{17}N_3O_7S_1$ (C, H, N) calcd: 43.54, 4.73, 11.70; found: 43.42, 4.45, 11.70.

5-*N*-*n*-Propyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 24. MS (FAB): $M+H^+$ peak at *m/z* 388. ^1H NMR (300 MHz, DMSO-d_6): δ 12.72 (s, 1H, COOH), 8.47 (d, 1H, *J* = 8.85, SO_2NH), 8.31 (d, 1H, *J* = 1.77, H-2′), 7.96 (dd, 1H, J_1 = 1.83, J_2 = 8.07, H-6′), 7.75 (m, 1H, CONH), 7.71 (d, 1H, *J* = 8.19, H-5′), 3.80 (m, 1H, H-2), 2.94 (m, 2H, N–CH_2-1′), 2.59 (s, 3H, Ar–CH_3), 2.11 (m, 2H, H_2-4), 1.89 (m, 1H, H_A-3), 1.68 (m, 1H, H_B-3), 1.36 (m, 2H, CH_2-2″), 0.81 (m, 3H, CH_3-3″). IR (KBr, cm^{-1}): 3311, 3188 (N–H str of CONH), 3027 (Ar–C–H str), 2886, 2829 (ali C–H str), 1694 (C=O str), 1563, 1519 (N=O str of Ar–NO_2) 1441 (ali C–H def), 1334 and 1164 (S=O str of SO_2NH), 972, 896, 882 (C–N str of Ar–NO_2), 797 and 753 (Ar–C–H def), 663. anal. $C_{14}H_{19}N_3O_7S_1$ (C, H, N) calcd: 45.04, 5.09, 11.26; found: 45.14, 4.82, 11.31.

5-*N*-*n*-Butyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 25. MS (FAB): $M+H^+$ peak at *m/z* 402. ^1H NMR (200 MHz, DMSO-d_6): δ 8.30 (m, 2H, H-2′, SO_2NH), 7.96 (dd, 1H, H-6′), 7.73 (m, 2H, H-5′ CONH-5), 3.82 (m, 1H, H-2), 3.00 (m, 2H, N-CH_2-1″), 2.56 (s, 3H, Ar–CH_3), 2.11 (m, 2H, H_2-4), 1.88 (m, 1H, H_A-3), 1.70 (m, 1H, H_B-3), 1.35 (m, 2H, CH-2″), 1.25 (m, 2H, CH_2-3″), 0.85 (m, 3H, CH_3-4″). IR (KBr, cm^{-1}): 3309, 3188 (N–H str of CONH), 3026 (Ar–C–H str), 2882, 2825 (ali C–H str), 1695 (C=O str), 1558, 1517 (N=O str of Ar–NO_2), 1443 (ali C–H def), 1333 and 1162 (S=O str of SO_2NH), 973, 880 (C–N str of Ar–NO_2), 798 and 754 (Ar–C–H def). Anal. $C_{15}H_{21}N_3O_7S_1$ (C, H, N) calcd: 46.51, 5.43, 10.85; found: 46.34, 5.11, 10.90.

5-*N*-*n*-Pentyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 26. MS (FAB): $M+H^+$ peak at *m/z* 416. ^1H NMR (200 MHz, DMSO-d_6): δ 8.42 (m, 1H, SO_2NH), 7.96 (d, 1H, H-2′), 7.74 (dd, 1H, H-6′), 7.52–7.38 (m, 2H, H-5′, CONH), 3.85 (m, 1H, H-2), 3.16 (m, 2H, N–CH_2-1″), 2.56 (s, 3H, Ar–CH_3), 2.28 (m, 2H, H_2-4), 2.08 (m, 1H, H_A-3), 1.92 (m, 1H, H_B-3), 1.50–1.20 (m, 6H, CH_2-2″, CH_2-3″, CH_2-4″), 0.89 (m, 3H, CH_3-5′). IR (KBr, cm^{-1}): 3313, 3191 (N–H str of CONH), 3022 (Ar–C–H str), 2879, 2813 (ali C–H str.), 1693 (C=O str), 1572, 1519 (N=O str of Ar–NO_2), 1442 (ali C–H def.), 1332 and 1164 (S=O str of SO_2NH), 977, 897, 880 (C–N str of Ar–NO_2), 798, 753 (Ar–C–H def), 662. Anal. $C_{16}H_{23}N_3O_7S_1$ (C, H, N) calcd: 47.88, 5.73, 10.47; found: 47.99, 5.62, 10.12.

5-*N*-*n*-Hexyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 27. MS (FAB): $M+H^+$ peak at *m/z* 430. ^1H NMR (200 MHz, DMSO-d_6): δ 8.40 (m, 1H, SO_2NH), 7.95 (d, 1H, H-2′), 7.70 (dd, 1H, H-6′), 7.50–7.40 (m, 2H, H-5′, CONH), 3.83 (m, 1H, H-2), 3.14 (m, 2H, N–CH_2-1″), 2.56 (s, 3H, Ar–CH_3), 2.28 (m, 2H, H_2-4), 2.06 (m, 1H, H_A-3), 1.92 (m, 1H, H_B-3), 1.50–1.11 (m, 8H, CH_2-2″, CH_2-3″, CH_2-4″, CH_2-5″), 0.84 (m, 3H, CH_3-6″). IR (KBr, cm^{-1}): 3308, 3189 (N–H str of CONH), 3023 (Ar–C–H str), 2879, 2812 (ali C–H str), 1694 (C=O str), 1557, 1518 (N=O str of Ar–NO_2), 1444 (ali C–H def), 1331 and 1165 (S=O str of SO_2NH), 971, 898 (C–N str of Ar–NO_2), 832, 799 and 717 (Ar–C–H def), 662. Anal. $C_{17}H_{25}N_3O_7S_1$ (C, H, N) calcd: 49.16, 6.02, 10.12; found: 49.02, 5.94, 10.34.

5-*N*-*i*-Propyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 28. MS (FAB): $M+H^+$ peak at *m/z* 388. ^1H NMR (300 MHz, DMSO-d_6): 8.44 (d, 1H, *J* = 8.70, SO_2NH), 8.30 (d, 1H, *J* = 1.90, H-2′), 7.94 (dd, 1H, J_1 = 1.91, J_2 = 8.10, H-6′), 7.70 (d, 1H, *J* = 8.10, H-5′), 7.64 (m, 1H, CONH), 3.82 (m, 1H, H-2), 2.92 (m, 1H, N–CH-1″), 2.59 (s, 3H, Ar–CH_3), 2.10 (m, 2H, H_2-4), 1.87 (m, 1H, H_A-3), 1.71 (m, 1H, H_B-3), 1.06–0.91 (m, 6H, CH_3-2″, CH_3-3″). IR (KBr, cm^{-1}): 3296, 3198 (N–H str of CONH), 3024 (Ar–C–H str), 2921, 2882 (ali C–H str), 1699 (C=O str), 1573, 1549, 1519 (N=O str of Ar–NO_2), 1436 (ali C–H def), 1334 & 1164 (S=O str of SO_2NH), 975, 898 (C–N str of Ar–NO_2), 757 and 723 (Ar–C–H def), 675, 662. Anal. $C_{18}H_{27}N_3O_7S_1$ (C, H, N) calcd: 50.35, 6.29, 9.79; found: 50.32, 6.34, 10.02.

5-*N*-*i*-Butyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 29. MS (FAB): $M+H^+$ peak at *m/z* 402. ^1H NMR (300 MHz, DMSO-d_6): δ 12.72 (s, 1H, COOH), 8.47 (d, 1H, *J* = 8.73, SO_2NH), 8.30 (d, 1H, *J* = 1.84, H-2′), 7.96 (dd, 1H, J_1 = 1.90, J_2 = 8.07, H-6′), 7.77 (m, 1H, CONH), 7.70 (d, 1H, *J* = 8.16, H-5′), 3.80 (m, 1H, H-2), 2.81 (m, 2H, N–CH_2-1″), 2.59 (s, 3H, Ar–CH_3), 2.12 (m, 2H, H_2-4), 1.89 (m, 1H, H_A-3), 1.70 (m, 1H, H_B-3), 1.62

(m, 1H, CH-2″), 0.80 (m, 6H, CH_3-3″, CH_3-4″). IR (KBr, cm^{-1}): 3311, 3198 (N–H str of CONH), 3035 (Ar–C–H str), 2880, 2822 (ali C–H str), 1697 (C=O str), 1581, 1522 (N=O str of Ar–NO_2), 1434 (ali C–H def), 1332 and 1165 (S=O str of SO_2NH), 897, 881 (C–N str of Ar–NO_2), 796 and 756 (Ar–C–H def), 661. Anal. $C_{15}H_{21}N_3O_7S_1$ (C, H, N) calcd: 46.51, 5.43, 10.85; found: 46.49, 5.42, 10.39.

5-*N*-Cyclohexyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 30. MS (FAB):$M+H^+$ peak at *m/z* 428. ^1H NMR (300 MHz, DMSO-d_6): δ 12.70 (s, 1H, COOH), 8.46 (d, 1H, *J* = 8.62, SO_2NH), 8.30 (d, 1H, *J* = 1.55, H-2′), 7.96 (dd, 1H, J_1 = 1.62, J_2 = 8.09, H-6′), 7.71 (d, 1H, *J* = 8.15, H-5′), 7.66 (m, 1H, CONH), 3.79 (m, 1H, H-2), 3.43 (m, 1H, N–CH_2-1′), 2.59 (s, 3H, Ar–CH_3), 2.09 (m, 2H, H_2-4), 1.88 (m, 1H, H_A-3), 1.67–1.52 (m, 5H, H_B-3, CH-2′, CH_2-6″), 1.37–1.03 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″). IR (KBr, cm^{-1}): 3297, 3206 (N–H str of CONH), 3032 (Ar–C–H str), 2878, 2807 (ali C–H str), 1702 (C=O str), 1576, 1545, 1519 (N=O str of Ar–NO_2), 1437 (ali C–H def), 1334 and 1164 (S=O str of SO_2NH), 989, 879 (C–N str of Ar–NO_2), 797 and 754 (Ar–C–H def), 662. Anal. $C_{16}H_{23}N_3O_7S_1$ (C, H, N) calcd: 47.88, 5.73, 10.47; found: 47.79, 5.74, 10.46.

5-*N*-Benzyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 31. MS (FAB): $M+H^+$ peak at *m/z* 436. ^1H NMR (200 MHz, DMSO-d_6): δ 8.45 (m, 1H, SO_2NH), 7.95 (d, 1H, H-2′), 7.60–7.20 (m, 8H, H-6′, H-5′, ph. protons CONH), 4.26 (m, 2H, CH_{2t-}ph), 3.92 (m, 1H, H-2), 2.58 (s, 3H, Ar–CH_3), 2.36 (m, 2H, H_2-4), 2.16 (m, 1H, H_A-3), 1.99 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3301, 3197 (N–H str of CONH), 3014 (Ar–C–H str), 1695 (C=O str), 1577, 1559, 1518 (N=O str of Ar–NO_2), 1331 and 1154 (S=O str of SO_2NH), 982, 899, 882 (C–N str of Ar–NO_2), 799 and 737 (Ar–C–H def), 662, 620. Anal. $C_{18}H_{25}N_3O_7S_1$ (C, H, N) calcd: 50.58, 5.85, 9.84; found: 50.49, 5.68, 9.90.

5-*N*-Phenyl-2-(4′-methyl-3′-nitro benzenesulphonyl) glutamine 32. MS (FAB): $M+H^+$ peak at *m/z* 422. ^1H NMR (200 MHz, DMSO-d_6): δ 8.43 (m, 1H, SO_2NH), 7.98 (d, 1H, H-2′), 7.67–7.28 (m, 8H, H-6′, H-5′, ph. protons CONH), 3.94 (m, 1H, H-2), 2.58 (s, 3H, Ar–CH_3), 2.39 (m, 2H, H_2-4), 2.13 (m, 1H, H_A-3), 1.96 (m, 1H, H_B-3). IR (KBr, cm^{-1}): 3299, 3188 (N–H str of CONH), 3029 (Ar–C–H str), 1700 (C=O str), 1581, 1538, 1517 (N=O str of Ar–NO_2), 1433, 1331 and 1155 (S=O str of SO_2NH), 971, 897 (C–N str of Ar–NO_2), 751 and 715 (Ar–C–H def), 687, 661. Anal. $C_{19}H_{21}N_3O_7S_1$ (C, H, N) calcd: 52.41, 4.83, 9.65; found: 52.51, 4.89, 9.64.

5-*N*-Methyl-2-(4′-ethyl benzenesulphonyl) glutamine 33. MS (FAB): $M+H^+$ peak at *m/z* 329. ^1H NMR (200 MHz, $CDCl_3$): δ 7.81 (m, 2H, H-2′, H-6′), 7.34 (m, 2H, H-3′, H-5′), 6.19 (m, 1H, SO_2NH), 5.98 (d, 1H, CONH), 3.86 (m, 1H, H-2), 2.73 (m, 2H, CH_2 of C_2H_5ph), 2.60–2.40 (m, 2H, H_2-3), 2.20–2.10 (m, 2H, H_2-4), 1.49 (m, 3H, N–CH_3), 1.26 (t, 3H, CH_3 of C_2H_5ph). IR (KBr, cm^{-1}): 3298, 3168 (N–H str of CONH), 2919, 2883 (ali C–H str), 1676, 1581, 1443 (ali C–H def), 1320 and 1151 (S=O str of SO_2NH), 827, 784 and 724 (Ar C–H def), 690, 652. Anal. $C_{18}H_{19}N_3O_7S_1$ (C, H, N) calcd: 51.31, 4.51, 9.98; found: 51.22, 4.34, 9.98.

5-*N*-Ethyl-2-(4′-ethyl benzenesulphonyl) glutamine 34. MS (FAB): $M+H^+$ peak at *m/z* 343. ^1H NMR (200 MHz, $CDCl_3$): δ 7.70 (m, 2H, H-2′, H-6′), 7.32 (m, 2H, H-3′, H-5′), 6.19 (m, 1H, SO_2NH), 5.80 (d, 1H, CONH), 3.80 (m, 1H, H-2), 3.20 (m, 2H, N-CH_2-1″), 2.71 (m, 2H, CH_2 of C_2H_5ph), 2.50–2.35 (m, 2H, H_2-3), 2.15–2.10 (m, 2H, H_2-4), 1.28 (t, 3H, CH_3 of C_2H_5ph), 1.00 (t, 3H, CH_3-2″). IR (KBr, cm^{-1}): 3296, 3197 (N–H str of CONH), 2918, 2877 (ali C–H str), 1712 (C=O str), 1582, 1520, 1447 (ali C–H def), 1327 and 1156 (S=O str of SO_2NH), 1087, 966, 831, 783 (Ar C—H def), 679, 653. Anal. $C_{14}H_{20}N_2O_5S_1$ (C, H, N) calcd: 51.22, 6.10, 8.54; found: 51.11, 6.21, 8.37.

5-*N*-*n*-Propyl-2-(4′-ethyl benzenesulphonyl) glutamine 35. MS(FAB): $M+H^+$ peak at *m/z* 357. ^1H NMR (200 MHz, $CDCl_3$): δ 7.72 (m, 2H, H-2′, H-6′), 7.30 (m, 2H, H-3′, H-5′), 6.17 (m, 1H, SO_2NH), 5.83 (d, 1H, CONH), 3.82 (m, 1H, H-2), 3.23 (m, 2H, N–CH_2-1″), 2.73 (m, 2H, CH_2 of C_2H_5ph), 2.65–2.31 (m, 2H, H_2-3), 2.15–2.05 (m, 2H, H_2-4), 1.60–1.49 (m, 2H, CH_2-2″), 1.26 (t, 3H, CH_3 of C_2H_5ph), 0.93 (t, 3H, CH_3-3″). IR (KBr, cm^{-1}): 3311, 3191 (N–H str of CONH), 2995, 2907, 2878 (ali C–H str), 1697 (C=O str), 1585, 1555, 1442 (ali C–H def), 1328 1156 (S=O str of SO_2NH), 784 and 700 (Ar C–H def), 661. Anal. $C_{15}H_{22}N_2O_5S_1$ (C, H, N) calcd: 52.63, 6.43, 8.18; found: 52.59, 6.63, 6.78.

5-*N*-*n*-Butyl-2-(4′-ethyl benzenesulphonyl) glutamine 36. MS(FAB): $M+H^+$ peak at *m/z* 371. ^1H NMR (200 MHz, $CDCl_3$): δ 8.92 (m, 2H, H-2′, H-6′), 7.51 (m, 2H, H-3′, H-5′), 6.29 (m, 2H, SO_2NH), 5.88 (d, 1H, CONH), 3.89 (m, 1H, H-2), 3.46 (m, 2H, N–CH_2-1″), 2.90 (m, 2H, CH_2 of C_2H_5ph), 2.83–2.23 (m, 4H, H_2–4, H_2-3), 1.80–1.39 (m, 7H, CH_2-2″, CH_2-3″, CH_3 of C_2H_5ph), 1.10–0.98 (m, 3H, CH_3-4″). IR (KBr, cm^{-1}): 3306, 3190 (N–H str of CONH), 3000, 2904, 2876 (ali C–H str), 1696 (C=O str), 1584, 1553, 1441 (ali C–H def), 1327 and 1156 (S=O str of SO_2NH), 969, 784 and 704 (Ar C–H def), 662. Anal. $C_{16}H_{24}N_2O_5S_1$ (C, H, N) calcd: 53.93, 6.74, 7.86; found: 53.58, 6.71, 7.56.

5-*N*-*n*-Pentyl-2-(4′-ethyl benzenesulphonyl) glutamine 37. MS (FAB): $M+H^+$ peak at 385. ^1H NMR (200 MHz, $CDCl_3$): δ 8.90 (m, 2H, H-2′, H-6″), 7.46 (m, 2H, H-3′, H-5′), 6.31 (m, 2H, SO_2NH), 5.90 (d, 1H, CONH), 3.96 (m, 1H, H-2), 3.48 (m, 2H, N–CH_2-1″), 2.90 (m, 2H, CH_2 of C_2H_5ph), 2.81–2.11 (m, 4H, H_2-4, H_2-3), 1.85–1.35 (m, 9H, CH_2-2″, CH_2-3″, CH_2-4″, CH_3 of C_2H_5ph), 1.15–0.90 (m, 3H, CH_3-5″). IR (KBr, cm^{-1}): 3307, 3190 (N–H str of CONH), 3032 (Ar C–H str), 2902, 2876 (ali C–H str.), 1696 (C=O str), 1584, 1554, 1443 (ali C–H def.), 1329 and 1155 (S=O str of SO_2NH), 784, 704 (Ar C–H def), 662. Anal. $C_{17}H_{26}N_2O_5S_1$ (C, H, N) calcd: 55.13, 7.03, 7.57; found: 55.01, 7.21, 7.55.

5-*N*-*n*-Hexyl-2-(4′-ethyl benzenesulphonyl) glutamine 38. MS (FAB): $M+H^+$ peak at *m/z* 399. ^1H NMR (200 MHz, $CDCl_3$): δ 8.94 (m, 2H, H-2′, H-6′), 7.51 (m, 2H, H-3′,

H-5′), 6.35 (m, 2H, SO_2NH), 6.02 (d, 1H, CONH), 4.01 (m, 1H, H-2), 3.47 (m, 2H, N–CH_2-1″), 2.92 (m, 2H, CH_2 of C_2H_5ph), 2.79–2.31 (m, 4H, H_2-4, H_2-3), 1.85–1.42 (m, 8H, CH_2-2″, CH_2-3″, CH_2-4″, CH_2-5″), 1.33–1.25 (m, 3H, CH_3 of C_2H_5ph), 0.89 (m, 3H, CH_3-6″). IR KBr, cm^{-1}): 3309, 3191 (N–H str of CONH), 3005 (Ar C–H str), 2906, 2876 (ali C–H str), 1696 (C=O str), 1585, 1555, 1444 (ali C–H def), 1329 and 1156 (S=O str of SO_2NH), 826, 786, 703 (Ar C–H def), 664. Anal. $C_{18}H_{28}N_2O_5S_1$ (C, H, N) calcd: 56.25, 7.29, 7.29; found: 56.32, 7.36, 7.30.

5-*N*-*i*-Propyl-2-(4′-ethyl benzenesulphonyl) glutamine 39. MS (FAB): $M+H^+$ peak at *m/z* 357. ^1H NMR (200 MHz, $CDCl_3$): δ 8.03–7.84 (m, 2H, H-2′, H-6′), 7.65–7.4 (m, 2H, H-3′, H-5′), 6.18 (m, 1H, SO_2NH), 5.95 (d, 1H, CONH), 4.30 (m, 2H, N–CH), 4.00 (m, 1H, H-2), 2.96 (m, 2H, CH_2 of C_2H_5ph), 2.70–2.20 (m, 4H, H_2-4, H_2-3), 1.55–1.00 (m, 9H, CH_3-2″, CH_3-3″, CH_3 of C_2H_5ph). IR (KBr, cm^{-1}): 3295, 3196 (N–H str of CONH), 3033 (Ar C–H str), 2915, 2877 (ali C–H str), 1702 (C=O str), 1582, 1543, 1442 (ali C–H def), 1327 and 1156 (S=O str of SO_2NH), 825, 784, 724, 687 (Ar C–H def). Anal. $C_{19}H_{30}N_2O_5S_1$ (C, H, N) calcd: 57.29, 7.54, 7.03; found: 57.21, 7.61, 7.21.

5-*N*-Benzyl-2-(4′-ethyl benzenesulphonyl) glutamine 40. MS (FAB): $M+H^+$ peak at *m/z* 405. ^1H NMR (200 MHz, $CDCl_3$): δ 7.70 (m, 2H, H-2′, H-6′), 7.5–7.15 (m, 7H, H-3′, H-5′, ph-protons), 6.75 (m, 2H, SO_2NH), 5.85 (m, 1H, CONH), 4.45 (m, 2H, N–CH_2), 3.85 (m, 1H, H-2), 2.75 (m, 2H, CH_2 of C_2H_5ph), 2.50–2.10 (m, 4H, H_2-4, H_2-3), 1.30 (m, 3H, CH_3 of C_2H_5ph). IR (KBr, cm^{-1}): 3296, 3197 (N–H str of CONH), 2912, 2878 (ali C–H str), 1700 (C=O str), 1584, 1551, 1434 (ali C–H def), 1327 and 1152 (S=O str of SO_2NH), 981, 783, 719, 690 (Ar C–H def), 660, 618. Anal. $C_{16}H_{24}N_2O_5S_1$ (C, H, N) calcd: 53.93, 6.74, 7.86; found: 53.84, 6.68, 7.49.

5-*N*-Phenyl-2-(4′-ethyl benzenesulphonyl) glutamine 41. MS (FAB): $M+H^+$ peak at *m/z* 391. ^1H NMR (200 MHz, $CDCl_3$): δ 7.78 (m, 2H, H-2′, H-6′), 7.65–7.20 (m, 7H, H-3′, H-5′, ph-protons), 6.78 (m, 2H, SO_2NH), 5.89 (m, 1H, CONH), 3.87 (m, 1H, H-2), 2.74 (m, 2H, CH_2 of C_2H_5ph), 2.48–2.15 (m, 4H, H_2-4, H_2-3), 1.30 (m, 3H, CH_3 of C_2H_5ph). IR (KBr, cm^{-1}): 3307, 3185 (N–H str of CONH), 3034 (Ar C–H str), 2912, 2878 (ali C–H str), 1705 (C=O str), 1617, 1583, 1534, 1432 (ali C–H def), 1326, 1308 and 1152 (S=O str of SO_2NH), 826, 748 and 705 (Ar C–H def), 688. Anal. $C_{20}H_{24}N_2O_5S_1$ (C, H, N) calcd: 59.40, 5.94, 6.93; found: 59.39, 5.68, 7.21.

Pharmacology

Tumor cells. Ehrlich ascites carcinoma (EAC) cells were maintained in vivo in Swiss albino mice, by passage after every 10 days. EAC cells 9 days old were used for the screening of all final compounds **6**–**41**.

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

Screening protocol. Two groups of five mice each were kept in separate cages under identical conditions. One of these groups was served as control and other as test. Compound were dissolved or suspended (where and when necessary with 2% Tween 80) in phosphate buffered saline (PBS: pH 7.2). EAC cells were collected from the donor mouse and were suspended in sterile isotonic saline. The viable EAC cells were counted (Trypan blue indicator) under the microscope and were adjusted at 10×10^6 cells/mL. 0.1 mL of EAC cells per 10-g body weight of the animals was injected (ip) on day zero. A day of incubation was allowed for multiplication of the cells. Seven doses of compound (0.2 mmol/kg, 0.1 mL per 10-g body weight) were injected ip from the first day up to the seventh day with 24-h intervals. Control animals received only vehicle. Food and water were withheld 6 h before sacrificing the animals. On day eight all the animals were sacrificed, peritoneal fluid was collected and the cell count was noted. All the fluid in the peritoneal cavity was wiped off with absorbent cotton, the weight of the animals was taken before sacrificing and after removing the fluid from the peritoneal cavity. The difference in weight was considered as tumor weight. Mitomycin-C at a dose level of 1 mg per kg body weight was used as standard, which showed 100% inhibition.

QSAR methods

Data set and parameters. Physicochemical parameters for the substituents like Hansch–Fujita's substituent constant characterizing hydrophobicity (π), Hammet's constant (σ) representing electron withdrawing power of the substituent, steric parameter (Es), molar refractivity (MR), field effect ($\mathfrak{F}$), resonance effect ($\mathfrak{R}$), sterimol steric parameters (L, B1 and B5) were compiled from the literature.[15] Descriptors for the whole molecule like Clog P and CMR were calculated using software program ClogP of Biobyte corporation[16] (http://www.biobyte.com/). Partition coefficients (πR_5) and molar refractivity (MRR_5) for aliphatic substituents were calculated by subtracting the parent structure value from that of the respective compound. For example, π value of CH_3 group in compound **22** was obtained by subtracting Clog P value of parent structure **21** from compound **22** (i.e., 0.26–0.22 = 0.04). All the parameters are recorded in Tables 4 and 5. Antitumor activities of the compounds were subjected to multiple regression analyses.

Correlation analysis

Correlation analyses of various physicochemical parameters used in developing the equation were performed and the resultant correlation matrix is given in Table 6. Intercorrelated parameters were eliminated stepwise depending on their individual correlation with the biological activity. All possible combinations of parameters were considered.

Multiple regression analysis

Multiple regression analysis was carried out and statistical quality of the equations[17] were justified by parameters like *correlation coefficient* (r or R), *standard error*

of the estimate (SEE), *variance ratio* (F) at specified degrees of freedom (df) and constant terms of regression equations: regression coefficients and intercepts. Significance of the regression coefficients was justified by *t*-test.[18] Predictor variables with the higher *p* values were removed in developing the equation to get the more acceptable equation with statistical quality.

QSAR validation

Robustness of the QSAR model was done by cross validation[21] by predicting the activities of the compounds in test set. Test set compounds were those which were not used in developing QSAR model. In this study, six compounds were grouped as the test set and 30 compounds were used in developing a QSAR model which were labeled as the training set. Calculation power of the final QSAR equations was validated by leave-one-out (LOO)[19] prediction. Each compound of the list was deleted once from the data set and the regression equation obtained thereby was used to calculate the activity of the deleted compound. These are listed in Table 4.

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