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1,5–*N*,*N*′–Disubstituted–2–(Substituted Benzenesulphonyl)–Glutamamides as Antitumor Agents. Part 2. Synthesis, Biological Activity and QSAR Study

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1,5–*N*,*N*′–Disubstituted–2–(Substituted Benzenesulphonyl)–Glutamamides as Antitumor Agents. Part 2. Synthesis, Biological Activity and QSAR Study[#]

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Abstract

Motivation. Cancer has been described as a nitrogen trap. Tumor cells are avid glutamine consumers. Glutamine (GLN), which is a glutamic acid derivative, supplies its amide nitrogen to tumor cells in the biosynthesis of purine and pyrimidine bases. Isoglutamines, which have 1–*N*–amide instead of 5–*N*–amide and 5–COOH instead of 1–COOH in glutamine, showed antitumor activities. Thus, compounds containing both 1– and 5–amido group (*i.e*. glutamamide) may have potential antitumor activity. In continuation of our complex program for the development of new potential anticancer agents through rational drug design, 28 new 1,5–*N*,*N*′–disubstituted–2–(3′,4′–disubstituted benzenesulphonyl)–glutamamides were selected for synthesis, biological evaluation and QSAR study.

Method. These compounds were synthesized and screened against Ehrlich Ascites Carcinoma (EAC) cells in Swiss Albino mice. A QSAR study was performed in order to design leads with increased effectiveness for antitumor activity using the Hansch approach.

Results. Some of the synthesized compounds showed excellent yields and several of then were found to have good antitumor activity. The QSAR study showed that low hydrophobic or hydrophilic substitution at benzene ring, electron withdrawing group at *para* and electron donating group at *meta* position of benzene ring and less bulky alkyl substitution at aliphatic side chain may result in appreciable antitumor activity.

Conclusions. This study throws some light in the future design of this type of compounds with better anticancer activity.

Keywords. Glutamamide; antitumor agents; synthesis; screening; Hansch approach; quantitative structure–activity relationships.

1 INTRODUCTION

Glutamine (GLN), which is easily converted into glutamic acid in cells, is one of the most abundant free amino acid in the human body. This non–essential amino acid is conditionally

[#] Dedicated to Professor Haruo Hosoya on the occasion of the 65th birthday.

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essential for normal cells and essential for the growth of tumor cells. Cancerous cells are reported to utilize more nitrogen compared with normal body cells [1]. The presence of a tumor produces great change in host glutamine metabolism in such a way that host nitrogen metabolism is accommodated to the tumor–enhanced requirements of glutamine.

Cancer patients often develop muscle glutamine depletion due to uptake by tumors and chronic protein metabolism. It is one of the major (if not the major) substrate for cancer. The other substrate is glucose which is the only circulatory sugar and no cell, either normal and cancerous, can survive without it, where as, non–essential amino acid glutamine is a must for tumor cells [2]. From various studies it was evident that tumor cells are avid GLN consumers *in vivo* and *in vitro* [3–5]. After glucose, GLN is assumed to be the main energy source in tumor cells [6–9]. GLN plays a key role in tumor cell growth by supplying its amide nitrogen in the biosynthesis of other amino acids, purines and pyrimidines [6], amino sugars and coenzymes, via a family comprised of 16 amidotransferases [10] with various mechanisms [11]. A glutamine derivative (thalidomide) was recently approved as a sedative–hypnotic by the U.S. FDA and is undergoing clinical trials for different types of cancer at National Cancer Institute [12]. Some reported isoglutamine derivatives are also used as anticancer agents [13]. So it is clear that glutamine and isoglutamine derivatives act as anticancer agents.

We have previously reported [14–17] the synthesis, biological evaluation and QSAR study of some glutamine analogues as possible anticancer agents as the part of our composite program of rational drug design. In this study, 28 new 1,5–*N*,*N*′–disubstituted–2–(3′,4′–disubstituted benzenesulphonyl)–glutamamides were synthesized in continuation of our earlier work [18] where both the amido moieties of glutamine and isoglutamine were retained. These compounds possess substituents on the phenyl ring as well as aliphatic side chain at 1 and 5–positions as shown in Figure 1. The compounds were screened for antitumor activity and a QSAR study was performed to explore the substitutional requirements to optimize leads to design for the improved anticancer activity. Some of the synthesized compounds were found to have good anticancer activity and the QSAR study showed the importance of hydrophilic substitution, electron–withdrawing group at *para* and electron donating group at *meta* position of the benzene ring. Less bulky alkyl substitution also played an important role in the QSAR study. This study throws some light on the future tailoring of this type of compounds with better anticancer activity.

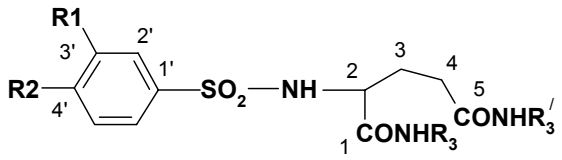


Figure 1. General structure for 1,5–*N*,*N*′–disubstituted–2–(3′,4′–disubstituted benzenesulphonyl)–glutamamides.

2 MATERIALS AND METHODS

Commercially available reagents and starting materials for the synthesis were obtained from Sigma, Aldrich, Spectrochem, Qualigens–Glaxo, Fluka, Merck, Ranbaxy and were used with no additional purification. Swiss albino mice breaded and maintained in our laboratory were used for antitumor evaluation. All the mice were kept in basal metabolic diet with water *ad libitum*. The QSAR study on the antitumor activity of the glutamamides with physicochemical parameters was performed using the Hansch approach. The statistical calculation was done with Statistica.

2.1 Synthesis

28 new 1, 5–*N*,*N*′–disubstituted–2–(3′,4′–disubstituted benzenesulphonyl)–glutamamides were synthesized and chemically characterized by IR, NMR and mass spectroscopy as well as C, H, and N composition. There were 3 different substituents at R_1 position and 2 substituents at R_2 position while R_3 was having eight different substituents.

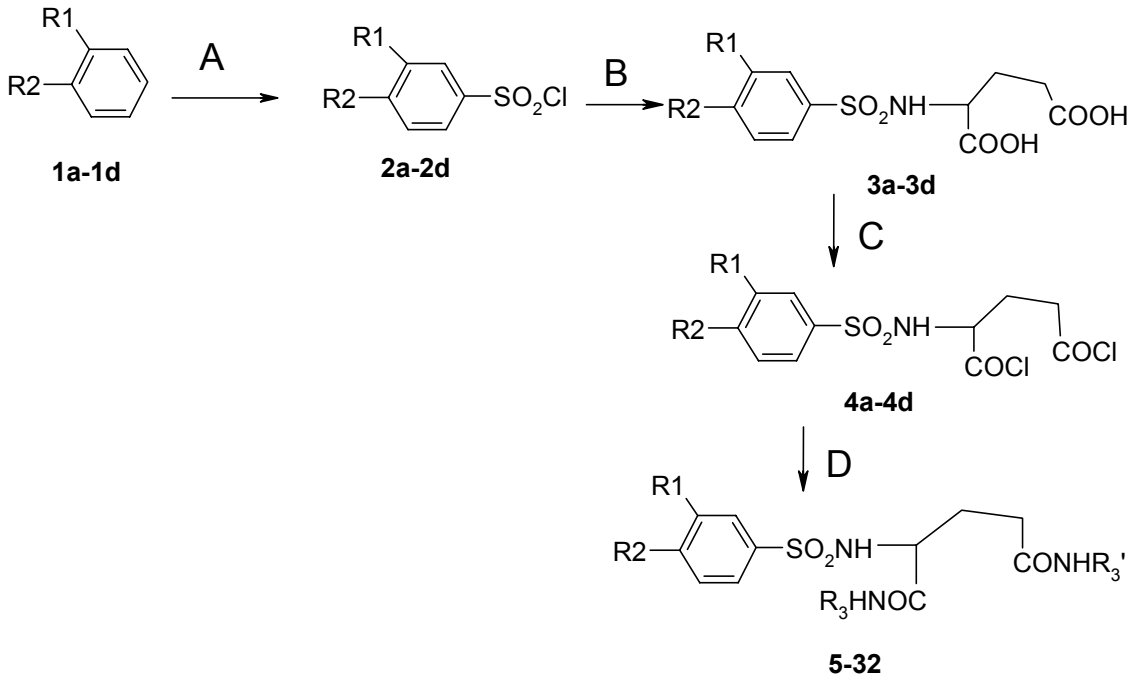
2.1.1 Chemistry

The route of the synthesis of 28 new 1,5–*N*,*N*′–disubstituted–2–(3′,4′–disubstituted–benzenesulphonyl)–glutamamides (**5–32**) is outlined in Scheme 1. The method employed involved four stages, viz., (*a*) direct introduction of a sulphonyl chloride group into the substituted benzene (**1a–1d**) nucleus by direct treatment with excess chlorosulphonic acid by the procedure described by Huntress and Carten [19] or a modification of that for 3–nitrobenzenesulphonyl chloride (**2a**); (*b*) condensation of the resulting substituted benzenesulphonyl chloride (**2a–2d**) with L–glutamic acid to yield 2–(3′, 4′–disubstituted benzenesulphonyl)–glutamic acid (**3a–3d**); (*c*) reflux of the resulting diacid (**3a–3d**) with thionyl chloride to give 2–(3′,4′–disubstituted benzenesulphonyl)–glutamic acid–1,5–dichloride (**4a–4d**); (*d*) condensation of the resulting acid dichloride (**4a–4d**) with different amines to obtain 1,5–*N*,*N*′–disubstituted–2–(3′,4′–disubstituted benzenesulphonyl)–glutamamides (**5–32**). The final products were recrystallized from dilute ethanol with charcoal treatment.

2.1.2 Synthetic procedure

Method A: 3,4–Disubstituted benzenesulphonyl chloride (2a–2d). To a mixture of substituted benzene (**1b–1d**; 0.1 mol) in chloroform (50mL), in a 250 mL round bottom flask fitted with a reflux condenser and calcium chloride guard tube, chlorosulphonic acid (sp. gr. 1.75, 0.25 mol) was added drop wise under anhydrous condition at 0–5^{0}C for 2 hrs followed by standing at room temp for 20 min. In case of 3–nitrobenzenesulphonyl chloride (**2a**), the reaction mixture of nitrobenzene (**1a;** 0.1 mol) and chlorosulphonic acid (0.25 mol) was heated slowly on an oil bath at 120–140^{0}C with occasional shaking under anhydrous condition. The shaking was continued till the evolution of hydrogen chloride gas ceased (for 2 hrs.). The reaction mass was allowed to cool to room temp. The

mixture was poured into crushed ice (500 gm.) and the precipitate (**2a–2d**) formed was filtered and extracted three times with 50mL portions of chloroform and dried over anhydrous sodium sulphate overnight. Chloroform was distilled off and the residual solid was dried in vacuum. The product was sufficiently pure which was not attempted for further purification. It was taken for the next step.



Scheme 1. Reagents: (**A**) HSO_3Cl; (**B**) L–Glutamic acid; (**C**) $SOCl_2$; (**D**) RNH_2

Method B: 2–(3′,4′–Disubstituted benzenesulphonyl)–glutamic acid (3a–3d). L–Glutamic acid (4.7g, 0.1 mol) was taken in a 250 ml conical flask fitted with a mechanical stirrer and placed on a water bath. Sodium hydroxide solution (2N) was added slowly till all the glutamic acid dissolved and the mass become distinctly alkaline to phenolphthalein. The water bath was maintained at 70–80 °°C and 3,4–disubstituted benzenesulphonyl chloride (**2a–2d**; 0.11mol) was slowly added with continuous stirring in small quantities at a time. Sodium hydroxide solution (2N) was added from time to time to keep the mass alkaline. The reaction was continued till clear homogeneous solution resulted. After the reaction was over, it was allowed to cool and filtered. The filtrate was acidified with concentrated hydrochloric acid to a pH of 2.0 and saturated with sodium chloride. The diacid (**3a–3d**) was extracted with three 50mL portions of ethyl acetate. The ethyl acetate layer was washed with brine solution (15mL) and dried overnight with anhydrous sodium sulphate. The solvent was removed by distillation to give the desired diacid (**3a–3d**).

Table 1. Physical data of the intermediate compounds (**2a–d**, **3a–d**, **4a–d**).

Cp	R_1	R_2	Molecular formula	MW	%Yield	MP (°C)
2a	NO_2	H	$C_6H_4NO_4SCl$	221.62	65	60–62
2b	Cl	Cl	$C_6H_3O_2SCl_3$	245.51	75	67–69
2c	H	Br	$C_6H_4O_2SBrCl$	255.52	64	73–75
2d	H	C_2H_5	$C_8H_9O_2SCl$	204.68	67	68–70
3a	NO_2	H	$C_{11}H_{12}N_2O_8S$	332.29	78	90–92
3b	Cl	Cl	$C_{11}H_{11}NO_6SCl_2$	356.18	73	87–89
3c	H	Br	$C_{11}H_{12}NO_6SBr$	366.19	75	77–79
3d	H	C_2H_5	$C_{13}H_{17}NO_6S$	315.34	73	110–112
4a	NO_2	H	$C_{11}H_{10}N_2O_6SCl_2$	369.18	68	105–107
4b	Cl	Cl	$C_{11}H_9NO_4SCl_4$	393.07	72	98–100
4c	H	Br	$C_{11}H_{10}NO_4SBrCl_2$	403.08	64	87–89
4d	H	C_2H_5	$C_{13}H_{15}NO_4SCl_2$	352.23	68	102–104

Table 2. Physical and biological data of the final compounds (**5–32**).

Cp	R_1	R_2	R_3	R_3'	Molecular Formula	MW	% Yield	MP (°C)	% TI (BA)	Log BA
5	NO_2	H	H	H	$C_{11}H_{14}N_4O_6S$	330.32	65	170–172	40.39	1.606
6	NO_2	H	CH_3	CH_3	$C_{13}H_{18}N_4O_6S$	358.37	67	165–167	64.29	1.808
7	NO_2	H	C_2H_5	C_2H_5	$C_{15}H_{22}N_4O_6S$	386.43	70	178–180	43.64	1.640
8	NO_2	H	*n*–C_3H_7	*n*–C_3H_7	$C_{17}H_{26}N_4O_6S$	414.48	85	156–158	18.84	1.275
9	NO_2	H	*n*–C_4H_9	*n*–C_4H_9	$C_{19}H_{30}N_4O_6S$	442.53	87	167–169	26.96	1.431
10	NO_2	H	*i*–C_3H_7	*i*–C_3H_7	$C_{17}H_{26}N_4O_6S$	414.48	67	178–180	49.29	1.693
11	NO_2	H	*n*–C_6H_{13}	*n*–C_6H_{13}	$C_{23}H_{38}N_4O_6S$	498.64	65	157–159	34.21	1.534
12	NO_2	H	C_6H_5	C_6H_5	$C_{23}H_{22}N_4O_6S$	482.51	75	175–177	53.49	1.728
13	Cl	Cl	H	H	$C_{11}H_{13}N_3O_4SCl_2$	354.21	64	142–144	42.11	1.624
14	Cl	Cl	CH_3	CH_3	$C_{13}H_{17}N_3O_4SCl_2$	382.26	53	156–158	74.85	1.874
15	Cl	Cl	C_2H_5	C_2H_5	$C_{15}H_{21}N_3O_4SCl_2$	410.32	87	157–159	59.42	1.774
16	Cl	Cl	*n*–C_3H_7	*n*–C_3H_7	$C_{17}H_{25}N_3O_4SCl_2$	438.37	76	166–168	28.70	1.458
17	Cl	Cl	*n*–C_4H_9	*n*–C_4H_9	$C_{19}H_{29}N_3O_4SCl_2$	466.42	83	163–165	37.19	1.570
18	Cl	Cl	*i*–C_3H_7	*i*–C_3H_7	$C_{17}H_{25}N_3O_4SCl_2$	438.37	69	172–174	55.47	1.744
19	Cl	Cl	*n*–C_6H_{13}	*n*–C_6H_{13}	$C_{23}H_{37}N_3O_4SCl_2$	522.53	74	173–175	28.95	1.462
20	Cl	Cl	C_6H_5	C_6H_5	$C_{23}H_{21}N_3O_4SCl_2$	506.40	67	177–179	55.04	1.741
21	H	Br	H	H	$C_{11}H_{14}N_3O_4SBr$	364.22	82	138–140	38.18	1.582
22	H	Br	CH_3	CH_3	$C_{13}H_{18}N_3O_4SBr$	392.27	83	143–145	91.52	1.962
23	H	Br	C_2H_5	C_2H_5	$C_{15}H_{22}N_3O_4SBr$	420.32	73	156–158	60.58	1.782
24	H	Br	*n*–C_3H_7	*n*–C_3H_7	$C_{17}H_{26}N_3O_4SBr$	448.38	77	153–155	69.70	1.843
25	H	Br	*n*–C_4H_9	*n*–C_4H_9	$C_{19}H_{30}N_3O_4SBr$	476.43	81	158–160	49.63	1.696
26	H	Br	*i*–C_3H_7	*i*–C_3H_7	$C_{17}H_{26}N_3O_4SBr$	448.38	91	165–167	69.09	1.839
27	H	Br	*n*–C_6H_{13}	*n*–C_6H_{13}	$C_{23}H_{38}N_3O_4SBr$	532.54	73	170–172	49.63	1.696
28	H	C_2H_5	H	H	$C_{13}H_{19}N_3O_4S$	313.37	83	120–122	38.06	1.580
29	H	C_2H_5	CH_3	CH_3	$C_{15}H_{23}N_3O_4S$	341.43	69	204–206	34.83	1.542
30	H	C_2H_5	C_2H_5	C_2H_5	$C_{17}H_{27}N_3O_4S$	369.48	71	180–182	43.87	1.642
31	H	C_2H_5	*n*–C_3H_7	*n*–C_3H_7	$C_{19}H_{31}N_3O_4S$	397.53	77	155–157	28.87	1.460
32	H	C_2H_5	*n*–C_4H_9	*n*–C_4H_9	$C_{21}H_{35}N_3O_4S$	425.59	82	198–200	58.75	1.769
Mitomycin C									100.00	2.000
Azesreine									100.00	2.000
DON									100.00	2.000

Method C: 2–(3′,4′–Disubstituted benzenesulphonyl) glutamic acid–1,5–dichloride (4a–4d). 2–(3′,4′–Disubstituted benzenesulphonyl) glutamic acid (**3a–3d**; 0.1 mol) was taken in a 250mL round bottom flask, fitted with a fork, condenser, separating funnel and calcium chloride guard tubes. The flask was heated on a steam bath and thionyl chloride (0.25 mol) was added from the separating funnel with vigorous shaking from time to time till the evolution of hydrogen chloride

gas ceased. The excess of thionyl chloride was removed by distillation on a steam bath under reduced pressure. Finally, three 10 mL portions of dry benzene was added to the flask and distilled under reduced pressure to remove the last traces of thionyl chloride. The liquid product thus obtained was sufficiently pure to proceed for the next step without further purification. Physical data of the intermediates are presented in Table 1.

Table 3. Spectral data of the final compounds (**5–32**).

Cp	Mass (FAB)	^{1}H–NMR (δ, ppm)	IR (KBr; cm^{-1})
5	M+H$^+$ peak at m/z 331	8.70 (d, 1H, H–6'), 8.30 (dd, 1H, H–4'), 7.55 (d, 1H, H–3'), 7.45 (m, 1H, SO_2NH), 7.10 (m, 2H, $CONH_2$–1), 6.71(m, 2H, $CONH_2$–5), 3.73 (m, 1H, H–2), 2.24 (m, 2H, H_2 – 4), 1.90 (m, 2H, H_2–3).	3270 (N–H str of CONH), 3028 (Ar–CH str), 1652 (C=O str), 1506 (N=O str of Ar–NO_2, asym), 1337 & 1150 (S=O str of SO_2NH), 883 (C–N str of Ar–NO_2)
6	M+H$^+$ peak at m/z 359	8.67 (d, 1H, H–6'), 8.26 (m, 1H, H–4'), 8.10 (m, 1H, SO_2NH), 7.60 (m, 1H, CONH–1), 7.43(m, 1H, CONH–5), 3.72 (m, 1H, H–2), 2.66 (m, 3H, N–CH_3 –1'"), 2.39 (m, 3H, N–CH_3 –1"), 2.19 (m, 2H, H_2 – 4), 1.85 (m, 2H, H_2 – 3).	3254(N–H str of CONH), 3021 (Ar–CH str), 1705 (C=O str), 1516 (N=O str of Ar–NO_2, asym), 1421 (Ali C–H def), 1343 & 1145 (S=O str of SO_2NH), 878 (C–N str of Ar–NO_2)
7	M+H$^+$ peak at m/z 387	8.72 (d, 1H, J =2.42, H–6'), 8.25 (dd, 1H, J_1 =2.44, J_2 =8.36, H–4'), 7.60 (d, 1H, J =8.46, H–3'), 7.50 (m, 1H, SO_2NH), 7.42 (m, 1H, CONH–1), 6.99(m, 1H, CONH–5), 3.73 (m, 1H, H–2), 3.23 (m, 2H, N–CH_2 –1'"), 2.95 (m, 2H, N–CH_2 –1"), 2.27 (m, 2H, H_2 – 4), 1.92 (m, 2H, H_2 –3), 1.13 (m, 3H, CH_3 – 2'"), 0.90 (m, 3H, CH_3 – 2").	3232 (N–H str of CONH), 3037 (Ar–C–H str), 2825 (Ali C–H str), 1627 (C=O str overlapped with N–H bend), 1511 (N=O str of Ar–NO_2,), 1427 (Ali C–H def), 1339 & 1154 (S=O str of SO_2NH), 884 (C–N str of Ar–NO_2), 734 (Ar–C–H def).
8	M+H$^+$ peak at m/z 415	8.44 (m, 1H, H–2′), 8.16–7.90 (m, 3H, H–4′, H–5′, H–6′), 7.78 (s, 1H, SO_2NH), 7.53 (m, 1H, CONH–1), 7.23 (m, 1H, CONH–5), 3.68 (m, 1H, H–2), 2.95(m, 2H, CH_2–1′′′), 2.68 (m, 2H, CH_2–1′′), 2.06 (m, 2H, H_2–4), 1.70–1.60 (m, 2H, H_2–3), 1.37(m, 2H, CH_2–2′′′), 1.14 (m, 2H, CH_2–2′′), 0.81 (t, 3H, CH_3–3′′′), 0.67 (t, 3H, CH_3–3′′)	3222 (N–H str of CONH), 3032 (Ar–CH str,), 2880 (Ali C–H str), 1695 (C=O str), 1506 (N=O str of Ar–NO_2), 1423 (Ali C–H def), 1337 & 1145 (S=O str of SO_2NH), 880 (C–N str of Ar–NO_2).
9	M+H$^+$ peak at m/z 443	8.71 (d, 1H, H–6'), 8.25 (dd, 1H, H–4'), 7.60 (d, 1H, H–3'), 7.49 (s, 1H, SO_2NH), 7.41 (m, 1H, CONH–1), 6.97 (m, 1H, CONH–5), 3.72 (m, 1H, H–2), 3.24 (m, 2H, CH_2 –1'"), 2.94(m, 2H, CH_2–1"), 2.30 (m, 2H, H_2–3), 1.94 (m, 2H, H_2–4), 1.52–1.20 (m, 8H, CH_2–2'", CH_2–3'", CH_2–2", CH_2–3"), 0.96–0.84 (m, 6H, CH_3–4'", CH_3–4").	3232(N–H str of CONH), 3031 (Ar–C–H str,), 2876 (Ali C–H str), 1628, 1505 (N=O str of Ar–NO_2, asym), 1423 (Ali C–H def), 1150 (S=O str of SO_2NH), 1113, 733 (Ar–C–H def).
10	M+H$^+$ peak at m/z 415	8.72 (d, 1H, J =1.96, H–6'), 8.26 (dd, 1H, J_1 =2.16, J_2 =8.32, H–4'), 7.49 (d, 1H, J =8.32, H–3'), 6.93 (m, 1H, SO_2NH), 6.61 (m, 1H, CONH–1), 5.52(m, 1H, CONH–5), 4.07 (m, 1H, N–CH –1'"), 3.81 (m, 1H, N–CH–1"), 3.64 (m, 1H, H–2), 2.43 (m, 1H, H_A–3), 2.27 (m, 1H, H_B–3), 1.94 (m, 2H, H_2–4), 1.25–1.16 (m, 6H, CH_3–2", CH_3–3"), 1.08–0.90 (m, 6H, CH_3–2'", CH_3–3'").	3228 (N–H str of CONH), 3030 (Ar–C–H str), 2822 (Ali C–H str), 1633 (C=O str overlapped with N–H bend), 1533, 1513 (N=O str of Ar–NO_2, asym), 1454 (Ali C–H def), 1337 & 1155 (S=O str of SO_2NH), 882 (C–N str of Ar–NO_2), 734 (Ar–C–H def).

Table 3. (Continued)

Cp	Mass (FAB)	^{1}H–NMR (δ, ppm)	IR (KBr; cm^{-1})
11	M+H$^+$ peak at m/z 499	8.72 (d, 1H, J =2.03, H–6'), 8.26 (dd, 1H, J_1 =2.18, J_2 =8.34, H–4'), 7.49 (d, 1H, J =8.31, H–3'), 7.13 (m, 1H, SO_2NH), 6.87 (m, 1H, CONH–1), 5.78(m, 1H, CONH–5), 3.69 (m, 1H, H–2), 3.25 (m, 2H, N–CH_2 –1'"), 3.04 (m, 2H, N–CH_2 –1"), 2.81 (s, 3H, Ar–CH_3), 2.36 (m, 2H, H_2–4), 1.94 (m, 2H, H_2–3), 1.52–1.21 (m, 16H, CH_2–2 ", CH_2–3 ", CH_2–4", CH_2–5", CH_2–2'", CH_2–3'", CH_2–4'", CH_2–5'"), 0.88 (m, 6H, CH_3–6", CH_3–6'").	3232 (N–H str of CONH), 3028 (Ar–C–H str), 2807 (Ali C–H str), 1633 (C=O str overlapped with N–H bend), 1504 (N=O str of Ar–NO_2), 1454 (Ali C–H def), 1313 & 1148 (S=O str of SO_2NH), 882 (C–N str of Ar–NO_2), 737 (Ar–C–H def).
12	M+H$^+$ peak at m/z 483	8.54 (s, 1H, H–2'), 7.94–7.55 (m, 3H, H–4′, H–5′, H–6'), 7.38–7.22 (m, 3H, SO_2NH, CONH–1, CONH–5), 7.12–6.99 (m, 10H, phenyl protons), 2.38 (m, 1H, H–2), 2.06–1.87 (m, 4H, H_2–3, H_2– 4).	3256 (N–H str of CONH), 3027 (Ar–CH str), 1705 (C=O str), 1506 (N=O str of Ar–NO_2), 1337 & 1166 (S=O str of SO_2NH), 883 (C–N str of Ar–NO_2), 734 (Ar C–H def)
13	M+H$^+$ peak at m/z 355	8.16 (s, 1H, SO_2NH), 7.87 (s, 1H, H–2'), 7.74 (s, 1H, H–5′), 7.58 (m, 1H, H–6'), 7.23–7.02 (m, 2H, CONH–1, CONH–5), 3.92 (m, 1H, H–2), 2.39 (m, 2H, H_2 – 4), 1.97 (m, 2H, H_2–3).	3301 (N–H str of CONH), 3049 (Ar–C–H str, assym), 1652 (C=O str), 1619, 1148 (S=O str of SO_2NH), 736, 713 (Ar–C–H def).
14	M+H$^+$ peak at m/z 383	8.22 (s, 1H, SO_2NH), 7.90 (s, 1H, H–2'), 7.82 (s, 1H, H–5′), 7.61 (m, 1H, H–6'), 7.27–7.10 (m, 2H, CONH–1, CONH–5), 3.83 (m, 1H, H–2), 2.74 (m, 3H, N–CH_3 –1'"), 2.52 (m, 3H, N–CH_3 –1"), 2.31 (m, 2H, H_2 – 4), 1.92 (m, 2H, H_2 – 3).	3371, 3032 (Ar–C–H str), 2875 (Ali C–H str), 1632 (C=O str overlapped with N–H bend), 1480, 1342 & 1158 (S=O str of SO_2NH), 1117, 757 & 702 (Ar–C–H def).
15	M+H$^+$ peak at m/z 411	8.18 (s, 1H, SO_2NH), 7.97 (s, 1H, H–2'), 7.78 (s, 1H, H–5′), 7.66 (m, 1H, H–6'), 7.32–7.18 (m, 2H, CONH–1, CONH–5), 3.76 (m, 1H, H–2), 3.37 (m, 2H, N–CH_2 –1'"), 2.97 (m, 2H, N–CH_2 –1"), 2.35 (m, 2H, H_2 – 4), 1.98 (m, 2H, H_2 –3), 1.26 (m, 3H, CH_3 – 2'"), 0.98 (m, 3H, CH_3 – 2").	3035 (Ar–C–H str), 2881 (Ali C–H str), 1634 (C=O str), 1440 (Ali C–H def), 1345 & 1164 (S=O str of SO_2NH), 1114, 765 & 722 (Ar–C–H def), 684.
16	M+H$^+$ peak at m/z 439	8.21 (s, 1H, SO_2NH), 7.93 (s, 1H, H–2'), 7.65 (s, 1H, H–5′), 7.51 (m, 1H, H–6'), 7.37–7.16 (m, 2H, CONH–1, CONH–5), 3.72 (m, 1H, H–2), 3.02 (m, 2H, CH_2–1′′′), 2.74 (m, 2H, CH_2–1′′), 2.16 (m, 2H, H_2–4), 1.72 (m, 2H, H_2–3), 1.42 (m, 2H, CH_2–2′′′), 1.19 (m, 2H, CH_2–2′′), 0.95–0.72 (m, 6H, CH_3–3′′′, CH_3–3′′).	3295, 3196 (N–H str of CONH), 2975, 2874, 1700 (C=O str of CONH), 1554, 1441, 1324 & 1154 (S=O str of SO_2NH), 981, 791, 719 (Ar–C–H def).
17	M+H$^+$ peak at m/z 467	8.71 (d, 1H, H–6'), 8.25 (dd, 1H, H–4'), 7.60 (d, 1H, H–3'), 7.49 (s, 1H, SO_2NH), 7.41 (m, 1H, CONH–1), 6.97 (m, 1H, CONH–5), 3.82 (m, 1H, H–2), 3.34 (m, 2H, CH_2 –1'"), 2.96 (m, 2H, CH_2–1"), 2.42 (m, 2H, H_2–3), 1.98 (m, 2H, H_2–4), 1.67–1.26 (m, 8H, CH_2–2'", CH_2–3'", CH_2–2", CH_2–3"), 0.99–0.81 (m, 6H, CH_3–4'", CH_3–4").	3250, 3176 (N–H str of CONH), 3035 (Ar–C–H str), 2875 (Ali C–H str), 1632, 1437 (Ali C–H def), 1328 & 1158 (S=O str of SO_2NH), 752 & 721 (Ar–C–H def).
18	M+H$^+$ peak at m/z 439	8.25 (s, 1H, SO_2NH), 7.91 (s, 1H, H–2'), 7.84 (s, 1H, H–5′), 7.68 (m, 1H, H–6'), 7.38–7.22 (m, 2H, CONH–1, CONH–5), 3.80 (m, 1H, N–CH –1'"), 3.67 (m, 1H, H–2), 3.48 (m, 1H, N–CH–1"), 2.10–1.87 (m, 4H, H_2–3, H_2– 4), 1.25–1.16 (m, 6H, CH_3–2", CH_3–3"), 1.08–0.90 (m, 6H, CH_3–2'", CH_3–3'").	3241, 3178 (N–H str of CONH), 3001 (Ar–C–H str), 2878 (Ali C–H str), 1624, 1437 (Ali C–H def), 1328 & 1152 (S=O str of SO_2NH), 748 & 714 (Ar–C–H def).

Table 3. (Continued)

Cp	Mass (FAB)	^{1}H–NMR (δ, ppm)	IR (KBr; cm^{-1})
19	M+H$^+$ peak at m/z 523	8.22 (s, 1H, SO_2NH), 7.81 (s, 1H, H–2'), 7.72 (s, 1H, H–5′), 7.60 (m, 1H, H–6'), 7.31–7.12 (m, 2H, CONH–1, CONH–5), 3.77 (m, 1H, H–2), 3.37 (m, 2H, N–CH_2 –1'"), 3.16 (m, 2H, N–CH_2 –1"), 2.95 (s, 3H, Ar–CH_3), 2.47 (m, 2H, H_2–4), 1.99 (m, 2H, H_2–3), 1.67–1.32 (m, 16H, CH_2–2 ", CH_2–3 ", CH_2–4", CH_2–5", CH_2–2'", CH_2–3'", CH_2–4'", CH_2–5'"), 0.92–0.82 (m, 6H, CH_3–6", CH_3–6'").	3232, 3170 (N–H str of CONH), 3021 (Ar–C–H str), 2897, 2877 (Ali C–H str), 1643(C=O str), 1625, 1444 (Ali C–H def), 1157 (S=O str of SO_2NH), 710, 672 (Ar–C–H def).
20	M+H$^+$ peak at m/z 507	8.18 (s, 1H, SO_2NH), 7.84 (s, 1H, H–2'), 7.74 (s, 1H, H–5′), 7.62 (m, 1H, H–6'), 7.28–7.18 (m, 2H, CONH–1, CONH–5), 7.07–6.87 (m, 10H, phenyl protons), 2.28 (m, 1H, H–2), 2.16–1.93 (m, 4H, H_2–3, H_2– 4).	3256 (N–H str of CONH), 3027 (Ar–C–H str), 2877, 1673(C=O str), 1332 & 1166 (S=O str of SO_2NH), 734 (Ar–C–H def).
21		8.12 (s, 1H, SO_2NH), 7.77–7.63 (m, 4H, H–2', H–3', H–5', H–6'), 7.52 (m, 2H, CONH–1, CONH–5), 3.87 (m, 1H, H–2), 2.43 (m, 2H, H_2 – 4), 1.82 (m, 2H, H_2–3).	3168 (N–H str of CONH), 3045 (Ar–C–H str), 2887 (Ali C–H str), 1637 (C=O str overlapped with N–H bend), 1447 (Ali C–H def), 1342 & 1168 (S=O str of SO_2NH), 752 (Ar–C–H def).
22	M+H$^+$ peak at m/z 393	8.02 (s, 1H, SO_2NH), 7.73–7.64 (m, 4H, H–2', H–3', H–5', H–6'), 7.53 (m, 2H, CONH–1, CONH–5), 3.93 (m, 1H, H–2), 2.82 (m, 3H, N–CH_3 –1'"), 2.48 (m, 3H, N–CH_3 –1"), 2.27 (m, 2H, H_2 – 4), 1.84 (m, 2H, H_2 – 3).	3179 (N–H str of CONH), 3039 (Ar–C–H str), 2897 (Ali C–H str), 1629 (C=O str overlapped with N–H bend), 1443 (Ali C–H def), 1338 & 1164 (S=O str of SO_2NH), 749 (Ar–C–H def).
23	M+H$^+$ peak at m/z 421	8.08 (s, 1H, SO_2NH), 7.83–7.74 (m, 4H, H–2', H–3', H–5', H–6'), 7.67 (m, 2H, CONH–1, CONH–5), 3.61 (m, 1H, H–2), 3.23 (m, 2H, N–CH_2 –1'"), 2.95 (m, 2H, N–CH_2 –1"), 2.27 (m, 2H, H_2 – 4), 1.92 (m, 2H, H_2 –3), 1.13 (m, 3H, CH_3 – 2'"), 0.90 (m, 3H, CH_3 – 2").	3037 (Ar–C–H str,), 2888 (Ali C–H str), 1640(C=O str overlapped with N–H bend), 1332 & 1164 (S=O str of SO_2NH), 736 (Ar–C–H def).
24	M+H$^+$ peak at m/z 449	8.18 (s, 1H, SO_2NH), 7.78–7.61 (m, 4H, H–2', H–3', H–5', H–6'), 7.52 (m, 2H, CONH–1, CONH–5), 3.43 (m, 1H, H–2), 2.87(m, 2H, CH_2–1′′′), 2.61 (m, 2H, CH_2–1′′), 2.02 (m, 2H, H_2–4), 1.71 (m, 2H, H_2–3), 1.28 (m, 2H, CH_2–2′′′), 1.19 (m, 2H, CH_2–2′′), 0.87 (t, 3H, CH_3–3′′′), 0.72 (t, 3H, CH_3–3′′).	3167 (N–H str of CONH), 3034 (Ar–C–H str), 2878(Ali C–H str), 1638, 1625, 1163 (S=O str of SO_2NH), 757 (Ar–C–H def).
25	M+H$^+$ peak at m/z 477	8.25 (s, 1H, SO_2NH), 7.65–7.52 (m, 4H, H–2', H–3', H–5', H–6'), 7.44 (m, 2H, CONH–1, CONH–5), 3.66 (m, 1H, H–2), 2.95 (m, 2H, CH_2–1′′′), 2.72 (m, 2H, CH_2–1′′), 2.11 (m, 2H, H_2–4), 1.82 (m, 2H, H_2–3), 1.52–1.20 (m, 8H, CH_2–2'", CH_2–3'", CH_2–2", CH_2–3"), 0.96–0.84 (m, 6H, CH_3–4'", CH_3–4").	3172 (N–H str of CONH), 3026 (Ar–C–H str), 2817 (Ali C–H str), 1638 (C=O str overlapped with N–H bend), 1442 (Ali C–H def), 1163 (S=O str of SO_2NH), 753 (Ar–C–H def).
26	M+H$^+$ peak at m/z 449	8.25 (s, 1H, SO_2NH), 7.65–7.52 (m, 4H, H–2', H–3', H–5', H–6'), 7.44 (m, 2H, CONH–1, CONH–5), 3.89 (m, 1H, N–CH –1'"), 3.78 (m, 1H, N–CH–1"), 3.52 (m, 1H, H–2), 2.56 (m, 2H, H_2–3), 1.87 (m, 2H, H_2–4), 1.32–1.20 (m, 6H, CH_3–2", CH_3–3"), 1.11–0.96 (m, 6H, CH_3–2'", CH_3–3'").	3270 (N–H str of CONH), 3028 (Ar–C–H str), 2876 & 2815 (Ali C–H str), 1662(C=O str), 1641 (N–H bend of CONH), 1441 (Ali C–H def), 1339 & 1169 (S=O str of SO_2NH), 711 (Ar–C–H def).

Table 3. (Continued)

Cp	Mass (FAB)	^{1}H–NMR (δ, ppm)	IR (KBr; cm^{-1})
27	M+H$^+$ peak at m/z 533	8.20 (s, 1H, SO_2NH), 7.76–7.61 (m, 4H, H–2', H–3', H–5', H–6'), 7.52 (m, 2H, CONH–1, CONH–5), 3.77 (m, 1H, H–2), 3.32 (m, 2H, N–CH_2 –1'"), 3.10 (m, 2H, N–CH_2 –1"), 2.48 (m, 2H, H_2–4), 1.98 (m, 2H, H_2–3), 1.66–1.42 (m, 16H, CH_2–2 ", CH_2–3 ", CH_2–4", CH_2–5", CH_2–2'", CH_2–3'", CH_2–4'", CH_2–5'"), 0.96 (m, 6H, CH_3–6", CH_3–6'").	3166 (N–H str of CONH), 3026 (Ar–C–H str), 2875 & 2810 (Ali C–H str), 1641 (C=O str overlapped with N–H bend), 1442 (Ali C–H def), 1337 & 1163 (S=O str of SO_2NH), 799 & 753 (Ar–C–H def).
28	M+H$^+$ peak at m/z 314	8.22–8.04 (m, 4H, H–2', H–3', H–5', H–6'), 7.24 (d, 1H, SO_2NH), 7.06 (t, 1H, CONH–1), 6.83 (t, 1H, CONH–5), 3.72 (m, 1H, H–2), 2.48 (m, 2H, H_2 – 4), 1.97 (m, 2H, H_2–3).	3234 (N–H str of CONH), 3032 (Ar–C–H str), 2876 & 2805 (Ali C–H str), 1626 (C=O str overlapped with N–H bend), 1436 (Ali C–H def), 1335 & 1162 (S=O str of SO_2NH), 796 & 755 (Ar–C–H def).
29	M+H$^+$ peak at m/z 342	8.16–7.98 (m, 4H, H–2', H–3', H–5', H–6'), 7.16 (d, 1H, SO_2NH), 7.06 (t, 1H, CONH–1), 6.64 (t, 1H, CONH–5), 3.67 (m, 1H, H–2), 2.72 (m, 3H, N–CH_3 –1'"), 2.38 (m, 3H, N–CH_3 –1"), 2.22 (m, 2H, H_2 – 4), 1.64 (m, 2H, H_2 – 3).	3165 (N–H str of CONH), 3031 (Ar–C–H str), 1625 (C=O str), 1337 & 1161 (S=O str of SO_2NH), 1103, 694 (Ar–C–H def).
30	M+H$^+$ peak at m/z 370	8.12–7.94 (m, 4H, H–2', H–3', H–5', H–6'), 7.12 (d, 1H, SO_2NH), 5.71 (t, 1H, CONH–1), 6.74 (t, 1H, CONH–5), 3.61 (m, 1H, H–2), 3.32 (m, 2H, N–CH_2 –1'"), 3.10 (m, 2H, N–CH_2 –1"), 2.90 (m, 2H, CH_2 of C_2H_5Ph), 2.42 (m, 2H, H_2–4), 2.02 (m, 2H, H_2–3), 1.42 (m, 3H, CH_3 of C_2H_5Ph), 1.06–0.92 (m, 6H, CH_3–2 ", CH_3–2'").	3184 (N–H str of CONH), 3013 (Ar–C–H str), 1679 (C=O str), 1335 & 1162 (S=O str of SO_2NH), 689 (Ar–C–H def).
31	M+H$^+$ peak at m/z 398	8.06–7.90 (m, 4H, H–2', H–3', H–5', H–6'), 7.22 (d, 1H, SO_2NH), 7.11 (t, 1H, CONH–1), 6.78 (m, 1H, CONH–5), 3.71 (m, 1H, H–2), 2.92(m, 2H, CH_2–1‴), 2.72 (m, 2H, CH_2–1″), 2.14 (m, 2H, H_2–4), 1.84 (m, 2H, H_2–3), 1.36 (m, 2H, CH_2–2‴), 1.26 (m, 2H, CH_2–2″), 0.93 (t, 3H, CH_3–3‴), 0.82 (t, 3H, CH_3–3″).	3165 (N–H str of CONH), 3032 (Ar–C–H str), 2823 (Ali C–H str), 1644 (C=O str overlapped with N–H bend), 1442 (Ali C–H def), 1335 & 1163 (S=O str of SO_2NH), 752 (Ar–C–H def).
32	M+H$^+$ peak at m/z 426	8.10–7.98 (m, 4H, H–2', H–3', H–5', H–6'), 7.18 (m, 1H, SO_2NH), 7.01 (t, 1H, CONH–1), 6.84 (t, 1H, CONH–5), 3.74 (m, 1H, H–2), 3.32 (m, 2H, CH_2 –1'"), 2.84(m, 2H, CH_2–1"), 2.36 (m, 2H, H_2–3), 1.97 (m, 2H, H_2–4), 1.47–1.22 (m, 8H, CH_2–2'", CH_2–3'", CH_2–2", CH_2–3"), 0.88–0.74 (m, 6H, CH_3–4'", CH_3–4").	3186 (N–H str of CONH), 3027 (Ar–C–H str), 2878 (Ali C–H str), 1444 (Ali C–H def), 1334 & 1164 (S=O str of SO_2NH), 754 (Ar–C–H def).

Method D: 1,5–*N*,*N*′–Disubstituted–2–(3′,4′–disubstituted benzenesulphonyl)–glutam–amides (5–32). 2–(3′,4′–Disubstituted benzenesulphonyl)–glutamic acid–1,5–dichloride (**4a–4d**; 0.1 mol) was taken in a 100mL round bottom flask. 50mL of dry benzene, the required amount of corresponding amine (0.5 mol) was added, and refluxed for 30 min. The excess of amine and benzene were removed by vacuum distillation. After evaporating the amine the residue was cooled in ice, filtered, washed with cold water and dried. Glutamamides (**5–32**) were recrystallized using ethanol as the solvent to get pure compound for chemical characterization and biological study. Physical data of these glutamamides (**5–32**) are presented in Table 2.

Table 4. Microanalysis data of the final compounds (**5–32**).

Cp	C		H		N	
	Calc.	Found	Calc.	Found	Calc.	Found
5	40.00	39.87	4.27	4.17	16.96	17.06
6	43.57	43.25	5.06	4.93	15.63	15.54
7	46.62	46.92	5.74	5.87	14.50	14.39
8	49.26	48.99	6.32	6.08	13.52	13.43
9	51.57	51.37	6.83	6.75	12.66	12.56
10	49.26	49.09	6.32	6.21	13.52	13.68
11	55.40	55.02	7.68	7.61	11.24	11.07
12	57.25	57.63	4.60	4.49	11.61	11.48
13	37.30	37.17	3.70	3.59	11.86	11.82
14	40.85	41.04	4.48	4.53	10.99	11.05
15	43.91	44.12	5.16	5.07	10.24	10.17
16	46.58	46.21	5.75	5.82	9.59	9.49
17	48.93	49.15	6.27	6.21	9.01	8.93
18	46.58	46.87	5.75	5.67	9.59	9.65
19	52.87	52.77	7.14	7.08	8.04	7.98
20	54.55	54.68	4.18	4.13	8.30	8.38
21	36.27	36.10	3.87	3.93	11.54	11.59
22	39.80	39.94	4.63	4.56	10.71	10.67
23	42.86	42.68	5.28	5.22	10.00	9.93
24	45.54	45.68	5.84	5.92	9.37	9.43
25	47.90	48.07	6.35	6.28	8.82	8.75
26	45.54	45.36	5.84	5.91	9.37	9.30
27	51.87	51.67	7.19	7.24	7.89	7.94
28	49.83	50.01	6.11	6.04	13.41	13.42
29	52.77	52.63	6.79	6.87	12.31	12.38
30	55.26	55.07	7.37	7.31	11.37	11.35
31	57.40	57.49	7.86	7.93	10.57	10.62
32	59.27	59.48	8.29	8.21	9.87	9.93

2.1.3 Characterization

Melting points were determined on Mel–Temp II melting point apparatus and were uncorrected. IR, ^{1}HNMR and mass spectra were recorded on Buck M500 IR spectrometer, Bruker 300 MHz spectrometer, with TMS as the internal standard and JEOL–JMS–SX–102 mass spectrometer at 70 eV respectively. The spectral data of the final compounds are shown in Table 3. Elemental analyses were performed from vacuum dried samples on a 2400 Series II microanalyzer of Perkin–Elmer and were within 0.4 % of theoretical values. Microanalysis data are shown in the Table 4.

2.2 Biological Evaluation

The glutamamides (**5–32**) were biologically evaluated for their possible antitumor activity in search of potential antitumor agents.

2.2.1 Antitumor screening

Antitumor activity of the final compounds (**5–32**) was evaluated against Ehrlich Ascites Carcinoma (EAC) cells in Swiss Albino mice (10 weeks old; 18–20gm). The compounds were administered intraperitoneally at a dose level of 0.2 mmol /kg body weight. EAC cells were

maintained *in vivo* in Swiss Albino mice, by passaging every 10 days. EAC cells of 9 day old were used for the screening. % Tumor weight inhibition (%TI) was taken as biological activity (BA) data.

2.2.2 Screening protocol

Control and test groups of five mice each were kept in separate cages under identical conditions. Compounds were suspended in phosphate buffer solution (PBS: pH 7.2) using 2% Tween 80. 0.1mL of EAC cells (10×10^6 cells/mL of isotonic saline) per 10gm body weight of the animals was injected (i.p.) on day 1. Seven doses of the final test compound (0.2 mmol/kg body weight, 0.1mL/10gm body weight) were injected i.p. from day 2 to day 8 in the test group. Control animals received only vehicle. After a 6 h fasting condition, on day 9, all animals were sacrificed. The fluid in the peritoneal cavity was wiped off with absorbent cotton. The weight of animals was taken before sacrifice and after removing the fluid from the peritoneal cavity. The difference in weight was considered as tumor weight. In comparison with the control, the % TI of the test compound was calculated. Mitomycin C as well as Azeserine and DON at a dose level of 1mg, 10mg and 10mg per kg body weight respectively were used as standard, which showed 100% inhibition at all times at all cases. The antitumor activity data is presented in Table 2.

2.3 QSAR Methodology

In order to find out the chemical and structural requirements for an efficient antitumor activity, a QSAR study was performed using the Hansch approach.

2.3.1 Data set and parameters

The physicochemical parameters for the substituents, namely the hydrophobic parameter π and the Hammett electronic constants σ_m and σ_p, representing the electron withdrawing power of a substituent, were collected from literature [20–22]. All parameters are presented in Table 5.

Table 5. Physicochemical parameters used for the QSAR study [20–22].

Substituents	π	σ_p	σ_m
NO_2	–0.28		0.71
Cl	0.71	0.23	0.37
Br	0.86	0.23	
C_2H_5	1.02	–0.15	

2.3.2 Statistical analysis

In order to obtain the QSAR models, correlation analysis and multiple regression analysis were carried out with the software Statistica. For the validation of the QSAR equations we used the leave–one–out (LOO) method [23] where each compound was deleted once from the data set and the regression equation obtained thereby was used to predict the activity of the deleted compound.

3 RESULTS AND DISCUSSION

The route of the synthesis of the compounds is economically quite viable. The yields of all synthesized compounds are good enough and all the final compounds are solid crystalline substances and can be easily characterized and biologically evaluated. The antitumor activity of all final compounds was evaluated *in vivo* against Ehrlich Ascites Carcinoma (EAC) in Swiss Albino mice. The percentage tumor weight inhibition (%TI) has been taken as biological activity (BA) and is presented in Table 2. Two compounds have a good antitumor activity, *i.e*. **14** and **22**.

Table 6. Correlation matrix for the physicochemical parameters and biological activity.

Variable	$\Sigma \pi_R$	σ_p	σ_m	I_1	log BA
$\Sigma \pi_R$	1.000	0.451	–0.592	0.010	0.194
σ_p	0.451	1.000	–0.121	0.009	0.352
σ_m	–0.592	–0.121	1.000	–0.038	–0.294
I_1	0.010	0.009	–0.038	1.000	–0.484
log BA	0.194	0.352	–0.294	–0.484	1.000

In order to design leads with increased antitumor activity, these compounds (**5–32**) were used in a QSAR study using the Hansch approach [16,24]. The physicochemical parameters used in QSAR analysis are presented in Table 5. The correlation analysis of these parameters was performed and the correlation matrix is presented in Table 6. Independent descriptors were used in a multiple linear regression analysis against the biological activity in log scale (*i.e*. log BA) to develop the QSAR:

$$\log BA = 1.796(\pm 0.068) - 0.067(\pm 0.053)\ \Sigma\pi_R + 0.438(\pm 0.178)\ \sigma_p - 0.234(\pm 0.108)\ \sigma_m - 0.158(\pm 0.048)\ I_1 \quad (1)$$

$n = 28 \quad r = 0.6864 \quad \%EV = 47.12 \quad F(4,23) = 5.12 \quad p < 0.0042 \quad SEE = 0.1233$

In this equation $\Sigma\pi_R$ is the sum of the hydrophobic constants of the substituents on the benzene ring, while σ_p and σ_m are the electronic constants for *para* and *meta* substituents on benzene ring. I_1 is the indicator variable for *n*–propyl, *n*–butyl and *n*–hexyl at position R_3 and R_3'. The value of I_1 is 1 for the presence of *n*–propyl, *n*–butyl and *n*–hexyl, otherwise is 0. Among the statistical parameters *n* represents number of data points, *r* is multiple correlation coefficient, EV is the explained variance, F is the ratio between the variances of observed and calculated activities, SEE is the standard error of estimate. The equation shows that a positive value of σ_p would increase the biological activity while a positive value reduces the activity. However, Eq. (1) explains 47.12% of the variance. Step by step deletion of the compound **32** as well as that of **32** and **21** gave Eq. 2 and Eq. 3, respectively. These compounds were deleted on the basis of their large residuals (predicted value – observed value). The compounds **32** and **21** supposedly did not fit the model, due to different mechanism of action for these two compounds or other unknown reasons.

$$\log BA = 1.785\ (\pm 0.060) - 0.087\ (\pm 0.047)\ \Sigma\pi_R + 0.620\ (\pm 0.168)\ \sigma_p - 0.209\ (\pm 0.095)\ \sigma_m - 0.190\ (\pm 0.043)\ I_1 \quad (2)$$

$n = 27 \quad DC=\mathbf{32} \quad r = 0.7783 \quad \%EV = 60.57 \quad F(4,22) = 8.45 \quad p < 0.0003 \quad SEE = 0.1078$

$$\log BA = 1.841\ (\pm 0.052) - 0.119\ (\pm 0.040)\ \Sigma\pi_R + 0.740\ (\pm 0.144)\ \sigma_p - 0.290\ (\pm 0.082)\ \sigma_m - 0.213\ (\pm 0.036)\ I_1 \quad (3)$$

$n = 26$ DC = **32**,**21** $r = 0.8605$ %EV = 74.05 $F(4,21) = 14.98$ $p < 0.00001$ SEE = 0.0892

where **DC** is the deleted compound. The values in the parenthesis were significant at 95 percent level. Eq. (3) was found to be predictive and statistically of good quality which explained 74.05% of variances in activity data. Predicted and residuals activities for Eq. (3) are presented in Table 7. Predictive value of Eq. (3) was confirmed by the LOO method [23]. LOO predicted values are also presented in Table 7.

Table 7. Observed, predicted and LOO predicted anticancer activities from Eq. (3).[a]

Cp	Obs	Pred	Res	L Pred	Cp	Obs	Pred	Res	L Pred
5	1.606	1.669	–0.063	1.680	**18**	1.744	1.736	0.008	1.734
6	1.808	1.669	0.139	1.645	**19**	1.462	1.523	–0.061	1.537
7	1.640	1.669	–0.029	1.674	**20**	1.741	1.736	0.005	1.735
8	1.275	1.456	–0.181	1.499	**22**	1.962	1.909	0.053	1.895
9	1.431	1.456	–0.025	1.462	**23**	1.782	1.909	–0.127	1.943
10	1.693	1.669	0.024	1.665	**24**	1.843	1.697	0.146	1.658
11	1.534	1.456	0.078	1.438	**25**	1.696	1.697	–0.001	1.697
12	1.728	1.669	0.059	1.659	**26**	1.839	1.909	–0.070	1.928
13	1.624	1.736	–0.112	1.755	**27**	1.696	1.697	–0.001	1.697
14	1.874	1.736	0.138	1.711	**28**	1.580	1.609	–0.029	1.619
15	1.774	1.736	0.038	1.729	**29**	1.542	1.609	–0.067	1.633
16	1.458	1.523	–0.065	1.538	**30**	1.642	1.609	0.033	1.598
17	1.570	1.523	0.047	1.512	**31**	1.460	1.397	0.063	1.363

[a] Cp –compound, Obs – observed activity, Pred – predicted activity, Res – residual, L Pred – LOO prediction

The negative contribution of $\Sigma\pi_R$ revealed that hydrophobic interactions at the benzene ring were detrimental to the activity or hydrophilic substitution might be advantageous to the activity. The positive coefficient of σ_p indicates that electron–withdrawing groups in the *para* position of the benzene ring might help to the activity and the negative coefficient of σ_m shows that electron–withdrawing groups in the *meta* position were detrimental to the activity or electron–donating group might increase the activity. The negative contribution of indicator parameter I_1 revealed that bulky alkyl substitution in positions 1 and 5 of the aliphatic side chain of glutamamide might be disadvantageous to high biological activity. The QSAR study showed that less bulky alkyl substitution were preferred for the high biological activity.

4 CONCLUSIONS

Compounds having electron–withdrawing groups in *para*, electron–donating groups in *meta*, low hydrophobic or hydrophilic substitution at the benzene ring and less bulky alkyl substitution in the aliphatic side chain of glutamamide may increase the antitumor activity. This QSAR study gives some important information about the structural requirements of the glutamamide moiety for increased antitumor activity.

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5 REFERENCES

[1] M. Medina, Glutamine and cancer, *J. Nutr*. **2001**, 131, 2539S–2542S.
[2] F. Kallinowski, S. Runkel, H. P. Fortmeyer, H. Forster and P. Vaupel, L–glutamine: a major substrate for tumor cells in vivo?, *J. Cancer Res. Clin. Oncol*. **1987**, *113*, 209–215.
[3] Y. J. Kang, Y. Feng, and E. L. Hatcher, Glutathione stimulates A549 cell proliferation in glutamine–deficient culture: the effect of glutamate supplementation, *J. Cell. Physiol*. **1994**, *161*, 589–596.
[4] Y. J. Kang, Buthionine sulfoximine spares intracellular glutamate: a possible mechanism for cell growth stimulation, *Cell. Mol. Biol. Res*. **1993**, *39*, 675–684.
[5] G. Ollenschlager, A. Simmel, and E. Roth, Availability of glutamine from peptides and acetylglutamine for human tumor–cell cultures, *Metabolism* **1989**, *38*, S40–S42.
[6] C. Costa, J. F. Huneau, and D. Tome, Characteristics of L–glutamine transport during Caco–2 cell differentiation, *Biochem. Biophys. Acta* **2000**, *1509*, 95–102.
[7] C. L. Collins, M. Wasa, W. W. Souba, and S. F. Abcouwer, Determinants of glutamine dependence and utilization by normal and tumor–derived breast cell lines, *J. Cell. Physiol*. **1998**, *176*, 166–178.
[8] S. Sukemori, and K. Sugimura, Inhibition of cultured leukemia cell growth by enhanced adriamycin cytotoxicity with reduction of glutamine or asparagine level in medium, *Cancer Biochem. Biophys*. **1994**, *14*, 99–105.
[9] F. Devreker, R. M. Winston, and K. Hardy, Glutamine improves human preimplantation development in vitro, *Fertil. Steril*. **1998**, *69*, 293–299.
[10] H. Zalkin, and J. L. Smith, Enzymes utilizing glutamine as an amide donor, *Adv. Enzymol. Relat. Areas Mol. Biol*. **1998**, *72*, 87–144.
[11] V. L. Rath, L. F. Silvian, B. Beijer, B. S. Sproat and T. A. Steitz, How glutaminyl–tRNA synthetase selects glutamine, *Structure* **1998**, *6*, 439–449.
[12] S. Singhal and J. Mehta, Thalidomide in cancer, *Biomed. Pharmacother*. **2002**, *56*, 4–12.
[13] H. Tsuda, A. Iemura, M. Sata, M. Uchida, K. Yamana and H. Hara, Inhibitory effect of antineoplaston A10 and AS2–1 on human hepatocellular carcinoma, *Kurume Med. J*. **1996**, *43*, 137–147.
[14] S. K. Purkayastha, T. Jha, D. K. Pal and A. U. De, Possible Antineoplastic Agents Part XIII– Synthesis, Biological Evaluation and QSAR Studies of some 1–(Substituted Benzenesulphonyl)–5–Oxopyrrolidine–2–Carboxylic Acid Derivatives, *Anticancer Drug Des*. **1993**, *8*, 95–100.
[15] K. Srikanth, Ch. A. Kumar, D. Goswami, A. U. De, and T. Jha, Quantitative Structure Activity Relationship (QSAR) studies of some substituted benzene sulphonyl glutamines as tumor suppressors, *Ind. J. Biochem. Biophys*. **2001**, *38*, 120–123.
[16] T. Jha, Rational Design of Glutamine analogs as possible anticancer agents; in: *Frontiers of Pharmaceutical Technology*, Eds. J. K. Gupta and T. Jha, Jadavpur University, Calcutta, 1999, pp. 87–95.
[17] K. Srikanth, Ch. A. Kumar, B. Ghosh and T. Jha, Synthesis, Screening and Quantitative Srtucture–Activity Relationship (QSAR) Study on Some Glutamine Analogues for Possible Anticancer Activity, *Bioorg. Med. Chem*. **2002**, 10, 2119–2131.
[18] K. Srikanth, B. Debnath, and T. Jha, Synthesis, Biological Evaluation and QSAR Study on Antitumor Activity of 1,5–N,N′–Disubstituted–2–(Substituted Benzenesulphonyl) Glutamamides, *Bioorg. Med. Chem*. **2002**, *10*, 1841–1854.
[19] E. H. Huntress and F. H. Carten, Identification of organic compounds: I. Chlorosulfonic acid as a reagent for the Identification of Aryl Halides, *J. Am. Chem. Soc*. **1940**, *62*, 511–514.
[20] C. Hansch, A. Leo and D. Hoekman, *Exploring QSAR: Hydrophobic*, *electronic*, *and steric constants*, American Chemical Society, Washington, DC, 1995.
[21] C. Hansch and A. Leo, *Exploring QSAR: Fundamentals and applications in chemistry and biology*, American Chemical Society, Washington, DC, 1995.
[22] C. Hansch and A. Leo, *Substituent constants for correlation analysis in chemistry and biology*, Wiley & Sons, New York, 1979.
[23] C. Hansch, J. Y. Fukunaga, and P. Y. Jow, Quantitative structure–activity relationships of antimalarial and dihydrofolate reductase inhibition by quinazolines and 5–substituted benzyl–2,4–diaminopyrimidines, *J. Med. Chem*. **1977**, *20*, 96–102.
[24] K. Srikanth, B. Debnath, and T. Jha, QSAR Study on Adenosine Kinase Inhibition of Pyrrolo [2,3–d]pyrimidine Nucleoside Analogs using Hansch Approach, *Bioorg. Med. Chem. Lett*. **2002**, *12*, 899–902.