Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 44 (2009) 70–82
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Possible anticancer agents: QSAR analogs of glutamamide: Synthesis and pharmacological activity of 1,5-*N*,*N*′-disubstituted-2-(substituted benzenesulphonyl) glutamamides

Soma Samanta, Sk. Mahasin Alam, Parthasarathi Panda[1], Tarun Jha*

Division of Medicinal and Pharmaceutical Chemistry, Department of Pharmaceutical Technology, P.O. Box 17020, Jadavpur University, Kolkata 700032, India

Received 30 January 2007; received in revised form 10 March 2008; accepted 13 March 2008
Available online 27 March 2008

Abstract

Based on our earlier QSAR prediction, a series of designed QSAR analogs of 1,5-*N*,*N*′-disubstituted-2-(substituted benzenesulphonyl) glutamamides were synthesized as possible anticancer agents. Inhibitions of tumor cell proliferation of the compounds were tested in tumor cell line IMR-32. Anticancer activities of these compounds were also evaluated on Swiss Albino mice against Ehrlich Ascites Carcinoma (EAC) cells. Tumor weight inhibition and tumor cell inhibition were considered as anticancer activity parameters. QSAR analysis of these compounds was performed on the basis of a set of descriptors like physicochemical, topological, quantum chemical and DRAGON whole molecular descriptors. The study showed that the increase of length of substituent at R_2 position and the increase of dipole moment of the molecule decrease the anticancer activity of these compounds, presence of bromine atom at R_3 position and hydrophilic substitution at R_2 position are advantageous to the activity. Nucleophilic attack at atom number 14 is advantageous and electrophilic attack at atom number 15 is detrimental to anticancer activity. Atom number 2 is important and may be involved in dispersive interactions of the compounds with enzymes. The results offer an opportunity for further tailoring of these analogs for an active member.

Keywords: Glutamamide; Anticancer activity; IMR-32; EAC; QSAR; Cluster analysis

1. Introduction

Glutamine is essential for rapidly proliferating tumor tissues as it is an important nutrient for rapidly growing cells [1–7]. Glutamine plays a key role in tumor cell growth by supplying its amide nitrogen atom in the biosyntheses of other amino acids, purines, pyrimidines, amino sugars and co-enzymes via a family comprised of 16 amidotransferase enzymes [8,9]. For cellular growth, glutamine provides multiple contributions by participating in protein, purine and pyrimidine metabolisms. Amide nitrogen of glutamine is utilized in a number of transfer reactions. Glutamine transports almost one third of the circulating amino acids and nitrogen; it is also the principle carrier of nitrogen from the skeletal muscles to the visceral organs [10]. As a principle metabolic fuel for the rapidly dividing cells including enterocytes, colonocytes, fibroblasts, lymphocytes, macrophages, neutrophils and tumor cells, it is as efficient as glucose. Through amidotransferase reactions, the amide nitrogen of glutamine serves as the precursor for the biosynthesis of many nitrogen-containing compounds and nucleotides such as pyridine nucleotide coenzymes, purines, pyrimidines, glucosamines, DPN and asparagine [10–13]. Amide nitrogen of glutamine is the precursor of nitrogen atom of carbamyl phosphate in many tissues [13]. It is reported that 2-(phenylacetyl) isoglutamine, which contains 1-*N*-amide, deserves anticancer

* Corresponding author. Tel.: +91 33 2414 6666x2495 (o), +91 33 24383814 (r), +91 9433187443 (m); fax: +91 33 2414 6927.
E-mail address: tjjupharm@yahoo.com (T. Jha).
[1] Present address: School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 634759, Singapore.

0223-5234/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2008.03.008

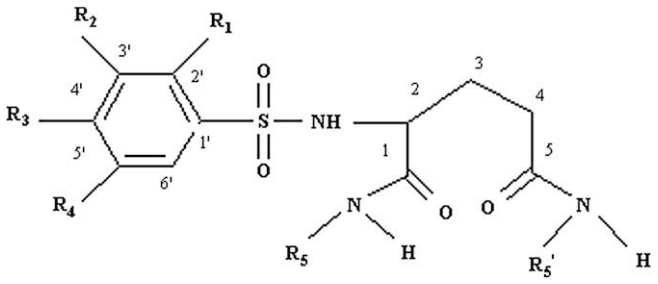

Fig. 1. General structure of 1,5-*N*,*N*′-disustituted-2-(substituted benzenesulphonyl) glutamamides (**26**–**57**).

activity [14]. As a part of the composite programme of drug design and discovery [15–20], our aim was to develop new compounds having possible anticancer activity. We have chosen to synthesize molecules that are structural variants of glutamine and/or glutamic acid as these variants may inhibit enzyme glutaminase and/or glutamine synthetase as well as glutamine receptor(s). Based on our earlier QSAR studies [15–19] on 1,5-*N*,*N*′-disubstituted-2-(substituted benzenesulphonyl) glutamamides, 32 new analogs of these glutamamides were designed and selected for synthesis and biological evaluation. The selected QSAR analogs were synthesized, characterized and biologically evaluated using in vitro and in vivo method. As these compounds were designed on the basis of earlier QSAR studies, these are termed as QSAR analogs to differentiate these analogs from the earlier synthesized non-designed analogs of Refs. [15–19]. To further explore the chemical structural features of these analogs, QSAR studies were performed on these newly synthesized QSAR analogs of 1,5-*N*,*N*′-disubstituted-2-(substituted benzenesulphonyl) glutamamides. The general structure of 1,5-*N*,*N*′-disubstituted-2-(substituted benzenesulphonyl) glutamamides is shown in Fig. 1.

Pharmacological evaluation showed that some of these compounds have good anticancer activities. The pharmacological activity data has been used as a preliminary biological activity dataset for QSAR study to find out the structural requirements of these compounds to improve the anticancer activity. For QSAR study, a set of descriptors like physicochemical, topological, quantum chemical and DRAGON whole molecular descriptors are used to build significant models. The study was performed on all these glutamamide analogs using percentage tumor weight inhibition (TWI) and tumor cell inhibition (TCI) as biological activity parameters.

2. Materials and method

2.1. Chemistry

2-(Substituted benzenesulphonyl)glutamic acids (**14**–**19**) were prepared by condensing L-glutamic acid (**13**) with substituted benzenesulphonyl chlorides (**7**–**12**) [21] which were synthesized by chlorosulphonylation of substituted benzenes (**1**–**6**) [22]. 2-(Substituted benzenesulphonyl)glutamic acid dichlorides (**20**–**25**) were obtained by treating the diacids (**14**–**19**) with thionyl chloride and title compounds were prepared by amination of acid chlorides (**20**–**25**) according to the methods described earlier [15–19]. The structures of the synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectra as well as by elemental analyses. Melting points of all synthesized compounds were determined in capillary tubes with a Mel-Temp Electrothermal apparatus and are uncorrected. The ^{1}H NMR spectra were determined with a BRUKER DRX 300 MHz and referenced to the solvent. Chemical shifts are expressed in ppm and coupling constants (J) are in Hz, (s) singlet, (d) doublet, (t) triplet, (m) multiplet. Position of hydrogen described in ^{1}H NMR interpretation are as per general structure (Fig. 1) and substitution at R_5 position are represented by the superscript ‘″’ (double dash) and the same for R_5' position are represented by the superscript ‘‴’ (triple dash). FAB+ mass spectra were obtained on a JEOL JMS-SX-102 mass spectrometer. *m*-Nitrobenzyl alcohol (MNBA) was used as the matrix (M^+) which showed the M + 1 peak at 154, 2M + 1 peak at 307. Elemental analyses were performed by 2400 Series-II CHN analyzer of Perkin–Elmer and gave combustion values for C, H, N within 0.4% of the theoretical values. Reactions were monitored by analytical thin layer chromatography performed on silica gel G plates.

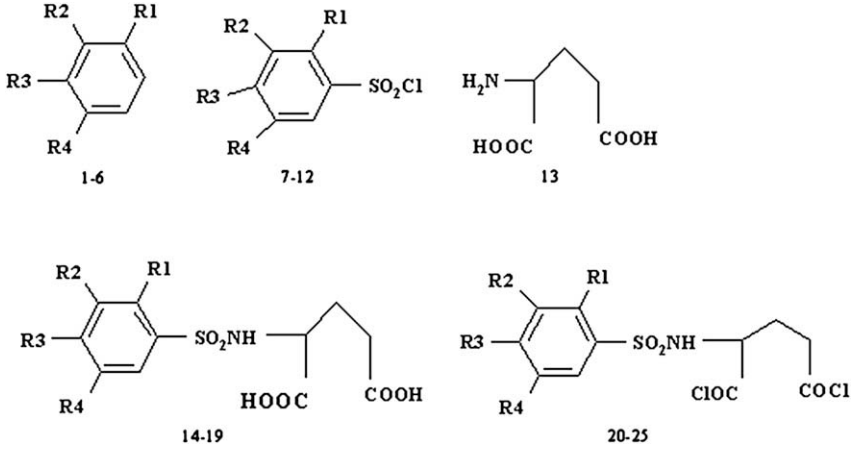

Fig. 2. General structures of starting materials and intermediates.

Table 1
Physical data for the intermediate compounds

Cpd[a]	R_1	R_2	R_3	R_4	Mp (°C)[b]	% Yield	Molecular formula
1	Cl	H	H	Cl	Liquid	80.0	$C_6H_3Cl_3O_2S$
2	H	H	Br	H	73–75	78.5	$C_6H_4BrClO_2S$
3	H	Cl	Cl	H	67–69	75.1	$C_6H_3Cl_3O_2S$
4	Cl	H	H	CH_3	Liquid	85.0	$C_7H_6Cl_2O_2S$
5	H	H	*t*-C_4H_9	H	56–58	72.1	$C_{10}H_{13}ClO_2S$
6	CH_3	H	CH_3	H	Liquid	70.6	$C_8H_9ClO_2S$
14	Cl	H	H	Cl	162–163	90.0	$C_{11}H_{11}Cl_2NO_6S$
15	H	H	Br	H	148–150	81.2	$C_{11}H_{12}BrNO_6S$
16	H	Cl	Cl	H	87–89	60.6	$C_{11}H_{11}Cl_2NO_6S$
17	Cl	H	H	CH_3	131–133	87.0	$C_{12}H_{14}ClNO_6S$
18	H	H	*t*-C_4H_9	H	162–164	44.2	$C_{15}H_{21}NO_6S$
19	CH_3	H	CH_3	H	109–111	68.2	$C_{13}H_{17}NO_6S$

[a] Compound number.
[b] Melting point.

The spots were located keeping the TLC plates in iodine chamber. All title compounds as well as the starting material L-glutamic acid showed optical activity when observed in a polarimeter.

The general structure of the starting materials and intermediates are given in Fig. 2.

Physical data of substituted benzenesulphonyl chlorides (**1–6**) and substituted glutamic acids (**14–19**) as well as spectral data of all substituted glutamic acids (**14–19**) are given in Tables 1 and 2, respectively.

Physical data and spectral data of all final compounds are given in Tables 3 and 4, respectively.

2.2. Pharmacological evaluation

Synthesized title compounds are pharmacologically evaluated by in vitro and in vivo method.

2.2.1. Inhibition of tumor cell proliferation in tumor cell line IMR-32

Anticancer activity of all final compounds was assessed against tumor cell line IMR-32 (Human Neuroblastoma Cell Line). Maintenance of the cell lines was carried out using RPMI-1640 media, the subcultures of the cell lines were maintained for optimum (<P25) and maintained in deep freezer (−80 °C). The cell counts were performed using trypan blue dye exclusion method on Neubaur slide (heamocytometer). MTT method [23] was used to study the cytotoxic action of these compounds on IMR-32 (Human Neuroblastoma Cell Line). Medium with DMSO concentration less than 1% was taken as the control and the cell viabilities in the test drug treated group was expressed as % viability with respect to

Table 2
Mass, IR and proton NMR spectroscopic as well as CHN analysis data of the intermediate compounds (**14–19**)

Cpd	Mass (FAB)	IR (KBr, cm^{-1})	^1H NMR (300 MHz, DMSO-d_6)	C, H, N % calcd/found		
				C	H	N
14	$M+H^+$peak at *m/z* 356	2972, 2946, 2850 (ali C–H str.), 1698 (C=O str. of COOH), 1432, 1387 (S=O str. of SO_2NH, asymmetric), 1160 (S=O str. of SO_2NH, symmetric), 1048 (Ar–Cl), 1582 (Ar C=C);	δ 7.95 (d, 2H, H-3′, H-4′), δ 7.86 (d, 1H, H-6′), δ 7.57 (s, 1H, SO_2NH), δ 3.68 (m, 1H, H-2), 2.93 (t, 2H, H-4), δ 2.83 (d, 2H, H-3)	37.09/37.18	3.11/3.25	3.93/3.99
15	$M+H^+$peak at *m/z* 366	2980, 2862 (ali C–H str.), 1705 (C=O str. of COOH), 1442,1380 (S=O str. of SO_2NH, asymmetric), 1158 (S=O str. of SO_2NH, symmetric), 1085 (Ar–Br), 1590, 1510 (Ar C=C),	δ 7.76 (d, 2H, H-2′, H-6′), δ 7.54 (d, 1H, H-3′, H-5′), δ 7.46 (s, 1H, SO_2NH), δ 3.72 (m, 1H, H-2), δ 2.86 (m, 2H, N–CH_2-1‴), δ 2.53 (m, 2H, N–CH_2-1″), δ 2.33 (m, 2H, H_2-4), δ 1.58 (m, 2H, H_2-3)	36.08/36.00	3.30/3.39	3.83/3.81
16	$M+H^+$peak at *m/z* 356	2981, 2888 (ali C–H str.), 1712 (C=O str. of COOH), 1430,1379 (S=O str. of SO_2NH, asymmetric), 1162 (S=O str. of SO_2NH, symmetric), 1048 (Ar–Cl), 1592, 1521 (Ar C=C)	δ 7.82 (d, 1H, H-2′), δ 7.65 (d, 1H, H-5′), δ 7.71 (d, 1H, H-6′), δ 7.59 (s, 1H, SO_2NH), δ 3.14 (m, 1H, H-2), δ 3.18 (m, 2H, N-CH_2-1‴), δ 3.64 (m, 2H, N-CH_2-1″), δ 2.35 (m, 2H, H_2-4), δ 1.93 (m, 2H, H_2-3)	37.09/37.15	3.11/3.21	3.93/3.84
17	$M+H^+$peak at *m/z* 335	2934, 2898 (ali C–H str.), 1721 (C=O str. of COOH), 1431,1380 (S=O str. of SO_2NH, asymmetric), 1188 (S=O str. of SO_2NH, symmetric), 1045 (Ar–Cl), 1498, 1532 (Ar C=C)	δ 7.75 (d, 2H, H-3′, H-4′), δ 7.66 (d, 1H, H-6′), δ 7.49 (s, 1H, SO_2NH), δ 3.83 (m, 1H, H-2), δ 2.72–1.30 (m, 4H, H_2-3, H_2-4)	42.93/42.99	4.20/4.25	4.17/4.32
18	$M+H^+$peak at *m/z* 343	2946, 2890 (ali C–H str.), 1698 (C=O str. of COOH), 1428, 1389 (S=O str. of SO_2NH, asymmetric), 1168 (S=O str. of SO_2NH, symmetric), 1492, 1526 (Ar C=C)	δ 7.51 (d, 2H, H-2′, H-6′), δ 7.62 (d, 1H, H-3′, H-5′), δ 7.43 (s, 1H, SO_2NH), δ 3.83 (m, 1H, H-2), δ 2.65 (m, 2H, H_2-4), δ 1.38 (m, 2H, H_2-3)	52.46/52.44	6.16/6.28	4.08/3.99
19	$M+H^+$peak at *m/z* 315	2952, 2892 (ali C–H str.), 1645 (C=O str. of COOH), 1425, 1386 (S=O str. of SO_2NH, asymmetric), 1158 (S=O str. of SO_2NH, symmetric), 1496, 1522 (Ar C=C)	δ 7.48 (d, 1H, H-3′), δ 7.29 d, 1H, H-5′), δ 7.37 (d, 1H, H-6′), δ 7.49 (s, 1H, SO_2NH), δ 3.77 (m, 1H, H-2), δ 2.64 (m, 2H, H_2-4), δ 1.59 (m, 2H, H_2-3)	49.51/49.48	5.43/5.45	4.44/4.40

Table 3
Physical data of final compounds and % inhibition of viable tumor cells of these synthesized compounds at the concentration of 60 μg/ml against IMR-32 cell line

Cpd[a]	R_1	R_2	R_3	R_4	R_5/R_5'	Mp (°C)	% Yield	Molecular formula	% Inhibition of viable cell (60 μg/ml)
26	Cl	H	H	Cl	C_2H_5	165–166	55.0	$C_{15}H_{21}Cl_2N_3O_4S$	12.9
27	Cl	H	H	Cl	*i*-C_3H_7	160–162	90.0	$C_{17}H_{25}Cl_2N_3O_4S$	16.7
28	Cl	H	H	Cl	*n*-C_4H_9	124–126	73.7	$C_{19}H_{29}Cl_2N_3O_4S$	15.8
29	Cl	H	H	Cl	*i*-C_4H_9	134–136	89.0	$C_{19}H_{29}Cl_2N_3O_4S$	20.4
30	Cl	H	H	Cl	C_5H_{11}	108–110	80.0	$C_{21}H_{33}Cl_2N_3O_4S$	12.2
31	Cl	H	H	Cl	*n*-C_6H_{13}	98–99	85.0	$C_{23}H_{37}Cl_2N_3O_4S$	12.4
32	Cl	H	H	Cl	C_6H_5	178–179	70.0	$C_{23}H_{21}Cl_2N_3O_4S$	22.9
33	Cl	H	H	Cl	$CH_2C_6H_5$	210–212	69.6	$C_{25}H_{25}Cl_2N_3O_4S$	13.4
34	H	H	Br	H	*i*-C_4H_9	213–215	75.4	$C_{19}H_{30}BrN_3O_4S$	17.1
35	H	H	Br	H	*c*-C_6H_{11}	248–250	79.2	$C_{23}H_{34}BrN_3O_4S$	18.4
36	H	H	Br	H	C_6H_5	218–220	80.0	$C_{23}H_{34}BrN_3O_4S$	10.0
37	H	H	Br	H	$CH_2C_6H_5$	208–210	54.8	$C_{25}H_{26}BrN_3O_4S$	ND[b]
38	H	Cl	Cl	H	C_5H_{11}	209–210	64.7	$C_{21}H_{33}Cl_2N_3O_4S$	20.8
39	H	Cl	Cl	H	*c*-C_6H_{11}	216–217	69.7	$C_{23}H_{33}Cl_2N_3O_4S$	24.0
40	H	Cl	Cl	H	$CH_2C_6H_5$	245–247	65.2	$C_{25}H_{25}Cl_2N_3O_4S$	11.4
41	Cl	H	H	CH_3	H	138–140	60.2	$C_{12}H_{16}ClN_3O_4S$	25.8
42	Cl	H	H	CH_3	C_2H_5	155–157	65.5	$C_{16}H_{24}ClN_3O_4S$	31.8
43	Cl	H	H	CH_3	*n*-C_3H_7	110–112	70.9	$C_{18}H_{28}ClN_3O_4S$	14.9
44	Cl	H	H	CH_3	*i*-C_3H_7	198–200	64.0	$C_{18}H_{28}ClN_3O_4S$	29.8
45	Cl	H	H	CH_3	*n*-C_4H_9	60–65	58.9	$C_{20}H_{32}ClN_3O_4S$	18.0
46	Cl	H	H	CH_3	*i*-C_4H_9	126–128	72.0	$C_{20}H_{32}ClN_3O_4S$	ND[b]
47	Cl	H	H	CH_3	C_5H_{11}	95–97	59.8	$C_{22}H_{36}ClN_3O_4S$	14.7
48	Cl	H	H	CH_3	*n*-C_6H_{13}	104–105	66.6	$C_{24}H_{40}ClN_3O_4S$	11.3
49	Cl	H	H	CH_3	*c*-C_6H_{11}	214–215	71.6	$C_{24}H_{36}ClN_3O_4S$	49.7
50	Cl	H	H	CH_3	C_6H_5	180–181	55.0	$C_{24}H_{24}ClN_3O_4S$	ND[b]
51	Cl	H	H	CH_3	$CH_2C_6H_5$	160–162	58.7	$C_{26}H_{28}ClN_3O_4S$	ND[b]
52	H	H	*t*-C_4H_9	H	H	179–181	63.2	$C_{15}H_{23}N_3O_4S$	ND[b]
53	H	H	*t*-C_4H_9	H	C_2H_5	83–85	69.7	$C_{19}H_{31}ClO_4S$	58.4
54	H	H	*t*-C_4H_9	H	*i*-C_3H_7	115–117	61.4	$C_{21}H_{35}N_3O_4S$	65.6
55	CH_3	H	CH_3	H	H	189–191	73.6	$C_{13}H_{17}NO_6S$	28.2
56	CH_3	H	CH_3	H	*n*-C_4H_9	144–145	88.3	$C_{21}H_{35}N_3O_4S$	46.1
57	CH_3	H	CH_3	H	*n*-C_6H_{13}	135–137	65.4	$C_{25}H_{43}N_3O_4S$	66.0
58			Cisplatin			–	–	–	98.2

[a] Compound number.
[b] Not determined due to poor solubility in appropriate solvent.

DMSO. Cisplatin (3 μg/ml) was used as the standard. The % inhibition of viable cells is given in Table 3.

2.2.2. Evaluation of anticancer activity by in vivo method

We faced great difficulties in dissolving these compounds in the solvent when performing the in vitro method. We also used in vivo method which is standardized in our laboratory [15–19,24]. All the final compounds were screened according to the earlier reported procedure [15–19]. In the present study, Mitomycin C as well as Azaserin and DON (1 mg/kg body weight) in sterile phosphate buffers (pH 7.2) were used as standards which showed 100% inhibition. Mitomycin C was chosen as the universal standard and azaserin, DON were used as the specific standard drugs to compare the activity of the test compounds. In vivo results of the final compounds are shown in Table 5.

2.3. QSAR methodology

QSAR studies of these 32 newly synthesized 1,5-*N*,*N*′-di-substituted-2-(substituted benzenesulphonyl) glutamamides (**26–57**) were performed using percentage tumor weight inhibition (%TWI) and tumor cell inhibition (%TCI) as the biological activity parameters. All these activities are calibrated to the logarithmic scale and are listed in Table 5.

2.3.1. Dataset and parameters

A large set of various descriptors like physicochemical, topological, geometrical, semi-empirical quantum chemical descriptors, were calculated by using software like HyperChem Release 7.0 Pro Package [25], DRAGON [26], Mouse [27] etc. The physicochemical parameters like hydrophobic constant (π), molar refractivity (MR), steric parameter (E_s), Verloop STERIMOL parameters like L, B_1, B_5 were collected from the literature [28]. Refractotopological state atom (RTSA) indices ($\mathcal{R}$) [29–31] were calculated using computer programme Mouse [27] developed in our laboratory. Before calculation, the atoms of the molecules were numbered arbitrarily (shown in Fig. 3) keeping the serial number of atoms same in all the molecules.

Semi-empirical quantum chemical descriptors were calculated by Hyperchem Release 7.0 Pro Package [25] according

Table 4
Mass, IR and proton NMR spectroscopic as well as CHN analysis data of the final compounds (**26**–**57**)

Cpd	Mass (FAB)	IR (KBr, cm^{-1})	^1H NMR (300 MHz, DMSO-d_6)	C, H, N % calcd/found		
				C	H	N
26	$M+H^{+\bullet}$peak at *m/z* 410	3321, 3299 (N−H str. of CONH), 3091 (Ar C−H str.), 2977, 2929 (ali C−H str.), 1649 (C=O str. of CONH), 1423,1377 (S=O str. of SO_2NH, asymmetric), 1163 (S=O str. of SO_2NH, symmetric), 1043 (Ar-Cl), 1452 (Ar C=C), 1564, 1548 (N−H bend of CONH)	δ 7.76 (d, 2H, H-3′, H-4′), δ 7.86 (d, 1H, H-6′), δ 7.47 (s, 1H, SO_2NH), δ 7.35 (m, 1H, CONH-1), δ 7.20 (m, 1H, CONH-5), δ 3.68 (m, 1H, H-2), δ 3.12 (m, 2H, N−CH_2-1‴), δ 2.18 (m, 2H, H_2-4), δ 1.78 (m, 2H, H_2-3), 1.16 (3H, CH_2-2‴), 1.02 (m, 3H, CH_3-2″)	43.91/43.56	5.16/5.02	10.24/10.05
27	$M+H^{+\bullet}$peak at *m/z* 438	3341, 3298 (N−H str. of CONH), 3080 (Ar C−H str.), 2975, 2925 (ali C−H str.), 1429, 1375 (S=O str. of SO_2NH, asymmetric), 1166 (S=O str. of SO_2NH, symmetric), 1641 (C=O str. of CONH), 1552, 1550 (N−H bend of CONH), 1452 (Ar C=C), 825 (Ar-disubstitution), 1043, 1099 (Ar−Cl),	δ 7.86 (d, 2H, H-3′, H-4′), δ 7.92 (d, 1H, H-6′), δ 7.53 (s, 1H, SO_2NH), δ 7.23 (m, 1H, CONH-1), δ 7.18 (m, 1H, CONH-5), δ 3.78 (m, 1H, H-2), δ 3.42 (m, 1H, N−CH-1‴), δ 3.28 (m, 1H, N−CH-1″), δ 2.66 (m, 2H, H_2-4), δ 1.78 (m, 2H, H_2-3), 1.16 (m, $2H_2$, H-4), 1.24−1.14 (m, 6H, CH_3-2′ CH_3-3″), 0.96−0.82 (m, 6H, CH_3-2‴, CH_3-3‴)	46.58/46.23	5.75/5.51	9.59/9.49
28	$M+H^{+\bullet}$peak at *m/z* 446	3376, 3296 (N−H str. of CONH), 3057 (Ar C−H str.), 2944, 2846 (ali C−H str.), 1324 (S=O str. of SO_2NH, asymmetric), 1158 (S=O str. of SO_2NH, symmetric), 1645 (C=O str. of CONH), 1553, 1548 (N−H bend of CONH)	δ 7.75 (d, 2H, H-3′, H-4′), δ 7.90 (d, 1H, H-6′), δ 7.49 (s, 1H, SO_2NH), δ 7.29 (m, 1H, CONH-1), δ 7.19 (m, 1H, CONH-5), δ 3.70 (m, 1H, H-2), δ 3.11 (m, 2H, N−CH_2-1‴), δ 3.53 (m, 2H, N-CH_2-1″), δ 2.17 (m, 2H, H_2-4), δ 1.79 (m, 2H, H_2-3), 1.36−1.06 (m, 8H, CH_2-2″, CH_2-3″, CH_2-2‴, CH_2-3‴), 0.98−0.81(m, 6H, CH_3-4″, CH_3-4‴)	48.93/48.62	6.27/6.11	9.01/8.92
29	$M+H^{+\bullet}$peak at *m/z* 466	3299 (N−H str. of CONH), 3097 (Ar C−H str.), 2958, 2927 (ali C−H str.), 1649 (C=O str. of CONH), 1452, 1375 (S=O str. of SO_2NH, asymmetric), 1164 (S=O str. of SO_2NH, symmetric), 1554 (N−H bend. of CONH) 1452 (Ar C=C), 823 (Ar-disubstitution), 1041 (Ar−Cl)	δ 7.58 (d, 2H, H-3′, H-4′), δ 7.56 (d, 1H, H-6′), δ 7.26 (s, 1H, SO_2NH), δ 7.10 (m, 1H, CONH-1), δ 7.09 (m, 1H, CONH-5), δ 2.62 (m, 1H, H-2), δ 2.46 (m, 2H, N−CH_2-1‴), δ 2.44 (m, 2H, N−CH_2-1″), δ 1.93 (m, 2H, H_2-4), δ 1.56 (m, 2H, H_2-3), 1.37−0.41 (m, 14H, CH-2″, CH-2‴, CH_3-3″, CH_3-3‴, CH_3-4″, CH_3-4‴)	48.93/48.71	6.27/6.13	9.01/8.96
30	$M+H^{+\bullet}$peak at *m/z* 494	3326 (N−H str. of CONH), 3045 (Ar C−H str.), 2966, 2892 (ali C−H str.), 1446, 1375 (S=O str. of SO_2NH, asymmetric), 1168 (S=O str. of SO_2NH, symmetric), 1651 (C=O str. of CONH), 1562, 1549 (N−H bend of CONH)	δ 8.03 (d, 2H, H-3′, H-4′), δ 8.02 (d, 1H, H-6′), δ 7.50 (s, 1H, SO_2NH), δ 7.49 (m, 1H, CONH-1), δ 7.30 (m, 1H, CONH-5), δ 3.16 (m, 1H, H-2), δ 3.13 (m, 2H, N−CH_2-1‴), δ 3.86 (m, 2H, N−CH_2-1″), δ 2.21 (m, 2H, H_2-4), δ 1.42 (m, 2H, H_2-3), 1.4−1.25 (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‴), 0.98−0.94(m, 6H, CH_3-6″, CH_3-6‴)	51.01/50.88	6.73/6.69	8.50/8.46
31	$M+H^{+\bullet}$peak at *m/z* 522	3294 (N−H str. of CONH), 3280 (N−H str. of SO_2NH), 3097 (Ar C−H str.), 2986, 293 (ali C−H str.), 1643, 1560, 1491 (ali C−H def.), 1315 (S=O str. of SO_2NH, asymmetric), 1153 (S=O str. of SO_2NH, symmetric), 1076, 984, 897 (Ar−C−H def.)	δ 7.73 (d, 2H, H-3′, H-4′), δ 7.72 (d, 1H, H-6′), δ 7.71 (s, 1H, SO_2NH), δ 7.20 (m, 1H, CONH-1), δ 7.10 (m, 1H, CONH-5), δ 2.91 (m, 1H, H-2), δ 3.53 (m, 2H, N−CH_2-1‴), δ 3.74 (m, 2H, N−CH_2-1″), δ 2.32 (m, 2H, H_2-4), δ 2.00 (m, 2H, H_2-3), 1.72−1.03 (m, 20H, CH_2-2″, CH_2-3″, CH_2-4″, CH_2-5″, CH_2-6″, CH_2-2‴, CH_2-3‴, CH_2-4‴, CH_2-5‴, CH_2-6‴), 0.99−0.95 (m, 6H, CH_3-7″, CH_3-7‴)	52.87/52.77	7.14/7.00	8.04/7.97
32	$M+H^{+\bullet}$peak at *m/z* 506	3323, 3274 (N−H str. of CONH), 3062 (Ar C−H str.), 2923 2854 (ali C−H str.), 1658 (C=O str. of CONH), 1444, 1379 (S=O str. of SO_2NH, asymmetric), 1150 (S=O str. of SO_2NH, symmetric), 1498 (Ar C=C), 1041 (Ar−Cl), 815 (Ar-disubstitution)	δ 7.84 (d, 2H, H-3′, H-4′), δ 7.79 (d, 1H, H-6′), δ 7.76 (s, 1H, SO_2NH), δ 7.28 (m, 1H, CONH-1), δ 7.15 (m, 1H, CONH-5), δ 2.95 (m, 1H, H-2), δ 2.36 (m, 2H, H_2-4), δ 2.08 (m, 2H, H_2-3, δ 7.19−6.91 (m, 10H, phenyl proton)	54.55/54.49	4.18/4.12	8.30/8.21.

33	$M+H^{+\bullet}$peak at *m/z* 534	3321, 3264 (N−H str. of CONH), 3059 (Ar C−H str.), 2942, 2896 (ali C−H str.), 1663 (C═O str. of CONH), 1448, 1389 (S═O str. of SO_2NH, asymmetric), 1156 (S═O str. of SO_2NH, symmetric), 1494 (Ar C═C), 1045 (Ar−Cl), 813 (Ar-disubstitution)	δ 7.55 (d, 2H, H-3′, H-4′), δ 7.69 (d, 1H, H-6′), δ 7.62 (s, 1H, SO_2NH), δ 7.38 (m, 1H, CONH-1), δ 7.25 (m, 1H, CONH-5), δ 2.85 (m, 1H, H-2), δ 3.66 (m, 2H, N−CH_2-1‴), δ 3.74 (m, 2H, N−CH_2-1″), δ 2.46 (m, 2H, H_2-4), δ 2.18 (m, 2H, H_2-3, δ 7.09−6.89 (m, 10H, phenyl proton)	C (calc.56.18, found 56.02)	H (Calc.4.71, found 4.68)	(Calc.7.86, found 7.66)
34	$M+H^{+\bullet}$peak at *m/z* 476	3311, 3236 (N−H str. of CONH), 3087 (Ar C−H str.), 2956, 2929 (ali C−H str.), 1641 (C═O str. of CONH.), 1434, 1388 (S═O str. of SO_2NH), 1454 (Ar C═C), 1166 (S═O str. of SO_2NH), 1554, 1467 (N−H bend of CONH), 649, 754 (Ar-monosubstitution), 1087 (Ar−Br)	δ 7.46 (d, 2H, H-2′, H-6′), δ 7.44 (d, 1H, H-3′, H-5′), δ 7.36 (s, 1H, SO_2NH), δ 7.34 (m, 1H, CONH-1), δ 7.26 (m, 1H, CONH-5), δ 3.82 (m, 1H, H-2), δ 2.76 (m, 2H, N−CH_2-1‴), δ 2.73 (m, 2H, N−CH_2-1″), δ 2.63 (m, 2H, H_2-4), δ 1.50 (m, 2H, H_2-3), 1.35−0.57 (m, 14H, CH-2″, CH-2‴, CH_3-3″, CH_3-3‴, CH_3-4″, CH_3-4‴)	47.9/47.86	6.35/6.32	8.82/8.73
35	$M+H^{+\bullet}$peak at *m/z* 528	3305, 3234 (N−H str. of CONH), 3062 (Ar C−H str.), 2925, 2852 (ali C−H str.), 1652, 1641 (C═O str. of CONH), 1573, 1539 (NH bend of CONH), 1468, 1388 (S═O str. of SO_2NH, asymmetric), 1168 (S═O str. of SO_2NH, symmetric), 1087 (Ar−Br.)	δ 7.56 (d, 2H, H-2′, H-6′), δ 7.64 (d, 1H, H-3′, H-5′), δ 7.38 (s, 1H, SO_2NH), δ 7.23 (m, 1H, CONH-1), δ 7.21 (m, 1H, CONH-5), δ 3.72 (m, 1H, H-2), δ 1.67−1.00 (m, 22H, cyclohexyl proton), δ 2.43 (m, 2H, H_2-4), δ 1.48 (m, 2H, H_2-3)	52.27/52.21	6.48/6.39	7.95/7.92.
36	$M+H^{+\bullet}$peak at *m/z* 516	3394 (N−H str. of CONH), 3280 (N−H str. of SO_2NH), 3067 (Ar C−H str.), 2956, 2832 (ali C−H str.), 1643, 1560, 1490 (ali C−H def.), 1315 (S═O str. of SO_2NH, asymmetric), 1153 (S═O str. of SO_2NH, symmetric), 1086, 984, 890 (Ar−C−H def.)	δ 7.62 (d, 2H, H-2′, H-6′), δ 7.56 (d, 1H, H-3′, H-5′), δ 7.48 (s, 1H, SO_2NH), δ 7.33 (m, 1H, CONH-1), δ 7.28 (m, 1H, CONH-5), δ 3.82 (m, 1H, H-2), δ 7.11−6.95 (m, 10H, phenyl proton), δ 2.42 (m, 2H, H_2-4), δ 1.52 (m, 2H, H_2-3)	53.49/53.31	4.29/4.22	8.14/8.10
37	$M+H^{+\bullet}$peak at *m/z* 544	3319, 3252 (N−H str. of CONH), 3092 (Ar C−H str.), 2961, 2893 (ali C−H str.), 1646 (C═O str. of CONH.), 1454, 1398 (S═O str. of SO_2NH), 1456 (Ar C═C), 1172 (S═O str. of SO_2NH), 1576, 1487 (N−H bend of CONH), 650, 761 (Ar-monosubstitution), 1091 (Ar−Br)	δ 7.56 (d, 2H, H-2′, H-6′), δ 7.64 (d, 1H, H-3′, H-5′), δ 7.38 (s, 1H, SO_2NH), δ 7.43 (m, 1H, CONH-1), δ 7.22 (m, 1H, CONH-5), δ 3.72 (m, 1H, H-2), δ 2.66 (m, 2H, N−CH_2-1‴), δ 2.83 (m, 2H, N−CH_2-1″), δ 2.53 (m, 2H, H_2-4), δ 1.48 (m, 2H, H_2-3), 7.17−6.86 (m, 10H, phenyl proton)	54.95/54.86	5.16/5.11	7.69/7.55
38	$M+H^{+\bullet}$peak at *m/z* 494	3312, 3233 (N−H str. of CONH), 3089 (Ar C−H str.), 2978, 2932 (ali C−H str.), 1657 (C═O str. of CONH), 1553, 1542 (N−H bend of CONH), 1423, 1378 (S═O str. of SO_2NH, asymmetric), 1169 (S═O str. of SO_2NH, symmetric), 1456 (Ar−C═C), 1041 (Ar−Cl)	δ 7.85 (d, 1H, H-2′), δ 7.55 (d, 1H, H-5′), δ 7.70 (d, 1H, H-6′), δ 7.58 (s, 1H, SO_2NH), δ 7.31 (m, 1H, CONH-1), δ 7.14 (m, 1H, CONH-5), δ 3.17 (m, 1H, H-2), δ 3.14 (m, 2H, N−CH_2-1‴), δ 3.74 (m, 2H, N−CH_2-1″), δ 2.25 (m, 2H, H_2-4), δ 1.89 (m, 2H, H_2-3), 1.49−1.28 (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‴), 0.92−0.86 (m, 6H, CH_3-6″, CH_3-6‴)	51.01/50.92	6.73/6.68	8.5/8.48
39	$M+H^{+\bullet}$peak at *m/z* 518	3309, 3242 (N−H str. of CONH), 3090 (Ar C−H str.), 2931 (ali C−H str.), 1643 (C═O str. of CONH.), 1541 (N−H bend of CONH), 1438, 1371 (S═O str. of SO_2NH, asymmetric), 1168 (S═O str. of SO_2NH, symmetric), 1454 (Ar−C═C.), 817 (Ar-disubstitution), 1031 (Ar−Cl)	δ 7.92 (d, 1H, H-2′), δ 7.65 (d, 1H, H-5′), δ 7.74 (d, 1H, H-6′), δ 7.58 (s, 1H, SO_2NH), δ 7.36 (m, 1H, CONH-1), δ 7.24 (m, 1H, CONH-5), δ 3.27 (m, 1H, H-2), δ 1.87−1.02 (m, 22H, cyclohexyl proton), δ 2.27 (m, 2H, H_2-4), δ 1.92 (m, 2H, H_2-3)	53.28/53.18	6.42/6.32	8.10/7.92
40	$M+H^{+\bullet}$peak at *m/z* 534	3318, 3238 (N−H str. of CONH), 3088 (Ar C−H str.), 2932 (ali C−H str.), 1652 (C═O str. of CONH.), 1543 (N−H bend of CONH), 1448, 1381 (S═O str. of SO_2NH, asymmetric), 1159 (S═O str. of SO_2NH, symmetric), 1464 (Ar−C═C.), 815 (Ar−disubstitution), 1032 (Ar−Cl)	δ 7.96 (d, 1H, H-2′), δ 7.56 (d, 1H, H-5′), δ 7.26 (d, 1H, H-6′), δ 7.21 (s, 1H, SO_2NH), δ 7.18 (m, 1H, CONH-1), δ 7.07 (m, 1H, CONH-5), δ 4.28 (m, 1H, H-2), δ 4.07 (m, 2H, N−CH_2-1‴), δ 3.58 (m, 2H, N−CH_2-1″), δ 2.53 (m, 2H, H_2-4), δ 2.07 (m, 2H, H_2-3), 7.07−6.76 (m, 10H, phenyl proton)	56.18/56.11	4.71/4.68	7.86/7.75

(*continued on next page*)

Table 4 (*continued*)

Cpd	Mass (FAB)	IR (KBr, cm^{-1})	^1H NMR (300 MHz, DMSO-d_6)	C, H, N % calcd/found		
				C	H	N
41	M + H$^{+\bullet}$peak at *m/z* 333	3380, 3292 (N−H str. of CONH), 3091 (Ar C−H str.), 2978, 2932 (ali C−H str.), 1668 (C=O str. of CONH.), 1429, 1387 (S=O str. of SO_2NH, asymmetric), 1154 (S=O str. of SO_2NH, symmetric), 1043 (Ar−Cl.)	δ 7.95 (d, 2H, H-3′, H-4′), δ 7.46 (d, 1H, H-6′), δ 7.40 (s, 1H, SO_2NH), δ 7.30 (m, 2H, $CONH_2$-1), δ 6.78 (m, 2H, $CONH_2$-5), δ 3.81 (m, 1H, H-2), δ 2.69−1.29 (m, 4H, H_2-3, H_2-4), δ 2.5 (s, 3H, Ar−CH_3)	43.18/43.02	4.83/4.83	12.59/12.48
42	M + H$^{+\bullet}$peak at *m/z* 389	3305, 3251 (N−H str. of CONH), 3093 (Ar C−H str.), 2979, 2933 (ali C−H str.), 1645 (C=O str. of CONH), 1556 (N−H bend of CONH), 1440, 1380 (S=O str. of SO_2NH, asymmetric), 1161 (S=O str. of SO_2NH, symmetric), 1460 (Ar−C=C), 819 (Ar−disubstitution), 1041 (Ar−Cl)	δ 7.91 (d, 2H, H-3′, H-4′), δ 7.90 (d, 1H, H-6′), δ 7.42 (s, 1H, SO_2NH), δ 7.30 (m, 1H, CONH-1), δ 7.25 (m, 1H, CONH-5), δ 3.15 (m, 1H, H-2), δ 3.14 (m, 2H, N−CH_2-1‴), δ 2.68 (m, 2H, H_2-4), δ 2.41 (m, 2H, H_2-3), 1.26 (3H, CH_2-2‴), 1.03 (m, 3H, CH_3-2″), δ 2.44 (s, 3H, Ar−CH_3)	49.29/49.12	6.20/6.11	10.78/10.56
43	M + H$^{+\bullet}$peak at *m/z* 417	3316, 3276 (N−H str. of CONH), 3089 (Ar C−H str.), 2972, 2923 (ali C−H str.), 1649 (C=O str. of CONH), 1562 (N−H bend of CONH), 1442, 1389 (S=O str. of SO_2NH, asymmetric), 1172 (S=O str. of SO_2NH, symmetric), 1465 (Ar−C=C), 812 (Ar−disubstitution), 1038 (Ar−Cl)	δ 7.87 (d, 2H, H-3′, H-4′), δ 7.41 (d, 1H, H-6′), δ 7.39 (s, 1H, SO_2NH), δ 7.27 (m, 1H, CONH-1), δ 6.97 (m, 1H, CONH-5), δ 3.65 (m, 1H, H-2), δ 3.24 (m, 2H, N−CH_2-1‴), δ 3.63 (m, 2H, N−CH_2-1″), δ 2.65 (m, 2H, H_2-4), δ 2.28 (m, 2H, H_2-3), 1.95−1.35 (m, 4H, CH_2-2″, CH_2-2‴, 0.97−0.80 (t, 6H, CH_3-3″, CH_3-3‴), δ 2.35 (s, 3H, Ar−CH_3)	51.73/51.70	6.75/6.68	10.05/9.99
44	M + H$^{+\bullet}$peak at *m/z* 417	3316, 3278 (N−H str. of CONH), 3068 (Ar C−H str.), 2980, 2911 (ali C−H str.), 1652 (C=O str. of CONH), 1554 (N−H bend of CONH), 1435, 1399 (S=O str. of SO_2NH, asymmetric), 1171 (S=O str. of SO_2NH, symmetric), 1458 (Ar−C=C), 822 (Ar-disubstitution), 1033 (Ar−Cl)	δ 7.67 (d, 2H, H-3′, H-4′), δ 7.50 (d, 1H, H-6′), δ 7.24 (s, 1H, SO_2NH), δ 7.21 (m, 1H, CONH-1), δ 7.06 (m, 1H, CONH-5), δ 3.55 (m, 1H, H-2), δ 2.41 (m, 1H, N−CH-1‴), δ 2.45 (m, 1H, N−CH-1″), δ 2.06 (m, 2H, H_2-4), δ 1.98 (m, 2H, H_2-3), 1.72 (m, 2H_2, H-4), 1.70−1.69 (m, 6H, CH_3-2″, CH_3-3″), 0.97−0.72 (m, 6H, CH_3-2‴, CH_3-3‴), δ 2.57 (s, 3H, Ar−CH_3)	51.73/51.66	6.75/6.59	10.05/9.98
45	M + H$^{+\bullet}$peak at *m/z* 446	3325, 3295 (N−H str. of CONH), 3085 (Ar C−H str.), 2962, 2895 (ali C−H str.), 1630 (C=O str. of CONH), 1549 (N−H bend of CONH), 1433, 1397 (S=O str. of SO_2NH, asymmetric), 1170 (S=O str. of SO_2NH, symmetric), 1459 (Ar−C=C), 829 (Ar-disubstitution), 1037 (Ar−Cl)	δ 7.90 (d, 2H, H-3′, H-4′), δ 7.89 (d, 1H, H-6′), δ 7.30 (s, 1H, SO_2NH), δ 7.28 (m, 1H, CONH-1), δ 6.45 (m, 1H, CONH-5), δ 3.71 (m, 1H, H-2), δ 3.30 (m, 2H, N−CH_2-1‴), δ 3.14 (m, 2H, N−CH_2-1″), δ 2.67 (m, 2H, H_2-4), δ 1.40 (m, 2H, H_2-3), 1.32−1.00 (m, 8H, CH_2-2″, CH_2-3″, CH_2-2‴, CH_2-3‴), 0.96−0.88 (m, 6H, CH_3-4″, CH_3-4‴), δ 2.62 (s, 3H, Ar−CH_3)	53.86/53.78	7.23/7.11	9.42/9.39
46	M + H$^{+\bullet}$peak at *m/z* 446	3312, 3286 (N−H str. of CONH), 3078 (Ar C−H str.), 2999, 2912 (ali C−H str.), 1628(C=O str. of CONH), 1542 (N−H bend of CONH), 1429, 1393 (S=O str. of SO_2NH, asymmetric), 1167 (S=O str. of SO_2NH, symmetric), 1492 (Ar−C=C), 832 (Ar-disubstitution), 1023 (Ar−Cl)	δ 7.91 (d, 2H, H-3′, H-4′), δ 7.90 (d, 1H, H-6′), δ 7.30 (s, 1H, SO_2NH), δ 7.28 (m, 1H, CONH-1), δ 7.19 (m, 1H, CONH-5), δ 3.15 (m, 1H, H-2), δ 2.98 (m, 2H, N−CH_2-1‴), δ 2.68 (m, 2H, N−CH_2-1″), δ 2.40 (m, 2H, H_2-4), δ 0.99 (m, 2H, H_2-3), 0.98−0.82 (m, 14H, CH-2″, CH-2‴, CH_3-3″, CH_3-3‴, CH_3-4″, CH_3-4‴), δ 2.51 (s, 3H, Ar−CH_3)	53.86/53.66	7.23/7.18	9.42/9.34
47	M + H$^{+\bullet}$peak at *m/z* 474	3354, 3276 (N−H str. of CONH), 3083 (Ar C−H str.), 2992, 2903 (ali C−H str.), 1665(C=O str. of CONH), 1565 (N−H bend of CONH), 1454, 1374 (S=O str. of SO_2NH, asymmetric), 1164 (S=O str. of SO_2NH, symmetric), 1464 (Ar−C=C), 814 (Ar-disubstitution), 1044 (Ar−Cl)	δ 7.715 (d, 2H, H-3′, H-4′), δ 7.409 (d, 1H, H-6′), δ 7.20 (s, 1H, SO_2NH), δ 7.08 (m, 1H, CONH-1), δ 7.06 (m, 1H, CONH-5), δ 2.98 (m, 1H, H-2), δ 2.96 (m, 2H, N−CH_2-1‴), δ 2.63 (m, 2H, N−CH_2-1″), δ 2.47 (m, 2H, H_2-4), δ 2.21 (m, 2H, H_2-3), 1.32−1.15 (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‴), 1.14−0.70 (m, 6H, CH_3-6″, CH_3-6‴), δ 2.49 (s, 3H, Ar−CH_3)	55.74/55.69	7.65/7.57	8.86/8.73

48	$M+H^{+\bullet}$peak at *m/z* 502	3355, 3282 (N−H str. of CONH), 3094 (Ar C−H str.), 2969, 2912 (ali C−H str.), 1642 (C═O str. of CONH), 1552 (N−H bend of CONH), 1438, 1382 (S═O str. of SO_2NH, asymmetric), 1162 (S═O str. of SO_2NH, symmetric),	δ 7.91 (d, 2H, H-3′, H-4′), δ 7.89 (d, 1H, H-6′), δ 7.41 (s, 1H, SO_2NH), δ 7.39 (m, 1H, CONH-1), δ 7.28 (m, 1H, CONH-5), δ 3.28 (m, 1H, H-2), δ 3.11 (m, 2H, N−CH_2-1‴), δ 3.07 (m, 2H, N−CH_2-1″), δ 2.67 (m, 2H, H_2-4), δ 2.21 (m, 2H, H_2-3), 1.96−1.26 (m, 20H, CH_2-2″, CH_2-3″, CH_2-4″, CH_2-5″, CH_2-6″, CH_2-2‴, CH_2-3‴, CH_2-4‴, CH_2-5‴, CH_2-6‴), 0.92−0.87 (m, 6H, CH_3-7″, CH_3-7‴), δ 2.38 (s, 3H, Ar−CH_3)	57.41/57.39	8.03/7.97	8.37/8.28
49	$M+H^{+\bullet}$peak at *m/z* 498	3487, 3284 (N−H str. of CONH), 3093 (Ar C−H str.), 2929 (ali C−H str.), 1639 (C═O str. of CONH), 1556 (N−H bend of CONH), 1380 (S═O str. of SO_2NH, asymmetric), 1159 (S═O str. of SO_2NH, symmetric), 1444 (Ar−C═C.), 833 (Ar−disubstitution), 1043 (Ar−Cl)	δ 7.77 (d, 2H, H-3′, H-4′), δ 7.76 (d, 1H, H-6′), δ 7.37 (s, 1H, SO_2NH), δ 7.36 (m, 1H, CONH-1), δ 7.26 (m, 1H, CONH-5), δ 3.53 (m, 1H, H-2), δ 2.56 (m, 2H, H_2-4), δ 2.34 (m, 2H, H_2-3), 1.77−1.08 (m, 22H, cyclohexyl proton), δ 2.26 (s, 3H, Ar−CH_3)	57.87/57.79	7.29/7.24	8.44/8.33
50	$M+H^{+\bullet}$peak at *m/z* 485	3325, 3255 (N−H str. of CONH), 3088 (Ar C−H str.), 2999, 2900(ali C−H str.), 1650 (C═O str. of CONH), 1561 (N−H bend of CONH), 1441, 1389 (S═O str. of SO_2NH, asymmetric), 1171 (S═O str. of SO_2NH, symmetric), 1472 (Ar−C═C), 832 (Ar-disubstitution), 1045 (Ar−Cl)	δ 7.87 (d, 2H, H-3′, H-4′), δ 7.65 (d, 1H, H-6′), δ 7.39 (s, 1H, SO_2NH), δ 7.28 (m, 1H, CONH-1), δ 7.16 (m, 1H, CONH-5), δ 3.43 (m, 1H, H-2), δ 2.55 (m, 2H, H_2-4), δ 2.24 (m, 2H, H_2-3), 7.01−6.89 (m, 10H, phenyl proton), δ 2.46 (s, 3H, Ar−CH_3)	59.31/59.22	4.98/4.85	8.65/8.58
51	$M+H^{+\bullet}$peak at *m/z* 514	3282 (N−H str. of CONH), 3089 (Ar C−H str.), 2939 (ali C−H str.), 1643 (C═O str. of CONH), 1544, 1512 (N−H bend of CONH), 1433,1382 (S═O str. of SO_2NH, asymmetric), 1161 (S═O str. of SO_2NH, symmetric), 1454 (Ar−C═C.), 825 (Ar-disubstitution), 1029(Ar−Cl)	δ 7.67 (d, 2H, H-3′, H-4′), δ 7.56 (d, 1H, H-6′), δ 7.47 (s, 1H, SO_2NH), δ 7.26 (m, 1H, CONH-1), δ 7.29 (m, 1H, CONH-5), δ 3.51 (m, 1H, H-2), δ 2.45 (m, 2H, H_2-4), δ 2.14 (m, 2H, H_2-3), 7.05−6.96 (m, 10H, phenyl proton), δ 3.97 (m, 2H, N−CH_2-1‴), δ 3.48 (m, 2H, N−CH_2-1″), δ 2.54 (s, 3H, Ar−CH_3)	60.75/60.67	5.49/5.45	8.17/8.08
52	$M+H^{+\bullet}$peak at *m/z* 341	3379 (N−H str. of CONH), 3149(Ar C−H str.), 2964 (ali C−H str.), 1663 (C═O str. of CONH), 1431, 1342 (S═O str. of SO_2NH, asymmetric), 1161 (S═O str. of SO_2NH, symmetric), 1460 (Ar−C═C)	δ 7.48 (d, 2H, H-2′, H-6′), δ 7.41 (d, 1H, H-3′, H-5′), δ 7.33 (s, 1H, SO_2NH), δ 7.23 (m, 2H, $CONH_2$-1), δ 7.20 (m, 2H, $CONH_2$-5), δ 3.71 (m, 1H, H-2), δ 2.53 (m, 2H, H_2-4), δ 1.48 (m, 2H, H_2-3)	52.77/52.76	6.79/6.77	12.31/12.29
53	$M+H^{+\bullet}$peak at *m/z* 397	3352, 3238 (N−H str. of CONH), 3088 (Ar C−H str.), 2948 (ali C−H str.), 1645, 1565, 1472 (ali C−H def.), 1354 (S═O str. of SO_2NH, asymmetric), 1155 (S═O str. of SO_2NH, symmetric), 1024, 976, 894 (Ar−C−H def.)	δ 7.42 (d, 2H, H-2′, H-6′), δ 7.49 (d, 1H, H-3′, H-5′), δ 7.35 (s, 1H, SO_2NH), δ 7.27 (m, 1H, CONH-1), δ 7.21 (m, 1H, CONH-5), δ 3.72 (m, 1H, H-2), δ 2.66 (m, 2H, N−CH_2-1‴), δ 2.73 (m, 2H, N−CH_2-1″), δ 2.63 (m, 2H, H_2-4), δ 1.50 (m, 2H, H_2-3), 1.35−0.57 (m, 6H, CH_3-2″, CH_3-2‴)	57.40/57.38	7.86/7.78	10.57/10.48
54	$M+H^{+\bullet}$peak at *m/z* 425	3307, 3261 (N−H str. of CONH), 3075 (Ar C−H str.), 2970, 2933, 2873 (ali C−H str.), 1645 (C═O str. of CONH), 1596, 1546 (NH bend of CONH), 1458 (Ar−C═C), 1323 (S═O str. of SO_2NH, asymmetric), 1155 (S═O str. of SO_2NH, symmetric)	δ 7.36 (d, 2H, H-2′, H-6′), δ 7.39 (d, 1H, H-3′, H-5′), δ 7.30 (s, 1H, SO_2NH), δ 7.24 (m, 1H, CONH-1), δ 7.16 (m, 1H, CONH-5), δ 3.42 (m, 1H, H-2), δ 2.66 (m, 2H, N−CH-1‴), δ 2.53 (m, 2H, N−CH-1″), δ 2.33 (m, 2H, H_2-4), δ 1.55 (m, 2H, H_2-3), 1.25−0.67 (m, 6H, CH_3-2″, CH_3-2‴)	59.27/59.22	8.29/8.15	9.87/9.77
55	$M+H^{+\bullet}$peak at *m/z* 313	3357, 3249 (N−H str. of CONH), 3191(Ar C−H str.), 2945 (ali C−H str.), 1654 (C═O str. of CONH), 1596 (N−H bend of CONH), 1421, 1323 (S═O str. of SO_2NH, asymmetric), 1155 (S═O str. of SO_2NH, symmetric), 1458 (Ar−C═C def.), 819 (Ar-disubstitution)	δ 7.38 (d, 1H, H-3′), δ 7.28 d, 1H, H-5′), δ 7.31 (d, 1H, H-6′), δ 7.43 (s, 1H, SO_2NH), δ 7.27 (m, 2H, $CONH_2$-1), δ 7.20 (m, 2H, $CONH_2$-5), δ 3.77 (m, 1H, H-2), δ 2.54 (m, 2H, H_2-4), δ 1.49 (m, 2H, H_2-3)	49.83/49.77	6.11/6.02	13.41/13.35
56	$M+H^{+\bullet}$peak at *m/z* 425	3307, 3240 (N−H str. of CONH), 3083 (Ar C−H str.), 2956, 2931 (ali C−H str.), 1660 (C═O str. of CONH), 1552 (N−H bend of CONH), 1436, 1380 (S═O str. of SO_2NH, asymmetric), 1155 (S═O str. of SO_2NH, symmetric), 1454 (Ar−C═C), 819 (Ar-disubstitution)	δ 7.56 (d, 1H, H-3′), δ 7.48 d, 1H, H-5′), δ 7.32 (d, 1H, H-6′), δ 7.63 (s, 1H, SO_2NH), δ 7.29 (m, 1H, CONH-1), δ 7.19 (m, 1H, CONH-5), δ 3.67 (m, 1H, H-2), δ 2.51 (m, 2H, H_2-4), δ 1.42 (m, 2H, H_2-3), δ 3.37 (m, 2H, N−CH_2-1‴), δ 3.24 (m, 2H, N−CH_2-1″), 1.36−1.09 (m, 8H, CH_2-2″, CH_2-3″, CH_2-2‴, CH_2-3‴), 0.98−0.82 (m, 6H, CH_3-4″, CH_3-4‴)	59.27/59.19	8.29/8.27	9.87/9.79

(*continued on next page*)

Table 4 (*continued*)

Cpd	Mass (FAB)	IR (KBr, cm^{-1})	^{1}H NMR (300 MHz, DMSO-d_6)	C, H, N % calcd/found		
				C	H	N
57	$M+H^+$peak at *m/z* 481	3379, 3309, (N–H str. of CONH), 3240 (N–H str. of SO_2NH), 3051 (Ar C–H str.), 2956, 2927, 2858 (ali C–H str.), 1660 (C=O str. of CONH), 1550, 1432 (NH bend of CONH) 1454 (Ar–C=C), 1436, 1380 (S=O str. of SO_2NH, asymmetric), 1155 (S=O str. of SO_2NH, symmetric)	δ 7.62 (d, 1H, H-3′), δ 7.58 d, 1H, H-5′), δ 7.42 (d, 1H, H-6′), δ 7.53 (s, 1H, SO_2NH), δ 7.32 (m, 1H, CONH-1), δ 7.24 (m, 1H, CONH-5), δ 3.72 (m, 1H, H-2), δ 2.57 (m, 2H, H_2-4), δ 1.49 (m, 2H, H_2-3), δ 3.31 (m, 2H, N–CH_2-1‴), δ 3.21 (m, 2H, N–CH_2-1″), 1.37–1.02 (m, 16H, CH_2-2″, CH_2-3″, CH_2-4″, CH_2-2‴, CH_2-3‴, CH_2-4‴), 0.97–0.80 (m, 6H, CH_3-5″, CH_3-5‴)	62.34/62.31	9.00/8.95	8.72/8.68

to AM1 method using Polok Ribiere (conjugate gradient) algorithm with RMS gradient of 0.1 kcal/Å mol. The Molecular Mechanics (MM+) force field was applied for the preliminary structure optimization to shorten the total time required for the energy minimization by AM1 method.

Various descriptors such as geometrical descriptors, constitutional descriptors, functional groups, properties and empirical descriptors were calculated by DRAGON [26] software – version 3, 2003.

Besides these, indicator parameters were also used in order to find out the role of the specific substituent at the specific position towards the biological activity. Parameters used to develop QSAR equation were standardized first by the following equation (1).

$$X_{ki} = \left(x_i - x_{i(\text{minimum})}\right) / \left(x_{i(\text{maximum})} - x_{i(\text{minimum})}\right) \quad (1)$$

The standardized values of these variables are provided as supplementary material.

2.4. Computational procedure

2.4.1. Correlation analysis

All descriptors were subjected to correlation analysis. Intercorreleted parameters were eliminated stepwise. All possible combinations of parameters were considered for the multiple linear regression analysis.

2.4.2. Multiple linear regression analysis

Multiple linear regression analysis [32–35] was carried out by ''Multi Regress'' [36] a computer programme developed in our laboratory. The statistical qualities of the regression equations were justified by parameters like correlation coefficient R, percentage of explained variance (% EV), adjusted R^2 (R^2_A), variance ratio (F), standard error of estimate (s).

2.4.3. Validation of the QSAR model

The predictive power of the equations was validated by Leave-One-Out (LOO) [37] cross-validation method. Predicted residual sum of square (PRESS), total sum of squares (SSY), cross-validated R^2 (R^2_{cv}), standard error of PRESS (S_{PRESS}) and predictive standard error of prediction (S_{DEP}) were considered for the validation of QSAR models.

2.4.4. k-Means cluster analysis

Designing of a test set and the training set was done with the help of *k*-means cluster analysis (*k*-MCA). A *k*-MCA splits all the synthesized compounds in four clusters with 1, 18, 3 and 9 members. Selection of the training and test set was carried out in a random way using compounds belonging to each cluster.

2.4.5. Prediction of the test set

On the basis of these models built using the training set, predicted values of the test set compounds were calculated.

Table 5
Pharmacological activities of glutamamide analogs (**26**–**57**) against EAC cells

Cpd[a]	Avg. wt. of Ascitic fluid in control[b]	Avg wt. of Ascitic fluid in Test[b]	% Inhibition of Ascitic fluid (TWI)	Avg. no of cells/ml in control[b] $\times 10^5$	Avg. no. of cells/ml in Test[b] $\times 10^5$	% Inhibition of ascetic cell (TCI)
26	3.2 (±0.3)	1.3 (±0.3)	60.5	345.6 (±6.3)	112.3 (±5.4)	67.5
27	2.8 (±0.7)	1.7 (±0.4)	38.5	298.2 (±5.5)	92.4 (±3.8)	73.3
28	3.2 (±0.3)	2.1 (±0.2)	34.0	345.6 (±6.3)	145.4 (±4.6)	57.9
29	3.2 (±0.3)	1.9 (±0.3)	42.5	345.6 (±6.3)	71.8 (±4.5)	79.3
30	4.2 (±0.5)	2.5 (±0.3)	41.2	507.4 (±6.7)	197.3 (±4.8)	61.1
31	4.2 (±0.5)	2.9 (±0.2)	31.9	507.4 (±6.7)	239.1(±3.6)	52.9
32	4.2 (±0.5)	2.7 (±0.3)	35.2	507.4 (±6.7)	146.5 (±3.8)	71.1
33	2.8 (±0.7)	1.4 (±0.2)	49.9	298.2 (±5.5)	238.0 (±3.1)	53.1
34	3.7 (±0.7)	1.7 (±0.1)	54.0	449.2 (±3.1)	220.9 (±4.0)	50.8
35	3.7 (±0.7)	2.1 (±0.5)	43.0	449.2 (±3.1)	238.2 (±5.4)	47.0
36	2.8 (±0.7)	1.0 (±0.2)	63.4	298.2 (±5.5)	150.3 (±4.2)	66.5
37	3.2 (±0.4)	1.0 (±0.2)	70.0	350.1 (±8.9)	229.6 (±4.8)	48.9
38	3.5 (±0.5)	2.5 (±0.5)	28.1	397.1 (±3.5)	357.4 (±7.8)	10.0
39	3.5 (±0.5)	2.9 (±0.3)	16.8	397.1 (±3.5)	328.1 (±4.7)	17.4
40	3.5 (±0.5)	2.1 (±0.3)	40.0	397.1 (±3.5)	217.1 (±5.6)	45.3
41	2.8 (±0.7)	1.8 (±0.3)	36.5	298.2 (±5.5)	283.7 (±3.1)	28.6
42	3.9 (±0.2)	2.8 (±0.3)	28.1	463.2 (±8.4)	313.8 (±4.2)	32.3
43			N.P.D[c]			N.P.D[c]
44	3.2 (±0.4)	0.6 (±0.3)	81.5	350.1 (±8.9)	217.6 (±4.6)	53.0
45	3.2 (±0.4)	2.4 (±0.4)	25.2	350.1 (±8.9)	261.7 (±3.2)	43.5
46	3.2 (±0.3)	2.3 (±0.3)	27.9	345.6 (±6.3)	278.0 (±2.6)	40.0
47	4.2 (±0.5)	2.8 (±0.2)	33.3	507.4 (±6.7)	252.7 (±2.1)	47.1
48	3.9 (±0.3)	3.0 (±0.2)	24.2	477.9 (±3.5)	318.5 (±2.8)	33.4
49	3.9 (±0.3)	2.4 (±0.3)	40.2	477.9 (±3.5)	310.7 (±3.3)	35.0
50	3.2 (±0.3)	1.9 (±0.2)	41.2	345.6 (±6.3)	260.6 (±4.4)	45.5
51	3.7 (±0.3)	2.3 (±0.3)	36.5	437.5 (±5.0)	216.7 (±5.0)	50.5
52	3.7 (±0.3)	2.7 (±0.2)	27.5	437.5 (±5.0)	253.5 (±3.1)	42.15
53	3.7 (±0.3)	2.5 (±0.2)	30.8	437.5 (±5.0)	342.9 (±6.0)	21.6
54	3.7 (±0.3)	2.0 (±0.4)	44.7	437.5 (±5.0)	288.4 (±6.7)	34.1
55	3.2 (±0.4)	2.2 (±0.3)	31.2	350.1 (±8.9)	204.8 (±2.3)	35.2
56	3.9 (±0.3)	2.1 (±0.3)	46.1	477.9 (±3.5)	203.7 (±2.3)	35.6
57	3.9 (±0.3)	2.4 (±0.3)	38.4	477.9 (±3.5)	151.6 (±5.0)	52.1
58[d]	3.9 (±0.2)	0.0 (±0.0)	100.0	463.2 (±8.4)	0.0 (±0.0)	100.0
59[e]	3.7 (±0.7)	0.0 (±0.0)	100.0	449.2 (±3.1)	0.0 (±0.1)	100.0
60[f]	3.5 (±0.5)	0.0 (±0.0)	100.0	397.1 (±3.5)	0.0 (±0.0)	100.0

[a] Compound number.
[b] Results represent mean of 6 data points and figure in parenthesis represent standard error of mean.
[c] Not possible to determine.
[d] Mitomycin C as the standard.
[e] Azaserin as the standard.
[f] DON as the standard.

3. Results and discussion

Synthesis of 1,5-*N*,*N*′-disubstituted-2-(substituted benzenesulphonyl) glutamamides was carried out according to the method starting with chlorosulphonylation of the substituted benzenes. Final compounds were obtained as the crystalline solids with appreciable yields.

In order to get the action of these synthesized analogs (**26**–**57**) as anticancer agents, compounds were tested against IMR-32 cell line and Ehrlich Ascites Carcinoma cells in Swiss Albino mice. All samples were dissolved in 100% DMSO but for MTT assay, 1% DMSO is allowed as it is highly toxic to the cell. It is found that some of these compounds are not soluble in 1% DMSO. Thus, inhibition of tumor cell proliferation in IMR-32 cell line cannot be observed for all these compounds. In vivo method of evaluation of anticancer activity for all these analogs against Ehrlich Ascites Carcinoma in Swiss Albino mice was done using percentage inhibition of tumor weight and percentage inhibition of ascites cells as activity

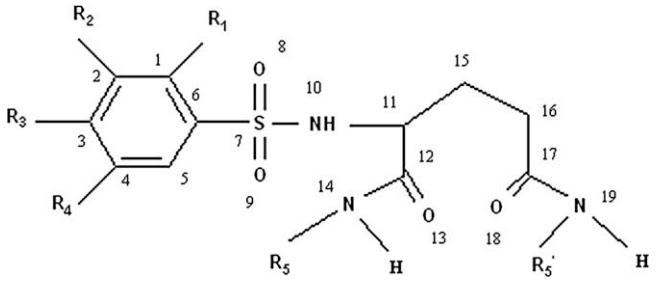

Fig. 3. Arbitrary numbering of 1,5-*N*,*N*′-disustituted-2-(substituted benzenesulphonyl) glutamamides (**26**–**57**).

parameters. QSAR studies of these 1,5-*N*,*N*′-disubstituted-2-(substituted benzenesulphonyl) glutamamides were performed. All parameters were subjected to correlation analysis. Intercorrelated parameters were eliminated depending on their individual correlation with the biological activity. All possible combinations of these parameters were considered and subjected to the multiple linear regression analysis. The correlation matrix is presented and provided as supplementary material.

k-Means cluster analysis (*k*-MCA) was used in designing the training set and the test set. From the total dataset, six compounds (compound nos. **28**, **32**, **34**, **36**, **46**, **55**) were chosen as the test set and the remaining 25 compounds were treated as the training set for QSAR study. The results of regressions of the antitumor activities are presented in Table 6.

Eqs. (2)–(5) resulted for the inhibition of tumor weight and Eqs. (6) and (7) were obtained for the inhibition of tumor cells (Table 8). The statistical parameters shown in Table 8 are correlation coefficient (R), explained variance (% EV), variance ratio (F), standard error of estimate (s) and cross-validated R^2 (R^2_{cv}). Eq. (2) explains 70.34% of variances in biological activity data. This equation suggests the importance of $L\mathrm{R}_2$ (STERIMOL length parameter for R_2 substituent), dipole moment (μ), number of benzene like ring (NBZ) and the indicator parameter (I_1 for the presence of bromine at R_3 position of benzene ring) for tumor weight inhibition of these glutamamide analogs. The negative coefficient of $L\mathrm{R}_2$ suggests that with the increase of the length of substituent at R_2 position the activity decreases. With the increase of dipole moment the activity decreases as evidenced by the negative coefficient of μ. The positive coefficient of NBZ implies that benzene like ring is important for the activity. The positive coefficient of I_1 suggests that the presence of bromine atom at R_3 position is conducive to the activity. Deletion of outliers (Compounds **49** and **56**), which might act through a different mechanism of action, improved the statistical qualities of Eq. (2) as shown in Eq. (3). This equation explains 81.90% of variances in the biological activity data. The significant value of R^2_{cv} (0.705) confirms the validity of the model and good predictivity.

In our efforts to further explore the relationship, another model was developed using $\pi\mathrm{R}_2$ (hydrophobic constant for substituent at R_2 position), μ (dipole moment) and $f^{(E)}15$ (frontier electron density for electrophilic attack at atom number 15) as shown in Eq. (4). This equation explains 73.66% variances of the anticancer activity data .The negative contribution of $\pi\mathrm{R}_2$ reveals that the hydrophobic interaction at this position of benzene ring is detrimental to the activity or hydrophilic substitution may be advantageous to the activity. Like previous model, this model also suggests that the increase of dipole moment is detrimental to the activity. The positive coefficient of I_1 suggests that the presence of bromine atom at R_3 position is favorable to the activity. The negative coefficient of $f^{(E)}15$ suggests that the higher probability of electrophilic attack at atom number 15 is detrimental to the activity. Stepwise deletion of compounds **27** and **48** improves statistical qualities of the equation as shown by Eq. (5) which explains 81.56% variances of the anticancer activity data. Here, this equation is also significantly predictive ($R^2_{cv} = 0.661$) in nature.

Table 6
Developed QSAR equations with statistics for training set

Eq. no.	Equations	n	DC	Statistics				
				R	% EV	F	s	R^2_{cv}
(2)	$\mathrm{Log(TWI)} = 1.801(\pm0.051) - 0.163\ (\pm0.055)L\mathrm{R}_2 - 0.351(\pm0.069)\mu + 0.095(\pm0.046)\mathrm{NBZ} + 0.161(\pm0.068)I_1$	25	–	0.839	70.34	11.857	0.088	0.561
(3)	$\mathrm{Log(TWI)} = 1.825(\pm0.042) - 0.145(\pm0.045)L\mathrm{R}_2 - 0.419(\pm0.060)\mu + 0.108(\pm0.038)\mathrm{NBZ} + 0.183(\pm0.056)I_1$	23	49, 56	0.905	81.90	20.361	0.072	0.705
(4)	$\mathrm{Log(TWI)} = 1.850(\pm0.048) - 0.141(\pm0.051)\pi\mathrm{R}_2 - 0.326(\pm0.066)\mu + 0.203(\pm0.062)I_1 - 0.230(\pm0.084)f^{(E)}15$	25	–	0.858	73.66	13.984	0.083	0.585
(5)	$\mathrm{Log(TWI)} = 1.850(\pm0.048) - 0.141(\pm0.051)\pi\mathrm{R}_2 - 0.326(\pm0.066)\mu + 0.203(\pm0.062)I_1 - 0.230(\pm0.084)f^{(E)}15$	23	27, 28	0.903	81.56	19.901	0.070	0.661
(6)	$(\mathrm{TCI}) = 1.415(\pm0.086) + 0.182(\pm0.075)\mathcal{R}_2 + 0.192(\pm0.051)\sigma\mathrm{R}_4 - 0.261(\pm0.083)\chi\mathrm{C}_3 + 0.263499(\pm0.087)f^{(N)}14$	25	–	0.875	76.53	16.302	0.101	0.587
(7)	$(\mathrm{TCI}) = 1.415(\pm0.086) + 0.182(\pm0.075)\mathcal{R}_2 + 0.192(\pm0.051)\sigma\mathrm{R}_4 - 0.261(\pm0.083)\chi\mathrm{C}_3 + 0.263499(\pm0.087)f^{(N)}14$	24	40	0.904	81.68	21.179	0.091	0.667

Another model was developed using the inhibition of tumor cells as the dependent parameter. For this case, we have also tried all possible combinations of descriptors to get significant models. The best equation obtained in combination of $\mathcal{R}_2$,σR_4, qC_3 and $f^{(N)}14$ was as shown in Eq. (6) which explains 76.53% of variances of activity data. The positive coefficient of $\mathcal{R}_2$ (RTSA index of atom number 2) indicates that the higher value of this index is advantageous to the activity. The positive coefficient of σR_4 indicates that electron-withdrawing groups in this position of the benzene ring may help to the activity. The negative coefficient of qC_3 (charge at atom number 3) indicates that the higher value of charge at 3 position reduces the activity. The positive coefficient of $f^{(N)}14$ (frontier electron density for nucleophilic attack at atom number 14) demonstrates that the higher probability of the nucleophilic attack at this position is favorable for the activity. On deletion of compound 40, statistical qualities of the previous equation are increased as shown by Eq. (7) which explains 81.68% variances of the anticancer activity data. Here, this equation is also significantly predictive ($R^2_{cv} = 0.667$) in nature. The student t-values and probability values of all equations are given in Table 7.

In deriving the above equations, some compounds were deleted, viz, compounds **49** and **56** for Eq. (3), compounds **27** and **48** for Eq. (5), compound **40** for Eq. (7). All these compounds exhibited aberrant behaviors. However, further study is necessary to find out convincing reasons behind such aberration of the excluded compounds.

Useful informations are obtained from QSAR study of the 1,5-*N*,*N*′-disubstituted-2-(substituted benzenesulphonyl) glutamamides for anticancer activity from the above three equations (Eqs. (3), (5) and (7)). The predictive powers of the equations were confirmed by Leave-One-Out (LOO) cross-validation method and details are avoided.

On the basis of these three models, the predicted values of the test set compounds were calculated. The observed and the predicted values of the test set compounds are given in Table 8. It shows significant R^2_{pred} for the test set as shown below:

$R^2_{pred} = 0.672$ for Eq. (3)
$R^2_{pred} = 0.655$ for Eq. (5)
$R^2_{pred} = 0.625$ for Eq. (7).

4. Conclusion

From the study, it is found that these 32 newly synthesized QSAR analogs of glutamamide have prominent anticancer activity. Amongst all these analogs, compound **44** showed good anticancer activity compared to the standard drugs against Ehrlich Ascites Carcinoma (EAC) cells. QSAR study showed that with the increase of the length of substituents at R_2 position and that of dipole moment the anticancer activity decrease when inhibition of tumor weight was considered as the dependent parameter. The presence of bromine atom at R_3 position is conducive to the activity. The presence of benzene like ring is also important for the activity. Hydrophobic interaction at R_2 position of benzene ring is detrimental to the activity or hydrophilic substitution may be advantageous to the activity. Even the higher probability of electrophilic attack at atom number 15 is detrimental to the activity.

Considering the inhibition of tumor cell as the biological activity parameter, it is found that the higher value of RTSA index of atom number 2 is advantageous to the activity. Atom number 2 may be involved in dispersive interactions of these compounds with enzymes. Electron-withdrawing groups at R_4 position of the benzene ring may help to increase the said activity. The higher value of charge at atom number 3 reduces the activity. Frontier electron density for nucleophilic attack at atom number 14 demonstrates that the higher probability of nucleophilic attack at this position is favorable for the activity.

This QSAR study gives some important information about the structural requirements of the glutamamide moiety for the increased antitumor activity. As the selected QSAR analogs of

Table 7
t-Statistics and *p*-values for all the equations

Eq. no.	Intercept/ parameter	*t*-Value	*p*-Value	Equation	Intercept/ parameter	*t*-Value	*p*-Value
(2)	Intercept	35.539	0.000	(6)	Intercept	16.442	0.000
	LR_2	−2.961	0.008		$\mathcal{R}_2$	2.414	0.026
	μ	−5.092	0.000		σR_4	3.748	0.001
	NBZ	2.080	0.051		qC_3	−3.164	0.005
	I_1	2.374	0.028		$f^{(N)}14$	3.022	0.007
(3)	Intercept	42.975	0.000	(7)	Intercept	16.012	0.000
	LR_2	−3.187	0.005		$\mathcal{R}_2$	3.537	0.002
	μ	−6.968	0.000		σR_4	4.155	0.001
	NBZ	2.869	0.010		qC_3	−3.635	0.002
	I_1	3.285	0.004		$f^{(N)}14$	3.087	0.006
(4)	Intercept	38.530	0.000				
	πR_2	−2.740	0.013				
	μ	−4.938	0.000				
	I_1	3.273	0.0038				
	$f^{(E)}15$	−2.720	0.013				
(5)	Intercept	43.395	0.000				
	πR_2	−3.559	0.002				
	μ	−5.890	0.000				
	I_1	3.583	0.002				
	$f^{(E)}15$	−3.571	0.000				

Table 8
Observed and predicted values of test set compounds

Cpd[a]	Observed		Predicted value		
	Log (TWI)	Log (TCI)	Eq. (3)	Eq. (5)	Eq. (7)
28	1.531	1.763	1.520	1.576	1.889
32	1.546	1.852	1.681	1.631	1.769
34	1.732	1.706	1.777	1.833	1.673
36	1.802	1.823	1.905	1.852	1.721
46	1.446	1.602	1.490	1.558	1.639
55	1.494	1.547	1.500	1.482	1.586

$R^2_{pred} = 0.672$ for Eq. (3); $R^2_{pred} = 0.655$ for Eq. (5); $R^2_{pred} = 0.625$ for Eq. (7).
[a] Compound number.

1,5-*N*,*N*′-disubstituted-2-(substituted benzenesulphonyl)glutamamide have shown good anticancer activity, the present QSAR study may help in further tailoring of these analogs to find an active member.

Acknowledgements

The authors are thankful to the University Grants Commission (UGC), New Delhi and All India Council for Technical Education (AICTE), New Delhi for providing financial support. One of the authors SS is grateful to University Grants Commission (UGC), New Delhi for awarding Junior Research Fellowship (JRF). Two of the authors PP and MA are grateful to UGC, New Delhi for awarding Post Graduate Fellowships. Authors also thank D.R. Krishna of Kakatiya University, Warangel for help in performing in vitro test.

References

[1] E.A. Newsholm, M. Parry-Billings, J. Parenter. Enteral. Nutr. 14 (1990) 63S–67S.
[2] E.A. Newsholm, B. Crabtree, M.S.M. Ardawi, Q. J. Exp. Physiol. 70 (1985) 473–489.
[3] M. Watford, Biochim. Biophys. Acta 120 (1994) 73–78.
[4] H.G. Windmueller, Adv. Enzymol. 53 (1982) 201–237.
[5] H.G. Windmueller, A.E. Spaeth, J. Biol. Chem. 249 (1974) 5070–5079.
[6] H. Krebs, Biochem. J. 29 (1935) 1951–1969.
[7] P. Bhattacharya, P. Maity, Pathol. Oncol. Res. 6 (2000) 217–223.
[8] H. Zalkin, J.L. Smith, Adv. Enzymol. Relat. Areas Mol. Biol. 72 (1998) 87–144.
[9] F. Massiere, M.A. Badet Denisot, Cell. Mol. Life. Sci. 54 (1998) 205–222.
[10] V.S. Klimberg, J.L. McClellan, Am. J. Surg. 172 (1996) 418–424.
[11] L. Taylor, N.P. Curthoys, Biochem. Mol. Biol. Ed. 32 (2004) 291–304.
[12] N.D. Miller, L. Alan, Altern. Med. Rev. 4 (1999) 239–248.
[13] Meister, in: P.D. Boyer (Ed.), Glutamine Synthetase of Mammals, third ed., The Enzymes, vol. X Academic Press, New York, 1974, pp. 699–754.
[14] H. Tsuda, A. Iemura, M. Sata, M. Uchida, K. Yamana, H. Hara, Kurume Med. J. 43 (1996) 137–147.
[15] K. Srikanth, B. Debnath, T. Jha, Bioorg. Med. Chem. 10 (2002) 1841–1854.
[16] B. Debnath, K. Srikanth, S. Banarjee, T. Jha, Internet Electron. J. Mol. Des. 1 (2002) 488–502.<http://www.biochempress.com>.
[17] S. Gayen, B. Debnath, S. Samanta, B. Ghosh, A. Basu, T. Jha, Internet Electron. J. Mol. Des. 4 (2005) 556–578.<www.biochempress.com>.
[18] Bikash Debnath, Ph.D. Dissertation, Jadavpur University, Kolkata, 2004.
[19] Shovanlal Gayen, M.Pharm. Dissertation, Jadavpur University, Kolkata, 2005.
[20] S. Samanta, B. Debnath, A. Basu, S. Gayen, K. Srikanth, T. Jha, Eur. J. Med. Chem. 41 (2006) 1190–1195.
[21] H.J. Barber, J. Chem. Soc. (1943) 102.
[22] E.H. Huntress, F.H. Carten, J. Am. Chem. Soc. 62 (1940) 511–514.
[23] M.C. Alley, D.A. Scudiero, A. Monks, M.L. Hursey, M.J. Czerwinski, D.L. Fine, B.J. Abbott, J.G. Mayo, R.H. Shoemaker, M.R. Boyd, Cancer Res. 48 (1988) 589–601.
[24] S.K. Purkayastha, T. Jha, D.K. Pal, A.U. De, Anticancer Drug Des. 8 (1993) 95–100.
[25] HyperChem Professional' software – version 7.0 of Hypercube Inc, 2002. <www.hyper.com>.
[26] R. Todeschini, V. Consonni, A. Mauri, M. Pavan, DRAGON Software for the Calculation of Molecualr Descriptors, Web version 3.0 for Windows, 2003.
[27] Mouse, a computer program written in C++ language by Departments of Computer Science and Engineering and Pharmaceutical Technology of Jadavpur University.
[28] C. Hansch, A. Leo, Substituent Constants for Correlation Analysis in Chemistry and Biology, John Wiley & Sons, NY, 1979, pp. 48–63.
[29] A.K. Ghose, A. Pritchett, G.M. Crippen, J. Comput. Chem. 9 (1988) 80–90.
[30] A.K. Ghose, G.M. Crippen, J. Chem. Inf. Comput. Sci. 27 (1987) 21–35.
[31] R. Carrasco, J.A. Padron, J.J. Galvez, J. Pharm. Pharm. Sci. 7 (2004) 19–26.
[32] G.W. Snedecor, W.G. Cochran, Multiple Regression in Statistical Methods, Oxford & IBH, New Delhi, India, 1967.
[33] J.O. Rawlings, Applied Regression Analysis, Wordsworth, CA, USA, 1988, p. 327.
[34] N.R. Darper, H. Smith, Applied Regression Analysis, John Wiley & Sons, New York, 1981, p. 294.
[35] E.J. Lien, in: E.J. Ariens (Ed.), Quantitative Correlations Using Multiple Regression and Substituent Constants, Drug Design, vol. V, Academic, New York, 1975, pp. 84–94.
[36] Multiregress, a computer program written in C++ language by Jadavpur University.
[37] I.V. Tetko, V.Y. Tanchuk, A.E. Villa, J. Chem. Inf. Comput. Sci. 41 (2001) 1407–1421.