Special Issue on "Medicinal Chemistry"

J. Indian Chem. Soc.,
Vol. 97, August 2020, pp. 1259-1264

Synthesis, biological evaluation, and enzyme assay of some 5-*N*-substituted-2-*N*-(arylsulphonyl)-L(+)glutamines as potential anticancer agents

Tarun Jha*[a], Soma Samanta[a], Amit Kumar Halder[a], Nilanjan Adhikari[a], Sk. Abdul Amin[a], Arpita Sanyal[b] and Tanmoy Mukherjee[b]

[a]Natural Science Laboratory, Division of Medicinal and Pharmaceutical Chemistry,
Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700 032, India

[b]Indian Institute of Chemical Biology, 4, Raja S. C. Mullick Road, Jadavpur, Kolkata-700 032, India

E-mail: tjupharm@yahoo.com

Manuscript received online 03 July 2020, accepted 30 July 2020

Thirty 5-*N*-substituted-2-*N*-(arylsulphonyl)-L(+)glutamines were synthesized and evaluated biologically for their anticancer activities. The best active compound of this series showed 92.92% inhibition of tumor weight against Ehrlich Ascites Carcinoma cells. The most active compound was proved to be a competitive inhibitor of glutaminase in the enzyme assay. The best active compound may be a starting point to generate 'lead' for further exploration.

Keywords: Anticancer agent, glutamine derivative, glutaminase inhibition, inhibition assay.

Introduction

Glutamine is one of the important nutrients for the rapid growing cells[1–3]. Notably, neoplastic cells also utilize glutamine[4,5]. Hence, glutamine is involved in the cancer cell growth. It supplies its nitrogen atom (amide) in the biosynthesis of nucleic acid bases (e.g. purine, pyrimidine), other amino acids, and amino sugar as well as coenzymes via different amidotransferases with versatile mechanisms. As far as the cellular growth is concerned, glutamine plays pivotal role in the metabolisms of protein and nucleic acid bases. Moreover, glutamine takes part in the transportation of few amino acids. It also serves as the pivotal carrier of nitrogen from the skeletal muscle to visceral organs. Glutamine levels are undetectable in malignant strain of Earlich ascite tumor cells (EATC). Additionally, literature reports suggested that glutamine modulates gene expression on various tissues. Glutamine stimulates antiapoptotic Bcl-2 and also inhibits proapoptotic CD95, thus, may aid in malignancy[6]. Furthermore, the increase of glutamine catabolism by the enzyme glutaminase has been reported recently. In consequence, an increased glutaminase activity is found to be the highest during the exponential stage of many cancer cell growth[7]. Overexpression of kidney type glutaminase (KGA) isoform was observed in rapidly proliferating cells of rodent and human hepatoma, EATCs, breast cancer cells as well as in leukemia. Moreover, inhibition of KGA in EATCs may activate apoptosis leading to the sensitization of these cells to hydrogen peroxide (H_2O_2) as well as methotrexate (MTX) toxicity. Therefore, glutaminase may be potential target for anticancer drug development[8,9]. Considering these, it can be anticipated that chemotypes those successfully lower down the glutamine uptake, utilization, and also may interrupt the glutaminase enzyme in cancer cells may exhibit antiproliferative properties. Herein, we report some new 5-*N*-substituted-2-*N*-(arylsulphonyl)-L(+)glutamines (Fig. 1) with antiproliferative potential and the affect of these compounds on glutaminase enzyme activity.

Experimental

Chemistry:

The synthesis of the titled compounds was carried out as depicted in Scheme 1. The synthetic work was initiated by the chloro-sulphonylation of substituted benzenes **(1-5)** to get compounds **6-10**[10]. These (**6**-**10**) were condensed with L(+) glutamic acid under alkaline medium (2 *N* NaOH) to obtain the compounds **11-15**. Next, the cyclization reaction of

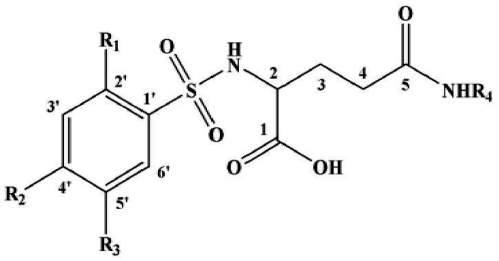
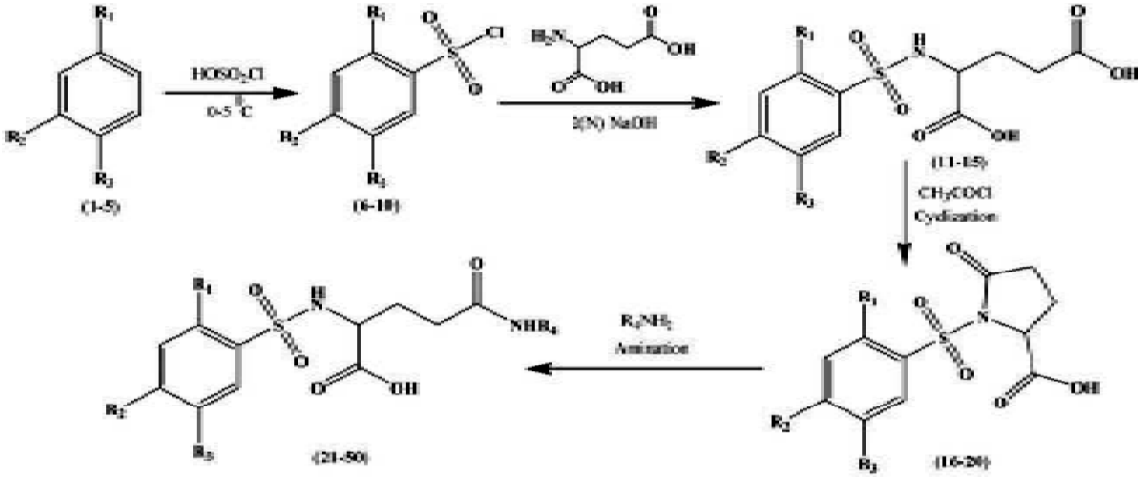


Fig. 1. Generalised structure of the glutamines investigated under this work.

the resulting compounds **11-15** with acetyl chloride produced **16-20**, respectively. Subsequently aminolysis of **16-20** with various amines led to the target compounds **21-50** (Scheme 1).

(20–25°C) was kept throughout the experiments. All mice were divided into two groups, i.e. a test and a control group. In each group, six mice were taken. The biological screening was done by dissolving the synthesized compounds (**21-50**) separately in sterile phosphate buffered saline (PBS). This was administered separately after 24 h incubation of 2×10^6 EAC cells to each mouse intraperitonially for 7 days. The cell count and tumor weight (in the form of ascetic fluid) were measured in the test groups against that of the control group. Numbers of tumor cells were counted under a microscope by using haemocytometer (Marienfeld, Germany). Percentage tumor weight inhibition (% TWI) was considered with respect to biological activity data. Mitomycin C (as the universal standard) and azaserin and DON (as the specific standard drugs) were taken as standards for comparison of the



Scheme 1. General synthetic route of 5-*N*-substituted-2-*N*-(arylsulphonyl)-L(+)glutamines **(21-50)**.

All the synthesized final compounds (**21-50**) were obtained as crystalline solids. The structural analyses of the final molecules were done by IR, Mass, NMR-spectroscopy along with elemental analyses. All the titled compounds and L(+)-glutamic acid were examined for their optical activity.

Pharmacological activity:

All the final products (**21-50**) were screened biologically for assessing the antitumor potential against EAC cells in mice model. The healthy Swiss Albino female mice (age: 10 weeks, average body weight: 18–20 g.) were considered for the *in vivo* study. Notably, the basal metabolic diet and water *ad libitum* was maintained. The ambient room temperature

biological activities with these investigated glutamines (**21-50**).

Enzyme assay:

Phosphate dependent kidney type glutaminase inhibition may retard the rapid proliferation of tumor cells of various origins; the best active compound **48** was selected for the enzyme inhibition study. Glutaminase was extracted from kidneys of Swiss Albino mice. For the study, all biochemicals were purchased from Sigma Chemicals, USA. Swiss Albino mice were anesthetized and subsequently, kidneys were excised. Tissue was trimmed of fat, weighed and chopped as much as possible in cold condition. For 1 kidney, 5 ml

homogenized solution (buffer containing 20 mM HEPES, 0.33 *M* sucrose, 0.2 mM EDTA of pH 7.5) was used to homogenize the chopped kidney. Homogenization was done with sonication (15 pulses, each of for 15 seconds in 30 seconds gap in ice bath) by a sonicator followed by cold centrifugation with 12000 r.p.m. for 45 min. The homogenate was used for the enzyme assay. Phosphate dependent glutaminase was reported as relatively stable and it showed promising results in preliminary tests as well as for the biochemical role. Hence, the enzyme was considered for biochemical characterizations. Results represented three separate individual experiments in triplicate conducted on three different times to get reliable and reproducible data. To study the characteristic property of the intracellular phosphate dependent glutaminase, the enzyme activity was determined with varied incubation time and varied enzyme concentration. The obtained glutaminase showed a linear relationship with incubation time for 30 min with a fixed amount of the enzyme (details avoided).

The phosphate dependent glutaminase was assayed[11,12] using spectrophotometric stop rate determination method with some modifications. The initial incubation mixture for glutaminase contained 20 mM glutamine, 50 mM Tris-Cl, 0.15 *M* potassium phosphate, 0.2 mM EDTA of pH 8.6 at 37°C.

For the assay, 100 μL of the appropriate reagent was pipetted into an eppendorf tube. The reaction was initiated by adding the enzyme solution (1–20 μL). After a varied period of time (10–30 min), the reaction was quenched with 10 μL of 3 *N* HCl. 1 ml of freshly prepared solution of 2 mM NAD, 0.25 mM ADP, 100 μg of glutamine dehydrogenase. 0.03% H_2O_2 and 80 mM Tris-Cl of pH 9.4 was used. After 30 min (at room temperature), the absorbance against a blank arranged by adding the acid immediately after the addition of the enzyme solution was determined at 340 nm.

Results and discussion

Chemistry:

The synthetic work was initiated with the chlorosulphonylation of the substituted benzenes **(1-5)** to get compounds **6-10**[10,13]. These compounds **6**-**10** were reacted with glutamic acid under alkaline medium (2 *N* aq. NaOH) to obtain the compounds **11-15**, respectively. This was followed by the cyclization of **11-15** with acetyl chloride to form produced compounds **16-20**, respectively. Finally, aminolysis of **16-20** separately with various amines led to corresponding glutamines **21-50** (Scheme 1). The physical data of **6**-**20** and those of the final glutamines **21-50** are provided in Table 1 and Table 2, respectively.

Table 1. The structural details, physical data, and yields of the intermediates **6-20**

Entry	R_1	R_2	R_3	m.p. (°C)	% yield
6	CH_3	H	CH_3	Liquid	75.32
7	H	F	H	Liquid	76.27
8	CH_3	CH3	H	Liquid	79.85
9	H	*i*-C_4H_9	H	Liquid	73.54
10	H	OCH_3	H	Liquid	71.42
11	CH_3	H	CH_3	102–104	50.82
12	H	F	H	125–127	55.92
13	CH_3	CH3	H	109–111	72.34
14	H	*i*-C_4H_9	H	132–134	61.05
15	H	OCH_3	H	75–77	58.66
16	CH_3	H	CH_3	82–84	90.25
17	H	F	H	78–80	72.13
18	CH_3	CH3	H	167–175	85.55
19	H	*i*-C_4H_9	H	112–114	86.12
20	H	OCH_3	H	95–97	81.53

Table 2. The structural details, physical data, and yield of compounds **21-50**

Entry	R_1	R_2	R_3	R_4	m.p. (°C)	% yield
21	CH_3	H	CH_3	H	153–155	60.50
22	CH_3	H	CH_3	C_2H_5	93–95	66.31
23	CH_3	H	CH_3	*n*-C_3H_7	108–110	70.00
24	CH_3	H	CH_3	*i*-C_3H_7	185–187	65.14
25	CH_3	H	CH_3	*n*-C_4H_9	99–101	71.23
26	CH_3	H	CH_3	*i*-C_4H_9	110–113	62.22
27	CH_3	H	CH_3	C5H11	104–106	75.80
28	CH_3	H	CH_3	*n*-C_6H_{13}	113–115	79.62
29	CH_3	H	CH_3	*c*-C_6H_{11}	114–116	73.23
30	CH_3	H	CH_3	C_6H_5	150–152	85.63
31	CH_3	H	CH_3	$CH_2C_6H_5$	163–165	81.25
32	H	F	H	H	158–160	78.93
33	H	F	H	C_2H_5	178–180	82.34
34	H	F	H	*n*-C_3H_7	185–187	86.33
35	H	F	H	*i*-C_3H_7	196–198	80.56
36	H	F	H	*n*-C_4H_9	170–172	76.89
37	H	F	H	*i*-C_4H_9	175–177	78.94
38	H	F	H	C5H11	158–160	89.84
39	H	F	H	*n*-C_6H_{13}	128–130	91.22
40	H	F	H	*c*-C_6H_{11}	213–215	66.78

Table-2 (contd.)

41	H	F	H	C_6H_5	195–197	79.96
42	H	F	H	$CH_2C_6H_5$	174–175	87.41
43	CH_3	CH_3	H	*n*-C_4H_9	148–150	88.16
44	CH_3	CH_3	H	*c*-C_6H_{11}	165–168	70.59
45	CH_3	CH_3	H	$CH_2C_6H_5$	175–176	68.84
46	H	*i*-C_4H_9	H	C_2H_5	177–178	90.83
47	H	*i*-C_4H_9	H	*n*-C_3H_7	168–170	88.78
48	H	*i*-C_4H_9	H	*n*-C_6H_{13}	172–174	77.27
49	H	OCH_3	H	*i*-C_3H_7	92–94	68.46
50	H	OCH_3	H	C_6H_5	208–210	73.45

All synthesized compounds **21-50** were characterized extensivly[13]. Melting points (m.p.) of final products were carefully examined on Mel-Temp electrothermal apparatus and these were further scrutinized in CTRONICS-a digital MP apparatus. Infrared (IR) spectra were collected on FTIR - 8400S Model of SHIMADZU by the aid of KBr pellets. The ^{1}H NMR data were taken on Bruker DRX 300 MHz NMR spectrometer as mentioned in our earlier communication[13]. Notably, the chemical shifts relative to tetramethylsilane (Me_4Si) as a standard for solutions in deuterated dimethylsulfoxide (DMSO-d_6).

The positions of hydrogen were described in ^{1}H NMR interpretation by following the common structure as illustrated in Fig. 1. Besides, the substitutions at the R_4 position have been depicted as the superscript '‴ '.

The mass spectra were recorded on API 200 mass spectrophotometer. The reactions were monitored by analytical thin layer chromatography (TLC), on silica gel G plates. Structural analysis derived information of a proto type compound **21** is shown below:

*5-N-2-(2′,5′-Dimethylbenzenesulphonyl)-L(+)glutamine (**21**):*

0.56 g, 60.50%, white solid, m.p. 153–155°C, MS (FAB): M+H$^+$ peak at *m/z* 314. IR (KBr, cm^{-1}): 3325 (N-H str. of $CONH_2$), 3277 (N-H str. of SO_2NH), 2961 (ali C-H str.), 1704 (C=O str. of COOH), 1642 (C=O str. of CONH), 1576, 1492, 1459 (C=C str. of phenyl), 1331 (S=O str. of SO_2 asym.) and 1149 (S=O str. of SO_2 sym); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (d, 1H, H-3′, *J* 8.34), 7.94 (dd, 1H, J_1 2.14, J_2 7.96, H-4′), 7.46 (d, 1H, *J* 1.9, H-6′), 7.40 (d, 1H, *J* 8.34, SO_2NH), 7.30 (m, 2H, $CONH_2$-5), 3.81 (m, 1H, H-2), 2.69–1.29 (m, 4H, H_2-3, H_2-4), 2.76 (m, 3H, Ar-CH_3). Anal. (C, H, N) calculated: 49.67, 5.77, 8.91; observed: 49.63, 5.43, 8.88.

Pharmacological activity:

The *in vivo* pharmacological activities of all final molecules (**21**-**50**) are provided in Table 3.

Table 3. *In vivo* pharmacological activities of titled compounds (**21**-**50**)

Cpd[a]	Avg. wt. of AF in control	Avg. wt. of AF in test	%TWI[b]	log TWI[c]
21	3.359 (±0.217)	1.483 (±0.226)	55.85	1.747
22	3.359 (±0.217)	1.866 (±0.224)	44.45	1.648
23	2.280 (±0.312)	1.377 (±0.118)	39.60	1.598
24	2.280 (±0.312)	1.245 (±0.156)	45.39	1.657
25	2.280 (±0.312)	0.947 (±0.128)	58.46	1.767
26	3.359 (±0.217)	2.097 (±0.428)	37.57	1.575
27	3.359 (±0.217)	1.869 (±0.216)	44.36	1.647
28	3.359 (±0.217)	1.426 (±0.258)	57.54	1.760
29	2.280 (±0.312)	1.349 (±0.232)	40.83	1.611
30	2.280 (±0.312)	1.475 (±0.208)	35.31	1.548
31	3.240 (±0.421)	1.508 (±0.197)	53.46	1.728
32	2.120 (±0.218)	1.490 (±0.356)	29.72	1.473
33	2.120 (±0.218)	1.013 (±0.114)	52.22	1.718
34	2.345 (±0.336)	1.690 (±0.126)	27.93	1.446
35	2.345 (±0.336	1.813 (±0.142)	22.69	1.356
36	3.140 (±0.412)	2.049 (±0.384)	34.75	1.541
37	3.140 (±0.412)	2.502 (±0.410)	20.32	1.308
38	3.140 (±0.412)	2.003 (±0.391)	36.21	1.559
39	2.345 (±0.336	1.517 (±0.186)	35.31	1.548
40	2.345 (±0.336	1.590 (±0.198)	32.20	1.508
41	2.345 (±0.336	1.664 (±0.136)	29.04	1.463
42	3.240 (±0.421)	2.180 (±0.444)	32.72	1.515
43	2.120 (±0.218)	1.060 (±0.109)	50.00	1.699
44	2.120 (±0.218)	1.560 (±0.128)	26.42	1.422
45	2.120 (±0.218)	1.140(±0.136)	46.23	1.665
46	3.140 (±0.412)	1.559 (±0.148)	50.35	1.702
47	3.140 (±0.412)	1.807 (±0.156)	42.45	1.628
48	3.240 (±0.421)	0.230 (±0.228)	92.90	1.968
49	3.240 (±0.421)	2.279 (±0.484)	29.66	1.472
50	3.240 (±0.421)	2.264 (±0.514)	30.12	1.479

[a]Compound number; [b]% Tumor weight inhibition; [c]Negative logarithm of tumor weight inhibition; AF, Ascitic fluid.

Enzyme assay:

The mechanism of action ((MOA) of the investigated molecules was not known earlier. To know the MOA of these compounds, the enzyme assay was performed with the enzyme glutaminase. It was hypothesized that glutamine derivatives and analogs might be acting through alteration of metabolism of glutamine or through prevention of the uptake and/or utilization of glutamine in neoplastic cells by using the enzyme glutaminase. The best active compound **48** (inhibitor 1) was selected for the enzyme inhibition study. The same enzyme also showed a linear relationship with varying concentration of glutamine upto 70.87 μg/ml with fixed time of incubation (data not shown). Glutaminase activity was measured in a range of substrate concentrations (1–20 mM). The velocity of the reaction was plotted against the substrate concentration. It resulted in a hyperbolic curve and that is shown in Fig. 2.

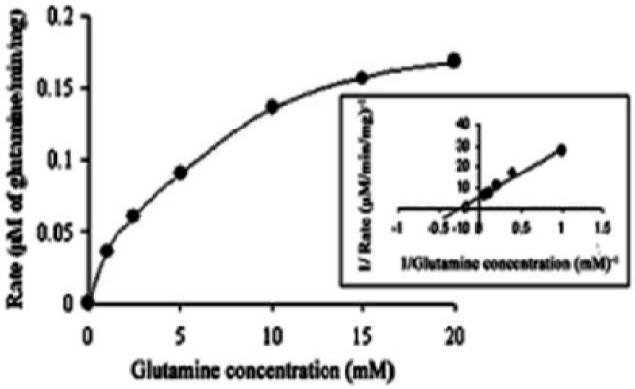


Fig. 2. Enzyme kinetics of the sample: the sample containing intracellular glutaminase incubated in a varying substrate concentration and its activity measured in the usual procedure. Velocity plotted against substrate concentration. K_m or the Michelis-Menten constant estimated to be (4.16 mM) while V_{max} 0.175 μmole/min/mg as shown in the inset.

The Lineweaver plot of gutaminase showed that K_m was 4.16 mM while V_{max} was 0.175 μmole/min/mg (Fig. 2). Kinetic analysis revealed that the compound **48** (inhibitor 1) acted as a strictly competitive inhibitor of the enzyme glutaminase (Fig. 3). The binding affinity of the compound **48** (inhibitor 1) was found to 1.057 μM (K_i = 1.057 μM).

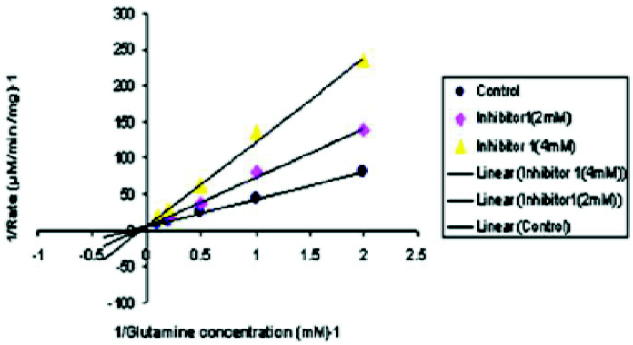


Fig. 3. Enzyme activity determination: requisite amount of enzyme 70.87 μg/ml used for the assay. Fixed concentration of compound **48** (inhibitor 1) (2 mM, 4 mM) used with varied concentrations of glutamine as substrate.

Conclusions

The present study describes the synthesis and biological evaluation of thirty 5-*N*-substituted-2-*N*-(arylsulphonyl)-L(+)glutamines for their anticancer activities. The best active compounds (**48**) that showed 92.90% tumor weight inhibitory activity against EAC cells *in vivo* competitively inhibits phosphate dependent glutaminase enzyme, claimed to be an important player in tumor formation and metastasis. Thus, the probable mechanism of action of these series of compounds could be to act as glutamine antagonists.

Acknowledgements

We are very much thankful to the Jadavpur University, Kolkata for providing the research facilities.

References

1. Z. Li, Y. Wang, H. Wu, L. Zhang, P. Yang and Z. Li, *Oncotarget*., 2014, **5**, 5369.
2. D. Daye and K. E. Wellen, *Semin. Cell. Develop. Biol*., 2012, **23**, 362.
3. S. A. Amin, N. Adhikari, S. Gayen and T. Jha, *J. Mol. Struct*., 2017, **1143**, 49.
4. R. DeBerardinis, J. J. Lum, G. Hatzivassiliou and C. B. Thompson, *Cell. Metabol*., 2008, **7**, 11.
5. R. DeBerardinis, N. Sayed, D. Ditsworth and C. B. Thompson, *Curr. Opin. Genet. Dev*., 2008, **18**, 54.
6. W. K. Chang, K. D. Yang, H. Chuang, J. T. Jan and M. F. Shaio, *Clin. Immunol.,* 2002, **104**, 151.
7. J. B. Wang, J. W. Erickson, R. Fuji, S. Ramachandran, P. Gao, R. Dinavahi, K. F. Wilson, A. L. B. Ambrosio, S. M. G. Dias, C. V.

Dang and R. A. Cerione, *Cancer. Cell*., 2010, **18**, 207.

8. W. Lu, H. Pelicano and P. Huang, *Cancer. Cell*., 2010, **18**, 199.
9. M. Szeliga and M. Obara-Michlewska, *Neurochem. Int*., 2009, **55**, 71.
10. E. H. Huntress and F. H. Carten, *J. Am. Chem. Soc*., 1940, **62**, 511.
11. N. P. Curthoys and O. H. Lowry, *J. Biol. Chem*., 1973, **248**, 162.
12. N. P. Curthoys and R. F. Weiss, *J. Biol. Chem*., 1974, **249**, 3261.
13. T. Jha, S. Basu, A. K. Halder, N. Adhikari and S. Samanta, *Med. Chem. Res*., 2017, **26**, 1437.