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Possible antineoplastic agents, part 15: synthesis, biological activity and quantitative structure activity relationship of substituted-2-(4′-methoxybenzenesulphonamido) glutaric acid analogs against Ehrlich ascites carcinoma

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Some substituted-2-(4′-methoxybenzenesulphonamido) glutaric acid analogs (**5a**–**m**, **7a**–**d**) have been synthesized and tested for their possible antineoplastic activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice using tumour (ascitic fluid) weight as activity parameter. Some of these compounds possess encouraging antitumour activity. A QSAR study, performed by the classical Hansch method, explains the significance of hydrophobic binding and electronic influence in the mechanism of antineoplastic action in this group of compounds.

1. Introduction

We hypothesise that aberrations in the structures of L-glutamic acid and L-glutamine might be the epicenter of formation and uncontrolled proliferation of neoplastic cells, notwithstanding the prevailing immunosurveillance [1, 2], virogen-oncogen [3, 4], free radical [5] theories. This is supported by the importance of L-glutamic acid and L-glutamine in the following instances – (i) occurrence of glutamic acid in many different types of malignant tumour [6, 7], (ii) biosynthesis of deoxyribonucleotides and ribonucleotides involving the 3- and 9-nitrogen atoms of purine bases [8], 2-amino group of guanine [8], 3-nitrogen atom and amino group of cytosine [9], (iii) presence of L-glutamic acid moiety in folic acid, and the antifolic antineoplastic, methotrexate, (iv) the teratogenic activity of thalidomide and the detection, isolation and characterization of its several metabolities, such as, *N*-(*o*-carboxybenzoyl)-DL-glutamic acid imide, *N*-phthalyl-DL-glutamine, *N*-phthalyl-DL-isoglutamine, all derived from D-glutamic acid, an unnatural amino acid, instead of natural L-glutamic acid presumably interfering with the biosynthesis of DNA and RNA causing teratogenicity [10, 11], (v) the structural similarity between *N*-(*o*-carboxybenzoyl) glutamic acid and folic acid and the consequent interference with the folic acid function [11], (vi) high metabolic reactivity of L-glutamic acid as amino donor in the transamination reaction and consequent protein synthesis [11], (vii) the higher demand for glutamine compared to other amino acids in certain neoplasms [12], (viii) L-glutamic acid and L-glutamine being components of protein, (ix) the interconvertibility of L-glutamic acid and L-glutamine by usual biotransformation processes, (x) the structural analogy between the teratogenic thalidomide, antineoplastic antibiotics like cycloheximide, streptovitacins characterized by a common glutarimide moiety on the one hand [13–16], and the antineoplastic antibiotic, tenuazonic acid, characterized by an oxopyrrolidine moiety on the other [17, 18], both the moieties may be viewed as cyclized variants of glutamic acid, (xi) glutamine antagonism by antineoplastic antibiotics like azaserine and DON [19–21], (xii) the role of glutamic acid in preventing sickle cell anemia [22], (xiii) occurrence of γ-glutamyl transpeptidase in many human carcinomas [23]. Based on our hypothesis we have been synthesizing and biologically evaluating various structural variants of L-glutamic acid and L-glutamine, such as glutarimides, glutaramides, glutamines, isoglutamines, oxopyrrolidines, etc. for the last three decades together with QSAR studies of them [24–37]. These screenings are also being done in collaboration with the National Cancer Institute, Bethesda, Maryland, USA, who evaluate promising compounds according to their own protocols. Whatever the validity of our hypothesis or rationale might be, we have found meanwhile, irrespective of our rationale or mode of action, a number of glutamic acid and glutamine analogs or their structural variants have been established as antineoplastic agents or are under advanced stages of clinical trial or claimed as potential antineoplastic agent, e. g., aminoglutethimide [38, 39], antineoplastons [40–42], γ-glutamyl analogs of phenylhydrazine, p-nitroanilides, etc. [23], glutamic acid-γ-anilides [43], L-*threo*-(2*S*,4*S*)-4-fluoroglutamic acid and DL-3,3-difluoroglutamic acid analogs [44], DL-4,4-difluoroglutamic acid and DL-γ,γ-difluoromethotrexate [45]. Lastly, it should be pointed out that thalidomide itself, one of the starting points of our hypothesis, has been claimed to have shown antineoplastic activity against AIDS related Kaposi's sarcoma (Phase II clinical trial) [46], which might be viewed as an indirect proof for our proposition.

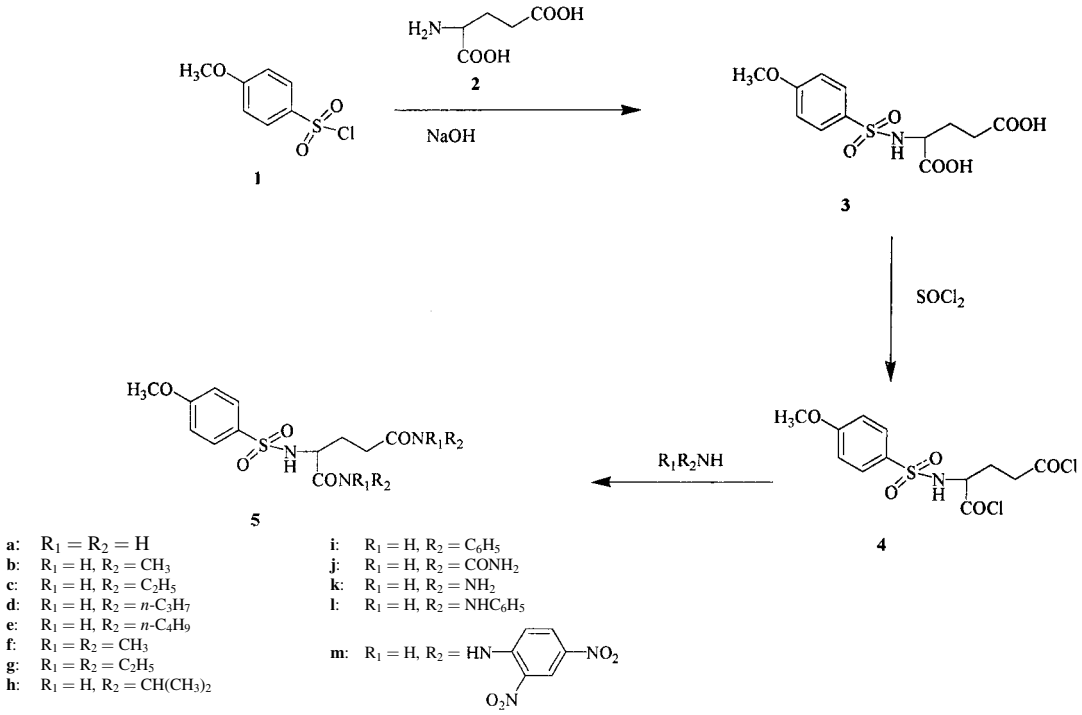
In continuation of this search we report here the synthesis, biological activity against Ehrlich ascites carcinoma in Swiss albino mice and preliminary QSAR analysis of some substituted 2-(4′-methoxybenzenesulphonamido) glutaric acids (**5a**–**m**, **7a**–**d**) as structural variants of glutamic acid. From our earlier observation [26, 27], and our QSAR analysis and Topliss operational scheme [47] as well as those of others [48] showing better antineoplastic activity of methoxy derivatives than their hydroxy congeners, we have included a methoxy group in the benzene ring in the target compounds. Different amines were chosen to include some randomness in the structure, a necessity for QSAR studies. Ureides and hydrazines being themselves groups of antineoplastic agents, some urea and hydrazine analogs were also studied.

2. Investigations, results and discussion

2.1. Synthesis of the compounds

The procedure for the preparation of the target compounds **5a**–**m** is summarized in Scheme 1. 4-Methoxybenzenesulphonyl chloride (**1**) was prepared from anisole and chlorosulphonic acid [49]. Condensation of **1** with L-glutamic

Scheme 1



acid (**2**) in alkaline medium yielded 2-*N*-(4′-methoxybenzenesulphonyl)-L-glutamic acid (**3**) in excellent yield and purity. The diacid **3** was refluxed with thionyl chloride to yield the corresponding dichloride **4**. Amination of **4** with different amino group bearing compounds yielded the target compounds **5a**–**m**. The compounds **7a**–**d** were prepared according to Scheme 2. Compound **3** was cyclized to 1-*N*-(4′-methoxybenzenesulphonyl)-5-oxopyrrolidine-2-carboxylic acid (**6**) with acetyl chloride. Aminolysis of **6** with different amines resulted in the target compounds **7a**–**d**, the substituted glutamines. Compound **4** was not crystallized because the acid halide hydrolysed when exposed. The physical properties and yields of the target compounds are shown in Table 1.

2.2. Biological activity

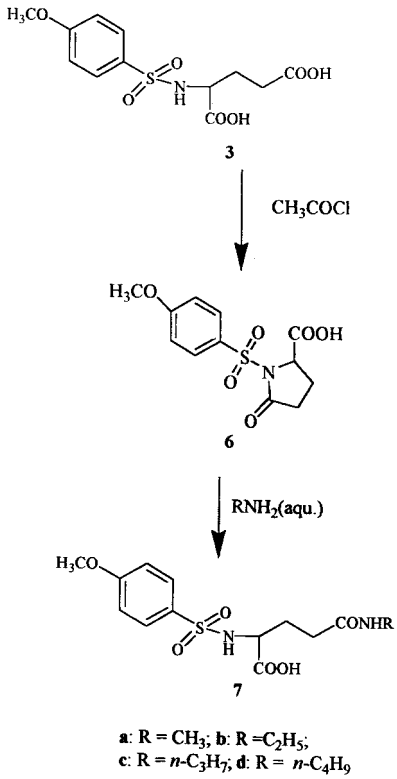
The target compounds (**5a**–**h**, **7a**–**d**) were tested *in vivo* against Ehrlich ascites carcinoma (EAC) cells in Swiss albino female mice as per the procedure described earlier [24, 25]. The EAC tumour (ascitic fluid) weight was used as the activity parameter. The results of the biological ac-

Table 1: Activities of substituted-2-(4′methoxybenzenesulphonamido) glutaric acid analogs against Ehrlich ascites carcinoma in Swiss albino mice

Compd.	Yield (%)	Melting point (°C)	Molecular formula[a]	T[b] (g)	C[b] (g)	Percentage inhibition of EAC fluid (P)
5a	86	216–218	$C_{12}H_{17}N_3O_5S$	0.609 (±0.137)	1.20 (±0.114)	50.00
5b	85	205–207	$C_{14}H_{21}N_3O_5S$	1.25 (±0.223)	1.80 (±0.135)	30.55
5c	83	184–186	$C_{16}H_{25}N_3O_5S$	0.75 (±0.074)	1.80 (±0.135)	58.33
5d	87	142–144	$C_{18}H_{29}N_3O_5S$	1.34 (±0.196)	1.80 (±0.135)	25.92
5e	81	124–126	$C_{20}H_{33}N_3O_5S$	1.00 (±0.226)	1.80 (±0.135)	44.44
5f[c]	77	144–146	$C_{16}H_{25}N_3O_5S$	1.20 (±0.345)	2.00 (±0.700)	40.00
5g[c]	81	140–142	$C_{20}H_{33}N_3O_5S$	0.50 (±0.152)	2.00 (±0.700)	75.00
5h[c]	85	196–198	$C_{18}H_{29}N_3O_5S$	2.00 (±0.566)	2.50 (±0.530)	20.00
5i[c]	74	224–226	$C_{24}H_{25}N_3O_5S$	1.00 (±0.100)	4.00 (±0.651)	75.00
5j[c]	79	220–222	$C_{14}H_{19}N_5O_7S$	0.20 (±0.057)	1.20 (±0.114)	83.33
5k[c]	83	218–220	$C_{12}H_{19}N_5O_5S$	1.00 (±0.353)	1.20 (±0.114)	16.67
5l	57	210–212	$C_{24}H_{27}N_5O_5S$	0.25 (±0.092)	4.60 (±0.689)	94.56
5m	83	91–93	$C_{24}H_{23}N_9O_{13}S$	0.25 (±0.067)	4.60 (±0.689)	94.56
7a	81	183–185	$C_{13}H_{18}N_2O_6S$	1.20 (±0.212)	1.80 (±0.135)	33.34
7b	85	158–160	$C_{14}H_{20}N_2O_6S$	0.41 (±0.148)	1.80 (±0.135)	60.00
7c	80	140–142	$C_{15}H_{22}N_2O_6S$	0.55 (±0.130)	1.80 (±0.135)	44.45
7d	83	144–146	$C_{16}H_{24}N_2O_6S$	0.60 (±0.137)	1.80 (±0.135)	66.67
Mitomycin C				0.00 (±0.010)	1.80 (±1.135)	100.00

[a] Microanalyses are within ±0.4% of the theoretical values for C, H and N
[b] Results represent the mean tumour weight from 5 data points. Figures within parenthesis are the standard error of the mean tumour weight. All the tumour weight recorded are significant with "t" statistics at 95% level
[c] These compounds were selected by NCI, Bethesda, Maryland, USA for further screening

Scheme 2



tivity study are reported in Table 1. The methoxy group in the benzene nucleus of the parent molecule resulted in a marked improvement in tumour weight inhibition compared to the chloro, methyl and unsubstituted benzene ring analogs having similar amino substituents, especially, those with urea and substituted hydrazines reported earlier from this laboratory [36, 37], which conforms to our rationale for choosing methoxy substituents in the target compounds. The ureide **5j** and the substituted hydrazine analogs **5l** and **5m** showed promising activity although the unsubstituted hydrazine analog **5k** showed no encouraging activity. The free carboxylic groups in the glutamines **7a–d** do not enhance the activity to any greater extent than was expected. Some of these compounds will be sent to the National Cancer Institute, Bethesda, Maryland, USA for further screening in accordance with our standing convention.

2.3. Quantitative structure activity relationship

Quantitative structure activity relationship (QSAR) studies were performed by the classical Hansch method [50]. π, σ^* and MR of the aliphatic substituents X and Y were used as the physicochemical descriptors of the biological activity, B_A, of the analogs studied. The biological activity term B_A denotes the logit transform of the percentage inhibition of EAC fluid weight [51]. π is defined here as the total hydrophobic contribution of the aliphatic substituents X and Y (structure **A** in Table 2). i.e.,

$$\pi = \pi_{Xali} + \pi_{Yali}$$

The π^2 term was also included in order to study the importance of transport to the site of action. σ^* is defined by Taft as the polar substituent constant for the aliphatic systems exclusively for its inductive nature [52], i.e.,

$$\sigma^* = \sigma^*_X + \sigma^*_Y$$

MR is the total molar refractivity of the same substituents and characterizes the size of the substituents as well as their polarizability, i.e.,

$$MR = MR_X + MR_Y$$

The multiple regression analyses were performed on a PC using the statistical package of Microsoft Excel 97. In the Hansch analyses we have used stepwise regression to obtain the best fit equation. The equation of the hyperplane with all the data points in Table 2 is given in eq. 1:

$$B_A = 1.3978\,(\pm 2.928)^{\ddagger}\,\pi + 0.2255\,(\pm 0.735)^{\ddagger}\,\pi^2 + 1.3068\,(\pm 1.118)^{\ddagger}\sigma^* + 0.2594\,(\pm 1.059)^{\ddagger}MR - 5.1729\,(\pm 6.528)^{\ddagger} \quad (1)$$

$$n = 17;\quad R = 0.7190;\quad R_a^2 = 0.3559;\quad SEE = 2.829;\quad s = 2.4497;\quad EV = 0.4054;\quad F(4, 12) = 3.210$$

The figures in parenthesis show the confidence intervals of the regression constants at 95% level, n is the number of observations in the run, R is the multiple regression coefficient, R_a^2 is adjusted R square, SEE is the standard error of estimate, s is the standard devivation of the regression, EV is the explained variance which is the proportion of the data explained by the regression hyperplane, F (k, n-k-1) is the variance ratio with k and n-k-l degrees of freedom where k is the number of independent variables. "t" statistics of the regression coefficient when insignificant at 95% level are marked with a double dragger (‡). As eq. 1 shows poor statistical parameters, in the next step the variables π^2 and MR were omitted one at a time in the next two equations:

$$B_A = 0.5809\,(\pm 1.190)^{\ddagger}\,\pi + 1.3109\,(\pm 1.083)\,\sigma^* + 0.4170\,(\pm 0.899)^{\ddagger}\,MR - 6.0728\,(\pm 5.646) \quad (2)$$

$$n = 17;\quad R = 0.7063;\quad R_a^2 = 0.3831;\quad SEE = 2.768;\quad s = 2.495;\quad EV = 0.4273;\quad F(3, 13) = 4.313$$

$$B_A = 1.8617\,(\pm 2.149)^{\ddagger}\,\pi + 0.3124\,(\pm 0.618)^{\ddagger}\,\pi^2 + 1.3871\,(1.028)\,\sigma^* - 4.0707\,(\pm 4.553)^{\ddagger} \quad (3)$$

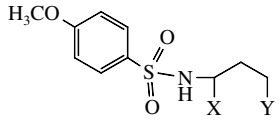
$$n = 17;\quad R = 0.7109;\quad R_a^2 = 0.3913;\quad SEE = 2.7498;\quad s = 2.4787;\quad EV = 0.4348;\quad F(3, 13) = 4.4288$$

The poor correlations obtained in the above equations were due to poor fit of several glutamic acid analogs of which residuals (difference of observed B_A and the calculated B_A) deviate by approximately 2 SEE and these are termed as outliers. Dropping the outlier **5g** eq. 4 is obtained which shows certain improvement in statistical parameters like R, EV, and F-test.

$$B_A = 2.0345\,(\pm 1.883)\,\pi + 0.3851\,(\pm 0.545)^{\ddagger}\,\pi^2 + 1.574\,(\pm 0.916)\,\sigma^* - 5.2693\,(\pm 4.131) \quad (4)$$

$$n = 16;\quad R = 0.7978;\quad R_a^2 = 0.5456;\quad SEE = 2.379;\quad s = 2.128;\quad EV = 0.5806;\quad F(3, 12) = 7.0044$$

Table 2: Physicochemical parameters[a] and logit transform antineoplastic activities of the target compounds



(A)

Cpd.	Substituents		π	σ*	MR	B_A^{obs}	$^bB_A^{calc1}$	$^cRes^1$	$^bB_A^{calc2}$	$^cRes^2$	$^bB_A^{calc3}$	$^cRes^3$
	X	Y										
5a[d]	$CONH_2$	$CONH_2$	−2.34	3.32	1.96	0	−2.6974	2.6974	−2.6122	2.6122	–	–
5b	CONHMe	CONHMe	−2.54	3.32	2.92	−1.7833	−2.7285	0.9452	−2.4666	0.6833	−3.1408	1.3575
5c[d,e]	CONHEt	CONHEt	−1.42	3.12	4.02	0.73034	−2.4723	3.2027	–	–	–	–
5d	CONHPr	CONHPr	−0.32	3.08	4.96	−2.2803	−1.0343	−1.2460	−1.5367	−0.744	−1.8797	−0.4006
5e	CONHBu	CONHBu	0.84	3.06	5.88	−0.4849	1.5265	−2.0114	1.6827	−2.168	1.7490	−2.2339
5f	$CONMe_2$	$CONMe_2$	−3.02	3.88	4.2	−1.306	−1.7962	0.4902	−1.0021	−0.304	−1.6103	0.3043
5g[d,e,f]	$CONEt_2$	$CONEt_2$	−0.64	2.92	6.04	4.075	–	–	–	–	–	–
5h	$CONHCHMe_2$	$CONHCHMe_2$	−0.98	2.94	4.96	−4.8044	−2.2670	−2.5374	−2.8267	−1.978	−3.3533	−1.4511
5i	$CONHC_6H_5$	$CONHC_6H_5$	0.98	3.12	7.08	4.45631	2.0038	2.4525	2.2907	2.1656	2.4247	2.0316
5j	$CONHCONH_2$	$CONHCONH_2$	−4.28	6.64	3.64	5.6049	3.5252	2.0796	6.3528	−0.748	6.2050	−0.6001
5k[d,e]	$CONHNH_2$	$CONHNH_2$	−3.8	4.56	5	−4.8221	−0.2647	−4.5574	–	–	–	–
5l	$CONHNHC_6H_5$	$CONHNHC_6H_5$	0.5	5.76	10.08	6.20054	4.9080	1.2926	4.8946	1.306	5.1640	1.0366
5m	$CONHNHC_6H_3(NO_2)_2$	$CONHNHC_6H_3(NO_2)_2$	−0.62	8.32	6.52	6.20054	6.7094	−0.5089	6.3457	−0.145	6.5802	−0.3796
7a	COOH	CONHMe	−1.59	4.6	2.15	−1.5044	−0.2923	−1.2121	−0.6313	−0.873	−1.0585	−0.4459
7b	COOH	CONHEt	−1.03	4.5	2.7	0.88046	0.1248	0.7557	−0.3724	1.2528	−0.7133	1.5938
7c	COOH	CONHPr	−0.48	4.48	3.17	−0.48406	0.8924	−1.3765	0.4070	−0.891	0.1953	−0.6794
7d	COOH	CONHBu	0.1	4.47	3.63	1.5055	1.9719	−0.4663	1.6754	−0.17	1.6387	−0.1332

[a] Values obtained from ref. [53]
[b] Calc1, calc2 and calc3 are calculated biological activity using eq. (4), (5) and (6) respectively
[c] $Res^1 = B_A^{obs} - B_A^{calc1}$; $Res^2 = B_A^{obs} - B_A^{calc2}$; $Res^3 = B_A^{obs} - B_A^{calc3}$
Compounds superscripted with *d*, *e* and *f* were excluded while deriving eqs. (6), (5) and (4) respectively

Similarly, dropping the other outliers, **5c** and **5k**, one by one we arrive at the following regression eq. 5, which explains 79.85% of variance having no coefficients insignificant by the t-test:

$$B_A = 2.463\,(\pm 1.365)\,\pi + 0.6539\,(\pm 0.413)\,\pi^2 + 1.6101\,(\pm 0.658)\,\sigma^* - 5.7746\,(\pm 2.999) \qquad (5)$$

$$n = 14; \quad R = 0.9108; \quad R_a^2 = 0.7784; \quad SEE = 1.6332; \quad s = 1.4325; \quad EV = 0.7985; \quad F(3,10) = 16.219$$

When another possible outlier **5a** is omitted, the best eq. 6 is obtained:

$$B_A = 2.7885\,(\pm 1.254)\,\pi + 0.7108\,(\pm 0.368)\,\pi^2 + 1.7359\,(\pm 0.594)\,\sigma^* - 6.4067\,(\pm 2.728) \qquad (6)$$

$$n = 13; \quad R = 0.9400; \quad R_a^2 = 0.8448; \quad SEE = 1.4188; \quad s = 1.2287; \quad EV = 0.8603; \quad F(3,9) = 22.776$$

The autocorrelation matrix of the parameters used to develop eq. 6 is given in Table 3 which indicates that the parameters π, π^2 and σ* are reasonably orthogonal in nature except MR. The square of the correlation coefficients between MR and π ($r^2 = 0.3025$) may be the cause for the insignificant regression coefficient of MR. The larger coefficient of π in eq. 6 supports the significance of hydrophobic binding with the site of action. Omission of the π^2 term led to an insignificant regression equation. The positive coefficient of π^2 is in conformity with our experimental design where the analogs are injected directly in the intraperitoneal cavity so excluding transport phenomena. Inclusion of the MR term led to regression equations with insignificant coefficients (t-statistics) of MR, both including and excluding the outliers. Thus molecular refractivity, and hence the bulk, may not be playing any significant role in this class of compounds. The statistical significance of the electronic term, Taft's substituent constant σ*, indicates significant electronic influences on the mechanism of action of the compounds studied. The positive coefficient of σ* suggests that the substituents are electron withdrawing and possibly bind with the electron-rich (nucleophilic) site. Thus, the electronic environment of the aliphatic substituent at the critical reaction site should be considered with care in preparing further analogs in this series of glutamic acids.

In summary, the QSAR study by the classical Hansch analysis shows that hydrophobicity, π, and electronic parameter, σ*, play a significant role in eliciting antineoplastic activity in this class of compounds. This findings will help us to synthesize more effective glutamine and glutamic acid analogs with judicious modification of the substituents at the aliphatic centre.

Table 3: Autocorrelation matrix of the physicochemical descriptors used to develop eqn. 6

	π^2	π	σ*	MR
π^2	1			
π	−0.88058	1		
σ*	0.243624	−0.23536	1	
MR	−0.29506	0.550008	0.187522	1

3. Experimental

3.1. Chemistry

All the m.p. ranges reported are uncorrected. C H N analyses, neutralization equivalent and IR spectra were used to characterise the structures. Microanalytical results, indicated by atomic symbols, were within ±0.4%

of the calculated values. The IR spectra were recorded using KBr discs. The abbreviations used are sym = symmetrical, antisym = antisymmetrical and str = stretching.

3.1.1. 2-N-(4′-Methoxybenzenesulphonyl)-L-glutamic acid (**3**)

To L-(+)-glutamic acid (**2**: 14.7 g, 0.1 mol) dissolved in 2 N NaOH (50 ml), 4-methoxybenzenesulphonyl chloride (**1**: 20.6 g, 0.1 mol) was added slowly with constant stirring, maintaining the internal temperature at 70 °C. The reaction was continued until a clear homogeneous solution resulted. After the reaction was complete, it was allowed to cool, acidified to Congo red with conc. HCl, saturated with NaCl, extracted with ethyl acetate, washed with saturated brine solution and allowed to stand overnight with anh. $MgSO_4$. Ethyl acetate was removed to yield the desired crude sticky product **3**. It was refluxed with dry benzene for 5 min and finally recrystallized from ethyl acetate-dry benzene to yield 26.2 g (83%) of analytically pure **3**; m.r. 114–116 °C; neutralization equivalent found 158.56; calc. 158.50.

3.1.2. 2-N-(4′-Methoxybenzenesulphonyl)-L-glutamic acid dichloride (**4**)

2-*N*-(4′-Methoxybenzenesulphonyl)-L-glutamic acid (**3**: 2 g, 0.006 mol) was refluxed with thionyl chloride in a steam bath for 2 h. The excess thionyl chloride was removed by codistillation with dry benzene to obtain **4**. This was sufficiently pure to be used in the subsequent steps.

3.1.3. General procedure for preparation of substituted 2-(4′-methoxybenzenesulphonamido)-L-glutaramides (**5a–i, 5l, 5m**). *Example 2-(4′-methoxybenzenesulphonamido)-L-glutaric acid diamide* (**5a**)

2-*N*-(4′-Methoxybenzenesulphonyl)-L-glutamic acid dichloride (**4**) was dissolved in dry benzene (10 ml) placed in a 100 ml round bottom flask fitted with a reflux condenser on a steam bath. Liquor NH_3 (15 ml) was added to it slowly under cold conditions with frequent shaking and refluxed for 15 min. The excess benzene was distilled off. The reaction mass was washed with dilute HCl to remove the excess amine, $NaHCO_3$ to remove the acid, and finally with H_2O and was recrystallized from dilute EtOH with charcoal treatment to obtain 86% of pure **5a**; m.r. 216–218 °C. IR 3450 (N–H str of CONH), 3320 (N–H str of CONH), 3260 (N–H str of SO–2NH), 1650 (C=O str of CONH), 1300 (SO_2 str of SO_2NH antisym), 1150 (SO_2 str of SO_2NH sym), 1015 (C–O str of CH_3OAr), 800 (phenyl) cm^{-1}.
$C_{12}H_{17}O_5N_3S$

3.1.4. 2-(4′-Methoxybenzenesulphonamido)-L-glutaric acid diureide (**5j**)

To the solution of **4** in benzene (10 ml), powdered urea (2 g, 0.03 mol) was added and refluxed for 1 h. A hard crust was formed after removal of benzene which was washed with dilute HCl, $NaHCO_3$, and H_2O and recrystallized from dilute EtOH with charcoal treatment to yield 2 g (79%) of pure **5j**; m.r. 220–222 °C.
IR 3365 (N–H str of CONH), 3150 (N–H str of SO_2NH), 1680 (C=O of CONH), 1355 (SO_2 of SO_2NH), 1255 (C–O of CH_3OAr), 1160 (SO_2 of SO_2NH), 1085 (C–O of CH_3OAr), 800 (phenyl) cm^{-1}.
$C_{14}H_{19}O_7N_5S$

3.1.5. 2-(4′-Methoxybenzenesulphonamido)-L-glutaric acid dihydrazide (**5k**)

To the solution of **4** in benzene (10 ml), cooled in an ice water bath, hydrazine hydrate (80%, 5 ml) was added and mixed well. The solid mass obtained after removal of benzene was recrystallized from dilute EtOH with charcoal treatment to yield 83% of pure **5k**; m.r. 218–220 °C. IR 3340 (N–H str of $CONH_2$), 3300 (N–H str of CONH), 3240 (N–H str of SO_2NH), 1650 (C=O of CONH), 1340 (SO_2 str antisym of SO_2NH), 1155 (SO_2 str sym of SO_2NH), 800 (phenyl) cm^{-1}.
$C_{12}H_{19}O_5N_5S$

3.1.6. 1-N-(4′-Methoxybenzenesulphonyl)-5-oxopyrrolidine-2-carboxylic acid (**6**)

To 10 g (0.03 mol) of **3**, acetyl chloride (20 ml) was added and refluxed on a steam bath for 2 h. The reaction mass was cooled and poured on to crushed ice slowly with continuous stirring. It was kept cold overnight when the semisolid mass solidified. It was filtered, washed with H_2O and recrystallized from dilute EtOH with charcoal treatment to yield 85% of pure **6**; m.r. 95–97 °C. Neutralization equivalent found 300, calc 299.

3.1.7. General procedure for preparation of 5-N-alkyl-2-N-(4′-methoxybenzenesulphonyl) glutamine (**7a–d**). *Example, 5-N-ethyl-2-N-(4′-methoxybenzenesulphonyl) glutamine* (**7b**)

To a suspension of **6** (3 g, 0.01 mol) in 30 ml H_2O, 15 ml monoethylamine (50%) was added and placed in a stoppered conical flask, shaken, and left overnight. The excess amine was evaporated out on a steam bath, water (30 ml) was added, and it was cooled and acidified with 6 N HCl to Congo red paper. The precipitate obtained was filtered, washed with H_2O and dried. The crude mass was recrystallized from H_2O with charcoal treatment to yield 85% of pure **7b**; m.r. 158–160 °C. Neutralization equivalent found 341.22, calc 344.00. IR 3359.39 (N–H str of CONH), 3270.68 (N–H str of CONH), 3101.94 (C–H str of *p*-subst. phenyl), 3017.57 (C–H str of *p*-subst. phenyl), 2974.18 to 2844.97 (O–H str of COOH), 1352.82 (S=O antisym str of SO_2NH), 1160.45 (S=O sym str of SO_2NH), 803.69 (phenyl) cm^{-1}.

3.2. In vivo antineoplastic activity evaluation

The target compounds were tested *in vivo* for their possible antineoplastic activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. Two groups of female mice containing 5 mice in each group, having average weight between 18–20 g and approximately the same age, were selected at random. 2×10^6 EAC cells per 0.2 ml were inoculated, i. p., on day 1. One group served as controls and other as the test group. After 24 h of incubation the target compounds suspended in phosphate buffer (pH 7.2), were injected, i. p., on days 2–8 to the test group. The animals (both test and control) were sacrificed on day 9 and individual weights of tumours were noted. The percentage inhibition of the tumour weight (P) was calculated as follows:

$$P = (1 - T/C) \times 100$$

where T and C are the mean weight of EAC fluid (tumour weight) in the test and control groups respectively. The compounds administered i. p. for assessing antineoplastic activity were at approximately fifty times the dose level of our standard, mitomycin C, in our reported protocol. The percentage inhibitions of tumour weight are given in Table 1. The dose level injected in each case was well within the toxic level as observed from our earlier LD_{50} studies in this class of compounds.

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