Synthesis and Biological Evaluation of Some Substituted-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) Glutamic Acid Derivatives against Prostate Cancer Cell Line PC3

Ghaneya Sayed Hassan and Doaa Ezzat Abdel Rahman*

Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University; Cairo 11562, Egypt.
Received October 7, 2012; accepted November 1, 2012

New series of substituted glutamine 5a–l and glutamic acid diamides, diureide and dihydrazide 7a–e were synthesized from parent glutamic acid compound 3 and evaluated for their cytotoxic activity against tumor cell line PC3 (prostate cancer cell line). Most of the tested compounds exploited potent growth inhibitory activity with IC_{50} values ranging 0.034–3.97 μM. Particularly, compounds 5a, 3, 5j, 5b, 7c, 7e, 5l, and 5k exhibited superior potency (IC_{50}=0.034, 0.04, 0.05, 0.074, 0.25, 0.4, 0.49, 0.522 μM, respectively) to the reference drug Doxorubicin (IC_{50}=0.63 μM), while compound 7b showed IC_{50}, 0.71 μM, comparable to that of Doxorubicin. In summary, the newly synthesized compounds provided promising new lead for the future design and development of glutamine and glutamic acid derivatives as novel antitumor agents. The quantitative structure–activity relationship (QSAR) study was applied to find a mathematical correlation between the structures of compounds and their activity against PC3 cell line expressed as IC_{50} values.

Key words glutametergic system; glutamic acid; glutamine; prostate cancer (PC); PC3 cell line

Prostate cancer (PC) is the most commonly diagnosed cancer among men around the world and it is considered the second most common cause of cancer-related deaths.[1,2] Human PC cell lines have been classified into androgen-dependant line established from metastatic PC in the lymph-node such as LNCaP cell line and androgen-independent line established from a patient with bone or brain metastatic PC such as PC3 cell line or DU145 cell line respectively.[3] However, after PC makes metastases, chemotherapy plays an extremely important role. With the pass of the time, PC cell lines become resistant to the current antiprostate cancer drugs. Therefore, there is a necessity to develop new antiprostate cancer agents with the ability to be active against several PC cell lines.[2]

Glutamine (Gln) is the most abundant free amino acid in the human body, it is essential for the growth of normal and neoplastic cells.[4] A continuous supply of glutamine may be necessary for cancerous cell growth since cancer has been described as a nitrogen trap.[4,5] In addition, L-glutamic acid provided an important role in formation and uncontrolled proliferation of neoplastic cells. This was attributed to obtain L-glutamic acid as product of hydrolysis of different types of malignant tumors.[6,7] Therefore, L-glutamic acid and glutamine gained great importance in formation and proliferation of different PC cell lines.

Furthermore, glutamate carboxypeptidase II (GCPII), a membrane bound cell surface peptidase, is identical to prostate-specific membrane antigen (PSMA). PSMA is a putative class II transmembranous glycoprotein and functionally serves as transporter or binding protein or has hydrolytic activity.[8–11] Isolated membrane fractions from human PC cell lines (LNCaP, PC3 and DU145) can be tested for hydrolase activity. Unlike LNCaP cell membranes, which highly express PSMA, membranes isolated from other human prostate adenocarcinoma cells (PC3 and DU145) did not exhibit comparable hydrolase activity.[11] PSMA serves as an attractive target for cancer immunotherapy by virtue of its abundant and restricted expression on the surface of prostate carcinoma and it is considered as a tumor marker for fast and reliable diagnosis of

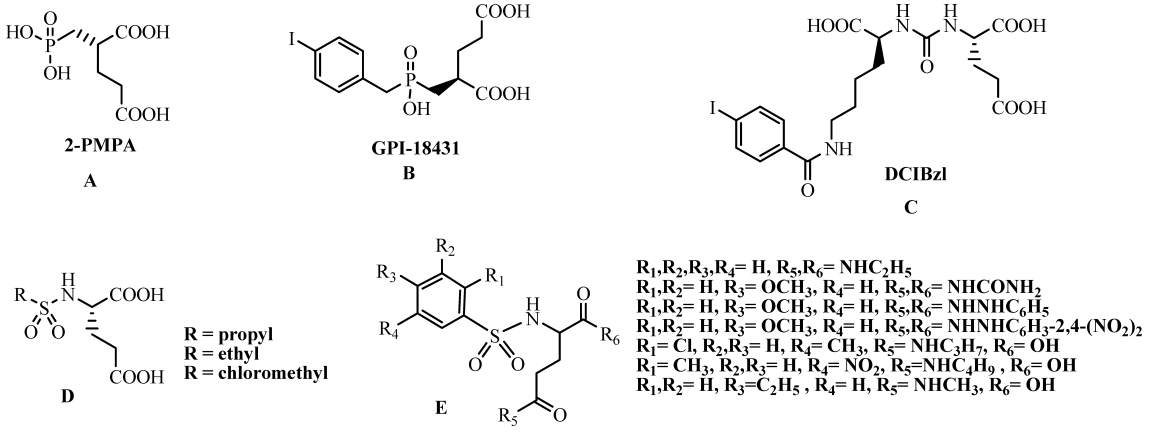
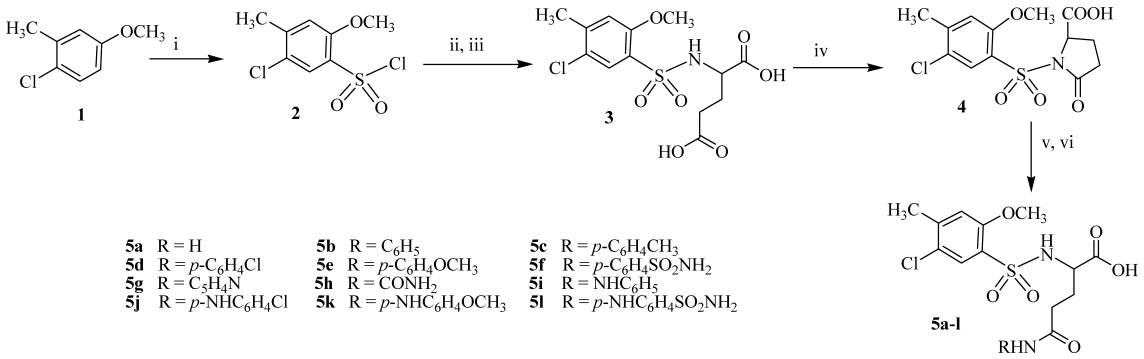


Fig. 1. Representative Inhibitors of GCPII and Anti-tumor Active Compounds

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: doaaezzat2004@yahoo.com



Reagents and conditions: (i) $ClSO_3H$, 0°C; (ii) L-(+)-glutamic acid 2 M NaOH, 70°C, 1 h; (iii) conc. HCl; (iv) CH_3COCl, 5°C, 1 h, reflux 2 h, stir room temperature 24 h; (v) R-NH_2, stir room temperature 24 h; (vi) 6 M HCl.

Chart 1

PC.[10,12] There are a number of GCPII inhibitors, characterized by a functional group connected to either a glutaryl moiety or the glutamyl amino group, which either terminates the structure or serves as a linker to another molecular fragment. GCPII inhibitors include 2-PMPA (**A**), GPI-18431 (**B**), DCIBzl (**C**) and *N*-substituted glutamyl sulfonamides (**D**) (Fig. 1).

In most inhibitors, glutarate/glutamate moiety appears to occupy the specificity pocket of GCPII while phosphonate, phosphinate, urea or sulfonamide moiety ligates to the catalytic zinc ion at the active site.[12–15] Structure–activity relationship for the glutamate binding site revealed that both carboxylic acid groups of glutamate moiety should be free. Esterification of terminal carboxylic group reduces the activity and of both carboxylic groups drastically reduces activity.[12]

Recently, there is increasing evidence to suggest that the glutamatergic system (Glu system) may be involved in cancer biology. The Glu system comprises the Glu receptors (GluRs), the Glu transporters (GluTs) and glutamine synthetase (GS). The expression of Glu system components were assessed in human androgen-independent PC3 and androgen dependent LNCaP PC cells.[16] Therefore, due to detection of the expression of mGLuR5 (metabotropic GluRs, mGluRs promote intracellular signal transduction pathways *via* activation of G proteins)[16,17] and GS (the metabolism of Glu by GS completes the Glu system, controlling intracellular glutamate signaling pathways by the conversion of glutamate to glutamine)[16,18–20] at the protein level in both PC3 and LNCaP cells, it is possible that PC3 and LNCaP prostate cancer cells possess ion-dependent channel activity and intracellular G protein-dependent signaling pathways controlled by glutamate. The possible importance of the Glu system in PC cells can be foreseen based on its relationship to prostate-specific membrane antigen (PSMA) expression, expression of PSMA in transfected PC3 cells (PC3 cells do not express PSMA) reduced the invasion potential of PC3 cells, suggesting that this reduction in the invasion capability was due to PSMA expression and not to intrinsic properties of transfected PC cell lines.[16]

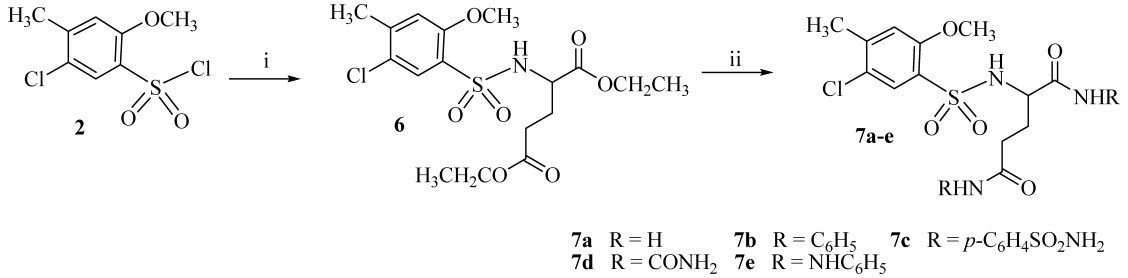
Moreover, relative specific amino acid dependency is one of the metabolic abnormalities of cancer cells, and restriction of specific amino acids may induce apoptosis of PC cells. Specific amino acid dependency differentially regulates glucose metabolism, oxidation-reduction reactions of mitochondria and mitochondrial damage in DU145 and PC3 cell lines.[21] Restriction of Gln in PC3 cell lines inhibits ATP synthesis, induces generation of reactive oxygen species (ROS), reduces glucose consumption and lactate production but did not induce apoptosis.[21,22] In addition, Gln deprivations modulate directionality and motility to inhibit invasion in PC3 cells.[23]

Substituted benzenesulphonylglutamic acid analogs (**E**) (Fig. 1) were prepared and biologically evaluated against Ehrlich ascites carcinoma. Results revailed that, introduction of methoxy group to benzene nucleus of the disubstituted glutamic acid analogs resulted in marked improvement in tumor weight inhibition compared to the chloro, methyl and unsubstituted benzene ring analogs having similar amino substituents[24] while unsubstituted ring analogs showed activity with different amino substituents.[25] In case of glutamine analogs presence of chloro, nitro, methyl and ethyl substituent provided more reactive compounds compared with un substituted benzene ring analog.[26,27]

Based on these, we report the synthesis of some substituted-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) glutamic acid derivatives and the antitumor evaluation of the prepared compounds against PC3 cell line. Synthesis of substituted-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) glutamic acid derivatives was proposed to study the effect of presence of chloro, methyl and methoxy substituents all together on activity. Compounds **5a**–**l**, were designed as structural analogs of glutamine, the most reactive amino acid especially in the tumor cells, while compounds **7a**–**e** were designed as disubstituted glutamic acid analogs. PC3 cell line was selected for biological antitumor evaluation for many reasons. First, structure of the prepared compounds is similar with glutamate and glutamine. Second, PC3 cells do not express PSMA, to exclude the effect on PSMA as the prepared compounds have one carboxylic group or both carboxylic groups substituted and expected to be inactive as PSMA inhibitor. Quantitative structure–activity relationship (QSAR) study was also performed for understanding and validating the cytotoxic activities.

Results and Discussion

Chemistry The procedure for the preparation of targeted compounds **5a**–**l** and **7a**–**e** were summarized in (Charts 1, 2). 5-Chloro-2-methoxy-4-methylbenzene-1-sulphonyl chloride **2** (Chart 1) was prepared by chlorosulphonation[28] of 1-chloro-4-methoxy-2-methylbenzene **1** with chlorosulphonic acid and melting point of the obtained product was as reported.[29] With the application of Schotten–Baumann reaction,[30] 2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) glutamic



7a R = H **7b** R = C_6H_5 **7c** R = *p*-$C_6H_4SO_2NH_2$
7d R = $CONH_2$ **7e** R = NHC_6H_5

Reagents and conditions: (i) diethyl glutamate, triethylamine, dry benzene, 24 h; (ii) R-NH_2, ethanol, reflux 16 h.
Chart 2

acid **3** (Chart 1) was prepared by one step condensation of 5-chloro-2-methoxy-4-methylbenzene-1-sulphonyl chloride **2** with solution of L-glutamic acid in 2 M NaOH. Structure of compound **3** was confirmed on the bases of elemental analysis and spectroscopic data (IR, ^{1}H-NMR, MS). IR spectra showed NH and OH carboxylic stretching bands at 3425 cm^{-1} and 2943, 2849 cm^{-1}, respectively, two carboxylic carbonyls at 1728, 1670 cm^{-1} and SO_2 group at 1369, 1118 cm^{-1}. ^{1}H-NMR spectra showed the aliphatic protons of glutamic acid in addition to methyl and methoxy protons at the region δ=2.05, 2.31, 3.71, 3.75 ppm, aromatic protons at 6.98 and 7.61 ppm. The NH and two carboxylic OH protons appeared at δ=9.71, 10.52, 10.93 ppm, exchanged with D_2O. Mass spectra showed molecular ion peak. Cyclization of compound **3** with acetyl chloride provided 1-(5-chloro-2-methoxy-4-methylphenylsulphonyl)-5-oxopyrrolidine-2-carboxylic acid **4** (Chart 1). Disappearance of NH group from IR and ^{1}H-NMR spectra proved structure of compound **4**.

The oxopyrrolidine-2-carboxylic acid **4** was reacted with appropriate amino derivative and gave 2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-substituted glutamine **5a**–**l** (Chart 1). The structure assignment for the prepared **5a**–**l** was deduced by elemental and spectral analysis. IR spectra showed additional NH bands at 3587–3120. ^{1}H-NMR spectra of the showed additional aromatic protons at δ=6.42–8.10 ppm (except for **5a**, **5h**). ^{13}C-NMR spectra for compounds **5d** and **5j** showed bands at δ: 19.70–19.72, 26.00, 31.00–36.00, 55.80–55.83, 171.10–174.00 and 175.00–176.00 for CH_3, $\underline{C}H_2CH_2CO$, $\underline{C}H_2CO$, CH and OCH_3, CONH, and COOH respectively.

Attempts to prepare the disubstituted compounds were done *via* different methods. Reaction of compound **3** with different aniline derivatives using phosphorus trichloride in chlorobenzene or preparation of the acid dichloride derivative of compound **3** followed by reaction with different amino compounds gave the monosubstituted compounds **5b**–**f** and **5b**, **h**, **i** respectively, supported by the measured melting points and IR spectral data.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl) glutamic acid diethyl ester **6**, (Chart 2) was prepared by reaction of the sulphonyl chloride derivative **2** and diethyl glutamate. IR spectra showed NH at 3441 cm^{-1}, absence of OH carboxylic stretching band, two ester carbonyls at 1720, 1670 cm^{-1} and SO_2 group at 1377, 1172 cm^{-1}. ^{1}H-NMR spectra showed the aliphatic protons of glutamic acid in addition to methyl, methoxy and ethyl protons at the region δ=1.20, 1.40, 2.30, 2.36, 3.09, 3.64–3.80 ppm, aromatic protons at 6.96 and 7.59 ppm. The NH appeared at δ=9.75 ppm, exchanged with D_2O and absence of the two carboxylic OH protons. Mass spectra showed molecular ion peak.

N^1,N^5-Bis substituted-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) glutamic acid diamides **7a**–**c**, diureide **7d** and dihydrazide **7e**, (Chart 2) were achieved by refluxing compound **6** with appropriate amino compounds in ethanol. The proposed structure of **7a**–**e** was confirmed by micro analysis and spectroscopic data. IR spectra showed additional NH bands at 3448–3101 and disappearance of OH carboxylic stretching bands. ^{1}H-NMR spectra showed absence of ethyl protons and appearance of additional aromatic protons at δ= 6.81–7.84 ppm (except for **7a**, **7d**) and additional NH at δ= 3.93–10.10 ppm, exchanged with D_2O. The ^{13}C-NMR spectra for compound **7e** showed bands at δ: 19.79, 20.04, 36.00, 55.78, 56.49, 172.00 and 176.00 for CH_3, $\underline{C}H_2CH_2CO$, $\underline{C}H_2CO$, CH, OCH_3, and 2 CONH, respectively.

Antitumor Screening All newly synthesized compounds were evaluated for their cytotoxicity against PC3 (prostate cancer cell line) by Skehan's method[31] as they may be good predictors of clinically useful drugs. In this protocol, cell line was inoculated and incubated in plate for 24 h. Test compounds were then added with different concentrations (0.001, 0.01, 0.1, 1, 10, 100 μM) and incubated for 48 h. Surviving curves were plotted as a relation between concentration and the surviving fraction to calculate IC_{50} (concentration that reduce the surviving fraction to 50%)[32] using known drug Doxorubicin (Dox) as a positive control (Table 1).

It was interesting to notice that, most of the tested compounds exhibited promising activity where IC_{50} ranged from 0.034–3.97 μM for compounds **3**, **5a**, **5b**, **5e**, **5i**, **5j**, **5k**, **5l**, **7a**, **7b**, **7c**, **7d** and **7e**.

Parent intermediate compound 2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) glutamic acid **3** exhibited potent cytotoxic activity where the IC_{50} 0.04 μM. Replacement of one carboxylic function with amide, anilide, uride or hydrazide function resulted in glutamine derivative with change in activity to more potent or potent derivatives and some were less active.

Glutamine derivative **5a** was very promising and more potent than the parent compound (IC_{50}, 0.034 μM). Anilide derivative **5b** showed more potency than substituted anilide derivative **5e** as substitution with methoxy group at position 4 slightly affect activity where IC_{50}, 0.074 and 3.97 μM, respectively. Substitution at position 4 of the anilide with methyl or chloro or sulphonamido group reduced activity. Heteroaromatic anilide and uride substitution greatly reduced activity.

Hydrazide derivatives **5i**–**l** showed good activity as IC_{50}, ranging from 0.05–3.23 μM where the chloro substitution at

Table 1. IC_{50} of Tested Compounds for Antitumor Screening against PC3 (Prostate Cancer Cell Line)

Cpd. No.	IC_{50} (μM)
3	0.04
5a	0.034
5b	0.074
5c	17.4
5d	22.03
5e	3.97
5f	17.9
5g	76.7
5h	33.8
5i	3.23
5j	0.05
5k	0.522
5l	0.49
7a	1.07
7b	0.71
7c	0.25
7d	2.18
7e	0.4
Dox	0.63

position 4 of phenylhydrazide was most active one among substituted hydrazide derivatives and the unsubstituted derivative was the least active one which explore the importance of substitution among these active compounds.

Replacement of both carboxylic functions with diamide, diuride and dihydrazide function **7a**–**e** resulted in active compounds with IC_{50}, ranging from 0.25–2.18 μM. It was noticed that diamide **7a** and dianilide **7b** were active compounds but activity decreased 30 and 10 folds from the amide **5a** and anilide **5b**, respectively. Dianilide substituted with sulphonamido group **7c** and diuride **7d** were active irrespective that monosubstituted derivatives **5f** and **5h** had reduced activity. Dihydrazide **7e** showed increased activity 8 folds than the monosubstituted one **5i**.

Compounds **3**, **5a**, **5b**, **5j**, **5k**, **5l**, **7c**, **7e** were found to be more active than Dox while compounds **7a** and **7b** were nearly active as Dox.

QSAR Study In an attempt to correlate the antiproliferative activity with the structure conformation of the synthesized glutamic acid derivatives, QSAR study was undertaken. Descriptors of the molecular modeling software, Molecular Operating Environment (MOE version 2008.10.2),[33] were used. The structural descriptors used in the generation of these models include; Kier molecular flexibility index (Flex), Water accessible surface area (ASA), the ionization potential (IP or mndo_IP), Value of the potential energy (E) and van der Waals component of the potential energy (E_vdw) as shown in Table 2.

To test the best structural predictors for activity, stepwise linear regression analysis (SLRA) technique was used.

The preliminary regression analysis has indicated that out of the 18 tested compounds, four compounds (**5d**, **f**–**h**) were outliers. These outliers were recognized by having high preliminary Z-score values (>2.5) against the selected descriptors.[34] For better statistical results these compounds were deleted from further regression procedure.

For the current dataset of 14 compounds, the QSAR model development was restricted to a maximum of three variables as one should select one parameter for five compounds data set (5 : 1 for compounds: descriptor).

The simple linear regression analysis yielded one statistically significant correlation (Eq. 1), that was the correlation between the activity and the ionization potential (IP) of the tested compounds.

$$IC_{50} = 69.9(\pm 21.2) - 7.15(\pm 2.24)\ IP$$
$$n = 14,\ S_e = 3.49,\ r = 0.678,\ r^2 = 0.460,\ F = 10.22 \quad (1)$$

n: Number of compounds. S_e: Standard error of estimation. r: Simple (multiple) correlation coefficient. r^2: Fraction of the variance. F: Fisher's statistics.

Stepwise regression analyses using different combinations of IP and other structural descriptors resulted into bi-parametric models including: IP, E; IP, E_vdw; IP, ASA and IP, Flex which show better statistics than the mono-parametric model discussed above.

Trials were made to correlate three combined descriptors with the biological activity aiming to improve the statistical parameters of the obtained models. Two tri-parametric models were obtained from stepwise regression analyses (Models 1 and 2).

Table 2. The Molecular Descriptor Values of the Studied Compounds

Cpd. No.	Descriptors				
	Kierflex	ASA	IP	E	E_vdw
3	7.2453	568.8266	10.1247	4.6182	35.3791
5a	6.9065	575.6415	10.0520	8.3250	36.0493
5b	7.4378	689.5095	9.5634	38.7099	53.3627
5c	7.6659	708.0336	8.8231	36.9720	53.3364
5e	8.2508	714.5831	9.4067	44.3930	56.6556
5i	7.7329	719.5165	9.2009	54.2075	53.0716
5j	8.7610	741.2696	9.2893	52.5998	52.2035
5k	8.5475	748.0812	9.2917	61.9106	57.5350
5l	9.5130	767.4078	9.8423	23.7034	56.1462
7a	6.5811	572.9178	9.8209	7.1049	39.0610
7b	7.8414	792.5010	9.0720	65.3133	71.0572
7c	11.2955	917.0554	9.7339	6.6719	74.2773
7d	7.7464	655.1626	9.6383	−42.5792	43.4123
7e	8.4134	789.6392	9.1544	94.1508	66.7716

$$IC_{50} = 121.4(\pm 22.4) - 11.7(\pm 2.2)\ IP - 0.06(\pm 0.02)\ E - 0.11(\pm 0.07)\ E\_vdw$$
$$n = 14,\ S_e = 2.631,\ r = 0.862,\ r^2 = 0.743,\ F = 9.667 \quad \text{(Model 1)}$$

$$IC_{50} = 150.3(\pm 28.6) - 13.9(\pm 2.7)\ IP - 0.08(\pm 0.02)\ ASA + 4.8(\pm 1.8)\ Flex$$
$$n = 14,\ S_e = 2.604,\ r = 0.865,\ r^2 = 0.749,\ F = 9.940 \quad \text{(Model 2)}$$

Fraction of the variance (r^2): Represent the goodness of fit. The value of r^2 may vary between 0 and 1, when multiplied by 100 gives explained variance in biological activity, where 1 means a perfect model explaining 100% of the variance in the data, and 0 means a model without any explanatory power. It has already been suggested that the only QSAR model having $r^2 > 0.6$ will be considered for validation.[35] The value of r^2 for Models 1 and 2 were 0.743 and 0.749 respectively.

The observed activities (Obs. IC_{50}) together with the predicted activities (Pred. IC_{50}) for the tested compounds calculated using multi-linear regression (MLR) are listed for Model 2 in Table 3.

From Model 1 equation, cytotoxic activity was negatively correlated with IP, E and E_vdw. The high coefficient value of IP and the comparatively lower value of E_vdw suggested that the decrease in ionization potential and van der Waals component of the potential energy lead to enhancement of activity. This was in good agreement with the obtained experimental data, for the most active compounds **3** and **5a**, slight decrease in ionization potential and slight increase in van der Waals component of the potential energy for compound **5a** (Table 2) led to comparable activity 0.04 and 0.034 μM, respectively. In case of compounds **5b** and **5j** decrease in their ionization potential and increase in van der Waals component of the potential energy (Table 2) led to slight decrease in activity as IC_{50} increased, 0.074 and 0.05 μM, respectively.

From Model 2 equation, cytotoxic activity was negatively correlated with IP, ASA and positively correlated with Flex. The high coefficient value of IP and the comparatively lower value of Flex suggested that the decrease in ionization potential and increase in Kier molecular flexibility index lead to enhancement of activity. This was in good agreement with the obtained experimental data, for the most active compounds **3** and **5a**, slight decrease in ionization potential and increase in Kier molecular flexibility index (Table 2) led to comparable activity IC_{50} 0.04 and 0.034 μM, respectively. In case of compounds **5b** and **5j** decrease in ionization potential and increase in Kier molecular flexibility index while increase in Water accessible surface area (ASA) (Table 2) led to slight decrease in activity IC_{50} 0.074 and 0.05 μM, respectively.

Conclusion

According to cytotoxic activity of tested compounds against PC3 (prostate cancer cell line), it is apparent from the results that:

Parent intermediate compound with both free carboxylic groups **3** exhibited promising activity.

Some monosubstituted glutamic acid derivatives as amide, anilide and hydrazide showed promising activity. Substitution of anilide derivative reduced activity while heteroaromatic anilide and uride were inactive.

Disubstituted glutamic acid derivatives as diamide, dianilide, diuride and dihydrazide showed good activity.

Compound with carboxylic groups free, compounds with monosubstituted carboxylic group and compounds with disubstituted carboxylic groups showed promising activity. These results supported the suggestion that these compounds are not active as PSMA inhibitors as PC3 do not express PSMA[11] and structure–activity relationship study of PSMA inhibitors[12] supported decreased activity of monosubstituted compounds and inactivity of disubstituted compounds relative to parent compound with both carboxylic groups free.

Most active compounds had structure similarity with glutamine **5a**, IC_{50}, 0.034 μM (34 nM), **5j**, IC_{50}, 0.05 μM (50 nM), **5b**, IC_{50}, 0.074 μM (74 nM) and glutamic acid **3**, IC_{50}, 0.04 μM (40 nM). It may be suggested that, these compounds may act through inhibition of GS, antagonistic effect on mGluR5 (as both of them are expressed in PC3) or may cause restriction of Gln amino acid that result in several consequent effects.

Briefly, the glutamic acid derivatives serve as promising

Table 3. The Observed Activities for PC3 Cell Line (Obs. IC_{50}) Together with the Predicted Activities (Pred. IC_{50}) for the Tested Compounds Calculated Using Multi-linear Regression (MLR) for (Model 3)

Cpd. No.	Obs. IC_{50}	MLR validation		
		Pred. IC_{50}	Residual	*Z*-Score
3	0.0400	0.0281	0.0119	0.0054
5a	0.0340	−1.1047	1.1387	0.5134
5b	0.0740	−0.6888	0.7628	0.3439
5c	17.4000	13.4354	3.9646	1.7876
5e	3.9700	3.4769	0.4931	0.2223
5i	3.2300	3.4821	−0.2521	0.1137
5j	0.0500	5.4647	−5.4147	2.4414
5k	0.5220	3.8902	−3.3682	1.5187
5l	0.4900	−0.6355	1.1255	0.5075
7a	1.0700	0.7576	0.3124	0.1408
7b	0.7100	0.1382	0.5718	0.2578
7c	0.2500	−2.2307	2.4807	1.1185
7d	2.1800	2.4675	−0.2875	0.1296
7e	0.4000	1.9390	−1.5390	0.6939

molecule for subsequent molecular modification in the search for novel anti-tumors. Furthermore, the result of the QSAR studies performed made clear that ionization potential of a molecule is the key for antiproliferative activity.

Experimental

Chemistry Melting points were determined by open capillary tube method using Gallen Kamp melting point apparatus MFB-595-010M (Gallen Kamp, London, England) and were uncorrected. Microanalysis was carried out at The Regional Center for Mycology and Biotechnology, Al-Azhar University and at Organic Microanalyses Section, Central Laboratory, National Research Center. Infrared Spectra were recorded as potassium bromide discs on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan) and expressed in wave number (cm^{-1}). The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^1H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in deuterated chloroform ($CDCl_3$) or dimethylsulphoxide (DMSO-d_6). Chemical shifts are quoted in δ as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard. Mass spectra were recorded using Hewlett Packard Varian (Varian, Polo, U.S.A.) and Shimadzu Gas Chromatograph Mass Spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan). TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents were benzene–methanol (4 : 1) and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France).

5-Chloro-2-methoxy-4-methylbenzene-1-sulphonyl Chloride (2), Chart 1 1-Chloro-4-methoxy-2-methylbenzene **1** (1.56 g, 10 mmol) was added to chlorosulphonic acid (5.8 g, 50 mmol), magnetically stirred at 0°C, over a period of 30 min. The stirring was continued for further 1 h at room temperature then the mixture poured onto crushed ice. The obtained solid was filtered, dried and crystallized from methanol. mp 126–128°C (as reported 126–128°C).[29] IR (KBr) cm^{-1}: 3425 (NH), 3109 (CH Ar), 2985, 2951 (CH aliphatic), 1597, 1554 (NH, C=C), 1377, 1172 (SO_2).

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl) Glutamic Acid (3), Chart 1 To L-(+)-glutamic acid (1.47 g, 10 mmol) dissolved in 2 M NaOH (5 mL), 5-chloro-2-methoxy-4-methylbenzene-1-sulphonyl chloride **2** (2.55 g, 10 mmol) 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 as showed by TLC, it was allowed to cool, acidified to pH 3.0 with HCl, saturated with NaCl. The obtained solid product **3** was filtered, dried and finally crystallized from ethyl acetate-benzene. Yield 80%. mp 254–255°C. ^1H-NMR (DMSO-d_6) δ: 2.05 (2H, q, J=6.5 Hz, $C\underline{H_2}CH_2CO$), 2.31 (5H, t, J=9.7 Hz, CH_2CO and CH_3), 3.71 (1H, t, J=9.3 Hz, CH), 3.75 (3H, s, OCH_3), 6.98 (1H, s, H-3 Ar), 7.61 (1H, s, H-6 Ar), 9.71 (1H, s, NH exch. D_2O), 10.52 (1H, s, COOH exch. D_2O), 10.93 (1H, s, COOH exch. D_2O). IR (KBr) cm^{-1}: 3425 (NH), 3085 (CH Ar), 2981 (CH aliphatic), 2943, 2854 (OH carboxylic), 1728, 1670 (2 C=O), 1597, 1562 (NH, C=C), 1369, 1118 (SO_2). MS *m/z*: 365 (M^+). *Anal.* Calcd for $C_{13}H_{16}ClNO_7S$ (365.79): C, 42.69; H, 4.41; N, 3.83. Found: C, 42.82; H, 4.38; N, 4.11.

1-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-5-oxopyrrolidine-2-carboxylic Acid (4), Chart 1 To 3.65 g (10 mmol) of compound **3**, acetyl chloride (10 mL) was added slowly with stirring at 5°C then refluxed on steam bath for 2 h. The reaction mass was poured onto crushed ice slowly with continuous stirring. It was kept cold overnight when the semi-solid mass solidified. It was filtered, dried and crystallized from ethanol. Yield 85%. mp >300°C. ^1H-NMR (DMSO-d_6) δ: 2.26–2.36 (5H, m, $COCH_2C\underline{H_2}CHCOOH$ and CH_3), 3.33 (2H, t, J=13.4 Hz, $COC\underline{H_2}CH_2CHCOOH$), 3.73 (4H, t, J=11.8 Hz, CH and OCH_3), 6.96 (1H, s, H-3 Ar), 7.59 (1H, s, H-6 Ar), 11.00 (1H, s, COOH exch. D_2O). IR (KBr) cm^{-1}: 3085 (CH Ar), 2981 (CH aliphatic), 2939, 2850 (OH carboxylic), 1774, 1735 (2 C=O), 1597, 1562 (NH, C=C), 1369, 1118 (SO_2). MS *m/z*: 344 (M^+-3). *Anal.* Calcd for $C_{13}H_{14}ClNO_6S$ (347.77): C, 44.90; H, 4.06; N, 4.03. Found: C, 45.13; H, 4.19; N, 4.38.

General Procedure for Synthesis of 2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-substituted Glutamine (5a–l), Chart 1 To a suspension of **4** (3.47 g, 10 mmol) in water (30 mL), the appropriate amino derivative (10 mmol) was added and stirred at room temperature for 24 h. Water (30 mL) was added, cooled and acidified with 6 M HCl to pH 3.0. The precipitate obtained was filtered, washed with water and dried. The crude mass was crystallized from the appropriate solvent to yield 60–85%.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl) Glutamine (**5a**): General procedure was adopted to prepare **5a** employing **4** (3.47 g, 10 mmol) in water (30 mL), and liquor ammonia (15 mL, 33%). After the reaction is complete, the excess ammonia was evaporated and water (30 mL) was added, cooled and acidified with 6 M HCl to pH 3.0 to yield 65%. The crude mass was crystallized from ethanol. mp 295–296°C. ^1H-NMR (DMSO-d_6) δ: 1.15 (2H, q, J=6.3 Hz, $C\underline{H_2}CH_2CO$), 2.39 (5H, t, J=7.9 Hz, CH_3 and CH_2CO), 3.66 (4H, t, J=7.9 Hz, CH and OCH_3), 7.21 (1H, s, H-3 Ar), 7.85 (1H, s, H-6 Ar), 8.10 (3H, s, NH and NH_2 exch. D_2O), 11.0 (1H, s, COOH exch. D_2O). IR (KBr) cm^{-1}: 3533, 3240 (NH_2, NH), 3059 (CH Ar), 2927, 2858 (OH carboxylic), 1735, 1665 (2 C=O), 1600, 1562 (NH, C=C), 1373, 1180 (SO_2). MS *m/z*: 364 (M^+). *Anal.* Calcd for $C_{13}H_{17}ClN_2O_6S$ (364.80): C, 42.80; H, 4.70; N, 7.68. Found: C, 43.11; H, 4.82; N, 8.04.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-phenylglutamine (**5b**): Yield 65%. The crude mass was crystallized from ethanol. mp 251–254°C. ^1H-NMR (DMSO-d_6) δ: 2.25–2.35 (5H, m, $C\underline{H_2}CH_2CO$ and CH_3), 2.38 (2H, t, J=10.2 Hz, CH_2CO), 3.65 (1H, t, J=5.7 Hz, CH), 3.72 (3H, s, OCH_3), 5.65 (1H, s, NH exch. D_2O), 6.96–7.84 (7H, m, Ar H and H-3,6 Ar), 7.79 (1H, s, NH exch. D_2O), 10.63 (1H, s, COOH exch. D_2O). IR (KBr) cm^{-1}: 3437 (2 NH), 3105 (CH Ar), 2960 (CH aliphatic), 2920, 2642 (OH carboxylic), 1700, 1660 (2 C=O), 1600, 1546 (NH, C=C), 1373, 1168 (SO_2). MS *m/z*: 442 (M^++2). *Anal.* Calcd for $C_{19}H_{21}ClN_2O_6S$ (440.90): C, 51.76; H, 4.80; N, 6.35. Found: C, 52.13; H, 4.97; N, 6.49.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-(4-methyphenyl) Glutamine (**5c**): Yield 74%. The crude mass was crystallized from ethanol. mp 248–249°C. ^1H-NMR (DMSO-d_6) δ: 2.27–2.35 (8H, m, $C\underline{H_2}CH_2CO$ and 2×CH_3), 2.38 (2H, t, J=10.5 Hz, CH_2CO), 3.65 (1H, t, J=6.9 Hz, CH), 3.72 (3H, s, OCH_3), 6.96–7.84 (6H, m, Ar H and H-3,6 Ar), 9.74 (3H, br s, 2×NH and COOH exch. D_2O). IR (KBr) cm^{-1}: 3425 (2 NH), 3101 (CH Ar), 2960 (CH aliphatic), 2924, 2642 (OH carboxylic), 1700, 1670 (2 C=O), 1600, 1560, 1540 (NH, C=C), 1373, 1165 (SO_2). MS *m/z*: 456 (M^++2). *Anal.* Calcd for

$C_{20}H_{23}ClN_2O_6S$ (454.92): C, 52.80; H, 5.10; N, 6.16. Found: C, 53.14; H, 5.28; N, 6.29.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-(4-chlorophenyl) Glutamine (**5d**): Yield 75%. The crude mass was crystallized from ethanol. mp 236–237°C. ^{1}H-NMR (DMSO-d_6) δ: 2.27–2.38 (5H, m, C$\underline{H}_2$CH_2CO and CH_3), 2.60 (2H, t, J=7.3 Hz, CH_2CO), 3.67 (1H, t, J=7.4 Hz, CH), 3.71 (3H, s, OCH_3), 5.63 (1H, s, NH exch. D_2O), 6.98 (1H, s, H-3 Ar), 7.16 (2H, dd, J=11.1, 3.0 Hz, H-2′,6′ Ar), 7.39 (2H, dd, J=11.1, 3.0 Hz, H-3′,5′ Ar), 7.60 (1H, s, H-6 Ar), 8.40 (1H, brs, NH exch. D_2O), 10.20 (1H, s, COOH exch. D_2O). ^{13}C-NMR (DMSO-d_6) δ: 19.70 (CH_3), 26.00 (C$\underline{H}_2$CH_2CO), 31.00 (C$\underline{H}_2$CO), 55.80 (CH and OCH_3), 114.81 (C-3), 122.29 (C-2′,6′), 122.78 (C-1), 128.27 (C-5) 128.60 (C-6), 129.35 (C-3′,5′), 134.35 (C-4′), 135.69 (C-1′), 137.56 (C-4), 154.74 (C-2), 171.10 (CONH), 175.00 (COOH). IR (KBr) cm^{-1}: 3433 (2 NH), 3101 (CH Ar), 2940 (CH aliphatic), 2924, 2638 (OH carboxylic), 1680, 1640 (2 C=O), 1597, 1550 (NH, C=C), 1373, 1149 (SO_2). MS *m*/*z*: 475 (M^+). *Anal.* Calcd for $C_{19}H_{20}Cl_2N_2O_6S$ (475.34): C, 48.01; H, 4.24; N, 5.89. Found: C, 48.36; H, 4.45; N, 5.98.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-(4-methoxyphenyl) Glutamine (**5e**): Yield 67%. The crude mass was crystallized from ethanol. mp 210–211°C. ^{1}H-NMR (DMSO-d_6) δ: 2.26–2.36 (5H, m, C$\underline{H}_2$CH_2CO and CH_3), 2.38 (2H, t, J=7.9 Hz, CH_2CO), 3.65 (4H, t, J=11.8 Hz, CH and OCH_3), 3.72 (3H, s, OCH_3), 5.65 (1H, s, NH exch. D_2O), 6.68–6.80 (4H, m, Ar H), 6.96 (1H, s, H-3 Ar), 7.59 (1H, s, H-6 Ar), 7.73 (1H, s, NH exch. D_2O), 10.20 (1H, s, COOH exch. D_2O). IR (KBr) cm^{-1}: 3450, 3410 (2 NH), 3100 (CH Ar), 2924, 2638 (OH carboxylic), 1700, 1680 (2 C=O), 1620, 1597, 1570 (NH, C=C), 1380, 1165 (SO_2). MS *m*/*z*: 467 (M^+−3). *Anal.* Calcd for $C_{20}H_{23}ClN_2O_7S$ (470.92): C, 51.01; H, 4.92; N, 5.95. Found: C, 51.23; H, 5.19; N, 6.18.

N^5-[4-(Aminosulphonyl)phenyl]-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) Glutamine (**5f**): Yield 60%. The crude mass was crystallized from ethanol. mp 209–210°C. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (2H, q, J=5.5 Hz, C$\underline{H}_2$CH_2CO), 2.39 (3H, s, CH_3), 2.79 (2H, t, J=6.8 Hz, CH_2CO), 3.53 (1H, t, J=5.5 Hz, CH), 3.65 (3H, s, OCH_3), 5.77 (2H, brs, 2×NH exch. D_2O), 6.58 (2H, d, J=8.1 Hz, H-2′,6′ Ar), 6.85 (2H, brs, NH_2 exch. D_2O), 7.20 (1H, s, H-3 Ar), 7.45 (2H, d, J=7.8 Hz, H-3′,5′ Ar), 7.85 (1H, s, H-6 Ar), 9.60 (1H, brs, COOH exch. D_2O). IR (KBr) cm^{-1}: 3464, 3379, 3340, 3248 (NH_2, 2 NH), 3101 (CH Ar), 2940 (CH aliphatic), 2920, 2850 (OH carboxylic), 1680, 1660 (2 C=O), 1620, 1597, 1560 (NH, C=C), 1311, 1149 (SO_2). MS *m*/*z*: 519 (M^+). *Anal.* Calcd for $C_{19}H_{22}ClN_3O_8S_2$ (519.98): C, 43.89; H, 4.26; N, 8.08. Found: C, 44.19; H, 4.60; N, 8.23.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-(pyridin-4-yl) Glutamine (**5g**): Yield 80%. The crude mass was crystallized from acetone. mp 251–252°C. ^{1}H-NMR (DMSO-d_6) δ: 2.20–2.35 (5H, m, C$\underline{H}_2$CH_2CO and CH_3), 2.60 (2H, t, J=8.6 Hz, CH_2CO), 3.71 (4H, t, J=8.6 Hz, CH and OCH_3), 6.79 (2H, d, J=7.8 Hz, H-2′,6′ Ar), 6.96 (1H, s, H-3 Ar), 7.59 (1H, s, H-6 Ar), 8.02 (2H, s, 2×NH exch. D_2O), 8.10 (2H, d, J=7.2 Hz, H-3′,5′ Ar), 13.00 (1H, brs, COOH exch. D_2O). IR (KBr) cm^{-1}: 3390, 3209 (2 NH), 3105 (CH Ar), 2962 (CH aliphatic), 2939, 2796 (OH carboxylic), 1662, 1612 (2 C=O), 1600, 1535 (NH, C=C), 1369, 1184 (SO_2). MS *m*/*z*: 441 (M^+). *Anal.* Calcd for $C_{18}H_{20}ClN_3O_6S$ (441.89): C, 48.92; H, 4.56; N, 9.51. Found: C, 49.07; H, 4.63; N, 9.85.

N^5-(Aminocarbonyl)-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) Glutamine (**5h**): Yield 73%. The crude mass was crystallized from ethanol. mp 270–272°C. ^{1}H-NMR (DMSO-d_6, D_2O) δ: 2.20–2.30 (5H, m, C$\underline{H}_2$CH_2CO and CH_3), 3.71 (2H, t, J=7.9 Hz, CH_2CO), 4.07 (4H, t, J=7.9 Hz, CH and OCH_3), 6.96 (1H, s, H-3 Ar), 7.56 (1H, s, H-6 Ar). IR (KBr) cm^{-1}: 3390, 3305, 3217, 3120 (NH_2, 2 NH), 3008 (CH Ar), 2954 (CH aliphatic), 2854, 2546 (OH carboxylic), 1705 (3 C=O), 1600, 1570 (NH, C=C), 1373, 1145 (SO_2). MS *m*/*z*: 407 (M^+). *Anal.* Calcd for $C_{14}H_{18}ClN_3O_7S$ (407.83): C, 41.23; H, 4.45; N, 10.30. Found: C, 41.10; H, 4.45; N, 10.10.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-phenylglutamic Acid Hydrazide (**5i**): Yield 80%. The crude mass was crystallized from ethanol. mp 262–263°C. ^{1}H-NMR ($CDCl_3$) δ: 1.83–2.0 (7H, m, C$\underline{H}_2$C$\underline{H}_2$CO and CH_3), 3.44 (1H, t, J=10.2 Hz, CH), 3.53 (3H, s, OCH_3), 5.0 (1H, s, NH exch. D_2O), 6.23 (1H, s, NH exch. D_2O), 6.42–7.42 (7H, m, Ar H and H-3,6 Ar), 8.10 (1H, s, NH exch. D_2O), 9.50 (1H, s, COOH exch. D_2O). IR (KBr) cm^{-1}: 3290 (3 NH), 2993 (CH aliphatic), 2951, 2735 (OH carboxylic), 1710, 1630 (2 C=O), 1604, 1546 (NH, C=C), 1369, 1176 (SO_2). MS *m*/*z*: 455 (M^+). *Anal.* Calcd for $C_{19}H_{22}ClN_3O_6S$ (455.91): C, 50.05; H, 4.86; N, 9.22. Found: C, 50.28; H, 4.70; N, 9.20.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-(4-chlorophenyl) Glutamic Acid Hydrazide (**5j**): Yield 85%. The crude mass was crystallized from ethanol. mp 248–250°C. ^{1}H-NMR (DMSO-d_6) δ: 2.25–2.35 (7H, m, C$\underline{H}_2$C$\underline{H}_2$CO and CH_3), 3.73 (4H, t, J=7.3 Hz, CH and OCH_3), 6.92 (1H, s, H-3 Ar), 6.95 (2H, dd, J=12.3, 3.0 Hz, H-2′,6′ Ar), 7.22 (2H, dd, J=12.3, 3.0 Hz, H-3′,5′ Ar), 7.61 (1H, s, H-6 Ar), 8.31 (1H, brs, NH exch. D_2O), 10.01 (3H, s, 2×NH and COOH exch. D_2O). ^{13}C-NMR (DMSO-d_6) δ: 19.72 (CH_3), 26.00 (C$\underline{H}_2$CH_2CO), 36.00 (C$\underline{H}_2$CO), 55.83 (CH and OCH_3), 114.80 (C-3), 115.96 (C-2′,6′), 122.76 (C-1), 125.19 (C-4′), 128.30 (C-5), 128.79 (C-3′,5′), 134.54 (C-6), 137.47 (C-4), 144.37 (C-1′), 154.79 (C-2), 174.00 (CONH), 176.00 (COOH). IR (KBr) cm^{-1}: 3282 (3 NH), 2993 (CH aliphatic), 2931, 2735 (OH carboxylic), 1700, 1630 (2 C=O), 1597, 1546 (NH, C=C), 1369, 1170 (SO_2). MS *m*/*z*: 491 (M^++1). *Anal.* Calcd for $C_{19}H_{21}Cl_2N_3O_6S$ (490.36): C, 46.54; H, 4.32; N, 8.57. Found: C, 46.74; H, 4.41; N, 8.86.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^5-(4-methoxyphenyl) Glutamic Acid Hydrazide (**5k**): Yield 85%. The crude mass was crystallized from ethanol. mp 186–187°C. ^{1}H-NMR (DMSO-d_6) δ: 2.25–2.35 (5H, m, C$\underline{H}_2$CH_2CO and CH_3), 2.56 (2H, t, J=7.9 Hz, CH_2CO), 3.65–3.80 (7H, m, CH and 2xOCH_3), 6.88–6.95 (5H, m, Ar H and H-3 Ar), 7.59 (1H, s, H-6 Ar), 9.75 (4H, s, 3×NH and COOH exch. D_2O). IR (KBr) cm^{-1}: 3444, 3421, 3271 (3 NH), 3060 (CH Ar), 2997 (CH aliphatic), 2927, 2731 (OH carboxylic), 1680, 1660 (2 C=O), 1620, 1593, 1525 (NH, C=C), 1369, 1176 (SO_2). MS *m*/*z*: 485 (M^+). *Anal.* Calcd for $C_{20}H_{24}ClN_3O_7S$ (485.94): C, 49.43; H, 4.98; N, 8.65. Found: C, 49.78; H, 5.17; N, 8.92.

N^5-[4-(Aminosulphonyl)phenyl]-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) Glutamic Acid Hydrazide (**5l**): Yield 61%. The crude mass was crystallized from ethanol. mp 241–243°C. ^{1}H-NMR (DMSO-d_6) δ: 2.25–2.35 (5H, m, C$\underline{H}_2$CH_2CO and CH_3), 2.38 (2H, t, J=6.8 Hz, CH_2CO), 3.73 (4H, t, J=9.0 Hz, CH and OCH_3), 6.96 (1H, s, H-3 Ar), 6.98 (2H, d, J=9.0 Hz, H-2′,6′ Ar), 7.21 (2H, brs, NH_2, exch. D_2O), 7.59 (1H, s, H-6 Ar), 7.72 (2H, d, J=8.7 Hz, H-3′,5′ Ar,), 8.70

(1H, s, NH exch. D_2O), 10.09 (3H, brs, 2×NH and COOH exch. D_2O). IR (KBr) cm^{-1}: 3325, 3294, 3236 (NH_2, 3 NH), 3060 (CH Ar), 2960 (CH aliphatic), 2927, 2735 (OH carboxylic), 1680, 1660 (2 C=O), 1597, 1543 (NH, C=C), 1346, 1172 (SO_2). MS *m/z*: 534 (M^+). *Anal.* Calcd for $C_{19}H_{23}ClN_4O_8S_2$ (534.99): C, 42.66; H, 4.33; N, 10.47. Found: C, 43.08; H, 4.68; N, 10.73.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl) Glutamic Acid Diethyl Ester (6), Chart 2 A mixture of sulphonyl chloride derivative **2** (2.55 g, 10 mmol) and diethyl glutamate (2.03 g, 10 mmol) in dry benzene (50 mL) and triethylamine (few drops) was refluxed for 24 h. The solvent was evaporated and residue was triturated with water. The obtained solid product was filtered, dried and crystallized from ethanol. Yield 85%. mp 136–138°C. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (2H, q, J=7.5 Hz, C$\underline{H_2}$CH$_2$CO), 1.40 (6H, t, J=7.9 Hz, 2×CH$_2$C$\underline{H_3}$), 2.30 (3H, s, CH_3), 2.36 (2H, t, J=11.8 Hz, CH_2CO), 3.09 (1H, t, J=7.9 Hz, CH), 3.64–3.80 (7H, m, OCH_3 and 2×C$\underline{H_2}$CH$_3$), 6.96 (1H, s, H-3 Ar), 7.59 (1H, s, H-6 Ar), 9.75 (1H, s, NH exch. D_2O). IR (KBr) cm^{-1}: 3441 (NH), 3109 (CH Ar), 2985, 2947, 2927 (CH aliphatic), 1720, 1670 (2 C=O), 1597, 1562 (NH, C=C), 1377, 1172 (SO_2). MS *m/z*: 421 (M^+). *Anal.* Calcd for $C_{17}H_{24}ClNO_7S$ (421.89): C, 48.40; H, 5.73; N, 3.32. Found: C, 48.56; H, 5.88; N, 3.71.

General Precedure for Synthesis of N^1,N^5-Bis Substituted-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) Glutamic Acid Diamides (7a–c), Diureide (7d) and Dihydrazide (7e), Chart 2 A mixture of diester derivative **6** (4.21 g, 10 mmol), liquor ammonia, aniline, 4-aminobenzenesulphonamide, urea or phenylhydrazine (20 mmol) in absolute ethanol (50 mL) was refluxed for 16 h. The solvent was evaporated under reduced pressure and water was added. The separated solid was filtered and dried. The crude mass was crystallized from the appropriate solvent to yield 70–80%.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl) Glutamic Acid Diamide (**7a**): Yield 78%. The crude mass was crystallized from ethanol. mp 268–270°C. ^{1}H-NMR (DMSO-d_6) δ: 2.26–2.40 (5H, m, C$\underline{H_2}$CH$_2$CO and CH_3), 2.61 (2H, t, J=11.8 Hz, CH_2CO), 3.69 (1H, t, J=7.9 Hz, CH), 3.74 (3H, s, OCH_3), 6.97 (1H, s, H-3 Ar), 7.20 (5H, broad, NH and 2×NH_2 exch. D_2O), 7.59 (1H, s, H-6 Ar). IR (KBr) cm^{-1}: 3448, 3425, 3170 (2NH_2, NH), 3051 (CH Ar), 2985 (CH aliphatic), 1670, 1640 (2 C=O), 1597, 1562 (NH, C=C), 1369, 1118 (SO_2). MS *m/z*: 363 (M^+). *Anal.* Calcd for $C_{13}H_{18}ClN_3O_5S$ (363.82): C, 42.92; H, 4.99; N, 11.55. Found: C, 43.18; H, 5.17; N, 11.69.

N^1,N^5-Bis Phenyl-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) Glutamic Acid Diamide (**7b**): Yield 80%. The crude mass was crystallized from ethanol. mp 201–202°C. ^{1}H-NMR (DMSO-d_6) δ: 2.28–2.44 (7H, m, C$\underline{H_2}$C$\underline{H_2}$CO and CH_3), 3.64 (1H, t, J=9.0 Hz, CH), 3.86 (3H, s, OCH_3), 6.95–7.84 (12H, m, Ar H and H-3,6 Ar), 10.10 (3H, s, 3×NH exch. D_2O). IR (KBr) cm^{-1}: 3263 (3NH), 3080 (CH Ar), 2978 (CH aliphatic), 1680, 1670 (2 C=O), 1597, 1562 (NH, C=C), 1369, 1161 (SO_2). MS *m/z*: 517 (M^++1). *Anal.* Calcd for $C_{25}H_{26}ClN_3O_5S$ (516.01): C, 58.19; H, 5.08; N, 8.14. Found: C, 58.41; H, 5.09; N, 8.43.

N^1,N^5-Bis [4-(Aminosulphonyl)phenyl]-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) Glutamic Acid Diamide (**7c**): Yield 78%. The crude mass was crystallized from ethanol. mp 248–249°C. ^{1}H-NMR (DMSO-d_6) δ: 1.10 (2H, q, J=7.9 Hz, C$\underline{H_2}$CH$_2$CO), 2.30 (3H, s, CH_3), 2.60 (2H, t, J=9.0 Hz, CH_2CO), 3.44 (1H, t, J=9.0 Hz, CH), 3.65 (3H, s, OCH_3), 5.40 (5H, broad, NH and 2×NH_2, exch. D_2O), 6.81 (5H, d, J=8.1 Hz, 2×H-2′,6′ Ar and NH exch. D_2O), 6.95 (1H, s, H-3 Ar), 7.55 (5H, d, J=8.1 Hz, 2×H-3′,5′ Ar and NH exch. D_2O), 7.57 (1H, s, H-6 Ar). IR (KBr) cm^{-1}: 3350, 3250, 3101 (2NH_2, 3 NH), 3078 (CH Ar), 2916 (CH aliphatic), 1680, 1670 (2 C=O), 1597, 1558 (NH, C=C), 1311, 1149 (SO_2). MS *m/z*: 674 (M^+). *Anal.* Calcd for $C_{25}H_{28}ClN_5O_9S_3$ (674.17): C, 44.54; H, 4.19; N, 10.39. Found: C, 44.69; H, 4.10; N, 10.57.

2-*N*-(5-Chloro-2-methoxy-4-methylphenylsulphonyl)-N^1,N^5-glutamic Acid Diuride (**7d**): Yield 75%. The crude mass was crystallized from ethanol. mp 281–283°C. ^{1}H-NMR (DMSO-d_6, D_2O) δ: 1.16 (2H, q, J=7.9 Hz, C$\underline{H_2}$CH$_2$CO), 2.38 (3H, s, CH_3), 2.80 (2H, t, J=7.9 Hz, CH_2CO), 3.64 (4H, t, J=11.8 Hz, CH and OCH_3), 7.20 (1H, s, H-3 Ar), 7.84 (1H, s, H-6 Ar). IR (KBr) cm^{-1}: 3406, 3275, 3213, 3101 (2NH_2, 3 NH), 3078 (CH Ar), 2951 (CH aliphatic), 1710, 1700, 1680 (4 C=O), 1597, 1562 (NH, C=C), 1311, 1149 (SO_2). MS *m/z*: 449 (M^+). *Anal.* Calcd for $C_{15}H_{20}ClN_5O_7S$ (449.87): C, 40.05; H, 4.48; N, 15.57. Found: C, 40.23; H, 4.51; N, 15.78.

N^1,N^5-Bis Phenyl-2-*N*-(5-chloro-2-methoxy-4-methylphenylsulphonyl) Glutamic Acid Dihydrazide (**7e**): Yield 70%. The crude mass was crystallized from ethanol. mp 220–223°C. ^{1}H-NMR (DMSO-d_6) δ: 2.28–2.39 (7H, m, C$\underline{H_2}$C$\underline{H_2}$CO and CH_3), 3.72 (1H, t, J=7.5 Hz, CH), 3.79 (3H, s, OCH_3), 3.93 (2H, s, 2×NH, exch. D_2O), 6.42 (2H, brs, 2×NH, exch. D_2O), 6.81–7.84 (12H, m, Ar H, H-3,6 Ar), 9.31 (1H, s, NH exch. D_2O). ^{13}C-NMR (DMSO-d_6) δ: 19.79 (CH_3), 20.04 ($\underline{C}H_2CH_2CO$), 36.0 ($\underline{C}H_2CO$), 55.78 (CH), 56.49 (OCH_3), 112.51 (C-3), 113.57 (2×C-2′,6′), 114.35 (C-1), 120.20 (2×C-4′), 123.55 (C-5), 124.43 (C-6), 128.39 (C-4), 128.76 (2×C-3′,5′), 147.04 (2×C-1′), 155.15 (C-2), 172.00 (CONH), 176.00 (CONH). IR (KBr) cm^{-1}: 3441, 3290, 3201 (5 NH), 3016 (CH Ar), 2924 (CH aliphatic), 1640, 1620 (2 C=O), 1604, 1562 (NH, C=C), 1369, 1161 (SO_2). MS *m/z*: 545 (M^+−1). *Anal.* Calcd for $C_{25}H_{28}ClN_5O_5S$ (546.04): C, 54.99; H, 5.17; N, 12.83. Found: C, 55.21; H, 5.32; N, 13.14.

Antitumor Screening All newly synthesized compounds were tested against the tumor cell line PC3 (prostate cancer cell line) at Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University using the Sulfo Rhodamine B stain (SRB) assay by the method of Skehan *et al.*[31]

Procedure Cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted to the appropriate volume. Different concentrations of the compound under test (0.001, 0.01, 0.1, 1, 10, 100 μM) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (w/v) Sulfo Rhodamine B dissolved in 1% acetic acid. Excess stain was washed with acetic acid and attached stain was recovered with Tris ethylenediaminetetraacetic acid (Tris EDTA) buffer. Colour intensity was measured in an enzyme-linked immunosorbent assay (ELISA) reader. The relation between the surviving fraction and drug concentration was plotted to get the survival curve of each tumor cell line after the specified compound. IC_{50} and R fraction of the tested compounds were illustrated in Table 1.

The dose response curve of compounds was analyzed using E_{max} model.

$$\% \text{ Cell viability} = (100 - R) \times \left\{ 1 - \frac{[D]^m}{K_d^m + [D]^m} \right\} + R$$

Where R is the residual unaffected fraction (the resistance fraction), $[D]$ is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient. IC_{50} was defined as the drug concentration required to reduce fluorescence to 50% of that of the control (*i.e.*, K_d=IC_{50} when R=0 and E_{max}=100−R).[32]

QSAR. Computational Method All the computational works were performed on Molecular Operating Environment software (MOE version 2008.10.2).[33] The structures of 18 compounds used as training set were sketched using molecular builder of MOE and each structure was subjected to energy minimization up to 0.01 kcal/mol Å using the MMFF94x force field. Optimization methods were used followed by conformational search of each energy-minimized structure. The most stable conformer of each structure was selected and saved into database to generate the common descriptors. QuaSAR descriptor module of MOE was used to calculate descriptors for each molecule. The probability density functions used are Gaussian. The root mean square deviation (RMSD) tolerance was set to 0.5 Å. Regression analysis was performed using PC3 IC_{50} as dependent factor and the calculated descriptors as predictable variables.

In this study, the pool of descriptors was optimized using principal components analysis (PCA). The optimization started with the reduction in the number of molecular descriptors by the determination of the highly inter-correlated descriptor pairs and only one from each pair was selected; then the descriptors with insignificant variance through the data set were also rejected. QSAR model was then constructed after ensuring reasonable correlation of cytotoxic activity with the individual descriptors and minimum inter-correlation among the descriptors used in the derived model. The quality of the model was assessed using the statistical parameter r^2.

Molecular Descriptors Flex: Kier molecular flexibility index (Kier and Hall Connectivity and Kappa Shape Indices): calculated using (KierA1) (KierA2)/n.

ASA: Water accessible surface area (Surface Area, Volume and Shape Descriptors): calculated using a radius of 1.4 Å for the water molecule. A polyhedral representation is used for each atom in calculating the surface area.

IP or mndo_IP: Ionization potential (MOPAC Descriptors): The ionization potential (kcal/mol) calculated using the MNDO Hamiltonian [MOPAC].

E: Value of the potential energy (Potential Energy Descriptors): The state of all term enable flags will be honored (in addition to the term weights). This means that the current potential setup accurately reflects what will be calculated.

E_vdw: van der Waals component of the potential energy (Potential Energy Descriptors): In the Potential Setup panel, the term enable flag is ignored, but the term weight is applied (Table 2).

Validation of the Model Validation technique has been applied to estimate the quality with regard to predictive ability of the generated model. The observed activities (Obs. IC_{50}) together with the predicted activities (Pred. IC_{50}) for the tested compounds calculated using multi-linear regression (MLR) are listed (Table 3). All compounds showed very good results with Z-scores not exceed the value of 2.5 indicating excellent predictive ability of the model.

Outliers Compounds **5d**, **5f**, **5g**, **5h** were considered as outliers as they were unable to fit in a QSAR model. Separating these outliers from the main data set and formulating another QSAR can resolve the problem. Outliers may be acting by a different mechanism.

Acknowledgment Authors are thankful to drug design laboratory, Pharmaceutical Chemistry Department, Faculty of Pharmacy, Assiut University, for assistance in performing the QSAR study.

References

1) American Cancer Society, "Cancer Facts & Figures.": ‹2010.http://www.cancer.org/acs/groups/content/@nho/documents/document/acspc-024113.pdf›. cited March 18, 2012.
2) Speck-Planche A., Kleandrova V. V., Luan F., Cordeiro M.-N. D. S., *Bioorg. Med. Chem.*, **19**, 6239–6244 (2011).
3) Leiblich A., Cross S. S., Catto J. W. F., Pesce G., Hamdy F. C., Rehman I., *Prostate*, **67**, 1761–1769 (2007).
4) Medina M. A´., *J. Nutr.*, **131**, 2539S–2542S (2001).
5) Collins C. L., Wasa M., Souba W. W., Abcouwer S. F., *J. Cell. Physiol.*, **176**, 166–178 (1998).
6) Graff S., *J. Biol. Chem.*, **130**, 13–17 (1939).
7) Graff S., Rittenberg D., Foster G. L., *J. Biol. Chem.*, **130**, 745–752 (1940).
8) Carter R. E., Feldman A. R., Coyle J. T., *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 749–753 (1996).
9) Tiffany C. W., Lapidus R. G., Merion A., Calvin D. C., Slusher B. S., *Prostate*, **39**, 28–35 (1999).
10) Schülke N., Varlamova O. A., Donovan G. P., Ma D., Gardner J. P., Morrissey D. M., Arrigale R. R., Zhan C., Chodera A. J., Surowitz K. G., Maddon P. J., Heston W. D. W., Olson W. C., *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 12590–12595 (2003).
11) Pinto J. T., Suffoletto B. P., Berzin T. M., Qiao C. H., Lin S., Tong W. P., May F., Mukherjee B., Heston W. D. W., *Clin. Cancer Res.*, **2**, 1445–1451 (1996).
12) Oliver A.-J., Wiest O., Helquist P., Miller M. J., Tenniswood M., *Bioorg. Med. Chem.*, **11**, 4455–4461 (2003).
13) Tsukamoto T., Wozniak K. M., Slusher B. S., *Drug Discov. Today*, **12**, 767–776 (2007).
14) Mesters J. R., Barinka C., Li W., Tsukamoto T., Majer P., Slusher B. S., Konvalinka J., Hilgenfeld R., *EMBO J.*, **25**, 1375–1384 (2006).
15) Blank B. R., Alayoglu P., Engen W., Choi J. K., Berkman C. E., Anderson M. O., *Chem. Biol. Drug Des.*, **77**, 241–247 (2011).
16) Pissimissis N., Papageorgiou E., Lembessis P., Armakolas A., Koutsilieris M., *Anticancer Res.*, **29**, 371–377 (2009).
17) Minakami R., Jinnai N., Sugiyama H., *J. Biol. Chem.*, **272**, 20291–20298 (1997).
18) Fahrner J., Labruyere W. T., Gaunitz C., Moorman A. F. M., Gebhardt R., Lamers W. H., *Eur. J. Biochem.*, **213**, 1067–1073 (1993).
19) Gao P., Tchernyshyov I., Chang T.-C., Lee Y.-S., Kita K., Ochi T., Zeller K. I., De Marzo A. M., Van Eyk J. E., Mendell J. T., Dang C. V., *Nature*, **458**, 762–765 (2009).
20) Labow B. I., Souba W. W., Abcouwer S. F., *J. Nutr.*, **131** (Suppl.), 2467S–2474S, discussion, 2486S–2487S (2001).
21) Liu X., Fu Y.-M., Meadows G. G., *Oncol. Lett.*, **2**, 349–355 (2011).
22) Fu Y.-M., Zhang H., Ding M., Li Y.-Q., Fu X., Yu Z.-X., Meadows G. G., *J. Cell. Physiol.*, **209**, 522–534 (2006).
23) Fu Y.-M., Yu Z.-X., Lin H., Fu X., Meadows G. G., *J. Cell. Physiol.*, **217**, 184–193 (2008).

24) Goswami D., Sen S., De A. U., *Pharmazie*, **56**, 366–371 (2001).
25) Srikanth K., Debnath B., Jha T., *Bioorg. Med. Chem.*, **10**, 1841–1854 (2002).
26) Samanta S., Srikanth K., Banerjee S., Debnath B., Gayen S., Jha T., *Bioorg. Med. Chem.*, **12**, 1413–1423 (2004).
27) Srikanth K., Kumar Ch A., Ghosh B., Jha T., *Bioorg. Med. Chem.*, **10**, 2119–2131 (2002).
28) Furniss B. S., Hannaford A. J., Smith P. W. G., Tatchell A. R., "Vogel's text book of practical organic chemistry," 5th ed., Longman Group UK Limited, 1989, p. 877.
29) ‹http://www.chemspider.com/Chemical-Structure.21242251.html?rid=dad93766-f235-489d-8463-0f1954a83c38›.
30) Smith M., March J., "March's Advanced Organic Chemistry, Part A: Reactions, mechanisms and structure," 5th ed., John Wiley & Sons, 2007, p. 417.
31) Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J. T., Bokesch H., Kenney S., Boyd M. R., *J. Natl. Cancer Inst.*, **82**, 1107–1112 (1990).
32) Al-Abd A. M., Lee J.-H., Kim S. Y., Kun N., Kuh H.-J., *Cancer Sci.*, **99**, 423–431 (2008).
33) Chemical Computing Group, Inc., MOE.: Montréal ‹http://www.chemcomp.com›.
34) Jamloki A., Karthikeyan C., Hari Narayana Moorthy N. S., Trivedi P., *Bioorg. Med. Chem. Lett.*, **16**, 3847–3854 (2006).
35) Golbraikh A., Tropsha A., *J. Mol. Graph. Model.*, **20**, 269–276 (2002).