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Original Article

SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME SUBSTITUTED GLUTAMIC ACID ANALOGS AS ANTICANCER AGENT

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ABSTRACT

Objective: Other than glucose, glutamic acid is one of the major substrates, if not the major substrate, for the energy metabolism of rapidly growing tumor cells. Glutamine acts as a nitrogen donor in the nucleotide and amino acid biosynthesis. Thus, the structural variants of glutamine attracted our attention to develop possible anticancer agents, which may act through glutamine and/or folic acid antagonism.

Methods: Fifteen compounds, five from each category of 2-(4-acetamidophenylsulfonamido) pentanediamide, 1-((4-acetamidophenyl) sulfonyl)-5-oxopyrrolidine-2-carboxamide and 2-(4- acetamidophenylsulfonamido) pentanedioic acid amide analogs and the basic compound 2-(4-acetamidophenylsulfonamido)pentanedioic acid have been synthesized, characterized and screened for anticancer activity both *in vitro* and *in vivo*. The *in vitro* activity was performed against five human cell lines like human breast cancer (MCF-7), leukemia (K-562), ovarian cancer (OVACAR-3), human colon adenocarcinoma (HT-29) and Human kidney carcinoma (A-498). The *in-vivo* activity was performed in female swiss albino mice against *Ehrlich Ascites Carcinoma* (EAC).

Results: It is observed that 2-(4-acetamidophenylsulfonamido)pentanedioic acid, 2-(4-acetamidophenylsulfonamido)-N^1,N^5-bis(3-bromophenyl)pentanediamide, 1-((4-acetamidophenyl)sulfonyl)-N-(3-bromophenyl)-5-oxopyrrolidine-2-carboxamide, 1-((4-acetamidophenyl)sulfonyl)-N-(3-nitrophenyl)-5-oxopyrrolidine-2-carboxamide and 2-(4-acetamidophenylsulfonamido)-5-((3-bromophenyl)amino)-5-oxopentanoic acid analogs showed encouraging activity in both the *in-vitro* and *in-vivo* compared to other compounds.

Conclusion: Along with the fifteen analogs, 2-(4-acetamidophenylsulfonamido) pentanedioic acid was screened with an aim to evaluate the anticancer activity of the parent moiety. It was noticed that final analogs showing better activity than the parent compound and it may be due to the substituents present in those compounds. These observations encourage performing QSAR study in future.

Keywords: 2-(4-Acetamidophenylsulfonamido) pentanedioic acid, Glutamic acid, Anticancer, Ehrlich ascites carcinoma.

INTRODUCTION

Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasin that intrudes upon and destroys adjacent tissues, and sometimes metastasis, or spreading to other locations in the body via lymph or blood. These three malignant properties of cancers differentiate them from benign tumors, which do not invade or metastasize [1]. It is well established that the transformation of the normal cells to a cancerous phenotype is often associated with cognate changes in the transport and metabolism of nutrients such as glucose and glutamine. Cancer affects people at all ages with the risk for most types increasing with age deaths in 2007 (7.6 million) [2-4]. It was observed that tumors assimilate not only the nitrogen from the diet, but also the nitrogen from host proteins, raising the concept of tumors as "nitrogen traps", actively competing with the host for nitrogen compounds [5]. The causes of cancer are diverse, complex, and only partially understood.

Other than glucose, glutamine is one of the major substrates, if not the major substrate, for the energy metabolism of rapidly growing tumor cells [6]. Glutamic acid (2-amino pentanedioic acid) plays an important role in the biosynthesis of purine and pyrimidine bases of DNA and RNA [7]. Glutamine acts as a nitrogen donor in the nucleotide and amino acid biosynthesis, secondly glutamine helps in the uptake of essential amino acid and it maintains the activation of TORkinase. For normal maintenance of cells, metabolism of glutamic acid to L-glutamine by L-glutamine synthetase is essential. The synthesis of L-glutamine is hindered in neoplastic cells due to lower reactivity of L-glutamine synthetase. Thus antagonists of this enzyme can interfere with the metabolic role of L-glutamine and act as anti-cancer agents [8]. Azaserine and 6-diaza-5-oxo-L-norleucine antagonized the metabolic process involving L-glutamine and exhibited antitumor activity in animal models [9]. L-glutamic acid γ-(4-hydroxyanilide), a growth regulatory substance isolated from mushroom *Agaricus bisporous* was found to inhibit B16 mouse melanoma cells in culture.

The importance of non-essential amino acid glutamine in proliferation of human tumor cells was studied extensively to have a high activity of phosphate-dependent glutaminase utilizing glutamine from the medium during long-term culture several fold faster than the normal human hepatocytes [10]. L-glutamine is not only the precursor of purine and pyrimidine bases of DNA but also used as a building block of proteins. Cancer caused about 13% of all human [10, 11]. Human hepatoma cells take up glutamine at rates several fold faster than the normal human hepatocytes [10].

Since no cells, whether cancerous or normal, cannot survive without the only circulatory sugar glucose whereas glutamine is a nonessential amino acid which is required by most of the cells and tissues, points out that it may be the major substrate for cancer. It also plays a central role in multiple metabolic pathways and considered to be the most essential component of tissue/cell culture media [11] for not only as the nitrogen source but also as the carbon source. After a definite time interval, all cells start mutation in cell culture medium, which is also indicative for the role of glutamine in cancer [13].

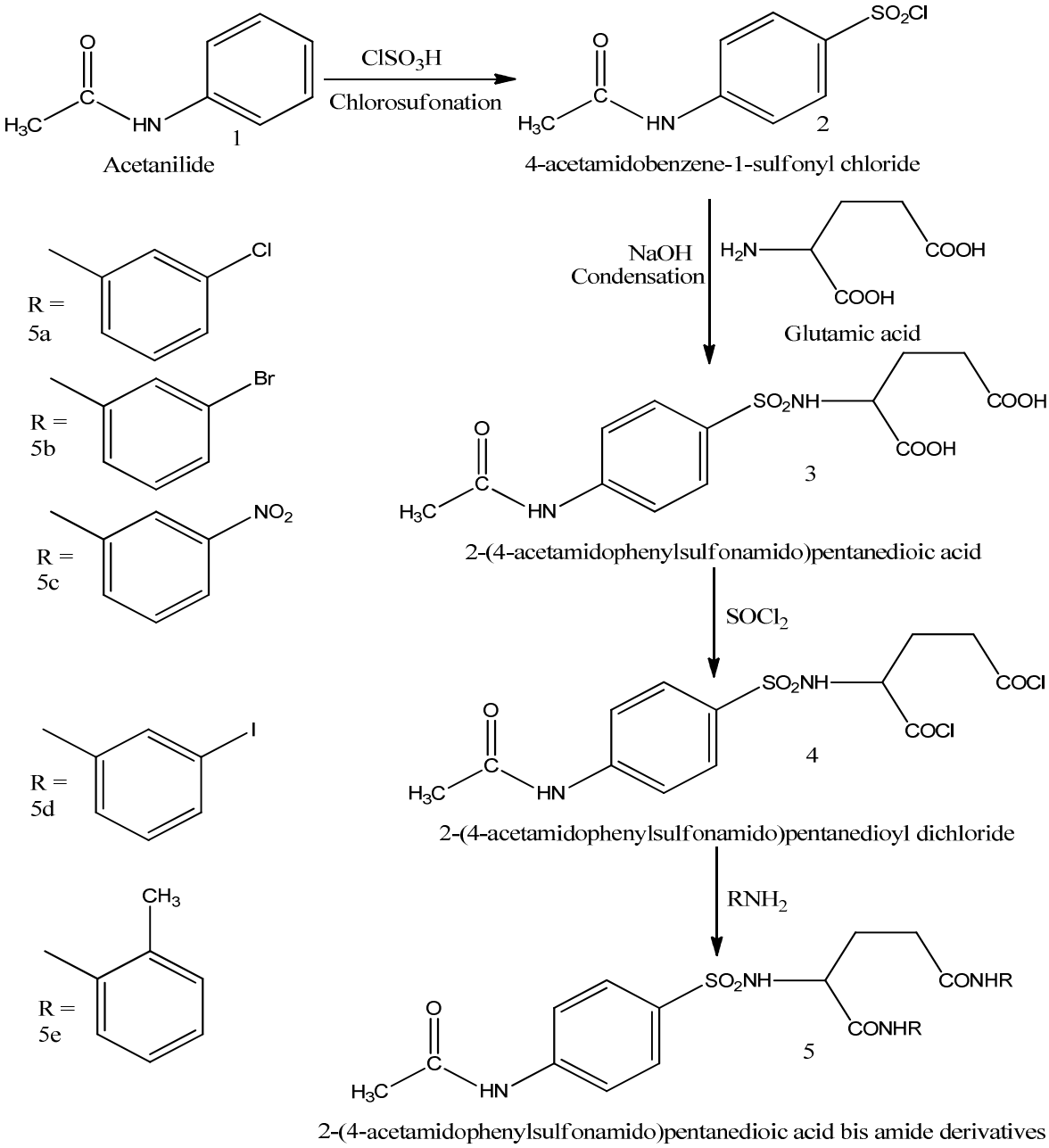
Aryl sulphatase C (ASC) family of transporters is involved in the mediation of glutamine uptake and glutamine, in the form of glutamate, and cysteine are supplied perhaps for glutathione synthesis [14]. Glutamic acid is used as a conjugate because it increases the efficacy of anticancer drug and decreases its toxicity toward normal cells. Thus, the structural variants of glutamine attracted our attention to develop possible anticancer agents, which may act through glutamine and/or folic acid antagonism.

MATERIALS AND METHODS

Commercially available reagents and starting materials for the synthesis were obtained from E. Merck, India, CDH, s.d. Fine Chem, India and Qualigens, India. Silica gel G used for TLC was obtained from E. Merck. The reaction was monitored by TLC using on 0.25 mm E. Merck silica gel $60F_{252}$ precoated plates, which were visualized under UV light. Melting points were determined in an open glass capillary using a Kjeldahl flask containing paraffin and are uncorrected. The proton and carbon magnetic resonance spectra (^{1}H NMR, ^{13}C NMR) were recorded on a Bruker 400 MHz instrument (Bruker, Germany) in dimethyl sulfoxide-d_6 (DMSO-d_6) using tetramethylsilane as internal standard. Chemical shifts (δ) are expressed in ppm and coupling constants (s) singlet, (d) doublet, (t) triplet, (m) multiplet.

The infrared spectra of compounds were recorded in KBr on Fourier Transform (FTIR-8400S, Shimadzu, Japan) infrared spectrophotometer. Mass spectra were recorded on LC-MS/MS (API-4000 TM, Applied BioSystems, MDS SCIEX, Canada). Elemental analyses were performed on a Perkin-Elmer model 240c analyzer (Perkin Elmer, USA).

General procedure for synthesis of substituted 2-(4-methylbenzenesulfonamido)pentanedioyl dichloroamides (5a-e)



Scheme 1: Synthetic pathway of substituted 2-(4-methylbenzenesulfonamido)pentanedioyl dichloroamides (5a-e)

Synthesis of 4-acetamidobenzene-1-sulfonyl chloride (2)

Acetanilide (15 g, 0.11 mole) was taken in a 250 mL three necked round bottom flask placed on an ice bath fitted with a mercury sealed mechanical stirrer, a calcium chloride guard tube and a 100 mL dropping funnel. Chlorosulfonic acid (25 mL, 3 equiv., 0.36 mole) was placed in the dropping funnel and was added drop wise slowly by continuous stirring. The temperature of the ice bath was

maintained at 0°C. After the addition of chlorosulfonic acid the mixture was heated to 80-85°C for two hours. Then the product was poured slowly into well stirred crushed ice. The crude 4-acetamidobenzene-1-sulfonyl chloride (**2**) was filtered and washed with water to remove the excess acid. The crude product was recrystalized from acetone. The yield was 18.60 g (79.35%) and mp: 94-96°C.

Synthesis of 2-(4-acetamidophenylsulfonamido)pentanedioic acid (3)

This was prepared form 4-acetamidobenzene-1-sulfonyl chloride (**2**) and L-(+)-glutamic acid according to the procedure reported below.

L-(+)-glutamic acid (20 g, 0.13 mole) was taken in a 250 mL conical flask and placed on a water both, fitted with a magnetic stirrer. Sodium hydroxide solution (2N) was added slowly till all the L-glutamic acid dissolved and the mass became distinctly alkaline to phenolphthalein. The water bath was maintained at 60-80°C and 4-acetamidobenzene-1-sulfonyl chloride (30 g, 0.13 mole) was added slowly with continuous stirring and simultaneous addition of sodium hydroxide solution (2N) to keep the mass alkaline. The reaction was continued till a clear homogeneous solution results. After the reaction was over, it was allowed to cool, acidified to congored with concentrated hydrochloric acid, saturated with sodium chloride, extracted with chloroform, and allowed to dry overnight with anhydrous magnesium sulfate. Chloroform was removed to yield the crude 2-(4-acetamido phenyl sulfonamido) pentanedioic acid (**3**). The crude product was recrystallized from hot water after charcoal treatment. Yield 57.89%, mp: 150-153°C. R_f 0.76. Neutral equivalent (found: 341.20, Calc. For $C_{13}H_{16}N_2O_7S$: 344). IR (KBr) ν_{max} (cm^{-1}) 3135.95 (C-H str. of phenyl ring), 3059.77-2620 (O-H str. of COOH), 1664.16, (C=O str.), 1500.34 (C=C str. of phenyl ring), 1322.65 (C-O str. or O-H def of COOH), 1369.06 (S=O str. antisym of SO_2N), 1263.80 (S=O str. sym of SO_2N), 825 (out of plane C-H def due to p-subs in phenyl ring), 3294.93 (N-H str.), 1664.16 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.519 (d, 2H, 2', 6' H of C_6H_4-NHCOCH$_3$), 7.071 (d, 2H, 3', 5' H of C_6H_4-NHCOCH$_3$), 3.7 (t, 3H, -OC-C.H-CH$_2$-), 2.152 (s, 1H, -CH$_3$), 2.14 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ = 129.12 (C-1 & 5), 118.14 (C-2 & 4), 143.76 (C-3), 168.32 (C-8), 57.78 (C-11), 27.43 (C-13), 176.20 (C-18), 173.27 (C-21). MS (FAB; *m/z*): 344. [M+1]. Elemental analysis ($C_{13}H_{16}N_2O_7S$); calcd. C, 45.34; H, 4.68; N, 8.14; found C, 45.40; H, 4.61; N, 8.29%.

Synthesis of 2-(4-acetamidophenylsulfonamido)pentanedioyl dichloride (4)

2-(4-Acetamidophenylsulfonamido)pentanedioic acid (**3**) (2 g, 0.006 mole) was taken in a 50 mL round bottom flask fitted with a reflux condenser and a calcium chloride guard tube. Thionyl chloride (5 mL) was added to it and refluxed in a stem bath for 2 h. The excess thionyl chloride was removed by distillation and hydrochloric acid fumes was removed by distillating it twice with dry benzene (10 mL). This product obtained was 2-(4-acetamido phenyl sulfonamido) pentanedioyl dichloride (**4**). This was later used in the subsequent steps. Yield: 2.10 g (95.45%); mp: 105-107°C.

Synthesis of 2-(4-Acetamidophenylsulfonamido)pentanedioyl dichloroamides (5a-5e)

The 2-(4-acetamidophenylsulfonamido)pentanedioyl dichloride (**4**) formed was dissolved in dry benzene (10 mL) and the whole mass was cooled by dipping in ice water. Aniline and its derivatives, previously cooled was added to it and mixed well. The whole mass was transferred in a mortar and pestle and triturated when the product was obtained. It was then acidified with dilute hydrochloric acid. The precipitate obtained was filtered and washed with distilled water to remove excess acid. The residue was dried and recrystallized from dilute ethanol after charcoal treatment.

2-(4-Acetamidophenylsulfonamido)-N^1,N^5-bis(3-chlorophenyl) pentanediamide (5a)

Yield 82.47%, mp: 142-144°C. R_f 0.87. IR (KBr) ν_{max} (cm^{-1}) 3267-3043 (O-H str. of COOH), 1662 (C=O str.), 1517 (C=C str. of phenyl ring), 1323 (S=O str. antisym of SO_2N), 1242 (S=O str. sym of SO_2N), 830 (out of plane C-H def due to p-subs in phenyl ring), 747 (C-Cl str. of phenyl ring), 3297 (N-H str.), 1554 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.814 (d, 2H, 2', 6' H of C_6H_4-NHCOCH$_3$), 7.412 (d, 2H, 3', 5' H of C_6H_4-NHCOCH$_3$), 7.247 (d, 2H, 2', 6' H of C_6H_4-Cl), 7.203 (s, 1H, 5' H of C_6H_4-Cl), 3.472 (t, 3H, -OC-C.H-CH$_2$-), 2.041 (s, 1H, -CH$_3$), 2.47 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ = 141.28 (C-1), 118.32 (C-2 & 6), 129.75 (C-3 & 5), 136.35 (C-4), 168.75 (C-8), 25.35 (C-10), 55.96 (C-11), 27.04 (C-12), 136.17 (C-14), 128.36 (C-17 & 23), 122.36 (C-21 & 25), 171.58 (C-33). MS (FAB; *m/z*): 562. [M+1]. Elemental analysis ($C_{25}H_{24}Cl_2N_4O_5S$); calcd. C, 53.29; H, 4.29; Cl, 12.58; N, 9.94; found C, 53.37; H, 4.27; N, 9.83%.

2-(4-Acetamidophenylsulfonamido)-N^1,N^5-bis(3-bromophenyl)pentanediamide (5b)

Yield 75.72%, mp: 201-203°C. R_f 0.75. IR (KBr) ν_{max} (cm^{-1}) 3142.22-2621.41 (O-H str. of COOH), 1687.15 (C=O str.), 1565.42 (C=C str. of phenyl ring), 1375.42 (S=O str. antisym of SO_2N), 1225.10 (S=O str. sym of SO_2N), 838.32 (out of plane C-H def due to p-subs in phenyl ring), 747.74 (C-Br str. of phenyl ring), 3315.87 (N-H str.), 1587.43 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.521 (d, 2H, 2', 6' H of C_6H_4-NHCOCH$_3$), 7.321 (d, 2H, 3', 5' H of C_6H_4-NHCOCH$_3$), 7.720 (d, 2H, 2', 6' H of C_6H_4-Br), 8.012 (s, 1H, 5' H of C_6H_4-Br), 3.541 (t, 3H, -OC-C.H-CH$_2$-), 2.241 (s, 1H, -CH$_3$), 2.713 (q, 4H,-H$_2$C-H$_2$C-), 2.171 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ = 140.58 (C-1), 117.23 (C-2 & 6), 130.58 (C-3 & 5), 136.78 (C-4), 167.36 (C-8), 25.78 (C-10), 54.36 (C-11), 28.36 (C-12), 136.02 (C-14), 128.78 (C-17 & 23), 121.36 (C-21 & 25), 169.35 (C-30), 171.25 (C-33). MS (FAB; *m/z*): 651. [M+1]. Elemental analysis ($C_{25}H_{24}Br_2N_4O_5S$); calcd. C, 46.03; H, 3.71; N, 8.59; found C, 45.97; H, 3.82; N, 8.68%.

2-(4-Acetamidophenylsulfonamido)-N^1,N^5-bis(3-nitrophenyl)pentanediamide (5c)

Yield 64.27%, mp: 178-180°C. R_f 0.72. IR (KBr) ν_{max} (cm^{-1}) 3121-2958 (O-H str. of COOH), 1654 (C=O str.), 1547 (C=C str. of phenyl ring), 1324 (S=O str. antisym of SO_2N), 1155 (S=O str. sym of SO_2N), 847 (out of plane C-H def due to p-subs in phenyl ring), 3358 (N-H str.), 1632 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.742 (d, 2H, 2', 6' H of C_6H_4-NHCOCH$_3$), 7.381 (d, 2H, 3', 5' H of C_6H_4-NHCOCH$_3$), 7.702 (d, 2H, 2', 6' H of C_6H_4-NO$_2$), 7.243 (s, 1H, 5' H of C_6H_4-NO$_2$), 3.814 (t, 3H, -OC-C.H-CH$_2$-), 2.127 (s, 1H, -CH$_3$), 2.071 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ = 140.21 (C-1), 118.78 (C-2 & 6), 130.25 (C-3 & 5), 134.63 (C-4), 168.78 (C-8), 25.02 (C-10), 55.55 (C-11), 28.96 (C-12), 134.69 (C-14), 129.68 (C-17 & 23), 122.09 (C-21 & 25), 172.57 (C-33). MS (FAB; *m/z*): 584. [M+1]. Elemental analysis ($C_{25}H_{24}N_6O_9S$); calcd. C, 51.37; H, 4.14; N, 14.38; found C, 51.48; H, 4.30; N, 14.41%.

2-(4-Acetamidophenylsulfonamido)-N^1,N^5-bis(3-iodophenyl)pentanediamide (5d)

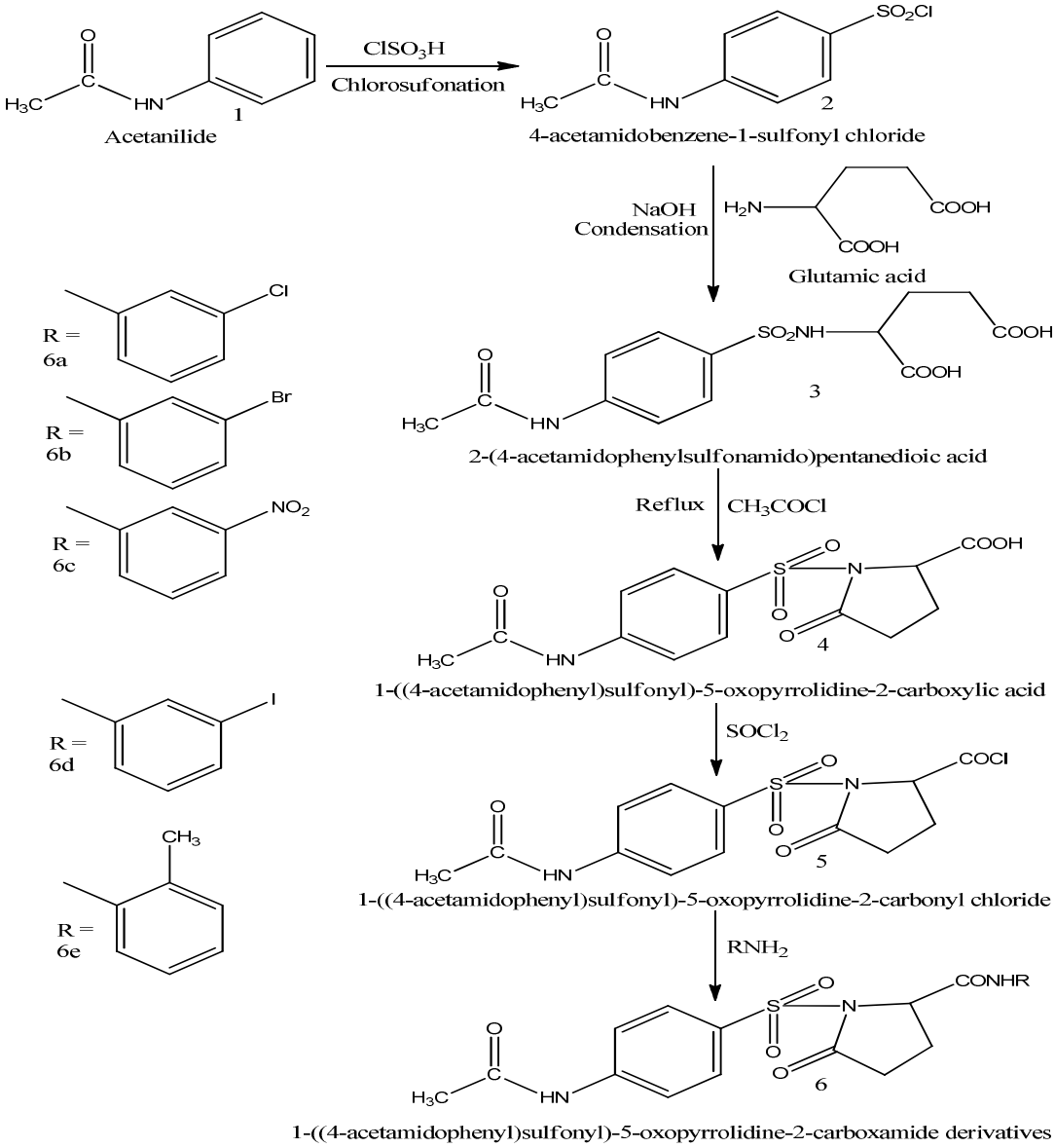
Yield 59.41%, mp: 210-212°C. R_f 0.78. IR (KBr) ν_{max} (cm^{-1}) 3072.01-2502.40 (O-H str. of COOH), 1652.44 (C=O str.), 1523.78 (C=C str. of phenyl ring), 1378.12 (S=O str. antisym of SO_2N), 1249.30 (S=O str. sym of SO_2N), 849.22 (out of plane C-H def due to p-subs in phenyl ring), 768.42 (C-I str. of phenyl ring), 3331.42 (N-H str.), 1589.41 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.511 (d, 2H, 2', 6' H of C_6H_4-NHCOCH$_3$), 7.121 (d, 2H, 3', 5' H of C_6H_4-NHCOCH$_3$), 7.704 (d, 2H, 2', 6' H of C_6H_4-I), 7.881 (s, 1H, 5' H of C_6H_4-I), 3.471 (t, 3H, -OC-C.H-CH$_2$-), 2.211 (s, 1H, -CH$_3$), 2.389 (q, 4H,-H$_2$C-H$_2$C-), 2.011 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ = 143.12 (C-1), 119.44 (C-2 & 6), 128.74 (C-3 & 5), 135.41 (C-4), 168.22 (C-8), 23.27 (C-10), 53.44 (C-11), 137.74 (C-14), 129.47 (C-16 & 18), 125.22 (C-17 & 23), 127.44 (C-22 & 24), 168.71 (C-30), 171.29 (C-33). MS (FAB; *m/z*): 746. [M+1]. Elemental analysis ($C_{25}H_{24}I_2N_4O_5S$); calcd. C, 40.23; H, 3.24; N, 7.51; found C, 40.22; H, 3.31; N, 7.42%.

2-(4-Acetamidophenylsulfonamido)-N^1,N^5-bis(2-methylphenyl) pentanediamide (5e)

Yield 67.22%, mp: 177-179°C. R_f 0.81. IR (KBr) ν_{max} (cm^{-1}) 3042.14-2966.78 (O-H str. of COOH), 1700.41 (C=O str.), 1597.75 (C=C str. of phenyl ring), 1375.41 (C-H bending of phenyl ring), 1335.11 (S=O str. antisym of SO_2N), 1170.23 (S=O str. sym of SO_2N), 832.12 (out of plane C-H def due to p-subs in phenyl ring), 3390.72 (N-H str.), 1642.77 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.621 (d, 2H, 2', 6' H of C_6H_4-NHCOCH$_3$), 7.444 (d, 2H, 3', 5' H of C_6H_4-

NHCOCH$_3$), 7.841 (s, 1H, 6' H of C_6H_4-CH$_3$), 7.411 (d, 2H, 3', 5' H of C_6H_4-CH$_3$), 3.642 (t, 3H, -OC-C.H-CH$_2$-), 2.240 (s, 1H, -CH$_3$), 2.041 (s, 1H, -SO$_2$NH-). ^{13}C NMR (DMSO-d_6) δ = 141.52 (C-1), 120.41 (C-2 & 6), 128.44 (C-3 & 5), 136.47 (C-4), 168.71 (C-8), 24.42 (C-10), 55.55 (C-11), 136.43 (C-14), 129.77 (C-16 & 18), 126.02 (C-17 & 23), 127.47 (C-22 & 24), 167.12 (C-30), 172.34 (C-33). MS (FAB; *m/z*): 522. [M+1]. Elemental analysis ($C_{27}H_{30}N_4O_5S$); calcd. C, 62.05; H, 5.79; N, 10.72; found C, 62.15; H, 5.68; N, 10.63%.

General procedure for synthesis of substituted 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carboxamides (6a-e)



Scheme 2: Synthetic pathway of substituted 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carboxamides (6a-e).

Synthesis of 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carboxylic acid (4)

2-(4-Acetamidophenylsulfonamido)pentanedioic acid (**3**) (32 g, 0.093 mole) was taken in a 500 ml round bottom flask, fitted with a reflux condenser and a calcium chloride guard tube. Acetyl chloride (70 mL) and benzene (40 mL) were added to it and refluxed in a steam bath for four hrs. After refluxing, benzene was removed by distillation and the reaction mass was cooled. After that chopped ice poured in the round bottom flask with continuous stirring. It was kept overnight in freeze, when the semisolid mass solidified. It was filtered, washed with distilled water. The crude product was recrystallized from dilute ethanol after charcoal treatment. Yield: 20.12 g (66%); mp: 178-180°C; Neutral equivalent (found: 322.32, Calc. For $C_{13}H_{14}O_6N_2S$: 326)

Synthesis of 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carbonyl chloride (5)

1-((4-Acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carboxylic acid (**4**) (3 g, 0.009 mole) was taken in a 50 mL round bottom flask fitted with a reflux condenser and a calcium chloride guard tube. Thionyl chloride (5 mL) was added to it and refluxed in a stem bath for 2 h. The excess thionyl chloride was removed by distillation and

hydrochloric acid fumes was removed by distillating it twice with dry benzene (10 mL).

This product obtained was 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carbonyl chloride (**5**).This was later used in the subsequent steps. Yield: 2.3 g (75%); mp: 105-107°C. R_f 0.79. IR (KBr) ν_{max} (cm^{-1}) 3238.09 (C-H str. of phenyl ring), 3058.82 (O-H str. of COOH), 1669.35 (C=O str.), 1495.91 (C=C str. of phenyl ring), 1314.54 (C-O str. or O-H def of COOH), 1395.80 (S=O str. antisym of SO_2N), 1246.06 (S=O str. sym of SO_2N), 830.10 (out of plane C-H def due to p-subs in phenyl ring), 3306.34 (N-H str.), 1669.35 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.541 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.247 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 3.45 (t, 3H, -OC-C.H-CH_2-), 2.201 (s, 1H, -CH_3), 2.12 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ = 129.14 (C-1 & 5), 118.17 (C-2 & 4), 143.19 (C-3), 138.32 (C-6), 24.60 (C-9), 171.23 (C-13), 24.99 (C-15), 168.48 (C-20). MS (FAB; *m/z*): 344. [M+1]. Elemental analysis ($C_{13}H_{16}N_2O_7S$); calcd. C, 45.34; H, 4.68; N, 8.14; found C, 45.40; H, 4.61; N, 8.29%.

Synthesis of 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carboxamides (6a-6e)

The 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carbonyl chloride (**5**) formed was dissolved in dry benzene (10 mL) and the whole mass was cooled by dipping in ice water. Aniline and its derivatives, previously cooled was added to it and mixed well. The whole mass was transferred in a mortar and pestle and triturated when the product was obtained. It was then acidified with dilute hydrochloric acid. The precipitate obtained was filtered and washed with distilled water to remove excess acid. The residue was dried and recrystallized from dilute ethanol after charcoal treatment.

1-((4-Acetamidophenyl)sulfonyl)-N-(3-chlorophenyl)-5-oxopyrrolidine-2-carboxamide (6a):

Yield 57.24%, mp: 225-227°C. R_f 0.91. IR (KBr) ν_{max} (cm^{-1}) 3124-2478 (O-H str. of COOH), 1647.41 (C=O str.), 1571.21 (C=C str. of phenyl ring), 1321.44 (S=O str. antisym of SO_2N), 1242.78 (S=O str. sym of SO_2N), 854.77 (out of plane C-H def due to p-subs in phenyl ring), 727.48 (C-Cl str. of phenyl ring), 3371.47 (N-H str.), 1674.28 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.741 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.352 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 7.608 (d, 2H, 2', 6' H of C_6H_4-Cl), 7.125 (s, 1H, 5' H of C_6H_4-Cl), 1.475 (s, 1H, -CH_3), 2.701 (s, 1H, -NH-). ^{13}C NMR (DMSO-d_6) δ = 141.23 (C-1), 118.32 (C-2 & 6), 129.78 (C-3 & 5), 138.32 (C-4), 167.25 (C-9), 25.23 (C-11), 171.77 (C-13), 30.32 (C-14), 63.63 (C-16), 135.85 (C-18), 120.12 (C-19 & 23), 127.78 (C-21), 170.32 (C-26). MS (FAB; *m/z*): 435. [M+1]. Elemental analysis ($C_{19}H_{18}ClN_3O_5S$); calcd. C, 52.35; H, 4.16; N, 9.64; found C, 52.37; H, 4.19; N, 9.78%.

1-((4-Acetamidophenyl)sulfonyl)-N-(3-bromophenyl)-5-oxopyrrolidine-2-carboxamide(6b):

Yield 77.15%, mp: 218-220°C. R_f 0.89. IR (KBr) ν_{max} (cm^{-1}) 3147-2543 (O-H str. of COOH), 1640 (C=O str.), 1552 (C=C str. of phenyl ring), 1371 (S=O str. antisym of SO_2N), 1377 (S=O str. sym of SO_2N), 870 (out of plane C-H def due to p-subs in phenyl ring), 741 (C-Br str. of phenyl ring), 3281 (N-H str.), 1608 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.457 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.241 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 7.747 (d, 2H, 2', 6' H of C_6H_4-Br), 7.241 (s, 1H, 5' H of C_6H_4-Br), 2.271 (s, 1H, -CH_3), 2.101 (s, 1H, -NH-). ^{13}C NMR (DMSO-d_6) δ = 144.47 (C-1), 118.27 (C-2 & 6), 131.23 (C-3 & 5), 135.63 (C-4), 170.21 (C-9), 23.45 (C-11), 171.85 (C-13), 31.71 (C-14), 61.25 (C-16), 137.45 (C-18), 122.23 (C-19 & 23), 128.47 (C-21), 169.36 (C-26). MS (FAB; *m/z*): 495. [M+1]. Elemental analysis ($C_{20}H_{20}BrN_3O_5S$); calcd. C, 48.59; H, 4.08; N, 8.50; found C, 48.39; H, 4.21; N, 8.43%.

1-((4-Acetamidophenyl)sulfonyl)-N-(3-nitrophenyl)-5-oxopyrrolidine-2-carboxamide (6c):

Yield 68.21%, mp: 231-233°C. R_f 0.79. IR (KBr) ν_{max} (cm^{-1}) 3071.72-2567 (O-H str. of COOH), 1671.73 (C=O str.), 1591.47 (C=C str. of phenyl ring), 1371.72 (S=O str. antisym of SO_2N), 1262 (S=O str. sym of SO_2N), 841.80 (out of plane C-H def due to p-subs in phenyl ring), 3285.07 (N-H str.), 1671.08 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.778 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.369 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 7.751 (d, 2H, 2', 6' H of C_6H_4-NO_2), 8.124 (s, 1H, 5' H of C_6H_4-NO_2), 2.007 (s, 1H, -CH_3), 2.074 (s, 1H, -NH-).^{13}C NMR (DMSO-d_6) δ = 143.77 (C-1), 118.30 (C-2 & 6), 131.45 (C-3 & 5), 137.78 (C-4), 168.72 (C-9), 23.78 (C-11), 171.58 (C-13), 31.45 (C-14), 64.32 (C-16), 138.78 (C-18), 120.32 (C-19 & 23), 127.78 (C-21). MS (FAB; *m/z*): 446. [M+1]. Elemental analysis ($C_{19}H_{18}N_4O_7S$); calcd. C, 51.12; H, 4.06; N, 12.55; found C, 50.92; H, 4.21; N, 12.63%.

1-((4-Acetamidophenyl)sulfonyl)-N-(3-iodophenyl)-5-oxopyrrolidine-2-carboxamide (6d):

Yield 71.42%, mp: 199-201°C. R_f 0.74. IR (KBr) ν_{max} (cm^{-1}) 3072-2650 (O-H str. of COOH), 1571 (C=O str.), 1522 (C=C str. of phenyl ring), 1341 (S=O str. antisym of SO_2N), 1242 (S=O str. sym of SO_2N), 871 (out of plane C-H def due to p-subs in phenyl ring), 674 (C-I str. of phenyl ring), 3271 (N-H str.), 1658 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.678 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.325 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 7.441 (d, 2H, 2', 6' H of C_6H_4-I), 7.674 (s, 1H, 5' H of C_6H_4-I), 2.224 (s, 1H, -CH_3), 2.012 (s, 1H, -NH-). ^{13}C NMR (DMSO-d_6) δ = 144.57 (C-1), 120.47 (C-2 & 6), 131.74 (C-3 & 5), 137.24 (C-4), 169.12 (C-9), 24.44 (C-11), 171.24 (C-13), 31.41 (C-14), 61.78 (C-16), 137.91 (C-18), 129.41 (C-20 & 22), 128.23 (C-21), 129.32 (C-20 & 22), 169.04 (C-26). MS (FAB; *m/z*): 527. [M+1]. Elemental analysis ($C_{19}H_{18}IN_3O_5S$); calcd. C, 43.28; H, 3.44; N, 7.97; found C, 43.39; H, 3.31; N, 7.93%.

1-((4-Acetamidophenyl)sulfonyl)-N-(2-methylphenyl)-5-oxopyrrolidine-2-carboxamide(6e):

Yield 66.71%, mp: 206-208°C. R_f 0.85. IR (KBr) ν_{max} (cm^{-1}) 3074.24-2577 (O-H str. of COOH), 1653.17 (C=O str.), 1614.18 (C=C str. of phenyl ring), 1374.12 (S=O str. antisym of SO_2N), 1252 (S=O str. sym of SO_2N), 844.55 (out of plane C-H def due to p-subs in phenyl ring), 3274.17 (N-H str.), 1642.22 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.724 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.217 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 7.966 (s, 1H, 6' H of C_6H_4-CH_3), 8.027 (d, 2H, 3', 5' H of C_6H_4-CH_3), 2.422 (s, 1H, -CH_3), 2.047 (s, 1H, -NH-).^{13}C NMR (DMSO-d_6) δ = 141.54 (C-1), 118.72 (C-2 & 6), 131.25 (C-3 & 5), 135.24 (C-4), 169.65 (C-9), 23.74 (C-11), 172.44 (C-13), 32.25 (C-14), 60.47 (C-16), 141.47 (C-18), 128.55 (C-20 & 22), 127.74 (C-21), 128.77 (C-20 & 22). MS (FAB; *m/z*): 415. [M+1]. Elemental analysis ($C_{20}H_{21}N_3O_5S$); calcd. C, 57.82; H, 5.09; N, 10.11; found C, 57.92; H, 5.11; N, 10.15%.

General procedure for synthesis of substituted 2-(4-acetamidophenylsulfonamido)pentanedioic acid amides (5A-E)

Synthesis of 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carboxylic acid (4)

2-(4-acetamidophenylsulfonamido)pentanedioic acid (**3**) (32 g, 0.093 mole) was taken in a 500 ml round bottom flask, fitted with a reflux condenser and a calcium chloride guard tube. Acetyl chloride (70 mL) and benzene (40 mL) were added to it and refluxed in a steam bath for four hrs. After refluxing, benzene was removed by distillation and the reaction mass was cooled. After that chopped ice poured in the round bottom flask with continuous stirring.

It was kept overnight in freeze, when the semisolid mass solidified. It was filtered, washed with distilled water. The crude product was recrystallized from dilute ethanol after charcoal treatment. Yield: 20.12 g (66%); mp: 78-80°C; Neutral equivalent (found: 329.32, Calc. For $C_{13}H_{14}O_6N_2S$: 326)

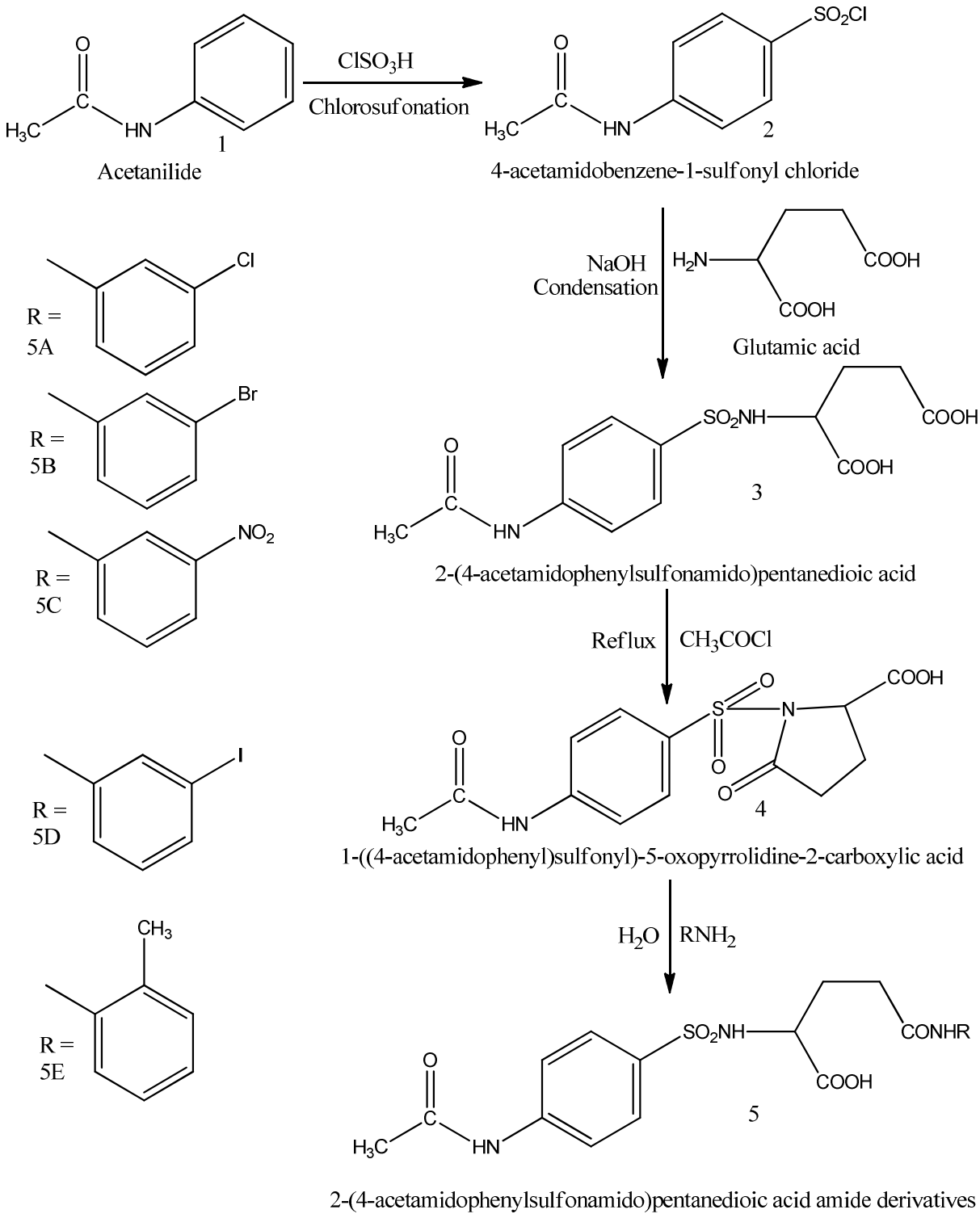
Synthesis of 2-(4-acetamidophenylsulfonamido)pentanedioic acid amides (5A-G)

The 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carboxylic acid **(4)** formed was dissolved in dry benzene (10 mL) and the whole mass was cooled by dipping in ice water. Aniline and its derivatives, previously cooled was added to it and mixed well. The whole mass was transferred in a mortar and pestle and triturated when the product was obtained. It was then acidified with dilute hydrochloric acid. The precipitate obtained was filtered and washed with distilled water to remove excess acid. The residue was dried and recrystallized from dilute ethanol after charcoal treatment.

2-(4-Acetamidophenylsulfonamido)-5-((3-chlorophenyl) amino)-5-oxopentanoic acid (5A):

Yield 84.76%, mp: 251-253°C. R_f 0.67. IR (KBr) ν_{max} (cm^{-1}) 3063.22-2582.15 (O-H str. of COOH), 1668.09 (C=O str.), 1563.27 (C=C str. of phenyl ring), 1323.07 (S=O str. antisym of SO_2N), 1293.77 (S=O str. sym of SO_2N), 913.24 (out of plane C-H def due to p-subs in phenyl ring), 757.21 (C-Cl str. of phenyl ring), 3301.21 (N-H str.), 1592.23 (N-H bending).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.724 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.400 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 7.684 (d, 2H, 2', 6' H of C_6H_4-Cl), 7.007 (s, 1H, 5' H of C_6H_4-Cl), 3.475 (t, 3H, -OC-C.H-CH_2-), 2.402 (s, 1H, -CH_3), 2.210 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ =144.20 (C-1), 119.02 (C-2 & 6), 137.78 (C-4), 168.48 (C-8), 23.25 (C-10), 58.32 (C-11), 36.78 (C-13), 136.32 (C-14), 120.78 (C-15 & 19), 127.78 (C-17), 170.23 (C-20), 170.78 (C-27). MS (FAB; *m/z*): 453. [M+1]. Elemental analysis ($C_{19}H_{20}ClN_3O_6S$); calcd. C, 50.28; H, 4.44; N, 9.26; found C, 50.22; H, 4.51; N, 9.33%.



Scheme 2: Synthetic pathway of substituted 2-(4-acetamidophenylsulfonamido)pentanedioic acid amides (5A-E)

2-(4-Acetamidophenylsulfonamido)-5-((3-bromophenyl) amino)-5-oxopentanoic acid (5B):

Yield 69.06%, mp: 193-195°C. R_f 0.83. IR (KBr) ν_{max} (cm^{-1}) 3121-2574 (O-H str. of COOH), 1624 (C=O str.), 1563 (C=C str. of phenyl ring), 1347 (S=O str. antisym of SO_2N), 1377 (S=O str. sym of SO_2N), 852 (out of plane C-H def due to p-subs in phenyl ring), 723 (C-Br str. of phenyl ring), 3320 (N-H str.), 1670 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.862 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.243 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 7.895 (d, 2H, 2', 6' H of C_6H_4-Br), 7.443 (s, 1H, 5' H of C_6H_4-Br), 3.75 (t, 3H, -OC-C.H-CH_2-), 2.214 (s, 1H, -CH_3), 2.121 (q, 4H,-H_2C-H_2C-), 2.041 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ =143.22 (C-1), 119.78 (C-2 & 6), 130.22 (C-3 & 5), 139.78 (C-4), 168.33 (C-8), 23.88 (C-10), 140.96 (C-14), 123.22 (C-15 & 19), 173.70 (C-20), 172.35 (C-27). MS (FAB; *m/z*): 499. [M+1]. Elemental analysis ($C_{19}H_{20}BrN_3O_6S$); calcd. C, 45.79; H, 4.05; N, 8.43; found C, 45.92; H, 3.91; N, 8.51%.

2-(4-Acetamidophenylsulfonamido)-5-((3-nitrophenyl)amino)-5-oxopentanoic acid (5C):

Yield 72.32%, mp: 187-189°C. R_f 0.77. IR (KBr) ν_{max} (cm^{-1}) 3134.71-2427.33 (O-H str. of COOH), 1672.44 (C=O str.), 1563.24 (C=C str. of phenyl ring), 1357.21 (S=O str. antisym of SO_2N), 1333.24 (S=O str. sym of SO_2N), 873.40 (out of plane C-H def due to p-subs in phenyl ring), 3373.05 (N-H str.), 1620.31 (N-H bending).^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.894 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.220 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 7.994 (d, 2H, 2', 6' H of C_6H_4-NO_2), 7.489 (s, 1H, 5' H of C_6H_4-NO_2), 2.147 (q, 4H, -H_2C-H_2C-), 2.047 (s, 1H, -CH_3), 2.072 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ =141.22 (C-1), 119.78 (C-2 & 6), 170.23 (C-8), 24.75 (C-10), 56.32 (C-11), 27.71 (C-12), 35.23 (C-13), 141.14 (C-14), 121.51 (C-15 & 19), 171.23 (C-20), 173.22 (C-27). MS (FAB; *m/z*): 464. [M+1]. Elemental analysis ($C_{19}H_{20}N_4O_8S$); calcd. C, 49.13; H, 4.34; N, 12.06; found C, 49.12; H, 4.21; N, 12.13%.

2-(4-Acetamidophenylsulfonamido)-5-((3-iodophenyl)amino)-5-oxopentanoic acid (5D):

Yield 68.27%, mp: 232-234°C. R_f 0.87. IR (KBr) ν_{max} (cm^{-1}) 3152-2545 (O-H str. of COOH), 1647 (C=O str.), 1622 (C=C str. of phenyl ring), 1342 (S=O str. antisym of SO_2N), 1347 (S=O str. sym of SO_2N), 847 (out of plane C-H def due to p-subs in phenyl ring), 744 (C-I str. of phenyl ring), 3274 (N-H str.), 1647 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.747 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.244 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 7.721 (d, 2H, 2', 6' H of C_6H_4-I), 7.447 (s, 1H, 5' H of C_6H_4-I), 3.177 (t, 3H, -OC-C.H-CH_2-), 2.112 (s, 1H, -CH_3), 2.147 (q, 4H,-H_2C-H_2C-), 2.110 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ =141.42 (C-1), 119.47 (C-2 & 6), 132.44 (C-3 & 5), 139.23 (C-4), 171.74 (C-8), 24.88 (C-10), 141.14 (C-14), 129.05 (C-16 & 18), 173.91 (C-20), 171.34 (C-27). MS (FAB; *m/z*): 541. [M+1]. Elemental analysis ($C_{19}H_{20}IN_3O_6S$); calcd. C, 41.85; H, 3.70; N, 7.71; found C, 41.92; H, 3.81; N, 7.66%.

2-(4-Acetamidophenylsulfonamido)-5-((2-methylphenyl) amino)-5-oxopentanoic acid (5E):

Yield 70.21%, mp: 201-203°C. R_f 0.70. IR (KBr) ν_{max} (cm^{-1}) 3145-2342.12 (O-H str. of COOH), 1645 (C=O str.), 1547 (C=C str. of phenyl ring), 1345 (S=O str. antisym of SO_2N), 1341 (S=O str. sym of SO_2N), 823 (out of plane C-H def due to p-subs in phenyl ring), 3374.21 (N-H str.), 1680.44 (N-H bending). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.142 (d, 2H, 2', 6' H of C_6H_4-$NHCOCH_3$), 7.547 (d, 2H, 3', 5' H of C_6H_4-$NHCOCH_3$), 8.012 (s, 1H, 6' H of C_6H_4-CH_3), 8.022 (d, 2H, 3', 5' H of C_6H_4-CH_3), 2.232 (q, 4H, -H_2C-H_2C-), 2.174 (s, 1H, -CH_3), 2.112 (s, 1H, -SO_2NH-). ^{13}C NMR (DMSO-d_6) δ =141.47 (C-1), 120.23 (C-2 & 6), 169.07 (C-8), 25.54 (C-10), 55.22 (C-11), 29.22 (C-12), 36.56 (C-13), 140.44 (C-14), 128.72 (C-16 & 18), 171.32 (C-20), 172.29 (C-27). MS (FAB; *m/z*): 433. [M+1]. Elemental analysis ($C_{20}H_{23}N_3O_6S$); calcd. C, 55.42; H, 5.35; N, 9.69; found C, 55.36; H, 5.28; N, 9.63%.

Biological evaluation

Experimental animals

Adult Swiss albino mice of same sex (female in this case) weighing 18 to 20 g maintained in our college animal house were used for the study. The selected animals were maintained by giving pelleted diet, water adlibitum and kept in 12 h/12 h light/dark cycle. The animals were divided into six groups each containing mice. All the animal experiments were performed following the approval of study protocols by the Institutional Animal Ethics Committee (BCRCP/IAEC/7/2012).

***In-vitro* anticancer assay**

Cell culture

Human breast cancer (MCF-7), Leukemia (K-562), Ovarian cancer (OVCAR-3), Human colon adeno carcinoma (HT-29) and Human kidney carcinoma (A-498) tumor cells were obtained from National Centre for Cell Sciences (Pune, India). The cultures were maintained in Dulbecco's Modified Eagles Medium (DMEM) containing 10% heat inactivated Fetal Bovine Serum (FBS), penicillin (100 units mL^{-1}) and streptomycin (100 µg mL^{-1}) at 37°C in 5% CO_2. Cells were grown in 25 cm^2 tissue cultures flask until confluent and used for cytotoxicity assays.

MTT assay

The MTT (3-(4,5-dimethylthiazole-2-yl)-2,5diphenyltetrazolium bromide) assay developed by Mosmann [15] was modified and used to determine the inhibitory effects of test compounds on cell growth in-vitro. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat bottomed tissue culture plate at a density of $5x10^3$ cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 48 h incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of both standard (Tamoxifen) and test compounds **5a-e**, **6a-e** and **5A-E** (8, 16, 32, 64, 128 and 256 µg mL^{-1}) in triplicates to achieve a final volume of 100 µL and then cultured for 48 h. The compound was prepared as 1.0 mg mL^{-1} concentration stock solutions in PBS. Culture medium and solvent are used as controls. Each well then received 5 µL of fresh MTT (0.5 mg mL^{-1} in PBS) followed by incubation for 2 h at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µL of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 570 nm on an ELISA reader, Anthos 2020 spectrophotometer. Both standard and test maintained in triplicate. The IC_{50} value refers to the drug concentration that produces a 50% reduction in cellular growth when compared to untreated control cells [16].

***In-vivo* anticancer assay**

The synthesized compounds were biologically evaluated against *Ehrlich ascites Carcinoma* (EAC) in Swiss Albino mice using tumor weight and cell count as activity parameters. Amongst various evaluation systems *in vogue*, this method has been standardized and numbers of screening results have been reported earlier [17-19]. Two groups of Swiss Albino mice, each containing five healthy mice of the same sex (female in this case), approximately of same age and body weight (18-20 g), were selected at random and kept in two different cages under identical condition. One of these two groups served as control while the other as test. *Ehrlich ascites Carcinoma* (EAC) cells were collected from the donor mice and were suspended in sterile isotonic solution (0.9% w/v NaCl). The numbers of tumor cells per mL of this suspension were counted under microscope with the help of haemocytometer. A definite number (about 2 x 10^6/0.2 mL) of these living viable cells was injected or implanted into the peritoneal cavity of each mouse. In this instance, the tumor cells multiplied relatively freely with in the peritoneal cavity and ascites developed. A day of incubation was allowed to establish the disease in the body before the drug administration started. From the second day of transplantation up to the eight day a suitable dose (0.2 mmole kg^{-1} body weight) of the drug solution/suspension in sterile phosphate buffer (pH 7.2) was injected intraperitoneally to each mouse in the test group at 24 h interval. Thus, seven doses of the drug were administered to each mouse in the test group. On the ninth day food and water were withheld or withdrawn 6 h before the testing operation started. The weight of all the animals was

recorded before they were sacrificed. The peritoneal cavity was dissected and the ascites fluid was drawn by a syringe to a suitable volume with sterile ice cold saline and preserved in ice bath. The total number of living cells/ml in the peritoneal fluid of the three mice in a group was calculated. The fluid was sucked by absorbent cotton. The weight of the five mice after sacrifice was recorded. The evaluation of the test drug was made by comparing the cell count and ascetic fluid weight of the test with that of the control. The percentage inhibition of ascetic cell count and ascetic fluid weight was obtained by the following expressions.

Percentage inhibition of Ascitic Cell = (1 - T/C) x 100

Percentage inhibition of Ascitic fluid = (1 - T′/C′) x 100

Where, T= Average no. of Ascitic cells per mL in test animals, C= Average no. of Ascitic cells per mL in control animals, T′= Average weight of Ascitic fluid in test animals and C′= Average weight of Ascitic fluid in control animals. Mitomycin-C (1 mg kg^{-1} body weight) in sterile phosphate buffer (pH 7.2) was used as standard.

RESULTS AND DISCUSSION

Chemistry

The synthetic strategies followed for the preparation of the substituted 2-(4-methylbenzenesulfonamido)pentanedioyl dichloroamide (**5a-e**), 1-((4-acetamidophenyl)sulfonyl)-5-oxopyrrolidine-2-carboxamide (**6a-e**) and 2-(4-acetamidophenylsulfonamido)pentanedioic acid amide (**5A-E**) analogs are depicted in Scheme 1, 2 and 3. Synthesis was started with chlorosulphonation of acetanilide, to get 2-(4-acetamidophenylsulfonamido)pentanedioic acid (**2**). This sulphonyl halide proved to be versatile synthon in the subsequent step in the preparation of substituted sulphonyl glutamic acids. With the application of Schotton-Bauman reaction, substituted sulphonyl glutamic acids (**3**) were prepared by one-step condensation of 2-(4-acetamidophenylsulfonamido)pentanedioic acid (**2**) with L-glutamic acid. In this reaction alkaline medium was maintained to remove the hydrochloric acid which was formed during condensation. Reaction of the resulting intermediates with thionyl chloride afforded corresponding acid chloride followed by the amination with various amines afford the corresponding amine **5a-e** (Scheme 1). In one such reaction, the resulting intermediates substituted sulphonyl glutamic acids (**3**) with acetyl chloride afforded cyclized acid intermediates substituted sulphonyloxopyrrolidine carboxylic acids (**4**). Treatment of **4** with thionyl chloride gives the corresponding acid chloride (**5**). Amination of **5** with various amines afford the corresponding amine **6a-e** (Scheme 2). In yet another reaction, the resulting intermediates **3** with acetyl chloride afforded cyclized acid intermediates substituted sulphonyloxopyrrolidine carboxylic acids (**4**). Aminolysis of the intermediates **4** with various amines afforded the corresponding glutamines **5A-E** (Scheme 3).

Formation of all compounds was confirmed by recording their IR, NMR, mass spectra and elemental analyses. The IR spectra of compounds **5a-e**, **6a-e** and **5A-E** revealed the presence of absorption bands from 3267 to 2320 cm^{-1} that indicate the presence of OH str. of COOH, from 1700.24 to 1598 cm^{-1} for C=O str., from 1631 to 1520 cm^{-1} for C=C str. of phenyl ring, from 3398.72 to 3255 cm^{-1} for N-H str., from 1681 to 1554 cm^{-1} for N-H bending, from 1387.21 to 1321 cm^{-1} for S=O str. anti-symmetric of SO_2N and 1377 to 1155 cm^{-1} for S=O str. symmetric of SO_2N vibrations. In addition to proton signals of the functional groups and both aromatic ring present in the respective molecule ^1H NMR spectra of these compounds contained one proton singlet from δ 1.475 to 2.471 ppm which was assigned to -SO_2NH- proton, δ 2.027 to 2.47 ppm for -NH- proton and from 1.475 to 2.471 ppm for -CH_3 proton. The ^1H NMR spectra of compounds **5a-e**, **6a-e** and **5A-E** showed doublets from *δ* 7.007 to 8.152 ppm for aromatic protons and triplets from *δ* 3.124 to 3.814 ppm for -OC-CH-CH_2- proton confirming the formation of compounds. The ^{13}C NMR spectra of compounds **5a-e**, **6a-e** and **5A-E** showed peaks from *δ* 117.23 ppm to 173.99 ppm for aromatic carbons, from *δ* 23.08 ppm to 25.78 ppm for -CH_3 carbon confirming the formation of compounds **5a-e**, **6a-e** and **5A-E**. The mass spectra of compounds **5a-e**, **6a-e** and **5A-E** showed molecular ion peaks [M+1] at m/z corresponding to their respective molecular masses, which is in consistency with their respective molecular formulas. For the compound **5a**, molecular weight 494 is consistent with the molecular formula $C_{25}H_{26}N_4O_5S$. The values for the remaining compounds have been presented under the experimental part.

Table 1: *In-vitro* anticancer activity (IC_{50}) of synthesized compounds against Human breast cancer (MCF-7), Leukemia (K-562), Ovarian cancer (OVCAR-3), Human colon adeno carcinoma (HT-29) and Human kidney carcinoma (A-498)

Compound	Human breast cancer (MCF-7)	Leukemia (K-562)	Ovarian cancer (OVCAR-3)	Human colon adeno carcinoma (HT-29)	Human kidney carcinoma (A-498)
3	14.71±0.23*	13.21±0.04*	13.35±0.31*	26.42±0.14*	24.45±0.14*
5a	59.24±0.04*	41.02±0.14*	54.32±0.33*	85.21±0.05*	42.21±0.21*
5b	17.12±0.14*	21.21±0.21*	13.74±0.12*	35.21±0.07*	28.21±0.11*
5c	23.73±0.12*	13.62±0.20*	33.42±0.14*	49.33±0.14*	17.25±0.06*
5d	69.19±0.21*	63.21±0.14*	81.23±0.21*	78.23±0.11*	87.21±0.042*
5e	87.34±0.04	92.32±0.17	98.21±0.11	> 100	89.25±0.121
6a	33.24±0.10*	49.21±0.20*	52.27±0.04*	61.21±0.07*	71.23±0.042*
6b	28.35±0.11*	35.21±0.11*	19.32±0.07*	36.44±0.11*	20.54±0.11*
6c	24.24±0.101*	38.21±0.14*	20.21±0.23*	42.21±0.131*	18.65±0.041*
6d	91.24±0.31	82.42±0.07	88.32±0.14	> 100	72.32±0.21
6e	82.32±0.41	78.32±0.21	96.39±0.17	> 100	86.32±0.24
5A	69.32±0.21*	84.21±0.03*	52.32±0.061*	80.74±0.13*	46.32±0.30*
5B	22.14±0.14*	32.21±0.24*	18.24±0.15*	36.32±0.14*	17.24±0.041*
5C	75.25±0.21	93.21±0.22	83.42±0.05	> 100	91.21±0.11
5D	72.20±0.14*	71.24±0.31*	47.94±0.04*	63.81±0.04*	81.24±0.21*
5E	66.35±0.11*	55.63±0.04*	72.35±0.13*	84.23±0.06*	69.33±0.032*
Tamoxifen (Standard)	8.21±0.004*	10.44±0.01*	9.30±0.021*	14.25±0.012*	12.22±0.02*

Compounds with IC_{50} >100 μM were considered not active; * p< 0.05 compared to Tamoxifen (Standard)

Biological evaluation

In-vitro anticancer assay

The cytotoxicity of the synthesized compounds (**5a-e**, **6a-e** and **5A-E**) were studied using the MTT assay in five human cancer cell lines, including Human breast cancer (MCF-7), Leukemia (K-562), Ovarian cancer (OVCAR-3), Human colon adeno carcinoma (HT-29) and Human kidney carcinoma (A-498). The results are listed in Table 1. Compounds 2-(4-acetamidophenylsulfonamido)-N^1,N^5-bis(2-methylphenyl)pentanediamide (**5e**), 1-((4-acetamidophenyl) sulfonyl)-N-(3-iodophenyl)-5-oxopyrrolidine-2-carboxamide (**6d**), 1-((4-acetamidophenyl)sulfonyl)-N-(2-methylphenyl)-5-oxopyrrolidine-2-carboxamide (**6e**) and 2-(4-acetamidophenylsulfonamido)-5-((3-nitrophenyl)amino)-5-oxopentanoic acid **(5C**) showed low cytotoxic

effects on all the cell lines. Compounds 2-(4-acetamidophenylsulfonamido)pentanedioic acid (**3**), 2-(4-acetamidophenylsulfonamido)-N^1,N^5-bis(3-bromo phenyl) pentanediamide (**5b**), 2-(4-acetamidophenylsulfonamido)-N^1,N^5-bis(3-nitrophenyl)pentanediamide (**5c**), 1-((4-acetamidophenyl)sulfonyl)-N-(3-bromophenyl)-5-oxopyrrolidine-2-carboxamide (**6b**), 1-((4-acetamidophenyl)sulfonyl)-N-(3-nitrophenyl)-5-oxopyrrolidine-2-carboxamide (**6c**) and 2-(4-acetamidophenylsulfonamido)-5-((3-bromophenyl)amino)-5-oxopentanoic acid (**5B**) showed high cytotoxicity in all cell lines with IC_{50} concentrations lines, except for the Human colon adeno carcinoma (HT-29) cell line as compare to standard drug, Tamoxifen. The primary antitumor activity of tamoxifen by inhibition protein kinase C and also ability to facilitate the apoptosis in cancer cell not expressing estrogen receptor is due to generation of oxidative stress resulting in thiol depletion and activation of the transcriptional factor NF-kappaB [20]. Many clinical studies explain the tamoxifen application in various kinds of maligamant diseases [21, 22].

***In-vivo* anticancer assay**

All the newly synthesized compounds (**5a-5e**, **6a-6e** and **5A-5E**) were screened for their anticancer activity against *Ehrlich Ascites Carcinoma* is summarized in Table 2 together with standard drug Mitomycin-C. Among the synthesized compounds, 2-(4-acetamidophenylsulfonamido)pentanedioic acid (**3**) showed encouraging activity in per cent inhibition of ascetic fluid weight (92.25%) and 2-(4-acetamidophenylsulfonamido)-N^1,N^5-bis(3-bromophenyl)pentanediamide (**5b**), 2-(4-acetamido phenyl sulfonamido)-N^1,N^5-bis(3-iodophenyl)pentanediamide (**5d**), 1-((4-acetamidophenyl)sulfonyl)-N-(3-bromo phenyl)-5-oxopyrrolidine-2-carboxamide (**6b**), 1-((4-acetamido phenyl) sulfinyl)-N-(3-nitrophenyl)-5-oxopyrrolidine-2-carboxamide (**6c**), 1-((4-acetamidophenyl)sulfonyl)-N-(3-iodophenyl)-5-oxopyrrolidine-2-carboxamide (**6d**) and 2-(4-acetamidophenylsulfonamido)-5-((3-bromophenyl)amino)-5-oxopentanoic acid (**5B**) showed encouraging activity in both the parameter, viz., ascetic fluid weight (96.01% for **5b**, 95.78% for **5d**, 95.80% for **6b**, 94.02% for **6c**, 92.42% for **6d** and 98.08% for **5B**) and ascetic cell count (95.47% for **5b**, 94.43% for **5d**, 97.36% for **6b**, 93.14% for **6c**, 96.41% for **6d** and 98.46% for **5B**) respectively as compare to Mitomycin-C (100% in both the parameters).

Hence, a detailed and prolonged study is necessary to establish their activity in other models.

Table 2: *In-vivo* anticancer activity of synthesized compounds against *Ehrlich Ascites Carcinoma*

Compound	AA	BB	CC	DD	EE	FF	GG
3	6	84.5	8.17±0.12*	90.33	5.32	0.412±0.04*	92.25
5a	6	84.5	7.87±0.04*	90.75	5.32	0.823±0.11*	84.53
5b	6	84.5	3.82±0.021*	95.47	5.32	0.212±0.12*	96.01
5c	6	84.5	6.13±0.41	93.74	5.32	1.330±0.04	75.00
5d	6	84.5	3.01±0.032*	96.43	5.32	0.224±0.17*	95.78
5e	6	84.5	11.95±0.14*	85.85	5.32	1.891±0.06*	64.45
6a	6	84.5	6.87±0.12*	91.86	5.32	0.951±0.02*	82.12
6b	6	84.5	2.23±0.07*	97.36	5.32	0.223±0.17*	95.80
6c	6	84.5	5.79±0.22*	93.14	5.32	0.318±0.041*	94.02
6d	6	84.5	3.84±0.16*	95.45	5.32	0.413±0.22*	92.23
6e	6	84.5	10.23±0.06	87.89	5.32	2.107±0.031	60.39
5A	6	84.5	4.32±0.044*	94.88	5.32	1.651±0.102	68.96
5B	6	84.5	1.3±0.10*	98.46	5.32	0.102±0.104*	98.08
5C	6	84.5	7.39±0.02	91.25	5.32	2.001±0.02	62.38
5D	6	84.5	4.92±0.041*	94.17	5.32	1.02±0.14*	80.82
5E	6	84.5	13.25±0.21*	84.31	5.32	2.98±0.04*	43.98
Mitomycin-C (Standard)	6	84.5	0.00	100.00	5.32	0.00	100.00

AA= Number of animals in each group; BB= Average no. of ascetic cells per mL in control, C (X 10^6 cells/ml); CC= Average no. of ascitic cells per mL in test±SD, T (X 10^6 cells/ml); DD= Percent inhibition of ascitic cells (1-T/C) X 100; EE=Average weight of ascitic fluid in control C′ (g); FF= Average weight of ascitic fluid in test±SD, T′ (g); GG= Percent inhibition of Ascitic fluid (1-T′/C′)X100; * p< 0.05 compared to Mitomycin-C (Standard)

Statistical analysis

Values are expressed as mean ± SD and data was analyzed by ANOVA followed by Dunnet's test. P<0.05 was considered as significant.

CONCLUSION

Thus, fifteen new compounds were synthesized and characterized by chemical and instrumental methods. All compounds were obtained in good yields. These compounds along with an intermediate compound (no 3) were biologically screened for *in-vitro* and *in-vivo* antitumor activity considering IC_{50} concentrations lines, percentage inhibition of ascetic cell count and ascetic fluid weight as the activity parameter. The compound number 3 was screened with an aim to evaluate the anticancer activity of the parent moiety. It was noticed that final analogs showing better activity than the parent compound and it may be due to the substituents present in those compounds. These observations encourage performing QSAR study in future.

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