Research Article

POSSIBLE ANTINEOPLASTIC AGENTS, PART XVI: SYNTHESIS, *IN VIVO* ANTITUMOR AND *INVITRO* ANTIANGIOGENIC STUDY OF FEW GLUTAMIC ACID ANALOGS

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ABSTRACT

Few series of glutamic acid analogs and its cyclised variants were designed, synthesized and characterized by FT-IR, ^{1}H NMR, Proton-decoupled ^{13}C NMR, MS and elemental analysis. Compounds were tested *in vivo* for antineoplastic activity in female Swiss albino mice against EAC (Ehrlich Ascites Carcinoma) strain. Cytotoxicity study was carried out on HUVEC and Vero cell lines by MTT assay method. Some promising results are noticed which show that few molecules are having selective inhibitory effect on EAC cell growth and probable antiangiogenic activity but there is no significant toxic effect on normal cell.

Keywords: Thalidomide, Glutamic acid, EAC, HUVEC, Vero.

INTRODUCTION

Inhibition of L-glutamic acid may arrest cancer cell growth. This hypothesis can be substantiated by the following premises.

i. Many malignant tumors are avid consumers of L-glutamine[1, 2] (Fig.1) which is biotransformed from the non-essential amino acid, L-glutamic acid (Fig.2) by amidotransferase.

ii. L-glutamine contributes 3- and 9- Nitrogen atoms of purine bases,[3] the precursors of DNA and RNA, 2-amino group of guanine,[3] 3- nitrogen atom and the amino group of cytosine.[4]

iii. Thalidomide (Fig.3) is a potential chemotherapeutic agent used against Multiple myeloma, myelo dysplastic syndrome, leprosy etc. it has multifarious mechanisms like inhibition of VEGF, TNF-α, GI growth factor, augmentation of apoptosis, proliferation of NK cells and stimulation of T cells.[5, 6]

The metabolites of thalidomide such as N-(o- Carboxy benzoyl)-DL-glutamic acid, (Fig. 4) N-phthalyl-DL-glutamine and N-phthalyl isoglutamine are the Antiglutamates containing the unnatural D-form besides the natural one.[7, 8]

iv. The antineoplastic antibiotic, e.g. streptovitacins, cyclohexidine are structurally analogous to thalidomide, having glutarimide moiety in common.[9, 10] Tenuazonic acid contains oxopyrrolidine moiety.[11, 12] Acivicin contains 4, 5-dihyroisooxazole moiety. All these moieties are bioisosteres of glutamic acid.[13]

v. Azaserine & DON are the examples of established glutamine antagonists.[14-16]

vi. Opulence of γ-glutamyl transpeptidase is observed in many different types of cancer cells.[17]

vii. Glutamic acid has significant role in prevention of sickle cell anemia.[18]

For last four decades the authors have synthesized many structural variants of L-glutamic acid as possible anticancer agents following QSAR studies.[19-33] Some of the molecules have shown promising results on different cancer strains.

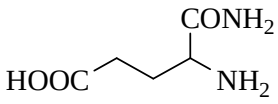


Figure 1: L-Glutamine

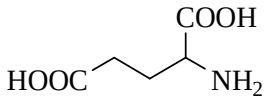


Figure 2: L-Glutamic acid

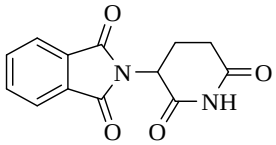


Figure 3: Thalidomide

MATERIALS AND METHODS

All chemicals were purchased either from Merk (India) or Loba Chemie (India). Melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR (300 MHz) spectra and ^{13}C-NMR (75 MHz) spectra were recorded on a Bruker DPX 300 MHz spectrometer using $CDCl_3$ and DMSO-d_6 as solvent and TMS as an internal standard. IR spectra were recorded on a Perkin Elmer Spectrum RX1 spectrometer using KBr (I.R. grade). Elemental analysis of the synthesized compounds was carried out on Carlo Erba 1108 analyzer.

Mass spectral analysis was carried out with MICROMASS Q-Tof micro ™. Compounds were purified by flash column chromatography using 230-400 mesh Silica Gel (Sigma-Aldrich make), ethyl acetate and benzene (at different ratio).

Synthesis of 3, 4-dimethoxyphenylsulfonyl chloride (A): Veratrole (13.8g, 0.1 mmole) was added drop wise to a stirred solution of chlorosulfonic acid (11.6 g, 0.1 mmole) in 25ml chloroform within 30 minutes at 0°C. After 30 minutes, the content was stirred at room temperature for another 45 minutes. Later, the content was poured on 100 g of crushed ice and extracted with chloroform (3x25 ml). The extract was dried with magnesium sulphate and kept overnight. The extract was distilled and the compound was dried and purified by recrystallization with ethanol.

Solid with light pink color, Yield 99.45 %, 34.53 g; mp 66-68°C(lit[34] mp 67-70°C).

Synthesis of 2-(3, 4-dimethoxyphenylsulfonamido) pentanedioic acid (1): To a stirred alkaline, aqueous solution of 7.35g (0.05 mol) of glutamic acid, 11.83g (0.05 mol) of 3, 4-Dimethoxy benzene-1-sulfonyl chloride was added drop wise within 30 minutes. After complete addition, 20 mg of DMAP (4-Dimethyl amino pyridine) was added as catalyst and stirred for another 30 minutes. The reaction was maintained throughout at pH 10 by adding sodium carbonate. The solution was filtered, acidified to pH 2-3, extracted with ethyl acetate and dried over anhydrous $MgSO_4$ overnight. After distillation the compound was purified by flash chromatography using ethyl acetate and benzene in 9:1 ratio and recrystallized using hydro-alcoholic solution (mixture of ethyl alcohol and water).

White solid, Yield 75%, 13.01 g; mp 214-216°C.

IR (KBr): 3269.72 (N-H str, -SO_2NH-), 1736.58 (C=O str, -COOH), 1334.5 (S=O str asym, -SO_2-), 1157.08 (S=O str sym, -SO_2-). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.3368 (br, 2H, -COOH), 7.9596 (s, 1H, -SO_2NH-), 7.3407 (d, 1H, *J* = 8.26 Hz, Ar-H), 7.2727 (s, 1H, Ar- H), 7.0780 (d, 1H, *J* = 8.26, Ar-H), 3.839 (s, 3H,-OCH_3), 3.8091 (s, 3H,-OCH_3), 3.7461 (m, 1H, -NHCHCOOH), 2.2204 (t, 2H, -CH_2CH_2COOH), 1.8279 (m, 1H, -CH_2CH_2COOH), 1.666 (m, 1H, -CH_2CH_2COOH).^{13}C-NMR (DMSO-d_6): δ 25.13, 30.28, 55.31, 58.2, 112.23, 115.63, 118.41, 133.42, 150.12, 153.02, 174.73, 178.43. Mass (*m/z*) ESI TOF: 348.09 (M+H), Anal. Calcd for $C_{13}H_{17}NO_8S$ (347.34): C, 44.95; H, 4.93; N, 4.03 %. Found: C, 45.10; H, 4.96; N, 4.37 %

Synthesis of 2-(3, 4-dimethoxyphenylsulfonamido) pentanediamide (1a): Thionyl chloride (10ml) was added to (5g, 14mmol) of compound (**1**), stirred for 2 hrs in an inert atmosphere and distilled with dry benzene (3x20ml).

Yellowish semisolid appeared and the compound was used for next step without purification. The compound recovered from the above reaction was dissolved in dry benzene and it was taken for amidation while DMAP (20mg in dry benzene) was added to the content and dry ammonia gas was passed through the solution in an inert atmosphere.

Solid precipitated out, after an hour, was filtered, dried and purified. The final product was purified by flash chromatography using ethyl acetate and benzene in 9:1 ratio and recrystallized using ethanol and water mixture.

Yellowish white solid; Yield 92.52%, 4.6 g; mp 214-216°C.

IR (KBr): 3450.03 (N-H str asym, -$CONH_2$), 3417.24 (N-H str sym, -$CONH_2$), 3262.97 (N-H str, -SO_2NH-); 1661.37 (amide- I), 1596.14 (amide- II), 1315.21 (S=O str asym, -SO_2-), 1154.19 (S=O str sym, -SO_2-); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.6838(d, 1H, *J*= 7.76, -SO_2NH-), 7.3385(d, 1H, *J* = 8.38, Ar-H), 7.2735 (s, 1H, Ar-H), 7.0657 (d, 1H, *J*= 8.38, Ar- H), 7.0082 (s, 1H, -$CONH_2$),6.7567(s, 1H, -$CONH_2$) 3.8193 (s, 3H,-OCH_3), 3.8059 (s, 3H,-OCH_3), 3.616 (m, 1H, -NHCHCONH$_2$), 2.039 (t, 2H, -CH_2CONH$_2$), 1.6858 (m, 2H, -CH_2CH_2CONH$_2$).^{13}C-NMR (DMSO-d_6): δ 26.72, 31.35, 54.86, 55.21, 55.29, 109.5, 110.96, 120.36, 132.57, 148.39, 151.65, 172.39, 174.27. Mass (*m/z*) ESI TOF: 346.3610 (M+H), Anal. Calcd for $C_{13}H_{19}N_3O_6S$ (345.3715): C, 45.21; H, 5.55; N, 12.17 %. Found: C, 45.30; H, 5.67; N, 11.87 %

Synthesis of 2-(3, 4-dimethoxyphenylsulfonamido)-N^1, N^5-dimethylpentanediamide (1b): The above process was repeated with same amount of compound **(1),** by passing dry methyl amine for an hour. The final product was purified by flash chromatography using ethyl acetate and benzene in 9:1 ratio and recrystallized using ethanol and water mixture.

White amorphous compound, Yield 83.71%, 4.5 g; mp 229-230°C

IR (KBr): 3318.28 (N-H str, -SO_2NH-), 1653.74 (amide- I), 1597.16 (amide- II), 1319.5 (S=O str, asym, -SO_2-), 1157.82 (S=O str, sym, -SO_2-). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.6923 (s, 1H, -SO_2NH-), 7.4013 (o, 2H, -CONH-), 7.3750 (d, 1H, *J*= 8.4 Hz, Ar-H), 7.253 (s, 1H, Ar- H), 7.1954 (d, 1H, *J*= 8.4 Hz, Ar-H), 3.8112 (s, 3H,-OCH_3), 3.8015 (s, 3H, -OCH_3), 3.579 (m, 1H, -SO_2NHCH-), 2.815 (s, 3H, -CONH-CH_3), 2,748 (s, 3H, -CONH-CH3) 1.7026 (m, 2H, -SO2NH-CH-CH_2-). ^{13}C-NMR (DMSO-d_6): δ 26.23, 26.92, 30.26, 54.16, 55.83, 55.91, 110.16, 111.19, 120.75, 132.83, 148.67, 151.93, 171.18, 172.64. Mass (*m/z*) ESI TOF: 374.3986 (M+H), Anal. Calcd for $C_{15}H_{23}N_3O_6S$ (373.4246): C, 48.25; H, 6.21; N, 11.25 %. Found: C, 48.36; H, 6.10; N, 11.59 %

Synthesis of 2-(3, 4-dimethoxyphenylsulfonamido)-N^1, N^5-diethylpentanediamide (1c): The above process was repeated with same amount of **(1),** by passing dry ethyl amine for one hour. The final product was purified by flash chromatography using ethyl acetate and benzene in 8:2 ratio and recrystallized using ethanol and water mixture.

Off white solid, Yield 84.78%, 4.9 g; mp 232-233°C

IR (KBr): 3255.34 (N-H str, -SO_2NH-), 1658.16 (amide- I), 1551.23 (amide- II), 1342.85 (S=O str, asym, -SO_2-),

1161.67 (S=O str, sym, -SO_2-). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.6103 (s, 1H, -SO_2NH-), 7.3980 (o, 2H, -CONH-), 7.3652 (d, 1H, J= 8.4 Hz, Ar-H), 7.236 (s, 1H, Ar- H), 7.1884 (d, 1H, J= 8.4 Hz, Ar-H), 3.816 (s, 3H,-OCH_3), 3.801 (s, 3H,-OCH_3), 3.564 (m, 1H, -SO_2NHCH-), 3.088 (q, 2H, J= 7.8 Hz -CONH-CH_2-CH_3), 2.8992 (q, 2H, J= 7.8 Hz -CONH-CH_2-CH_3),2.067 (t, 2H, -CH_2CONHCH$_2$CH$_3$) 1.68 (m, 2H, -SO_2NHCH-CH_2-), 1.042 (t, 1H, J= 7.4, -CONHCH$_2$-CH_3). 0.912 (t, 1H, J= 7.4, CONHCH$_2$-CH_3). ^{13}C-NMR (DMSO-d_6): δ 14.63, 14.89, 26.45, 30.43, 34.2, 34.92, 54.55, 109.64, 110.97, 120.45, 132.91, 148.88, 151.72, 172.23, 174.5. Mass (*m/z*) ESI TOF: 402.6987 (M+H), Anal. Calcd for $C_{17}H_{27}N_3O_6S$ (401.4778): C, 50.86; H, 6.78; N, 10.47 %. Found: C, 51.01; H, 6.90; N, 10.38 %

Synthesis of 2-(3, 4-dimethoxyphenylsulfonamido)-N^1, N^5-diphenylpentanediamide (1d): The above process was repeated with same amount of **(1),** by adding aniline (4 ml) drop wise for one hour. The final product was purified by flash chromatography using ethyl acetate and benzene in 7:3 ratio and recrystallized using propyl alcohol.

Lime yellow solid, Yield 55.85%, 4.0 g; mp 227-230°C

IR (KBr): 3248.27 (N-H str, -SO_2NH-), 1659.34 (amide- I), 1554.87 (amide- II), 1347.56 (S=O str, asym, -SO_2-), 1160.49 (S=O str, sym, -SO_2-). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.9696 (s, 1H, -SO_2NH-), 7.6421 (m, 2H, -CONH-), 7.487 (m, 4H, Ar-H), 7.324(m, 4H, Ar-H), 7.302 (o, 1H, Ar-H), 7.231 (d, 1H, J= 7.6 Hz, Ar-H), 7.142 - 7.113 (o, 3H, Ar-H), 3.836 (s, 3H,-OCH_3), 3.7856 (s, 3H,-OCH_3), 3.582 (m, 1H, -SO_2NHCH-), 2.11(t, 2H, J=7.4, -CH_2CH_2CONH$_2$), 1.682 (m, 2H, -SO_2NHCH-CH_2-).). ^{13}C-NMR (DMSO-d_6): δ 26.67, 31.08, 54.27, 55.66, 55.74, 109.82, 110.42, 120.11, 121.66, 121.97, 124.79, 125.03, 129.12, 129.83, 132.78, 138.66, 139.09, 148.77, 151.30, 172.86, 174.40.

Mass (*m/z*) ESI TOF: 498.4872 (M+H), Anal. Calcd for $C_{25}H_{27}N_3O_6S$ (497.5634): C, 60.35; H, 5.47; N, 8.44 %. Found: C, 60.55; H, 5.56; N, 8.36 %

Synthesis of 5-amino-4-(3, 4-dimethoxy phenylsulfonamido)-5-oxopentanoic acid: (2a): To a suspension of (**3**) 6g (18 mmol) in water (30 ml), excess liquor ammonia (54 mmol) was added, shaken and kept overnight in a conical flask with stopper. The excess amine was evaporated out on a steam bath, water 40 ml was added, cooled and acidified with HCl (2N) to congored paper. The precipitate was washed with water filtered and dried. The final product was purified by flash chromatography using ethyl acetate and benzene in 9:1 ratio and recrystallized using ethanol.

White solid, Yield 84.90%, 5 g; mp 183-185°C

IR (KBr): 3453.88 (N-H str, asym, -CONH$_2$), 3349.75 (N-H str, sym, -CONH$_2$), 3275.5 (N-H str, -SO_2NH-), 1715.37 (C=O str, -COOH), 1646.91 (amide- I), 1556.44 (amide- II), 1321 (S=O str, asym, -SO_2-), 1156.12 (S=O str, sym, -SO_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.82 (s, 1H, -COOH), 7.762(s, 1H, -SO_2NH-), 7.3012 (d, 1H, J = 8.4, Ar-H), 7.224 (s, 1H, Ar-H), 7.146 (d, 1H, J= 8.4, Ar- H), 6.986 (s, 2H, -CONH$_2$), 3.82 (s, 3H, -OCH_3), 3.7991 (s, 3H, -OCH_3), 3.648 (s, 1H, -NHCHCOOH), 2.061 (t, 2H, J= 7.6, Hz CH_2CH_2CONH$_2$), 1.7014 (m, 2H, -CH_2CH_2CONH$_2$).). ^{13}C-NMR (DMSO-d_6): δ 25.87, 32.05, 54.72, 55.31, 55.39, 109.46, 110.83, 121.1, 133.07, 148.56, 151.87, 172.46, 174.35. Mass (*m/z*) ESI TOF: 347.2964 (M+H), Anal. Calcd for $C_{13}H_{18}N_2O_7S$ (346.3582): C, 45.08; H, 5.24; N, 8.09 %. Found: C, 45.38; H, 5.32; N, 8.3 %

Synthesis of 4-(3, 4-dimethoxyphenylsulfonamido)-5-(methylamino)-5-oxopentanoic acid (2b): The above procedure was repeated using methyl amine (40% w/w, 5 ml). The final product was purified by flash chromatography using ethyl acetate and benzene in 9:1 ratio and recrystallized using propyl alcohol.

White crystalline solid, Yield 83.77%, 5.5 g; mp 175-177°C

IR (KBr): 3343.18 (N-H str, -CONH$_2$), 3281.19 (N-H str, -SO_2NH-), 1716.86 (C=O str, -COOH), 1648.01 (amide- I), 1557.26 (amide- II), 1322.64 (S=O str, asym, -SO_2-), 1157.19 (S=O str, sym, -SO_2-).

^{1}H NMR (300 MHz, DMSO-d_6): δ 11.74 (s, 1H, -COOH), 7.805 (s, 1H, -SO_2NH-), 7.713 (s,1H, CONHCH$_3$), 7.311 (d, 1H, J = 8.4, Ar-H), 7.277 (s, 1H, Ar-H), 7.169 (d, 1H, J= 8.4, Ar- H), 3.819 (s, 3H, -OCH_3), 3.7903 (s, 3H, -OCH_3), 3.542 (m, 1H, -NHCHCOOH), 2.856 (s, 3H, J= 7.6Hz, CONHCH$_3$), 2.0461 (t, 2H, J= 7.6 Hz, -CH_2CH_2CONH-), 1.689 (m, 2H, -NHCHCH$_2$-).). ^{13}C-NMR (DMSO-d_6): δ 26.15, 26.44, 30.33, 54.76, 55.66, 55.73, 109.55, 110.76, 120.39, 132.98, 148.88, 151.82, 172.71, 174.49. Mass (*m/z*) ESI TOF: 361.3645 (M+H), Anal. Calcd for $C_{14}H_{20}N_2O_7S$ (360.3828): C, 46.66; H, 5.59; N, 7.77 %. Found: C, 46.79; H, 5.60; N, 7.76 %

Synthesis of 2-(4-ethoxy-3-methoxyphenylsulfonamido)-5-(ethylamino)-5-oxopentanoic acid (2c): the above procedure was repeated using ethyl amine (40% w/w, 3 ml). The final product was purified by flash chromatography using ethyl acetate and benzene in 9:1 ratio and recrystallized using ethanol.

White amorphous solid, Yield 87.96%, 6 g; mp 163-165°C

IR (KBr): 3342.66 (N-H str, -CONH$_2$), 3282.81 (N-H str, -SO_2NH-), 1717.06 (C=O str, -COOH), 1647.19 (amide- I), 1558.46 (amide- II), 1324.54 (S=O str, asym, SO_2), 1158.26 (S=O str, sym, SO_2).

^{1}H NMR (300 MHz, DMSO-d_6): δ 11.79 (s, 1H, COOH), 7.746 (s, 1H, -SO_2NH-), 7.698 (s,1H, -CONHCH$_2$-), 7.298 (d, 1H, J = 8.4, Ar-H), 7.2052 (s, 1H, Ar-H), 7.182 (d, 1H, J= 8.4, Ar- H), 3.824 (s, 3H, -OCH_3), 3.745 (s, 3H, -OCH_3), 3.562 (m, 1H, -SO_2NHCH-), 3.026 (q, 2H, J= 7.6Hz, -CONHCH$_2$CH$_3$), 2.055 (t, 2H, J= 7.8 Hz, -CH_2CH_2CONH-), 1.772 (m, 2H, -NHCHCH$_2$-), 1.046 (t, 3H, J= 7.4 Hz, -CONHCH$_2$CH$_3$).). ^{13}C-NMR (DMSO-d_6): δ 14.79, 26.23, 30.98, 34.12, 54.68, 55.41, 55.49, 109.99, 111.13, 121.09, 133.15, 148.72, 151.76, 172.34, 174.77. Mass (*m/z*) ESI TOF: 375.3809 (M+H), Anal. Calcd for $C_{15}H_{22}N_2O_7S$ (374.4094): C, 48.12; H, 5.92; N, 7.48 %. Found: C, 48.43; H, 6.10; N, 7.51%.

Synthesis of 2-(4-ethoxy-3-methoxyphenylsulfonamido)-5-(butylamino)-5-oxopentanoic acid (2d): The above procedure was repeated using n-butyl amine (5.5 ml). The final product was purified by flash chromatography using ethyl acetate and benzene in 9:1 ratio and recrystallized using water ethanol mixture.

Light yellow solid, Yield 84.50%, 6.2 g; mp 195-197°C.

IR (KBr): 3343.63 (N-H str, -CONH-), 3282.33 (N-H str, -SO_2NH-), 1717.46 (C=O str, -COOH), 1649.22 (amide- I), 1558.44 (amide- II), 1323.14 (S=O str, asym, -SO_2-), 1158.49 (S=O str, sym, -SO_2-).

^{1}H NMR (300 MHz, DMSO-d_6): δ 11.72 (s, 1H, -COOH), 7.813 (s, 1H, -SO_2NH-), 7.754 (s,1H, -CONHCH$_2$-), 7.309 (d, 1H, *J* = 8.4, Ar-H), 7.226 (s, 1H, Ar-H), 7.099 (d, 1H, *J*= 8.4, Ar- H), 3.8152 (s, 3H, -OCH_3), 3.792 (s, 3H, -OCH_3), 3.499 (s, 1H, -SO_2NHCH-), 2.987 (t, 2H, *J*= 7.2 Hz, -CONHCH$_2$-), 2.104 (t, 2H, *J*= 7.2 Hz, -CH$_2$CH$_2$CONH-), 1.6652 (m, 2H, -NHCHCH$_2$-), 1.267 (m, 2H, -CONHCH$_2$CH$_2$-), 1.167 (m, 2H, -CONHCH$_2$CH$_2$CH$_2$-), 0.867 (t, 3H, *J*=7.6 Hz, -CONHCH$_2$CH$_2$CH$_2$CH3).

^{13}C-NMR (DMSO-d_6): δ 13.81, 19.93, 26.39, 30.78, 32.54, 40.13, 54.33, 55.29, 55.37, 109.14, 110.38, 120.87, 132.44, 148.77, 151.86, 171.8, 174.23. Mass (*m/z*) ESI TOF: 403.3985 (M+H), Anal. Calcd for $C_{17}H_{26}N_2O_7S$ (402.4625): C, 50.73; H, 6.51; N, 6.96 %. Found: C, 50.75; H, 6.66; N, 7.02 %

Synthesis of 2-(3, 4-dimethoxyphenylsulfonamido)-5-oxo-5-(phenylamino) pentanoic acid (2e): The above procedure was repeated using aniline (5 ml). The final product was purified by flash chromatography using ethyl acetate and benzene in 8:2 ratio and recrystallized using propyl alcohol.

White amorphous compound, Yield 84.45%, 6.5 g; mp 140-142°C

IR (KBr): 3345.86 (N-H str, -CONH$_2$), 3280.42 (N-H str, -SO_2NH-), 1716.36 (C=O str, -COOH), 1653.47 (amide- I), 1555.19 (amide- II), 1323.29 (S=O str, asym, -SO_2-), 1158.44 (S=O str, sym, -SO_2-).

^{1}H NMR (300 MHz, DMSO-d_6): δ 11.869 (s, 1H, -COOH), 7.823 (s, 1H, -SO_2NH-), 7.639 (s, 1H, -CONH-), 7.483 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.327-7.309 (o, 3H, Ar-H), 7.236 (d, 1H, *J*= 7.64 Hz, Ar- H), 7.19-7.11 (o, 2H, Ar-H), 3.854 (s, 3H,-OCH_3), 3.727 (s, 3H,-OCH_3), 3.561 (s, 1H, -SO_2NHCH-), 2.09 (t, 2H, *J*= 7.2 Hz, -CH$_2$CH$_2$CONH -), 1.701 (m, 2H, -NHCHCH$_2$-).). ^{13}C-NMR (DMSO-d_6): δ 26.34, 30.13, 54.71, 55.82, 55.89, 109.28, 110.39, 120.08, 121.59, 124.66, 129.08, 132.22, 138.48, 148.73, 151.69, 172.86, 174.87. Mass (*m/z*) ESI TOF: 423.3987 (M+H), Anal. Calcd for $C_{19}H_{22}N_2O_7S$ (422.4522): C, 54.02; H, 5.25; N, 6.63 %. Found: C, 54.25; H, 5.36; N, 6.79 %.

Synthesis of 1-(3, 4-dimethoxyphenylsulfonyl)-5-oxopyrrolidine-2-carboxylic acid (3): To 5g of compound (**1**) 10 ml of acetyl chloride was added and refluxed for 2 hrs. The content was poured in crushed ice and kept overnight. The compound was filtered and dried. The final product was purified by flash chromatography using ethyl acetate and benzene in 9:1 ratio and recrystallized using propyl alcohol solution.

White crystalline solid, Yield 86.5%, 4.1 g; mp 128-131°C.

IR (KBr): 3304.43 (O-H str, -COOH), 1771.30 (C=O str, ring C=O), 1707.66 (C=O str, -COOH), 1354.75 (S=O str, asym, -SO_2-), 1163.83 (S=O str, sym, -SO_2-). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.294 (br, 2H, -COOH), 7.9232 (s, 1H, -SO_2NH-), 7.3581 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.2403 (s, 1H, Ar-H), 7.113 (d, 1H, *J* = 8.4, Ar-H), 3.831 (s, 3H, -OCH_3), 3.762 (s, 3H, -OCH_3), 2.462 (m, 2H, -COCH$_2$C**H$_2$**-), 2.166 (m, 2H, -COC**H$_2$**-). ^{13}C-NMR (DMSO-d_6): δ23.4, 28.91, 47.23, 55.88, 55.96, 110.14, 111.39, 121.06, 133.2, 149.03, 151.88, 173.18, 174.50. Mass (*m/z*) ESI TOF: 330.3145 (M+H), Anal. Calcd for $C_{13}H_{15}NO_7S$ (329.3257): C, 47.41; H, 4.59; N, 4.25; %. Found: C, C, 47.69; H, 4.86; N, 4.48; %

Synthesis of N-butyl-1-(3, 4-dimethoxyphenylsulfonyl)-5-oxopyrrolidine-2-carboxamide (3a): To 5g (15 mmol) of compound (**3**) 10 ml of thionyl chloride was added and stirred for 2 hrs in room temperature. The excess thionyl chloride was distilled off. The compound was dissolved in dry benzene. DMAP (20mg in dry benzene) and excess butyl amide (approx. 30mmol) was added to the reaction mixture with stirring. The content was cooled and kept overnight for maturation. The compound was filtered and washed with dil.HCl (2N) to remove excess amine, sodium bicarbonate solution to remove excess acid and finally with water. The final product was purified by flash chromatography using ethyl acetate and benzene in 8:2 ratio and recrystallized using ethanol and water mixture.

Yellow solid, Yield 65.10%, 3.8g; mp 129-131°C

IR (KBr): 3341.34 (N-H str, -CONH-), 3280.42 (N-H str, -SO_2NH-), 1769.43 (C=O str, ring C=O), 1653.74 (amide- I), 1563.84 (amide- II), 1321.66 (S=O str, asym, -SO_2-), 1159.83 (S=O str, sym, -SO_2-). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.811 (s, 1H, -SO_2NH-),7.766 (s, 1H, -CONH-), 7.322 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.251 (s, 1H, Ar- H), 7.156 (d, 1H, *J* = 8.4, Ar-H), 3.815 (s, 3H, -OCH_3), 3.779 (s, 3H, -OCH_3), 3.012 (t, 2H, *J*= 7.6 Hz, -CONHCH$_2$-), 2.426 (m, 2H, -COCH$_2$CH$_2$-), 2.162 (m, 2H, -COCH$_2$-), 1.268 (m, 2H, -CONHCH$_2$CH$_2$-), 1.159 (-CONHCH$_2$CH$_2$CH$_2$-), 0.914 (t, 3H, *J*= 7.2, -CONHCH$_2$CH$_2$CH$_2$CH$_3$).^{13}C-NMR (DMSO-d_6): δ13.82, 18.92, 23.11, 28.09, 32.37, 40.18, 45.7, 55.77, 55.84, 109.56, 110.71, 120.26, 132.85, 148.66, 151.79, 171.19, 174.74. Mass (*m/z*) ESI TOF: 385.3986 (M+H), Anal. Calcd for $C_{17}H_{24}N_2O_6S$ (384.4473): C, 53.11; H, 6.29; N, 7.29 %. Found: C, 53.25; H, 6.37; N, 7.17 %

Synthesis of 1-(3, 4-dimethoxyphenylsulfonyl)-5-oxo-N-phenylpyrrolidine-2-carboxamide (3b): The above process was repeated taking aniline (3ml). The final product was purified by flash chromatography using ethyl acetate and benzene in 8:2 ratio and recrystallized using ethanol and water mixture.

White amorphous solid, Yield 78.17%, 4.8 g; mp 186-188°C.

IR (KBr): 3357.46 (N-H str, -CONH-), 3279.42 (N-H str, -SO_2NH-), 1768.88 (C=O str, ring C=O), 1652.62 (amide- I), 1562.34 (amide- II), 1341.25 (S=O str, asym, -SO_2-), 1136.83 (S=O str, sym, -SO_2-).^{1}H NMR (300 MHz, DMSO-d_6): δ 7.819 (s, 1H, -SO_2NH-), 7.645 (s, 1H, -CON**H**-), 7.422 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.323 (d, 2H, *J*= 8.7 Hz, Ar- H), 7.318-7.305 (o, 3H, Ar-H), 7.229 (d, 2H, *J*= 8.7 Hz, Ar- H), 7.165 -7.091 (o, 2H, Ar-H), 3.844 (s, 3H, -OCH_3), 3.741 (s, 3H, -OCH_3), 2.433 (m, 2H, -$COCH_2$ -), 2.193 (m, 2H, -CO**CH_2**CH_2-).^{13}C-NMR (DMSO-d_6): δ 23.45, 28.11, 44.91, 55.91, 56.01, 109.34, 110.65, 120.19, 121.66, 123.98, 128.87, 132.63, 138.5, 148.09, 151.27, 172.82, 174.89. Mass (*m/z*) ESI TOF: 405.3897 (M+H), Anal. Calcd for $C_{19}H_{20}N_2O_6S$ (404.4369): C, 56.43; H, 4.98; N, 6.93 %. Found: C, 56.55; H, 4.78; N, 6.65 %

RESULTS AND DISCUSSION

In total, 13 bioisosteres of thalidomide metabolites were synthesized by reaction of aryl Sulphonyl chlorides with glutamic acid followed by acid halide preparation with thionyl chloride in good yield. Amidation of acid halide was carried out using different amines and aniline. Cyclisation was carried out using acetyl chloride and mono substitution was performed by distilling the cyclised variant with amine. Elemental analysis, IR, ^{1}H NMR, ^{13}C NMR & Mass spectral data of the synthesized compounds were found in agreement with the designed molecular structures.

Some alterations were made in the conventional processes; like sulphonamidation was carried out using DMAP (dimethyl amino pyridine) which ensures good yield, purity and decreased reaction time. Acid halide was prepared with excess of thionyl chloride while the content was stirred at room temperature for 45 minutes to 2h. Amidation was performed catalytically. This nearly abolishes competing side reactions

Final compounds were purified by flash chromatography and further recrystallized with different solvents. Purity was confirmed by RP-HPLC using water, methanol, acetonitrile and phosphate buffer in solvent system.

The molecules reported in the present work are the isosteres of a part of Folic acid (Fig.5) comprised of PABA and glutamic acid. These are also analogs of N-(o- Carboxy benzoyl)-DL-glutamic acid, an active metabolite of thalidomide.

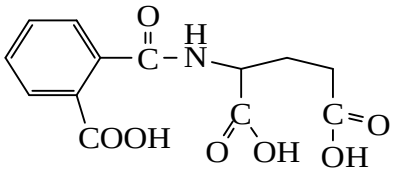


Figure 4: N-(o-Carboxy benzoyl)-DL-glutamic acid

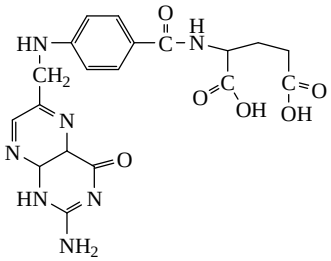
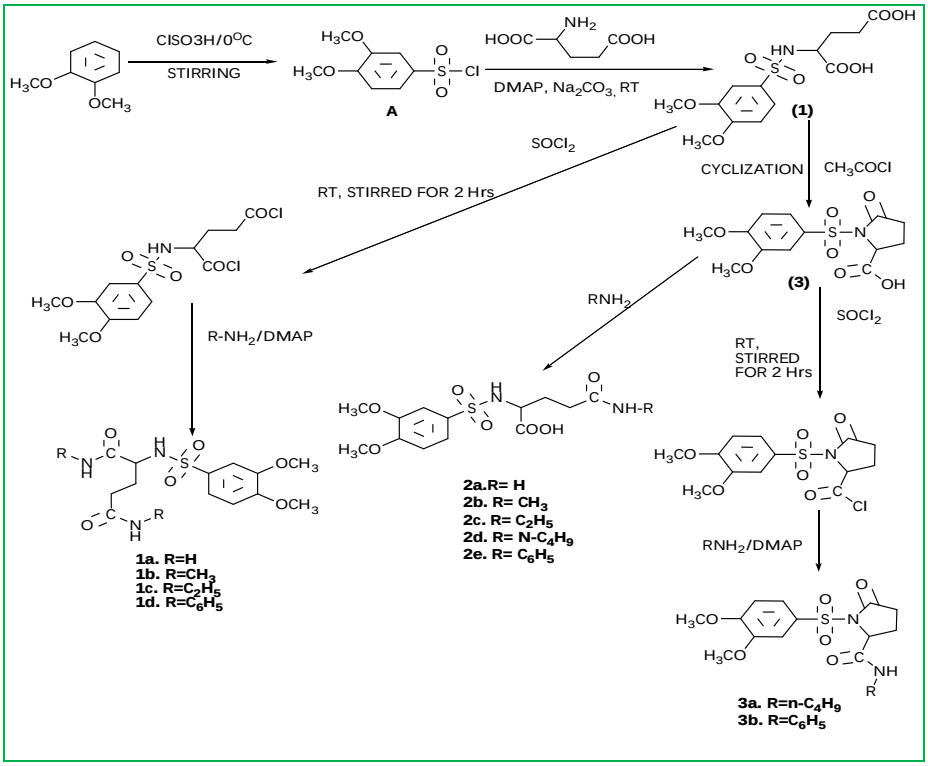


Figure 5: Folic acid



Scheme 1: Route of synthesis of designed compounds

Table 1: Biological Activity (% Inhibition) and cytotoxicity study (IC_{50}) Report

ENTRY	% INHIBITION ON EAC	IC_{50}* (µ molar) (Vero cell line)	IC_{50}* (µ molar) (HUVEC cell line)
1	25.19	86	60
1a	35.2	55	72
1b	32.8	76	55
1c	23.36	43	46
1d	29.6	08	09
2a	86.34	51	01
2b	97.01	21	02
2c	18.45	85	44
2d	43.69	31	65
2e	82.43	22	05
3	48.62	66	61
3a	13.79	73	30
3b	37.86	58	52
STANDARD	100 (Mitomycin C)	4.5 (Doxorubicin)	0.5 (Doxorubicin)

* 50% Inhibitory concentration

BIOLOGICAL EVALUATION RESULTS

Anticancer activity

The compounds (**1-3c)** were screened *in vivo* for % inhibition of ascitic cell against EAC and the results are depicted in Table 2. Mitomycin C was used as standard drug where cell inhibition is 100%. Biological experiments were carried out as described in the literature.[19, 20]

The study was conducted on Swiss albino mice having average weight of 18-20g. On day 1 approximately, 2 x 10^6 numbers of cells were inoculated, intraperitonially. One control group and test groups (each group contained six mice) were taken. A dose of 0.1 mmole/Kg body weight was administered for 7 days. On day 9 animals were sacrificed and ascitic cells were counted for all the groups. Percentage inhibition of ascitic cell was calculated w. r. t control group. The results are reported below.

This Animal experimentation is approved by Animal Ethical Committee bearing Registration no. 1170/ac/08/CPCSEA.

Cytotoxicity analysis of compounds against HUVEC/ Vero cell lines

Cytotoxicity study of the compounds were carried out on HUVEC/ Vero cell lines by MTT assay method with incubation period of 96 h, and the results are depicted in Table 1. The base medium for these cell lines was Dulbecco's Modified Eagle's medium. The components/ conditions applied to the base medium were as follows: foetal bovine serum to a final concentration of 20%; Temperature**:** 37.0°C; Atmosphere**:** 95% air; 5% carbon dioxide (CO_2).

Protocol

The volumes used in this protocol were for 75cm^2 Culture flask. Cell layer was rinsed with 0.05% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contain trypsin inhibitor. 1.0 to 2.0 ml of Trypsin-EDTA solution was added to the flask and the cells were observed under an inverted microscope until the cell layer was dispersed (usually within 5 to 15 minutes). The cells which were difficult to detach were placed at 37°C to facilitate dispersal. Approximately 6.0 to 8.0 ml of complete growth medium was added and the cells were aspirated by pipetting gently. Trypsin-EDTA solution was removed by transferring cell suspension to a centrifuge tube and spun at approximately 125 xg for 5 to10 minutes. Supernatant was discarded and cells were re-suspended in fresh growth medium. Culture vessels were placed in incubators at 37°C.

CONCLUSION

Since there is no significant toxic effect found on Vero cells (normal cell), it is inferred that the inhibition observed in proliferation of EAC cells is not due to non selective toxic effect.

It implies that the molecules **2a**, **2b, 2e** have anticancer activity on EAC cells. In another study, cytotoxicity of the same molecules on HUVEC cells (a primary test for antiangiogenic activity study) indicates that these molecules may have antiangiogenic activity.

So, it necessitates further investigation of antiangiogenic activity of the molecules of interest, which are under progress and subsequent 2D & 3D QSAR studies are being carried out for development of more potential molecule.

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