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# Synthesis and Biological Activity of Succinimidobenzenesulfonyl Oxopyrrolidine Analogs as Possible Antineoplastic Agents

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Some new succinimidobenzenesulfonyl oxopyrrolidine analogs were synthesized and screened for antitumor activity against *Ehrlich ascites Carcinoma* in female Swiss albino mice using percentage inhibition of tumor cell count and tumor weight as activity parameters. Few synthesized compounds show encouraging activity.

**Key Words: Succinimidobenzenesulfonyl oxopyrrolidine, *Ehrlich ascites Carcinoma*, Tumor.**

## INTRODUCTION

Cancer is a disease of worldwide importance because it is one of the major killer diseases in human history. Cancer has been described as a nitrogen trap[1,2]. The importance of non-essential amino acid glutamine in proliferation of human tumor cells was studied extensively[3]. Glutamine and glutamic acid play an important role in many biotransformation reactions. They are not only components of protein but also play an important role in the biosynthesis of deoxyribonucleotides and ribonucleotides, which are the direct precursors of DNA and RNA. Glutamic acid and glutamines are interconvertible by a typical biotransformation process in presence of the enzyme, amido transferase. Glutamine and glutamate are the donors of 3- and 9-nitrogen atom of purines and 2-amino group of guanine[4]. They also contribute the 3-nitrogen atom and the amino group of cytosine[5]. Thus, the analogs synthesized may interfere in any one of the steps and act as antineoplastic agents.

Glutamic acid is present in many malignant tumors[6] and in certain neoplasm the requirement of L-glutamine is higher than that of any other amino acid[7]. Many tumors are also dependent upon exogenous sources of

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it. γ-Glutamyl transpeptidase is present in human carcinoma[8]. It was reported that antineoplaston, a glutamic acid analog has activity on malignant brain tumor[9]. Glutamic acid-γ-anilides showed promising antineoplastic activity[10]. Thus, glutamic acid analogs are of interest in search of antineoplastic agents.

1-(Substituted benzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid may be regarded as precursor or cyclized version of glutamic acid. This compound has striking resemblance with tenuazoic acid, an antibiotic produced by the strain of fungus *Alternaria tenius* Auct., which inhibits protein synthesis by preventing its release[11]. N-Phenylsuccinimide and its derivatives are reported to show fungicidal activity with nephrotoxic potential[12,13]. So, this moiety has been incorporated in the target compounds. Hence, in this paper, the synthesis of 1-(4-succinimidobenzenesulfonyl)-5-oxopyrrolidine carboxylic acid and four new derivatives of the acid are reported. All the five compounds were screened for antitumor activity against *Ehrlich ascites Carcinoma* in female Swiss albino mice.

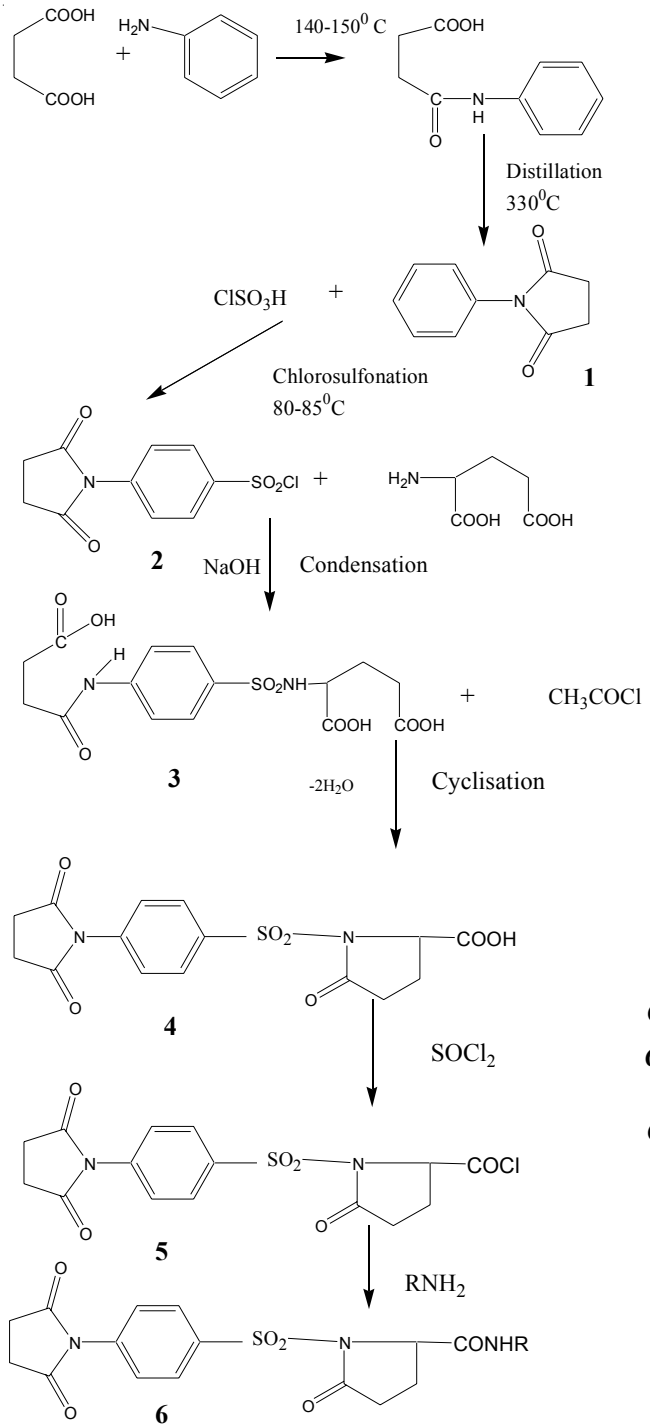
## EXPERIMENTAL

The synthesis of 1-(4-succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid and four new derivatives of the acid were carried out in accordance with the **Scheme-I**. Melting points were measured on a capillary melting point apparatus and were uncorrected. The compounds, which contain free carboxylic groups, were characterized by matching neutralization equivalent. The IR spectra were recorded on Buck M 500 quick scanning IR spectrophotometer using KBr pellets and the frequencies were expressed in $cm^{-1}$. $^1H$ NMR spectra were collected at 25 ºC in the pulsed Fourier transformation mode on Bruker DRX 300 MHz spectrophotometer. Chemical shifts are reported in δ ppm relative to tetramethyl silane for deuteriorated dimethylsulfoxide (DMSO-$d_6$). Elemental analysis (C, H, N) of the compounds was performed on 2400 series II CHN analyzer of Perkin-Elmer.

### Synthetic procedure

**N-Phenylsuccinimide (1):** A mixture of freshly distilled aniline (34 mL, 0.37 mol) and pulverized succinic acid (43.7 g, 0.37 mol) was heated on an oil bath at 140-150 ºC for 4 h. The mixture was then distilled at 330 ºC to obtain N-phenylsuccinimide. The distillate was recrystallized from 95 % alcohol after charcoal treatment.

**4-Succinimidobenzenesulfonyl chloride (2):** N-Phenyl-succinimide (15 g, 0.12 mol) was taken in a 250 mL three necked round bottom flask placed on a water 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 mol) was placed in the dropping funnel and




**6a** R = -$CH_3$

**6b** R = -$C_2H_5$

**6c** R = -$C_6H_5$

**6d** R = -$CONH_2$

**Scheme-I**

added dropwise. After the addition, the mixture was heated to 80-85 ºC for 2 h and the product poured slowly into well-stirred crushed ice. The crude sulfonyl chloride was filtered and washed with water to remove the excess acid. The crude product was recrystallized from acetone after charcoal treatment.

**2-N-(Succinic acid monoanilidino sulfonyl)-L-glutamic acid (3):** L-Glutamic acid (20 g, 0.13 mol) was taken in a 250 mL conical flask and sodium hydroxide solution (2 N) was added slowly till all glutamic acid dissolved and the mixture become distinctly alkaline to phenoloph-thalein. The reaction mixture was stirred on a magnetic stirrer and the temperature was maintained at 60 ºC. 4-Succinimidobenzenesulfonyl chloride (37 g, 0.13 mol) was added slowly with continuous stirring and sodium hydroxide solution (2 N) was added time-to-time to keep the reaction mixture alkaline. The reaction was continued until a clear homogeneous solution resulted and thin layer chromatography showed that the reaction was complete. It was allowed to cool to room temperature and filtered to separate undissolved solid matter, if any. The filtrate was acidified with conc. HCl and saturated with NaCl. The product was extracted with three 50 mL portions of ethyl acetate. Ethyl acetate layer was washed with brine solution (15 mL) and dried overnight over anhydrous magnesium sulphate. The solvent was distilled off to get the desired product. The crude product was recrystallized from dioxane-benzene after charcoal treatment.

**1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid (4):** 2-N-(Succinic acid monoanilidino sulfonyl)-L-glutamic acid (32 g, 0.079 mol) 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 and refluxed in a steam bath for 4 h. After refluxing, benzene was removed by distillation and the reaction mass was cooled. The completion of the reaction was tested by thin layer chromatography. After that crushed ice was poured in the round bottom flask with continuous stirring. It was kept overnight in freeze and the semisolid mass was solidified. The product was filtered and washed with distilled water. The crude product was recrystallized from dilute ethanol after charcoal treatment.

**1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid chloride (5):** 1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid (2 g, 0.005 mol) 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 steam 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 was used immediately in subsequent steps.

**1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid methyl amide/ethyl amide/anilide (6a-c):** 1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid chloride formed was dissolved in dry benzene (10 mL) and the whole mass was cooled by dipping in ice water. Then methylamine (5 mL, 40 %)/ethylamine (5.2 mL, 50 %)/aniline (1.2 mL), previously cooled was added and mixed well. The whole mass was refluxed in a steam bath for 10 min. The excess benzene was distilled off and the solid residue was washed with dilute HCl to remove excess amine, again filtered and the acid was washed with distilled water. The residue was dried and recrystallized from dilute alcohol after charcoal treatment.

**1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid ureide (6d):** 1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid chloride formed was dissolved in dry benzene (10 mL). Then urea (1 g, 3 mol) was added to it and the whole mass was refluxed in a steam bath for 2 h. The excess benzene was distilled off and the resulting mass first washed with bicarbonate solution and then with water. The crude mass was filtered, dried and recrystallized from dilute alcohol after charcoal treatment. Physical data of the intermediates and title compounds were given in Table-1.

TABLE-1
PHYSICAL DATA OF THE INTERMEDIATES AND FINAL COMPOUNDS

| Compd. | m.p. (ºC) | Yield (%) | Elemental analysis (%): Found (Calcd.) | | | Neutralization equivalent: Found (Calcd.) |
|---|---|---|---|---|---|---|
| | | | C | H | N | |
| **1** | 155-156 | 85.73 | 68.42 (68.56) | 5.12 (5.17) | 7.94 (7.99) | - |
| **2** | 194-195 | 79.35 | 43.70 (43.88) | 2.90 (2.94) | 5.07 (5.11) | - |
| **3** | 185-188 | 57.00 | 44.35 (44.77) | 4.41 (4.50) | 6.91 (6.96) | 137.33 (134.126) |
| **4** | 234-238 | 94.53 | 48.92 (49.17) | 3.79 (3.85) | 7.58 (7.64) | 364.95 (366.348) |
| **6a** | 264-267 | 53.14 | 50.52 (50.65) | 4.46 (4.51) | 11.01 (11.07) | - |
| **6b** | 288-290 | 77.35 | 51.43 (51.90) | 4.80 (4.86) | 10.63 (10.68) | - |
| **6c** | 240-241 | 95.80 | 56.85 (57.13) | 4.28 (4.33) | 9.46 (9.51) | - |
| **6d** | 215-217 | 91.00 | 46.82 (47.05) | 3.89 (3.94) | 13.67 (13.71) | - |

**Spectral data:**

**1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid (4):** IR (KBr, $\nu_{max}$, $cm^{-1}$): 3047 (C-H *str.* of phenyl ring), 2922-2547 (O-H *str.* of COOH), 1734, 1687 (C=O *str.*), 1586 (C=C *str.* of phenyl ring), 1391 (C-O *str.* or O-H def of COOH), 1352 (S=O *str.* antisym of $SO_2N$), 1172 (S=O *str.* sym of $SO_2N$), 844 (out of plane C-H def due to *p*-subst. in phenyl ring); $^1$H NMR (DMSO-$d_6$): δ 8.1191(d, 2H, 2', 6' H of $C_6H_4$), 7.543 (d, 2H, 3', 5' H of $C_6H_4$), 2.072 (s, 1H, -$CH_2$-CH.H-CH), 4.8852 (s, 1H, -$CH_2$-CH-COOH), 2.812 (q, 4H, O=C-$H_2$C-$H_2$C-C=O).

**1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid methyl amide (6a):** IR (KBr, $\nu_{max}$, $cm^{-1}$): 3292 (N-H *str.* of CONH), 3038 (C-H *str.* of phenyl ring), 2923, 2876 (C-H *str.* of methylene group), 1731, 1700 (C=O *str.*), 1585 (C=C *str.* of phenyl ring), 1554 (N-H def of CONH), 1346 (S=O *str.* antisym of $SO_2N$), 1169 (S=O *str.* sym of $SO_2N$), 839 (out of plane C-H def due to *p*-subst. in phenyl ring); $^1$H NMR (DMSO-$d_6$): δ 2.792 (q, 4H, O=C-$H_2$C-$H_2$C-C=O), 8.068 (d, 2H, 2', 6' H of $C_6H_4$), 7.532 (d, 2H, 3', 5' H of $C_6H_4$), 2.0721 (s, 1H, -$CH_2$-CH.H-HC(CO)N), 8.3307 (s, 1H, -$CONHCH_2$), 2.712 (t, 3H, $CH_3$).

**1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid ethyl amide (6b):** IR (KBr, $\nu_{max}$, $cm^{-1}$): 3292 (N-H *str.* of CONH), 3038 (C-H *str.* of phenyl ring), 2938, 2892 (C-H *str.* of methylene group), 1692, 1662 (C=O *str.*), 1584 (C=C *str.* of phenyl ring), 1538 (N-H def of CONH), 1346 (S=O *str.* antisym of $SO_2N$), 1162 (S=O *str.* sym of $SO_2N$), 846 (out of plane C-H def due to *p*-subst. in phenyl ring); $^1$H NMR (DMSO-$d_6$): δ 2.813 (q, 4H, O=C-$H_2$C-$H_2$C-C=O), 8.074 (d, 2H, 2', 6' H of $C_6H_4$), 7.5324 (d, 2H, 3', 5' H of $C_6H_4$), 2.0721 (s, 1H, -$CH_2$-CH.H-HC(CO)N), 8.3307 (s, 1H, -$CONHCH_2$), 3.1283 (d, 2H, -$CONHCH_2CH_3$), 1.073 (t, 3H, -$CH_2CH_3$).

**1-(4-Succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid anilide (6c):** IR (KBr, $\nu_{max}$, $cm^{-1}$): 3273 (N-H *str.* of CONH), 3038 (C-H *str.* of phenyl ring), 2977, 2923 (C-H *str.* of methylene group), 1723, 1700 (C=O *str.*), 1585 (C=C *str.* of phenyl ring), 1515 (N-H def of CONH), 1346 (S=O *str.* antisym of $SO_2N$), 1161 (S=O *str.* sym of $SO_2N$), 846 (out of plane C-H def due to *p*-subst. in phenyl ring); $^1$H NMR (DMSO-$d_6$): d 2.7925 (q, 4H, O=C-$H_2$C-$H_2$C-C=O), 7.956 (d, 2H, 2', 6' H of $C_6H_4$), 7.5324 (d, 2H, 3', 5' H of $C_6H_4$), 2.0721 (s, 1H, -$CH_2$-CH.H-HC(CO)N), 8.3024 (s, 1H, -CONH), 7.6452 (d, 2H, 2', 6' H of $C_6H_4$), 7.2453 (d, 2H, 3', 5' H of $C_6H_4$).

**1-(4-succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid ureide (6d):** IR (KBr, $\nu_{max}$, $cm^{-1}$): 3385 (N-H *str.* antisym of $CONH_2$), 3307 (N-H *str.* sym of $CONH_2$), 3269 (N-H *str.* of CONH), 3046 (C-H *str.* of phenyl ring), 2938, 2877 (C-H *str.* of methylene group), 1700 (C=O

*str.*), 1584 (C=C *str.* of phenyl ring), 1554 (N-H def of CONH), 1346 (S=O *str.* antisym of $SO_2N$), 1177 (S=O *str.* sym of $SO_2N$), 846 out of plane C-H def due to *p*-subst. in phenyl ring); $^1$H NMR (DMSO-$d_6$): δ 2.8144 (q, 4H, O=C-$H_2$C-$H_2$C-C=O), 8.1043 (d, 2', 6' H of $C_6H_4$), 7.562 (d, 2H, 3', 5' H of $C_6H_4$), 2.073 (s, 1H, -$CH_2$-CH.H-CH), 10.6298 (s, 1H, -CONHCO$NH_2$), 4.98654 (s, 1H, -$CH_2$-HC(CO)N).

**Biological activity:** 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[14-20]. 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 conditions. 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 the tumor cells per mL of this suspension were counted under microscope with the help of haemocytometer. A definite number (about $2 \times 10^6$/ 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 starting the drug administration. From the second day of transplantation up to the eighth day a suitable challenge dose (0.2 mmol/Kg 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 was withheld 18 h before the starting of the testing operation. The weights of all the animals were recorded before they were sacrificed. The peritoneal cavity was dissected and by a syringe the ascitic fluid was withdrawn to a suitable volume, collected in sterile ice-cold saline and preserved in ice bath. The total number of living cells/mL in the peritoneal fluid of the five mice in a group was calculated. The fluid was sucked by adsorbent cotton. The weight of the five mice after sacrifice was recorded. Comparing the cell count and tumor weight of the test with that of control made the evaluation of the test drug. The percentage inhibition of cell count and tumor weight of the ascitic fluid was obtained by the following expression:

$$\text{Percentage inhibition of ascitic cells} = (1 - T / C) \times 100$$

$$\text{Percentage inhibition of ascitic fluids} = (1 - T'/C') \times 100$$

where, T = average number of ascitic cells per mL in test animals, C = average number 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. Mytomycin-C (1 mg/kg body weight) in sterile phosphate buffer (pH 7.2) was used as standard. The results of the biological activity are shown in Table-2.

TABLE-2
BIOLOGICAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS AGAINST EHRLICH ASCITES CARCINOMA

| Compd. | AA | BB | CC | DD | EE | FF | GG |
|---|---|---|---|---|---|---|---|
| **4** | 5 | 766.5 | 340.05 | 55.64 | 2.70 | 0.10 | 96.30 |
| **6a** | 5 | 766.5 | 147.05 | 80.81 | 2.70 | 0.60 | 77.78 |
| **6b** | 5 | 766.5 | 357.24 | 53.39 | 2.70 | 0.70 | 74.07 |
| **6c** | 5 | 766.5 | 259.94 | 66.09 | 2.70 | 1.00 | 62.96 |
| **6d** | 5 | 766.5 | 90.75 | 88.16 | 2.70 | 0.30 | 88.89 |
| Mitomycin-C (standard) | 5 | 766.5 | 0.00 | 100.00 | 2.70 | 0.00 | 100.00 |

AA = Number of animals in each group; BB = Average no. of ascitic cells per mL in control, C ($\times 10^6$ cells/mL); CC = Average no. of ascitic cells per mL in test, T ($\times 10^6$ cells/mL); DD = Percent Inhibition of ascitic cells (1-T/C) × 100; EE = Average weight of ascitic fluid in control, C' (g); FF = Average weight of ascitic fluid in test, T' (g); GG = Percent inhibition of ascitic fluid (1-T'/C') × 100.

## RESULTS AND DISCUSSION

Among the synthesized compounds, 1-(4-succinimidobenzenesulfonyl)-5-oxopyrrolidine-2-carboxylic acid (**4**) showed encouraging activity in per cent inhibition of ascitic fluid weight (96.30 %) and its ureide derivative showed encouraging activity in both the parameter, *viz.*, ascitic fluid weight (88.89 %) (**6d**) and ascitic cell count (88.16 %). Hence, a detailed and prolonged study is necessary to establish their activity in other models.

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