Research Article

Synthesis of New Sulfonamide Derivatives-phenylalanine and Proline Ester Conjugate using Maleamide Spacer as Anticancer Agents

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Received: 25-02-2017; **Revised:** 28-03-2017; **Accepted:** 16-04-2017.

ABSTRACT

In view of potential biological activity of sulfonamide derivatives, and the importance of large amino acid transport system (LAT) in the body, and the presence of LAT1 in many malignant tissues, new compounds were designed and synthesized using sulfonamide derivatives of cytosine and 9-methyl adenine that conjugated with amino acids ester through maleamide spacer, and suspected to have anticancer activity. 4-acetamidobenzenesulfonyl chloride was reacted with cytosine and 9- methyl adenine to form N-substituted 4-acetamidobenzenesulfonamides that were deacetylated to N-substituted 4-aminobenzenesulfonamides compounds. Esterification of amino acids was done to obtain phenyl alanine methyl ester HCl and proline methyl ester HCl that were reacted with maleic anhydride to form carboxylic acid derivatives. Each one of the carboxylic acid derivatives was reacted with N-substituted 4-aminobenzenesulfonamides through formation of amide bond and leads to the formation of the targeting compounds (B, E, H, and K). The synthesized compounds were characterized and identified by I.R spectra, elemental microanalysis, and ^{1}HNMR study, and it was found that all the results shown good agreements with the proposed chemical structures of the synthesized compounds. The study has also employed an in vitro evaluation of the cytotoxic activity of the synthesized compounds (B and K) on two cancer cell lines: the murine mammary adenocarcinoma cell line (AMN3), the human breast cancer cell line (AMJ13), and one normal cell line: the rat embryo fibroblast cell line (Ref), at different concentrations of the tested compounds and different treatment exposure time (48 hrs. and 72 hrs.). The results indicate that both new designed compounds give cytotoxic effects against the tested cancer cell lines at different concentrations and no significant effect on the normal cell line. In conclusion, the new designed compounds have significant selective anticancer effect, and further investigations are needed to confirm their effects against other cancer cells.

Keywords: Cytosine, sulfonamide derivative, maleamide derivative, cytotoxicity.

INTRODUCTION

Sulfonamides bear -SO2NH- group are present in many pharmacologically important compounds. [1]A large number of structurally novel sulfonamide derivatives have been reported to show anticancer activity,[2] through a variety of mechanisms, such as cell cycle perturbation in the G1 phase, disruption of microtubule assembly,[3] and inhibition of carbonic anhydrase (CA) enzymes.[4]Many research endeavors have reported these agents to act through inhibition of CA IX,[5] Indisulam (*N*-(3-chloro-7-indolyl)-1, 4benzene disulfo namide) (E7070) is a novel sulfonamide anticancer agent that acts as inhibitor of carbonic anhydrase isoform IX.[6]Sulfonamides may be decrease the provision of bicarbonate to synthesis of nucleotides and membrane lipids. [7]Novel nonhydroxamate sulfonamide anilides have been designed and synthesized, to inhibit human histone deacetylase(HDAC)enzymes and cause hyperacetylation of histones in human cancer cells. These compounds selectively act in cancer cells to block the cell cycle but not that in normal cells.[8] The indole sulfonamides like ER-68384 and ER-68378 are shown to inhibit microtubule polymerization *in vitro*.[9] Pyrimidine is nitrogen containing heterocyclic ring,[10] many pyrimidine derivatives have been developed as anticancer agents and are widely used, such as 5-Fluorouracil (5-FU).[11] Purine ring system is one of the most heterocyclic ring systems in nature having the potential to develop more potent medicines, such as antineoplastic, antileukemic, anti-HIV, and antimicrobial.[12] The L-type amino acid transporter (LAT) family consists of four Na+-independent neutral amino acid transporters.[13] LAT1 has been found in many malignant cells,[14] suggesting that LAT1 has important prognostic significance and could be a potential target in the treatment of cancer.[15, 16]

MATERIALS AND METHODS

All chemicals were reagent grade and obtained from standard commercial sources. Acetanilide (Riedel-Dehaen, Germany), Chlorosulfonic acid (Alpha chemika, India), Cytosine (BDH, England), 9-Methyl adenine (Hangzhou hyper chemicals limited, China), L-phenyl alanine (SCR, China), L-proline (Chemical point, Germany), Maleic anhydride (Merk, Germany).Melting points of the compounds and their intermediates were determined by capillary tube method on Stuart (U.K) Electrical melting point apparatus, ascending thin layer chromatography (TLC) was run on silica gel GF254 (type 60) pre-coated aluminum sheets, Merck (Germany) to check the purity and the reactions progress, the products were detected by reacting with iodine vapor or by irradiation with UV light. Infrared spectra were recorded on F.T. IR Spectrophotometer Shimadzu (Japan), CHNS microanalysis was done using Elemental micro-analyzer Vario Micro (Germany), and the ^{1}HNMR spectra were

recorded on Bruker NMR Spectrometer 400 MHz (Germany) with TMS as an internal standard.

Chemical synthesis

Synthesis of compound 4-acetamidobenzenesulfonyl chloride (Ia) [17]

Acetanilide (18.49 mmole, 2.5gm) was put in dry conical flask and melted on a direct flame. The melted acetanilide was distribute in a thin layer, round and over the bottom of the flask, cooled the flask to let the acetanilide solidify again, with cooling in ice bath chlorosulfonic acid (94 mmole, 6ml) was added and immediately connect the flask to the gas trap. The flask was swirled after removing it from ice bath for 15 min. The mixture was heated on a water bath for 20 minutes to complete the reaction, the flask was cooled under the tap water and the mixture was poured in a thin stream into 75ml of crushed ice water mixture in a beaker with stirring. The precipitated p-acetamido benzene sulfonyl chloride in the form of granular white solid, was stirred, filtered off at the pump and washed it with a little cold water, pressed and drained well. The crude product was washed with toluene and recrystallized from chloroform. White to pale yellow solid, Yield 85%, melting point 142-145 °C.

General procedure for the synthesis of N-substituted 4-acetamidobenzenesulfonamides (Ib and Id) [18]

4-acetamidobenzene sulfonyl chloride (6.7 mmol, 1.56gm) was added gradually to a suspension of related amine (6.7 mmol) and pyridine (6.7 mmol, 0.54 ml) in DMF (25 ml), with stirring at room temperature for 15 min. Then the mixture was heated for 12 hrs, the solvent was evaporated to dryness under vacuum. The crude material was washed with chloroform, diethyl ether, and recrystallized from ethanol.

Compound(N-(4-(N-(2-oxo-1, 2-dihydropyrimidin-4-yl) sulfamoyl) phenyl) acetamide) (Ib)

Off white powder, yield 82%, melting point242-245°C.IR **(υ= cm^{-1}, KBr):** 3331(NH) of 2^{o} amide; 3190 (NH) of sulfonamide; 3057 (CH) aromatic; 2924 and 2885 (CH) asymmetric and symmetric respectively of CH_3; 1703 (C=O) anilide; 1660(C=O) of amide of cytosine; 1593, 1527, 1492 (C=C) aromatic; 1315, 1170 (O=S=O) asymmetric and symmetric respectively.

Compound (N-(4-(N-(9-methyl-9H-purin-6-yl) sulfamoyl) phenyl) acetamide) (Id)

Off white powder, yield 56%, melting point218-220 °C.IR (υ= cm^{-1}, KBr): 3298(NH) of anilide; 3182 (NH) of sulfonamide; 3105 (CH) aromatic; 3000 and 2794 (CH) asymmetric and symmetric respectively of CH_3; 1700 (C=O) anilide; 1597, 1535, 1496 (C=C) aromatic; 1319, 1172 (O=S=O) asymmetric and symmetric respectively.

General procedure for the synthesis of N-substituted 4-aminobenzenesulfonamides, compounds Ic and Ie) [18]

HCl (6N, 10 ml) was added to related N-substituted 4-acetamidobenzenesulfonamide (Ib, Id)(1gm) and refluxed for 4 hrs. After this time, the product was neutralized by 25% sodium hydroxide; the obtained product was filtered and washed with ethanol to give compound Ic and Ie respectively.

Compound Ic (Amino-N-(2-oxo-1, 2-dihydropyrimidin-4-yl) benzene sulfonamide)

Pale yellow powder, yield 64%, melting point215-218°C.IR (υ= cm^{-1}, KBr): 3483 and 3385(NH) asymmetric and symmetric stretch respectively of 1^{o} amine; 3331 (NH) of 2^{o} amide; 3174(NH) of sulfonamide; 3066 (CH) aromatic; 1664 (C=O) 2^{o} amide (cytosine); 1602, 1581, 1502 (C=C) aromatic; 1363, 1180 (O=S=O) asymmetric and symmetric respectively.

Compound Ie (4-Amino-N-(9-methyl-9H-purin-6-yl) benzene sulfonamide)

Pale brown powder, yield 60%, melting point198-200°C (decomposed).IR (υ= cm^{-1}, KBr): 3489 and 3383(NH) asymmetric and symmetric stretch respectively of 1^{o} amine; 3238(NH) of sulfonamide; 3103 (CH) aromatic; 2922 and 2870 (CH) asymmetric and symmetric stretch respectively of CH_3; 1600, 1575, 1502 (C=C) aromatic; 1303, 1175 (O=S=O) asymmetric and symmetric respectively.

General procedure for the synthesis of amino acid methyl ester hydrochloride (IIa and IIb)[19]

A suspension of related amino acid (30.26 mmol) in absolute methanol (75 ml) was cooled down to -10°Cand thionyl chloride (36.32 mmol, 2.6 ml) was added drop wise, the reaction mixture was stirred at 40°Cfor 3 hrs, then refluxed for 3 hrs, and left at room temperature overnight, the solvent was evaporated to dryness under vacuum, redissolved in methanol and evaporated several times. The resulting solid product was collected and dried under vacuum, to give crude methyl ester hydrochloride. The crude material was dissolved in minimum amount of hot methanol. Slow addition of excess of diethyl ether followed by cooling to 0 °C gave pure crystals. The crystals were collected on the following day and washed twice with diethyl ether: methanol (5:1) mixture and dried to get pure compound.

L-phenylalanine methyl ester HCl, compound (IIa)

White crystals, yield 90%, melting point158-160°C.IR (υ= cm^{-1}, KBr): 3003-2800(NH) stretch of ammonium include (CH) aromatic and CH_3; 1741(C=O) ester; 1627(NH) bending of ammonium; 1583 and 1496 (C=C) aromatic.

L-proline methyl ester HCl, compound (IIb)

Oily, yield 98%, IR (υ= cm^{-1}, KBr): 3425(NH) stretch of ammonium salt of 2^{o} amine; 2955 and 2740 (CH) of CH_3

and CH_2; 1743(C=O) ester; 1627(NH) bending of amine salt.

Synthesis of compound4-(1-methoxy-1-oxo-3-phenylpropan-2-ylamino)-4-oxobut-2-enoic acid (IIIb) [20]

To a suspension of IIa(4.6 mmol, 1 gm) in chloroform (25 ml), N-methyl morpholine (4.6 mmol, 0.5ml) was added drop wise, and stirring at room temperature until clear solution is predominant, then maleic anhydride (4.6 mmol, 0.45 gm) was added gradually to the reaction mixture, the reaction mixture was stirred at room temperature for about 2 hours. The organic layer was washed with distilled water (2 ×20ml), dried with anhydrous magnesium sulphate and filtered; the chloroform was evaporated to yield an oily residue. Yield 89 %, IR (υ= cm^{-1}, KBr): 3269(NH) stretch of 2° amide and (OH) stretch of carboxylic acid; 3030(C-H) stretch Aromatic; 2955 and 2858 (C-H) asymmetric and symmetric stretch of CH_3 and CH_2; 1741(C=O) stretch of ester; 1716 (C=O) stretch of carboxylic acid.

Synthesis of 4-(2-(methoxycarbonyl) pyrrolidin-1-yl)-4-oxobut-2-enoic acid (IVb) [20]

To a suspension of IIb(6 mmol, 1 gm) in ethyl acetate (25 ml), N-methyl morpholine (6 mmol, 0.66ml) was added drop wise, and stirring at room temperature, then maleic anhydride (6 mmol, 0.59 gm) was added gradually to the reaction mixture, the reaction mixture was stirred at room temperature for about 2 hours. N-methyl morpholine hydrochloride was filtered off and the filtrate was collected, ethyl acetate was evaporated to yield an oily residue. Yield 88%, IR (υ= cm^{-1}, KBr): 3522(O-H) stretch of carboxylic acid; 2991 and 2883(C-H) asymmetric and symmetric stretch of CH_3; 2956(C-H) asymmetric stretch of CH_2; 1739(C=O) stretch of ester; 1714(C=O) stretch of carboxylic acid; 1624(C=O) stretch of 3° amide and (C=C) stretch.

General procedure for synthesis of compounds B, E, H and K [21]

Thionyl chloride (0.82 mmole, 0.06ml) was added drop wise to a stirred solution of related carboxylic acid derivatives IIIb, IVb (0.82mmole) in dry chloroform (25ml) at −5 °C, and the reaction mixture was refluxed for 3 hrs. Chloroform was evaporated and the residue was redissolved in chloroform, and evaporated under vacuum. An oily residue of acid chloride was obtained and dissolved in dry THF (10 ml) and added drop wise to a stirred solution of related N-substituted 4-aminobenzenesulfonamides (Ic, Ie)(0.82mmole) and N-methyl morpholine (0.82mmole, 0.09ml) in DMF (10ml). The reaction mixture was stirred over night at room temperature. The solvent was evaporated and the residue was washed with chloroform, triturated with diethyl ether, and recrystallized from ethanol.

Methyl (4-oxo-4-((4-(N-(2-oxo-1, 2-dihydropyrimidin-4-yl) sulfamoyl) phenyl) amino) but-2-enoyl) phenylalaninate (Compound B)

Yellowish orange powder, yield 64%, melting point 80-82°C (decomposed), IR (υ= cm^{-1}, KBr): 3354(N-H) stretch of 2° amide; 3221(N-H) stretch of sulfonamide; 3066(C-H) stretch of aromatic; 2939 and 2880(C-H) asymmetric and symmetric stretch of CH_3; 1730(C=O) stretch of ester; 1678(C=O) stretch of 2° amide; 1631(C=C) stretch of maleic residue; 1600 and 1502(C=C) stretch of aromatic ring; 1321 and 1172(O=S=O) asymmetric and symmetric stretch of sulfonamide. ^{1}HNMR(400MHz), DMSO-d_6 , δ ppm):3(d, 2H, -CH_2-Ar), 3.8(s, 3H, CH_3-O), 4.4(t, 1H, -CH-), 5.1(s, 2H, -CH=CH-C=O), 6.7-6.8(m, 2H, pyrimidin-one), 7-8(m, 9H, Ar-), 11.5(s,1H, -NH-C=O), 12(s, 1H, -NH-Ar), 12.5(s, 1H, -NH-pyrimidin), 12.89(s, 1H, -NH-SO_2). CHNS Calculated for$C_{24}H_{23}N_5O_7S$: C, 54.85; H, 4.41; N, 13.33; S, 6.10. Found; C, 54.34; H, 4.47; N, 13.69; S, 5.79.

Methyl - (4-oxo-4-((4-(N-(2-oxo-1, 2-dihydropyrimidin-4-yl) sulfamoyl) phenyl) amino) but-2-enoyl) prolinate (Compound E)

Yellowish green powder, yield 70%, melting point 68-70°C (decomposed), IR (υ= cm^{-1}, KBr): 3336(N-H) stretch of 2° amide; 3255(N-H) stretch of sulfonamide; 3080(C-H) stretch of aromatic ring; 2980, 2951, 2877(C-H) asymmetric and symmetric stretch of CH_3 and CH_2; 1730(C=O) stretch of ester; 1680(C=O) stretch of amide; 1645(C=C) stretch of maleic residue;1602, 1544, 1510(C=C) stretch of aromatic ring; 1325 and 1174(O=C=O) asymmetric and symmetric stretch of sulfonamide respectively.

^{1}HNMR (400MHz), DMSO-d_6, δ ppm)

2.1(m, 2H, CH_2-cyclo), 2.3(m, 2H, CH_2-cyclo), 3.4(s, 3H, CH_3-O), 3.9(t, 2H, CH_2-cyclo), 4.3(t, 1H, -CH-), 5.25(s, 2H, CH=CH-C=O), 6.4-6.7(m, 2H, pyrimidin-one), 7-8(m, 4H, Ar-), 11.96(s, 1H, -NH-C=O), 12.4(s, 1H, -NH-pyrimidin), 12.95(s, 1H, -NH-SO_2).

Methyl (4-((4-(N-(9-methyl-9H-purin-6-yl) sulfamoyl) phenyl) amino)-4-oxobut-2-enoyl) phenylalaninate (Compound H)

Yellowish orange powder, yield 64%, melting point 85-88°C (decomposed), IR (υ= cm^{-1}, KBr):3325(N-H) stretch of 2° amide; 3226(N-H) stretch of sulfonamide; 3026(C-H) stretch of aromatic ring; 2945 and 2870(C-H) asymmetric and symmetric stretch of CH_3; 1731(C=O) stretch of ester;1691(C=O) stretch of 2° amide; 1629(C=C) stretch of maleic residue; 1600, 1510(C=C) stretch of aromatic ring; 1325 and 1186(O=S=O) asymmetric and symmetric stretch of sulfonamide respectively. ^{1}HNMR(400MHz), DMSO-d_6 , δ ppm):3(d, 2H, CH_2-Ar), 3.5(s, 3H, CH_3-N), 3.9(s, 3H, CH_3-O), 4.6(t, 1H, -CH-), 5.2(s, 2H, -CH=CH-C=O), 5.8(s, 1H, purine), 6.9(s, 1H, purine),7-8(m, 9H, Ar-), 11.8(s, 1H, -NH-C=O), 12.4(s, 1H, -NH-Ar), 12.7(s, 1H, -NH-SO_2).

Methyl (4-((4-(N-(9-methyl-9H-purin-6-yl) sulfamoyl) phenyl) amino)-4-oxobut-2-enoyl) prolinate(Compound K)

Mustard powder, yield 63%, melting point 63-65°C (decomposed), IR (υ= cm^{-1}, KBr):3331(N-H) stretch of 2° amide; 3111(N-H) stretch of sulfonamide; 3064(C-H) stretch of aromatic ring; 2953, 2877(C-H) asymmetric and symmetric stretch of CH_3 and CH_2; 1732(C=O) stretch of ester; 1631(C=O) stretch of amide; 1622(C=C) stretch of maleic residue; 1600, 1502(C=C) stretch of aromatic ring; 1309 and 1180(O=S=O) asymmetric and symmetric stretch of sulfonamide respectively.[1]HNMR(400MHz), DMSO-d_6 , δ ppm):1.8(m, 2H, CH_2-cyclic), 2(m, 2H, CH_2-cyclic), 3.6(s, 3H, CH_3-N), 3.8(s, 3H, CH_3-O), 4.2(t, 2H, -CH_2-N), 4.8(t, 1H, -CH-), 5.2(s, 2H, CH=CH-C=O), 6(s,1H, purine), 6.9(s,1H, purine), 7.6-8(m, 4H, Ar-), 12.7(s, 1H, -NH-C=O), 12.8(s, 1H, -NH-SO_2).CHNS Calculated for$C_{22}H_{23}N_7O_6S$: C, 51.46; H, 4.51; N, 19.09; S, 6.24. Found; C, 50.88; H, 4.83; N, 19.67; S, 6.42.

Cytotoxic Activity Study

The preliminary in vitro cytotoxicity study was done at the Iraqi Centre for Cancer and Medical Genetic Research (ICCMGR) using two types of tumor cell lines: murine mammary adenocarcinoma cell line (AMN3)[22]and primary tumour of a 70 years old Iraqi woman with a histological diagnosis of infiltrating ductal carcinoma (breast cancer cell line AMJ13)[23]and one type of normal cell line: rat embryo fibroblast cell line (Ref).[24]They were maintained in growth medium supplemented with 10% fetal calf serum and seeded on micro-titration (96- well plates at a concentration of 1×10^4 cells/well) and various concentrations of tested compounds (B and K) were added from (3.125 to 100 µg/ml) prepared by serial two fold dilutions using maintenance media from stock solution of test sample in triplicate form of each concentration. The negative control wells contained only the cells with culture media, then the 96-well cell culture plate incubated at 37°C in an incubator supplemented with 5% CO_2 for 2 different times (48, 72) hrs.[25] The cytotoxic activity of compounds were evaluated by Crystal violet assay, the optical density of each well was measured by using ELISA (Enzyme Linked Immuno Sorbent Assay) reader at a transmitting wave length on 492 nm. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as (A-B)/A ×100, where A is the mean optical density of untreated wells (control), and B is the optical density of treated wells.[26]Data were analyzed by 2-way analysis of variance with ANOVA. The level of significance ($p<0.05$) was used for analysis of the results.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the compounds (B, E, H and K) was outlined in the schemes (1)and (2) which are also illustrated the reactions sequences for the all synthesized compounds.

Reaction of acetanilide with chlorosulfonic acid resulted in the formation of 4-acetamidobenzenesulfonyl chloride (Ia), which was reacted with related amines (cytosine and 9- methyl adenine) to form compounds (Ib and Id) respectively.Removal of acetyl group afforded compounds (Ic and Ie) respectively. Esterification of amino acids was done by activation of the amino acid by thionyl chloride to get acyl chloride that attacks methanol producing methyl ester of the selected amino acid, compounds IIa and IIb were synthesized by this method. Reaction of maleic anhydride with compound IIa and IIb lead to the formation of compounds IIIb and IVb. Each one of the compounds (IIIb and IVb) was reacted with thionyl chloride in dry chloroform and refluxed for 3 hours to form acyl chloride that was reacted with compound Ic leads to the formation of the targeting compounds (B, E) respectively. The acyl chloride of the compounds (IIIb, IVb) was also reacted with compound Ie leads to the formation of the targeting compounds (H, K) respectively.

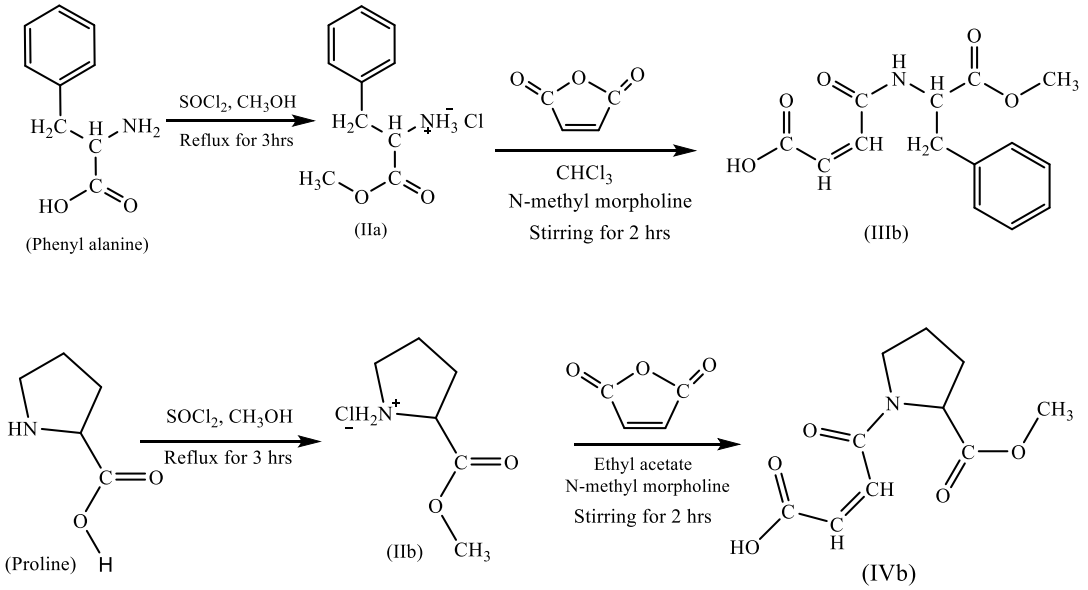
Cytotoxicity study

The cytotoxic activities (cell viability assay) of compounds (B and K) were evaluated by Crystal violet assay.[27] A set of six concentrations (3.125, 6.25, 12.5, 25, 50 & 100 µg/ml) was prepared for each product. Three cell lines were studied (AMN-3 passage no.117, AMJ-13 passage no. 50, Ref cell lines passage no. 120) at two times of exposure (48, 72 hours).

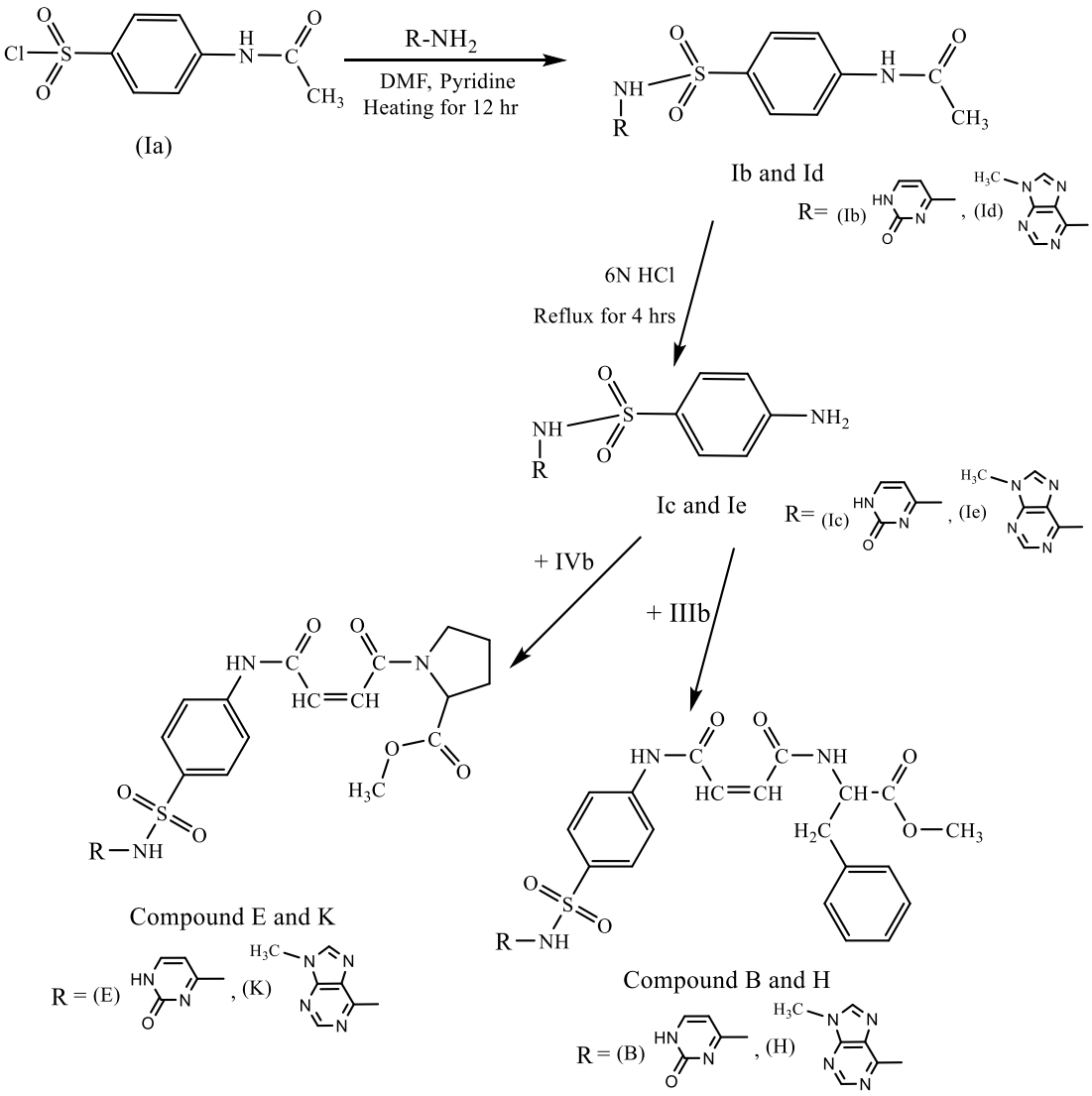
Table 1: The effects of different concentrations of compound B on growth of AMN3, AMJ13, and Ref cell lines

	48 hours				72 hours		
Conc. µg/ml	AMN3	AMJ13	Ref		AMN3	AMJ13	Ref
3.125	0.4 (1.3)	5.1 (1.4)	3.6 (3.6)		3.3 (2.1)	5.4(2.1)	-2.6(5.1)
6.25	1.8 (0.9)	6.2 (0.9)	6.1 (5.3)		0.9 (1.9)	16.2(2.2)	2.6 (2.7)
12.5	10.4 (2.4)	13.3(2.7)	7.0 (3.5)		1.6 (0.6)	21.6(2.6)	-0.3(1.1)
25	52.7 (8.7)	49.7(6.3)	7.0 (2.6)		40.0(1.2)	26.0(1.8)	5.1 (2.6)
50	55.9 (0.6)	53.8(1.8)	0.0(5.8)		50.2(1.0)	30.9(3.1)	-1.5(5.5)
100	61.6 (0.9)	80.2(1.6)	6.4(3.2)		51.1(4.8)	34.3(3.5)	-2.6(3.2)
Data presented using mean (standard error mean)							

Scheme 1: Synthesis of compound IIIb and IVb.



Scheme 2: Synthesis of compounds B, E, H and K.



Control= 0% inhibition.
Positive results of the % growth inhibition indicate anti-proliferation.
Negative results of the % growth inhibition indicates proliferation

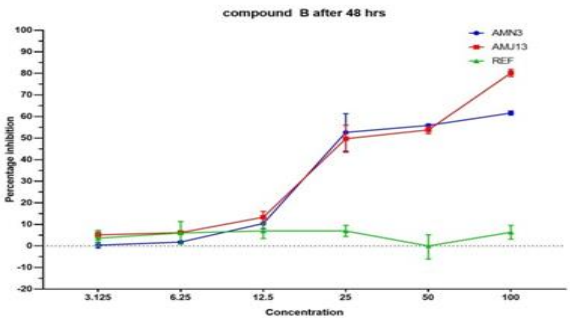


Figure 1: Inhibition rate (%IR) of different concentrations of compound B on three cell lines at 48 hrs.

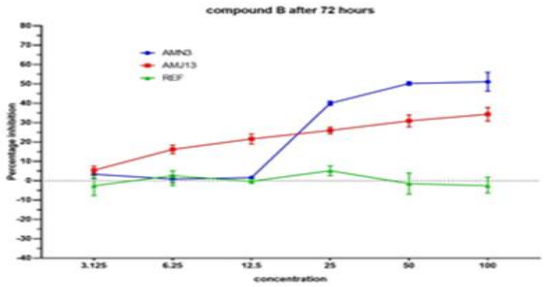


Figure 2: Inhibition rate (%IR) of different concentrations of compound B on three cell lines at 72 hrs.

Data were analyzed by 2-way analysis of variance with ANOVA. The level of significance ($p<0.05$) was used for analysis of the results.

Table 2: Post-hoc test of each pair of concentrations of compound B (columns comparison) showing their p values

	48 hours			72 hours		
Conc. µg/ml	AMN3	AMJ13	Ref	AMN3	AMJ13	Ref
3.125 vs. 6.25	NS	NS	NS	NS	NS	NS
6.25 vs. 12.5	NS	NS	NS	NS	NS	NS
12.5 vs. 25	<0.0001	<0.0001	NS	<0.0001	NS	NS
25 vs. 50	NS	NS	NS	NS	NS	NS
50 vs. 100	NS	0.0002	NS	NS	NS	NS
NS: non-significant						

Table 3: Post-hoc test of each pair of tissue line at fixed concentration of compound B (row comparison) showing their p values

	48 hours			72 hours		
Conc. µg/ml	AMN3 vs. Ref	AMJ13 vs. Ref	AMN3 vs. AMJ13	AMN3 vs. Ref	AMJ13 vs. Ref	AMN3 vs. AMJ13
3.125	NS	NS	NS	NS	NS	NS
6.25	NS	NS	NS	NS	NS	NS
12.5	NS	NS	NS	NS	0.0040	0.0087
25	<0.0001	<0.0001	NS	<0.0001	0.0062	NS
50	<0.0001	<0.0001	NS	<0.0001	<0.0001	0.0115
100	<0.0001	<0.0001	0.0027	<0.0001	<0.0001	0.0312

The results indicate that significant inhibitory effects appeared in the cancer cell lines (AMN3 and AMJ13) at different concentrations, while no significant inhibitory effect appear in normal cell line (Ref).

Table 4: The effects of different concentrations of compound K on growth of AMN3, AMJ13, and Ref cell lines

		48 hours			72 hours		
Conc.µg/ml		AMN3	AMJ13	REF	AMN3	AMJ13	REF
3.125		21.1 (5.1)	4.1 (5.3)	-5.1 (9.2)	30.2 (5.5)	3.7 (1.2)	0.0 (4.8)
6.25		17.5 (2.7)	7.3 (2.8)	0.0 (8.9)	27.1 (4.1)	3.7 (1.9)	0.0 (4.8)
12.5		24.0 (7.7)	36.6 (5.1)	2.6 (2.6)	22.9 (0.6)	28.2 (4.6)	-2.8 (2.8)
25		45.6 (2.0)	66.7 (2.2)	9.0 (7.1)	45.9 (3.1)	67.6 (3.3)	0.0 (4.8)
50		55.6 (1.5)	62.6 (3.5)	2.6 (2.6)	52.5 (1.0)	65.3 (1.6)	2.8 (2.8)
100		62.0 (3.1)	80.5 (3.7)	10.3 (6.8)	63.5 (1.9)	79.2 (3.5)	8.3 (4.8)
Data presented using mean (standard error mean)							

Control= 0% inhibition.
Positive results of the % growth inhibition indicate anti-proliferation.
Negative results of the % growth inhibition indicates proliferation

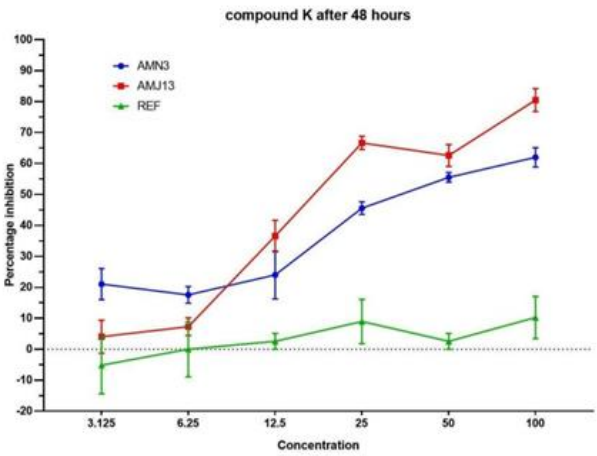


Figure 3: Inhibition rate (%IR) of different concentrations of compound K on three cell lines at 48 hrs.

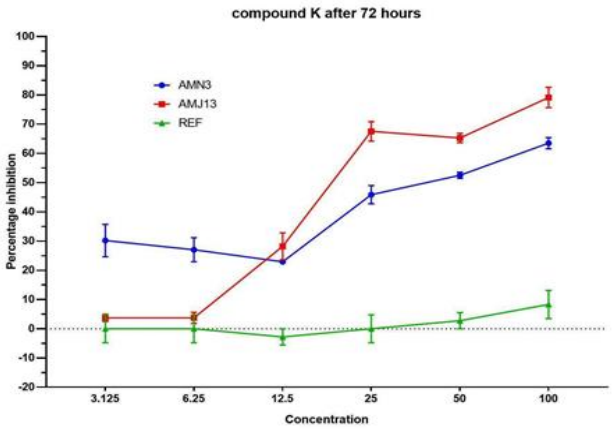


Figure 4: Inhibition rate (%IR) of different concentrations of compound K on three cell lines at 72 hrs.

Data were analyzed by 2-way analysis of variance with ANOVA. The level of significance ($p<0.05$) was used for analysis of the results.

Table 5: Post-hoc test of each pair of concentrations of compound K (columns comparison) showing their p values

	48 hours			72 hours		
Conc. µg/ml	AMN3	AMJ13	Ref	AMN3	AMJ13	Ref
3.125 vs. 6.25	NS	NS	NS	NS	NS	NS
6.25 vs. 12.5	NS	0.0036	NS	NS	0.0003	NS
12.5 vs. 25	NS	0.0026	NS	0.0006	<0.0001	NS
25 vs. 50	NS	NS	NS	NS	NS	NS
50 vs. 100	NS	NS	NS	NS	NS	NS
NS: non-significant						

Table 6: Post-hoc test of each pair of tissue line at fixed concentration for compound K (row comparison) showing their p values

	48 hours			72 hours		
Conc. µg/ml	AMN3 vs. Ref	AMJ13 vs. Ref	AMN3 vs. AMJ13	AMN3 vs. Ref	AMJ13 vs. Ref	AMN3 vs. AMJ13
3.125	0.0027	NS	NS	<0.0001	NS	<0.0001
6.25	NS	NS	NS	<0.0001	NS	0.0001
12.5	0.0152	0.0001	NS	<0.0001	<0.0001	NS
25	<0.0001	<0.0001	0.0172	<0.0001	<0.0001	0.0003
50	<0.0001	<0.0001	NS	<0.0001	<0.0001	0.0377
100	<0.0001	<0.0001	0.0401	<0.0001	<0.0001	0.0092
NS: non-significant						

The results indicate that significant inhibitory effects appeared in the cancer cell lines (AMN3 and AMJ13) at different concentrations, while no significant inhibitory effect appear in normal cell line (Ref).

CONCLUSION

New derivatives of sulfonamides-amino acids ester conjugate through maleamide spacer were synthesized and evaluated for their anticancer activities. The synthesized compounds were characterized and identified by I.R spectra, elemental microanalysis, and ^{1}HNMR study, and it was found that all the results shown good agreements with the proposed chemical structures of the synthesized compounds. A preliminary cytotoxicity study that evaluated by crystal violet assay indicate that

the tested compounds have considerable anticancer activity against two cancer cell lines at different concentrations with no significant inhibitory effect of tested compounds on normal cell line, so the synthesized compounds have selectivity in their action toward cancer cells.

Acknowledgment: We would like to thank Dept. of pharmaceutical chemistry in the college of pharmacy-Baghdad University for support in carrying out the research; also we thank the Iraqi Centre for Cancer and Medical Genetic Research (ICCMGR) for their help in the cytotoxicity study.

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Source of Support: Nil, **Conflict of Interest:** None.