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Antineoplastic Effect of New Synthesized Compounds of 2-Thiouracil Sulfonamide Derivatives against Ovarian and Breast Carcinoma Cells "In Vitro Study"

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ABSTRACT
Many studies have been focused on the cancer therapeutics through studying the cytotoxic activities of different compounds such as synthetic, semi synthetic and plant constituents, our study deal with synthesis of novel 2-Thiouracil-5-sulfonamide derivatives. The target compounds were prepared by chlorosulfonation of 2-thiouracil using chlorosulfonic acid at 120°C giving 2-Thiouracil-5-sulfonyl chloride 2, which in turn was reacted with p-halobenzyl amines namely; p-fluoro, p-chloro and p-bromo analogues respectively to yield sulfonamides 3a-c. Moreover, sulfonamides 3a-c was chlorinated by $POCl_3/PCl_5$ mixture to afford chloro derivatives 4a-c. In addition, the latter were hydrazinolysed by NH_2NH_2 yielding hydrazine derivatives 5a-c. In another pathway, hydrazine derivatives 5a-c was condensed with benzaldehyde yielding Schiff's bases 6a-c. Furthermore, hydrazine derivatives 5a-c was reacted with phenyl isocyanine affording compounds **7a-c.** The newly synthesized compounds were screened for in vitro anticancer activity against A-2780 ovarian and MCF-7 breast carcinoma cells. They showed variable response, the most active compounds were hydrazine carbothioamides 7a-c which showed wide spectrum activity against the two cell lines. Moreover, the prepared compounds were formulated as water soluble tablets in an attempt to improve the bioavailability of thiouracil containing compounds. The tablets were subjected to some quality control testing.

Keywords: Thiouracil Sulfonamide Derivatives, breast carcinoma cell, 5-FU, Ovarian and Breast carcinoma

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INTRODUCTION

Ovarian cancer is one of the most important diseases that cause death in women (1) where, in 2019 there are 22,530 new cases and 13,980 deaths in the United States (2). Breast cancer incidence is high relatively compared to ovarian cancer with 207.090 in 2011(3). Cytotoxic chemotherapy is administered at the maximum tolerated dose (MTD) with free intervals to lower side effects such as bone marrow suppression (4) and to avoid chemotherapy resistance which were considered the major problems with these drugs (5).

2-Thiouracil (TU) is one of the most important pharmacologically active ant metabolite pyramiding compounds (6). 2-thiouracil was chlorosulfonated by chlorosulfonic acid at the 5th position as an intermediate for the synthesis of a huge number of active and dispersible sulfonamides to widen the biological spectrum as antibacterial, antiviral, antithyroid, anticancer and others, for example 6-n-propyl-2-thiouracil is one of the mostly used antithyroid agents (7), 6–Thienyl–5–cyano-2–thiouracil derivatives have antiviral activity(8) and several novel 6-aryl-5-cyano thiouracil derivatives were synthesized as antibacterial, antifungal and anticancer agents(9). 5-FU is one of the most commonly and classic anticancer agent (10). It is a drug that used for both curative and adjuvant chemotherapy, it acts by interfering with RNA, DNA synthesis by inhibition of thymidine synthase (TS) causing inhibition of DNA de novo synthesis (11-13). There are multiple factors lead to 5-FU resistance such as change in drug influx, efflux, and mutation of the drug target (14), on the other hand some cancer cells increase synthesis of TS(15), and over expression of anti-apoptotic proteins such as Bcl-2 and Bcl-XL (16).

MATERIAL AND METHODS

All melting points were determined in capillary tube on a Boethius melting point microscope. Micro-analyses were performed by the micro analytical unit at Cairo University. IR spectra were recorded as KBr pellets on a Beckmann infra spectrophotometer PU9712 using KBr discs. HNMR spectra were determined on a Joel EX 270 MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Finnegan SSQ 7000 Mass spectrometer at 70 ev All reactions were followed and checked by TLC using Chloroform/Methanol (3:1) and spots were examined under a UV-lamp.

Experimental

Synthesis of 4-Oxo-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-sulfonyl chloride 2:

A mixture of 12.5 g (0.055 mol) of 2-thiouracil 1 and 51 ml (0.055 mol) of chlorosulfonic acid was heated at 120 oC for 8 hours. The reaction mixture was cooled and poured on a mixture of 1:1 ice and acetic acid, the precipitate was filtered off, dried under suction and used as crude for subsequent work. Yield: 61%, m.p 230 oC (as reported). Another procedure to improve the yield: A four necked flask equipped with a power-driven stirrer, a thermometer, a reflux condenser and a dropping funnel was charged with 7.6g (0.054 mol) of 2-thiouracil 1 dried in an oven at 110-115 oC for 3-4 hr. The flask was placed on a water bath, and

10 ml (16.5 g, 0.138 mol) of freshly distilled thionyl chloride was added with vigorous stirring. The contents were stirred for 20-30 min, after which 16 ml (28.6 g, 0.265 mol) of freshly distilled chlorosulfonic acid was added drop wise at such a rate that the reaction mixture temperature did not exceed 30-35 oC. The reflux condenser was connected through a CaCl2 tube with a beaker absorbing the released gases. The mixture was heated to 60-65 oC and kept at this temperature with vigorous stirring for 4.5-5 h, after which it was heated to 75-80 oC and kept for an additional 5.5-6 h. The reaction progress was monitored chromatographically on Silufol UV-254 plates, eluent ethyl acetate. After the reaction completion, the mixture was cooled to 0-5 oC and poured with vigorous stirring on to a mixture of 50 g of crushed ice and 35 ml of glacial acetic acid, keeping the temperature in the range from -5 to 0 oC. 2-Thiouracio-5-sulfonyl chloride precipitated as a buff powder substance; it was filtered off and washed several times with ice-cold water and acetic acid. Yield 9.97 g (82%), mp. 230 oC (as reported).

Synthesis of N-(4-Halobenzyl)-4-oxo-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-sulfonamides 3a-c:
A mixture of sulfonyl chloride 2 (1.13g, 0.005 mol), p-halobenzyl amine (0.005 mol) and pyridine (0.4 ml, 0.005 mol) in 50 ml absolute ethanol was refluxed for 12 hours, then cooled, filtered off, dried under suction and recrystallized from DMF / water.

N-(4-Fluorobenzyl)-4-oxo-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-sulfonamide 3a:
Yield: 77%: mp: 244-245 oC: IR (KBr cm-1): 3200 (NH) 3150 (CH, aromatic,), 1695(C= O), 1651(C=N), 1320, 1130 (SO2), 1270 (C=S). 1HNMR (DMSO-d6), δ: 3.5(s,2H, CH2-),7.2,7.4(dd,4H, aromatic), 8.1(s,1H, thiouracil),10.5, 11.0,11.6(s,3H, NH, D2O exchangeable), MS: m/z (%), 315.01 (M+,17.2%), Anal Calcd, for C11H10FN3O3S2: C, 41.90; H, 3.20; N, 13.33. Found: C, 41.82; H, 3.15; N, 13.45.

N-(4-Chlorobenzyl)-4-oxo-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-sulfonamide 3b:
Yield: 81%: mp: 256-257 oC: IR (KBr cm-1): 3205 (NH) 3170(CH, aromatic), 1683(C= O), 1655(C=N), 1325, 1136 (SO2), 1272 (C=S). 1HNMR (DMSO-d6),δ: 3.6(s,2H,CH2-),7.1,7.5(dd,4H, aromatic), 8.2(s,1H,thiouracil),10.4, 11.1,11.7(s,3H,NH,D2O exchangeable) , MS: m/z(%), 330.99 (M+,28.25%), 332.98 (M+2,9.42%),Anal. Calcd for C11H10ClN3O3S2: C,39.82; H, 3.04; N, 12.66. Found: C, 39.69; H, 3.05; N, 12.43.

N-(4-Bromobenzyl)-4-oxo-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-sulfonamide 3c:
Yield: 79%: mp: 270-271 oC: IR (KBr cm-1): 3208 (NH) 3166 (CH, aromatic), 1686 (C= O), 1659(C=N), 1322, 1131 (SO2), 1274 (C=S). 1HNMR (DMSO-d6),δ: 3.5 (s,2H,CH2-),7.2,7.4(dd,4H, aromatic), 8.0 (s,1H,thiouracil),10.5, 11.0,11.3(s,3H,NH ,D2O exchangeable) , MS: m/z (%) , 376.01 (M+,14.32%), 378.01(M+2,13.31%),Anal. Calcd, for C11H10BrN3O3S2: C, 35.11; H, 2.68; N, 11.17. Found: C, 35.12; H, 2.565; N, 11.18.

Synthesis of 4-Chloro-N-(4-halobenzyl)-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-sulfonamides 4a-c:
10 g of any compounds 3a-c was heated with 40 ml POCl3 and 5g of PCl5 on boiling water bath for 8 hours, then cooled and poured drop wise on ice /water and the produced chloro derivatives were filtered off, dried under suction and used as crude for subsequent work.

4-Chloro-N-(4-fluorobenzyl)-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-sulfonamide 4a:
Yield: 75%: mp: 279-280 oC: IR (KBr cm-1) : 3220 (NH) 3167 (CH, aromatic,), 1652(C=N), 1321,1137 (SO2),1275 (C=S). 1HNMR (DMSO-d6),δ: 3.6 (s,2H,CH2-),7.2,7.4 (dd,4H, aromatic), 8.1(s,1H,thiouracil) ,10.5, 11.1, (s,2H,NH, D2O exchangeable) , MS: m/z (%) , 332.98 (M+,100%),334.98(M+2,41.3%), Anal. Calcd for C11H9ClFN3O2S2: C, 39.58; H, 2.72; N, 12.59. Found: C, 39.52; H, 2.65; N, 12.43.

4-Chloro-N-(4-chlorobenzyl)-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-sulfonamide 4b:
Yield:71%: mp: 263-264 oC: IR (KBr cm-1): 3224 (NH) 3161 (CH, aromatic,), 1654(C=N), 1326,1130 (SO2),1270 (C=S). 1HNMR (DMSO-d6),δ: 3.5(s,2H,CH2-),7.3,7.3 (dd,4H, aromatic), 8.2(s,1H,thiouracil) ,10.5, 11.2, (s,2H,NH, D2O exchangeable) , MS: m/z(%), 348.95 (M+,100%),350.95 (M+2,73.3%), Anal Calcd for C11H9Cl2N3O2S2: C,37.72; H, 2.59; N, 12.00. Found: C, 37.81; H, 2.55; N, 12.13.

4-Chloro-N-(4-bromobenzyl)-2-thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-sulfonamide 4c:
Yield: 67%: mp: 286-287 oC: IR (KBr cm-1): 3227 (NH) 3157 (CH, aromatic,), 1653(C=N), 1325, 1130 (SO2),1271 (C=S). 1HNMR (DMSO-d6),δ: 3.4(s,2H,CH2-),7.3,7.3 (dd,4H, aromatic), 8.1 (s,1H,thiouracil),10.6, 11.1, (s,2H,NH, D2O exchangeable), MS: m/z(%), 394.90 (M+,100%),396.90(M+2,23.4%), Anal. Calcd for C11H9BrClN3O2S2: C, 33.47; H, 2.30; N, 10.65. Found: C, 33.51; H, 2.40; N, 10.63.

Synthesis of N-(4-halobenzyl)-4-hydrazinyl-2-thioxo-1,2-dihydropyrimidine-5-sulfonamides 5a-c:
A mixture of any of chloropyramines 4a-c (0.001 mol) and hydrazine hydrate (0.001 mol) in 30 ml methanol was refluxed for 15 min., then cooled and stirred for 24 hours at room temperature. The solution was poured into ice/water and the produced precipitate was filtered off, dried and recrystallized from DMF/water.

N-(4-Fluorobenzyl)-4-hydrazinyl-2-thioxo-1, 2-dihydropyrimidine-5-sulfonamide 5a:
Yield:73%: mp: 286-287 oC: IR (KBr cm-1): 3208 (NH) 3170(CH, aromatic, CH), 1651(C=N), 1320,1130 (SO2),1270 (C=S). 1HNMR (DMSO-d6),δ: 3.4(s,2H,CH2-),7.2,7.5(dd,4H, aromatic), 8.1(s,1H,thiouracil),2.1,4.3,10.5, 11.0, (s,5H,NH,NH2,D2O exchangeable) , MS: m/z(%), 329.04 (M+,18.2%), Anal. Calcd for C11H12FN5O2S2: C, 40.11; H, 3.67; N, 21.26. Found: C, 40.02; H, 3.65; N,21.35.

N-(4-Chlorobenzyl)-4-hydrazinyl-2-thioxo-1, 2-dihydropyrimidine-5-sulfonamide 5b:
Yield: 79%: mp: 296-297 oC: IR (KBr cm-1): 3210 (NH) 3179(CH, aromatic, CH), 1646(C=N), 1325,1137 (SO2),1272 (C=S). 1HNMR (DMSO-d6),δ: 3.5(s,2H,CH2-),7.1,7.4(dd,4H, aromatic), 8.2(s,1H,thiouracil),2.2,4.2,10.6, 11.1, (s,5H,NH,NH2,D2O exchangeable) , MS: m/z(%), 345.01 (M+,22.7%), 347.01(M+2, 7.3%) Anal Calcd, for C11H12ClN5O2S2: C, 38.20; H, 3.51; N, 20.25. Found: C, 38.12; H, 3.55; N, 20.19.

N-(4-Bromobenzyl)-4-hydrazinyl-2-thioxo-1, 2-dihydropyrimidine-5-sulfonamide 5c:
Yield: 70%: mp: 281-282 oC: IR (KBr cm-1): 3220 (NH) 3168(CH, aromatic, CH), 1637(C=N), 1320, 1136 (SO2), 1270 (C=S). 1HNMR (DMSO-d6),δ: 3.5 (s,2H,CH2-),7.2,7.5(dd,4H,aromatic),8.1(s,1H,thiouracil),2.3,4.3,10.6,11.2, (s,5H,NH,NH2,D2O exchangeable), MS: m/z(%), 390.96 (M+,20.7%), 392.96(M+2, 19.3%) Anal Calcd for C11H12BrN5O2S2: C, 33.85; H, 3.10; N, 17.94. Found: C, 33.92; H, 3.15; N, 17.81.

Synthesis of 4-(2-B enzylidenehydrazinyl)-N-(4-halobenzyl)- -thioxo-1,2-dihydropyrimidine-5-sulfonamides 6a-c:
A mixture of any of 5a-c (0.001 mol) and benzaldehyde (0.001 mol) in 30 ml methanol was refluxed for 8 hours, then cooled and the produced precipitate was filtered off, dried and recrystallized from DMF/water.
4-(2-Benzylidenehydrazinyl)-N-(4-fluorobenzyl)--thioxo-1,2-dihydropyrimidine-5-sulfonamides 6a:
Yield:70%: mp: 273-274 oC: IR (KBr cm-1): 3212 (NH) 3166(CH, aromatic, CH), 1656(C=N), 1320,1130 (SO2),1270 (C=S). 1HNMR (DMSO-d6),δ: 3.5(s,2H,CH2-),7.2-7.5(m,9H, aromatic), 8.1(s,1H,thiouracil), 8.5(s.1H,CH=N-) ,2.1, 10.5, 11.0, (s,3H,NH, D2O exchangeable) , MS: m/z(%), 417.07 (M+,15.2%), Anal Calcd, for C18H16FN5O2S2: C,51.79; H, 3.86; N, 16.78. Found: C, 51.62; H,3.75; N,16.85.

4-(2-Benzylidenehydrazinyl)-N-(4-chlorobenzyl)--thioxo-1,2-dihydropyrimidine-5-sulfonamides 6b:
Yield:76%: mp: 266-267 oC: IR (KBr cm-1): 3219 (NH) 3169(CH, aromatic, CH), 1664(C=N), 1320,1130 (SO2),1270 (C=S). 1HNMR (DMSO-d6),δ: 3.4(s,2H,CH2-),7.2-7.6(m,9H, aromatic), 8.2(s,1H,thiouracil), 8.6(s.1H,CH=N-) ,2.2, 10.4, 11.1, (s,3H,NH, D2O exchangeable) , MS: m/z(%), 433.04 (M+,14.2%), 435.04 (M+2, 4.7%) Anal Calcd, for C18H16ClN5O2S2: C, 49.82; H, 3.72; N, 16.14. Found: C, 49.72; H, 3.75; N,16.26.

4-(2-Benzylidenehydrazinyl)-N-(4-bromobenzyl)--thioxo-1,2-dihydropyrimidine-5-sulfonamides 6b:
Yield:72%: mp: 270-271 oC: IR (KBr cm-1): 3222 (NH) 3163(CH, aromatic, CH), 1667(C=N), 1320,1130 (SO2),1270 (C=S). 1HNMR (DMSO-d6),δ: 3.5(s,2H,CH2-),7.3-7.7(m,9H, aromatic), 8.1(s,1H,thiouracil), 8.5(s.1H,CH=N-) ,2.3, 10.5, 11.0, (s,3H,NH, D2O exchangeable) , MS: m/z(%), 478.99 (M+,10.2%), 480.99 (M+2, 9.2%) Anal Calcd for C18H16BrN5O2S2: C, 45.19; H, 3.37; N, 14.64. Found: C, 45.02; H, 3.45; N, 14.66.

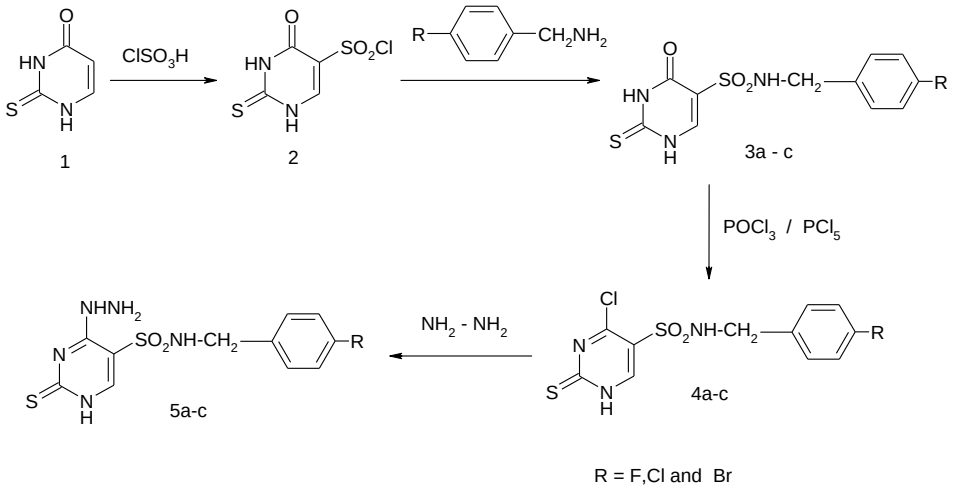
Synthesis of 2-(5-(N-(4-halobenzyl) sulfonyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)-N-phenyl hydrazine carbothioamides 7a-c:
A mixture of any of 5a-c (0.001 mol) and phenyl isothiocyanate (0.001 mol) in 30 ml DMF was refluxed for 10 hours, then cooled and the produced precipitate was filtered off, dried and recrystallized from DMF/water.

2-(5-(N-(4-fluorobenzyl) sulfonyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)-N-phenyl hydrazine carbothioamides 7a:
Yield: 79%: mp: 285-286 oC: IR (KBr cm-1): 3256 (NH) 3168(CH, aromatic, CH), 1657(C=N), 1320, 1130 (SO2),1270 (C=S). 1HNMR (DMSO-d6),δ: 3.5(s,2H,CH2-),7.3-7.6(m,9H, aromatic), 8.1(s,1H,thiouracil), 2.1, 2.5, 10.5, 11.0, 12.5 (s,5H,NH, D2O exchangeable) , MS: m/z(%), 464.06 (M+,11.2%), Anal. Calcd., for C18H17FN6O2S3: C,46.54; H, 3.69; N, 18.09. Found: C, 46.60; H,3.65; N,18.05.

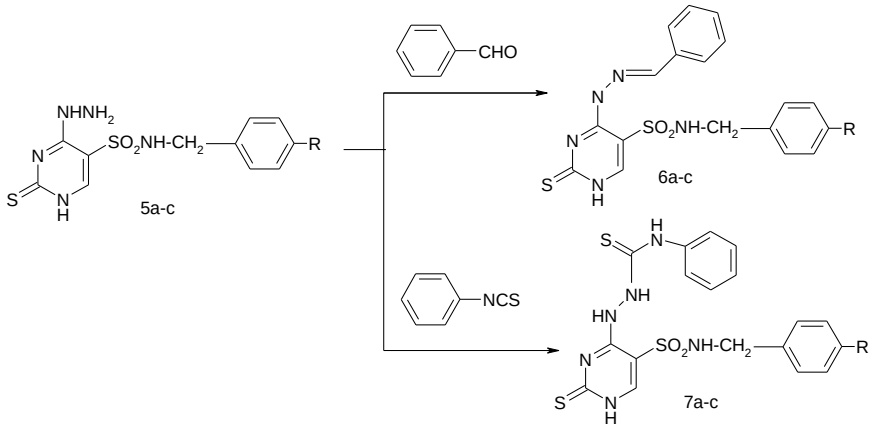
2-(5-(N-(4-chlorobenzyl) sulfonyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)-N-phenyl hydrazine carbothioamides 7b:
Yield: 73%: mp: 299-300 oC: IR (KBr cm-1): 3250 (NH) 3160(CH, aromatic, CH), 1650(C=N), 1320, 1130 (SO2),1270 (C=S). 1HNMR (DMSO-d6),δ: 3.5(s,2H,CH2-),7.2-7.6(m,9H, aromatic), 8.1(s,1H,thiouracil), 2.1, 2.5, 10.5, 11.0, 12.5(s,5H,NH, D2O exchangeable) , MS: m/z(%), 480.03 (M+,15.2%), 482.03(M+2, 5.06%),Anal Calcd, for C18H17ClN6O2S3: C,44.95; H, 3.56; N, 17.47. Found: C, 44.80; H,3.49; N,17.45.

2-(5-(N-(4-bromobenzyl) sulfonyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)-N-phenyl hydrazine carbothioamides 7c:
Yield: 75%: mp: 291-292 oC: IR (KBr cm-1): 3256 (NH) 3167(CH, aromatic, CH), 1652(C=N), 1320, 1132(SO2),1270 (C=S). 1HNMR (DMSO-d6),δ: 3.5(s,2H,CH2-),7.3-7.6 (m,9H, aromatic), 8.2 (s,1H,thiouracil), 2.1, 2.5, 10.5, 11.0, 12.5(s,5H,NH, D2O exchangeable) , MS: m/z(%), 525.97 (M+,23.2%), 527.97(M+2, 22.2%),Anal Calcd, for C18H17BrN6O2S3: C,41.14; H, 3.26; N, 15.99. Found:
C, 41.21; H, 3.39; N, 16.01

Scheme I:



Scheme II:



BIOLOGICAL RESULTS

Anticancer Activity Cells

We performed preliminary experiments with the human A-2780 ovarian and MCF-7 breast carcinoma cells. Stock cultures were grown in T-75 containing 50 MI of RPMI-1640 medium with glutamine, bicarbonate and 5% fetal calf serum. Medium was changed at 48-hours intervals. Cells were dissociated with 0.25% trypsin and 3Mm 1,2-cyclohexanediaminetetracetic acid in NKT buffer (137Mm NaCl, 5.4Mm KCl, and 10 Mm tris; pH 7.4) Experimental cultures were plated in microtiter plates (Costar and Cambridge, Ma) containing 0.2 mL of growth medium per well at densities of 1,000-200, 000 cells per well [17].

Dyes

Dyes were purchased from sigma chemical Co. Preliminary studies were conducted with each of many dyes to determine whether each stained cell more intensively at acidic, neutral or basic pH. One of these anionic dyes was sulforhodamine B (SRB) which was dissolved in 1% acetic acid for cell staining and extracted from cells with un buffered Tris base. The absorption maximum of the dye was determined with a DU-70 scanning spectrophotometer (Beckman Instruments, Inc., Fullerton, CA).

Cell Fixation

Washing cultures with buffer prior to fixation to remove serum protein commonly caused detachment and loss. To avoid this potential problem, cultured were fixed with TCA before washing. Cells attached to the plastic substratum were fixed by gently layering 50µL of cold50%TCA (4°C) on top of the growth medium in each well to produce a final TCA concentration of 10% The cultures were incubated at 4°C for 1 hour and then washed five times with tap water to remove TCA, growth medium and law-molecular weight metabolites and serum plates were air dried and then stored until use. Background optical densities were measured in wells incubated with growth medium without cells. Cells in suspension were allowed to settle out of solution. When these cells were physically resting on the bottom of the wells,50 µL of cold 80%TC. A (4°c) was gently layered on top of the overlying growth medium. The cultures were left undisturbed for 5 minutes and then refrigerated at 4°C for an additional hour of fixation. This procedure led to the attachment of single cell suspensions to the plastic substratum provided that cells were in contact with it when the fixative was applied. This method was as effective in promoting cell attachment as were cytospinning and using the macromolecular adhesive Cell-Talk (Biopolymers, Farmington, CT). However, it did

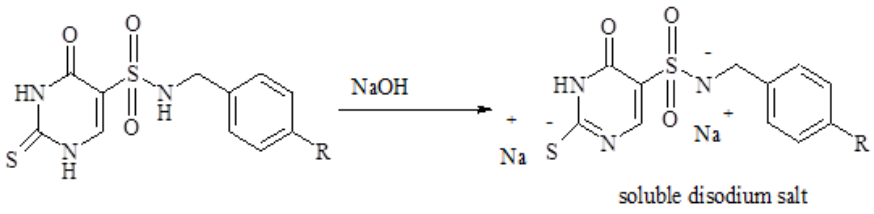
not adequately attach cells that grew as floating aggregates rather than as single cell suspensions. Small cell lung carcinoma lines were particularly unsuited to this method of fixation.

Procedure

Preparation of the sample: Compounds 3a-c,4a-c,5a-c and 6a-c were used as a sodium salt by dissolving in 20 % sodium carbonate then the aq. solution was evaporated to obtain the sodium salt as a solid. Cells were plated in 96-multiwell plate (104 cells | well) for 24 h before treatment with the test compound to allow attachment of cell to the wall of the plate. different concentrations of the compound under test (0, 2.5, 5, and 10 μg/ml) were added to the cell monolayer in triplicate wells individual dose, monolayer cells were incubated with the compounds for 48h at 37°C and in atmosphere of 5% CO_2, after 48h, cells were fixed, washed and stained with SRB stain, excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer, color intensity was measured in an ELISA reader, the relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line after the specified compound and the IC_{50} was calculated.

Formulation of 2-Thiouracil-5-Sulfonamides as water soluble tablets

Thiouracil is important class of pharmacologically active compounds. However, some of these compounds do not have the same profile of activity in vivo assays. This could be partially explained by the low bioavailability of these compounds. The main reasons for this low bioavailability may be low aqueous solubility, poor gastrointestinal stability and difficulty crossing membranes [18]. The rapid and extensive metabolism by the intestinal flora and by some liver enzymes of mercaptopyrimidines after oral administration, may explain the poor bioavailability. Given the importance of the topic, this thesis aims to address the impacts of metabolism of thiouracils on their pharmacological effects by improving their aq. solubility, and consequently their bioavailability. Additionally, we modified some strategies to improve bio efficacy of these compounds. Improving the aqueous solubility of our target compounds is achieved by obtaining the disodium salts by their stirring with 10% aqueous sodium hydroxide. Excess alkali solution was neutralized with diluted hydrochloric acid, and then evaporated to dryness. The solid was extra dried in an oven at100°C.



The dried crystals were compressed into soluble tablets according to the provided methods of literatures [19-21]. Firstly, each crystal was screened through a No. 16-22 sieve. This step is necessary to break lumps and to produce a uniform granular powder. The tablet machine was adjusted to 1/2 gm size tablet. The solid was filled into the feeder (hopper) of tablet machine whose dosing unit had been adjusted for compressing tablet having weight 0.5 gm. The weight of the tablets was checked and then the machine was adjusted accordingly. The prepared tablets were subjected to friability test to measure the ability of tablet to withstand handling and transportation using Roch Friabilator. 5 tablets were weighed (WO), then placed in friability drum, the apparatus was adjusted with the timer to 4min., and the speed at 25 r.p.m. At the end of the operation the tablets were removed, de-dusted and re-weighed (w). Friability B was expressed as a percentage loss in weight.

B = 100 (1-(w|wo)) = 1- (1.3 gm| 2.7 gm) = 0.52, thus the batch was accepted (the value should be 1.0 or less to be accepted).

Statistical analysis

We used mean ±SEM to express the data and One-way ANOVA followed by Tukey's post hoc. We also used $P < 0.05$ to find the difference in IC50 between different compounds. Analysis was done by using SPSS software update version and the graphs were draw by GraphPad Prism software v8.0.2.

Results of Anticancer assay of newly synthesized compounds

1. A-2780 ovarian carcinoma cells

The results of cytotoxicity assay (IC50) of newly synthesized compounds as compared with 5-FU against A-2780 ovarian carcinoma cells show that 5-FU, 7c, and 7a (0.50 ± 0.006, 0.50 ± 0.009, and 0.60 ± 0.009 respectively) have insignificant difference ($p>0.05$) and they have minimum IC50 as compared with others compounds. we found that 5b has lowered IC50 as compared with 5a and 5b and also it lowered IC50 than 3a-c, 4a-c, and 6a-c. See table (1) and figure (1)

3a-c compounds: There is significant difference ($p<0.05$) between 3a, 3b, and 3c as compared with other compounds.

4a-c compounds: There is insignificant difference ($p>0.05$) between 4a and 4b while there is significant difference ($p<0.05$) between them and other compounds except 5c where there is insignificant difference ($p>0.05$). There is significant difference ($p<0.05$) as compared 4c with other compounds.

5a-c compounds: There is significant difference ($p<0.05$) between 5a as compared with other compounds. There is significant difference ($p<0.05$) between 5b and other

compound except 7b where there is insignificant difference (p>0.05) between 5b and 7b. we found there are insignificant difference (p>0.05) between 5c, 4a, and 4b, while there is significant difference (p<0.05) between 5c and other compounds.

6a-c compounds: There is insignificant difference (p>0.05) between 6a and 6b, and significant difference (p<0.05) between 6a-c as compared with other compounds.

7a-c compounds: There is insignificant difference (p>0.05) between 7a and 7c and also between them and 5-FU. There is significant difference (p<0.05) between 7a-c and other compounds except 7b where is insignificant difference (p>0.05) between it and 5b.

See table (1) and figure (1)

2. MCF-7 breast carcinoma cells

The results of cytotoxicity assay (IC50) of newly synthesized compounds as compared with 5-FU against MCF-7 breast carcinoma cells show that 5-FU, 7c, and 7a (0.67 ± 0.006, 0.65 ± 0.003, and 0.65 ± 0.006 respectively) have insignificant difference (p>0.05) and they have minimum IC50 as compared with others compounds. we found that 5b has lowered IC50 as compared with 5a and 5b and also it lowered IC50 than 3a-c, 4a-c, 6a-c and 7b. See table (2) and figure (2)

3a-c compounds: There is insignificant difference (p>0.05) between 3b and 3c and also between them 5c. There is insignificant difference (p>0.05) between 3b with 5a but there is significant difference (p<0.05) between 3a-c and other than compounds.

4a-c compounds: There is significant difference (p<0.05) between 4a-c other compounds except there are insignificant difference (p>0.05) between 4b and 6c. we found that 4a has maximum IC50 as compared with 4b and 4c and also it higher IC50 than 3a-c, 5a-c, 6a-c and 7a-c.

5a-c compounds: There is significant difference (p<0.05) between 5a -c as compared with other compounds except There is insignificant difference (p>0.05) between 5a with 3b and 5c with 3c.

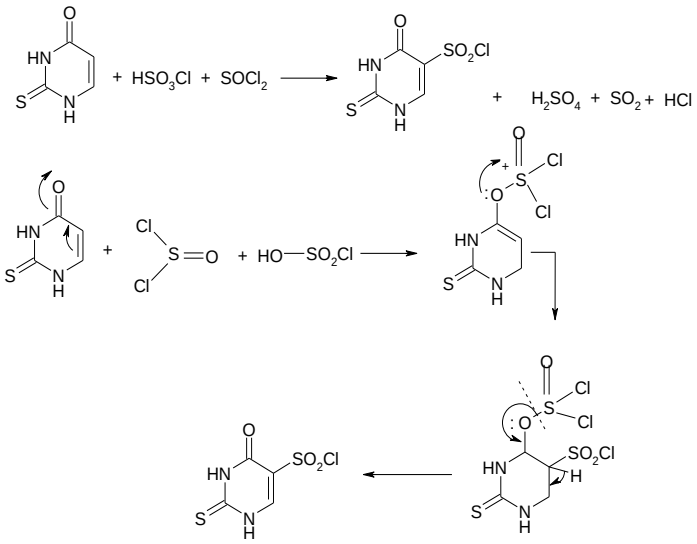
6a-c compounds: significant difference (p<0.05) between 6a-c as compared with other compounds.

7a-c compounds: There is insignificant difference (p>0.05) between 7a and 7c and also between them and 5-FU. There is significant difference (p<0.05) between 7a-c and another compounds.

See table (2) and figure (2)

DISCUSSION

2-Thiouracil is an activated pyrimidine compound, thus it suffers from the characteristic electrophilic substitution reactions of aromatic compounds, thus it could be chlorosulfonated at the 5th position using chlorosulfonic acid at 120 °C (17).To develop a more efficient procedure for preparing 2-thiouracil-5-sulfonyl chloride, gradient temperature technique is used, thus at 30°C chlorosulfonation was suppressed , the reaction started at about 40 °C. as the temperature was raised to 60,80,100, and 120 °C the reaction was optimized at 120 °C (61% yield). When the reaction mixture is poured on to ice to isolate the product, hydrolysis may occur thus lowering the yield.. To suppress the hydrolysis, the reaction mixture was poured on to a 1:1 mixture of ice and acetic acid. It was found that coupling of chlorosulfonic acid with thionyl chloride (volume ratio 1.5:1) increased the rate of formation and yield of 2. This augmented effect could be summarized as follow:



The produced sulfonyl chloride 2 was reacted with any of p-fluoro, chloro or bromobenzyl amine respectively in a S_N2 reaction in presence of pyridine as an acid scavenger yielding a moderately active anticancer agent against the used two-cell lines. Furthermore, these 2-thiouracilsulfonamides were chlorinated at the 4th position of 2-thiouracil by a mixture of $POCl_3$/ PCl_5 yielding chloropyrimidines 4a-c of weaker anticancer activity. Hydrazinolysis of 4a-c gave hydrazine derivatives 5a-c. Condensation of 5a-c with benzaldehyde gave Schiff's bases with weak activity, while their reaction with phenyl

isothiocyanate dramatically produced potent carbothioamide derivatives 7a-c of wide spectrum activity comparable to 5-fluorouracil. We measured the activity of new synthesized compounds by IC_{50} which defined as the concentration which results in 50% decrease in cell number as compared with that of the control structures in the absence of an inhibitor [22].

According to the foregoing statistical results, compounds 3a-c exhibited a moderate activity and nearly the same biological effect against ovarian carcinoma cells, where R= F,Cl and Br (IC_{50} 2.93, 3.13, and 3.77 respectively) but against breast carcinoma cells the three halogens of R =F, Cl, or Br modified the response. The IC_{50} (2.89, 3.88, and 3.95 respectively), when R = F activity was increased as compared to Cl and Br; therefore change of R group gave variable effect in the cytotoxic effect. all compounds were less active as compared to compounds 7a-c and 5-FU.

Compounds 4a-c and 6a-c showed the highest IC50 exhibiting the lowest activity against both ovarian and breast cancer. Thus, chlorination and formation of Schiff' bases at the 4th position may decrease or abolish the cytotoxic activity. Hydrazinolysis of chloropyrimidines 4a-c decreased IC_{50} (4.47, 1.60, and 4.98 respectively) and increased biological activity, however when R = Cl (5b) the compound become more effective than when R = F or Br. Compounds 7a-c exhibited a potent activity (IC_{50} 0.60, 1.50, and 0.50 respectively) vs 5-FU (IC_{50} 0.50) and nearly as potent as 5-FU against both ovarian and breast carcinoma cells. The biological activity of 7a-c against breast cancer were (IC_{50} 0.65, 3.51, and 0.65 respectively). Thus, 7c is more active than 7a and 7b.

CONCLUSION

2-Thiouracil-5-sulfonamides showed variable activity against a human A-2780 ovarian and MCF-7 breast carcinoma cells. Substitution at 4th position may decrease or inactivate the anticancer activity except in the case of introducing of a hydrazine carbothioamide group which potentiate the activity against carcinoma cells in comparison to 5-fluorouracil when used as a reference standard.

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Table 1: Anticancer activity (IC50) of new synthesized compounds and 5-FU against A-2780 ovarian carcinoma

Compound	Mean ± Std. Error	Std. Deviation	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
3a	2.93 ± 0.006	0.010	2.90	2.95
3b	3.13 ± 0.006	0.0100	3.11	3.15
3c	3.77 ± 0.006	0.010	3.74	3.79
4a	4.91 ± 0.018	0.032	4.83	4.99
4b	4.95 ± 0.03	0.043	4.84	5.06
4c	4.67 0.003	0.006	4.66	4.69
5a	4.47 ± 0.003	0.006	4.46	4.49
5b	1.60 ± 0.006	0.010	1.57	1.62
5c	4.98 0.006	0.010	4.95	5.01
6a	5.34 ± 0.003	0.006	5.33	5.36
6b	5.46 ± 0.006	0.010	5.43	5.48
6c	5.76 0.006	0.010	5.73	5.78
7a	0.60 ± 0.009	0.015	0.56	0.64
7b	1.50 ± 0.130	0.225	0.94	2.06
7c	0.50 ± 0.009	0.015	0.46	0.54
5-FU	0.50 ± 0.006	0.010	0.47	0.52

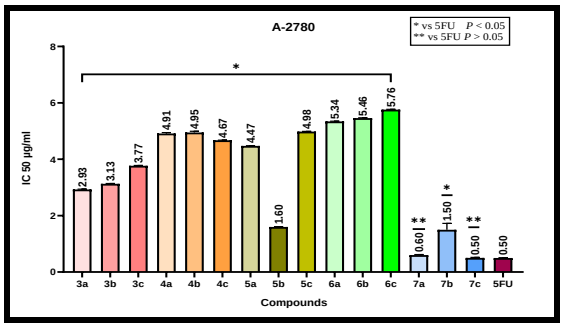

Figure 1: Anticancer activity (IC50) of new synthesized compounds and 5-FU against A-2780 ovarian carcinoma.

Table 2: Anticancer activity (IC50) of new synthesized compounds and 5-FU against MCF-7 breast carcinoma cells

Compounds	Mean ± Std. Error	Std. Deviation	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound
3a	2.89 ± 0.006	.01000	2.8652	2.9148
3b	3.88 ± 0.006	.01000	3.8552	3.9048
3c	3.95 ± 0.025	.04359	3.8417	4.0583
4a	5.98 ± 0.006	.01000	5.9552	6.0048
4b	4.18 ± 0.006	.01000	4.1552	4.2048
4c	4.60 ± 0.006	.01000	4.5752	4.6248

5a	3.80 ± 0.006	.01000	3.7752	3.8248
5b	2.75 ± 0.006	.01000	2.7252	2.7748
5c	3.92 ± 0.006	.01000	3.8952	3.9448
6a	4.07 ± 0.006	.01000	4.0452	4.0948
6b	4.89 ± 0.006	.01000	4.8652	4.9148
6c	4.24 ± 0.006	.01000	4.2152	4.2648
7a	0.65 ± 0.006	.01000	.6252	.6748
7b	3.51 ± 0.000	.00000	3.5100	3.5100
7c	0.65 ± 0.003	.00577	.6423	.6710
5-FU	0.67 ± 0.006	.01000	.6452	.6948

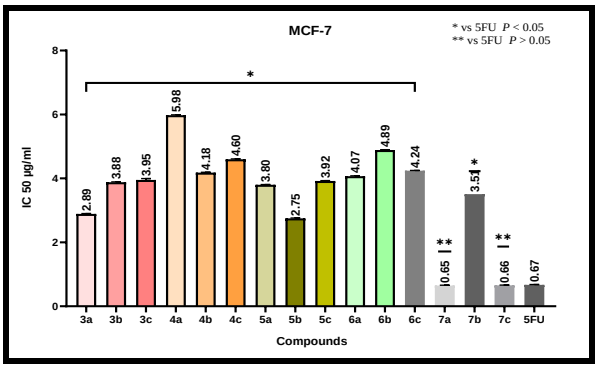

Figure 2: Anticancer activity (IC50) of new synthesized compounds and 5-FU against MCF-7 breast carcinoma cells