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SYNTHESIS, ANTICANCER ACTIVITY AND MOLECULAR DOCKING STUDY OF SOME NOVEL 2-THIOURACIL SULFONAMIDE DERIVATIVES

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ABSTRACT

A novel series of 2-thiouracil sulfonamide derivatives (3 - 13) were synthesized; and their chemical structures were confirmed by spectral and elemental analyses. All compounds were tested for anti-cancer activity by SRB assay method against Breast (MCF7) and Colon (CaCo-2) cell lines. Most of the tested compounds are active against two cell lines in comparison to 5- fluorouracil. Compound 9 was the most potent against CaCo-2 and MCF7 cell lines (IC50=2.82 and 2.92 µg/mL, respectively). Molecular docking studies for the best active compounds were performed on the active site of c-kit protein tyrosine kinase (PTK). All of the docked compounds exhibited proper fitting on the active site of the c-kit tyrosine kinas with fitness score range from 65.13±0.17 to 69.55±0.39 KJ/mol. The most active compound 9 showed high fitness score (69.55±0.39 KJ/mol).

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Introduction

Pyrimidine derivatives exhibited a wide range of biological activities. 5-Fluorouracil (5-FU) I, Tegafur, Thioguanine [1] are examples of drugs containing pyrimidine nucleus and were used as anticancer agents. Hydrazinopyrimidine-5-carbonitriles [2], pyrazolo [1,5-a] pyrimidine [3], thiazolo [5,4-d] pyrimidine[e] [4,5] and pyrido [3,2-d] pyrimidines [5] were reported as antitumor agents. Moreover, the thiouracil derivatives are of particular significance in medicinal chemistry due to their broad scope of remarkable antiviral, anticancer, and antimicrobial activities [7-15]. For example, S-alkylation and N^3-alkylation derivatives have been reported as novel cytotoxic agents [16, 17]. 2-{3-[4-(4-Chlorophenyl)-piperazin-1-yl]-3-oxo-propylsulfanyl}-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile II is displayed promising anticancer activity against breast cancer cell line (MCF-7), inhibited all five kinases, namely pim1 , pim2, GSK-3b, EGFR, and CDK5/p25 and showed selectivity for pim1 kinase [18]. Reports revealed that 5-cyano-2-thiouracils and thiouracils with sulfonamide exerted promising anticancer activity against most human cancers e.g. breast (MCF-7), colon (HCT-116, HT-29, and CaCo-2), leukemia (MOLT-4), liver (HEPG-2), renal cancer (HELa) and cervical cancer [19-22]. For example, novel (2-pyrimidinylthio) acetyl benzene sulfonamides III were reported as human carbonic anhydrases inhibitors [23]. Sulfonamides are a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications. A series of novel sulfonamide derivatives have been reported as antitumor agents [24-27]. For example, numerous sulfonamides IV and V were reported as tyrosine kinase (TK) inhibitors [28]. From the previous findings of biological effectiveness of derivatives containing thiouracil moiety, pyrimidine base, or sulfonamide (SO_2 NH_2) group and in continuation for our research program on the synthesis of novel compounds exhibiting anticancer activity, we have

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herein synthesized a new series of 2-thiouracil derivatives containing sulfonamide and evaluating the anticancer activities against two human cancer cell lines Breast (MCF7) and Colon (CaCo-2) using SRB assay.

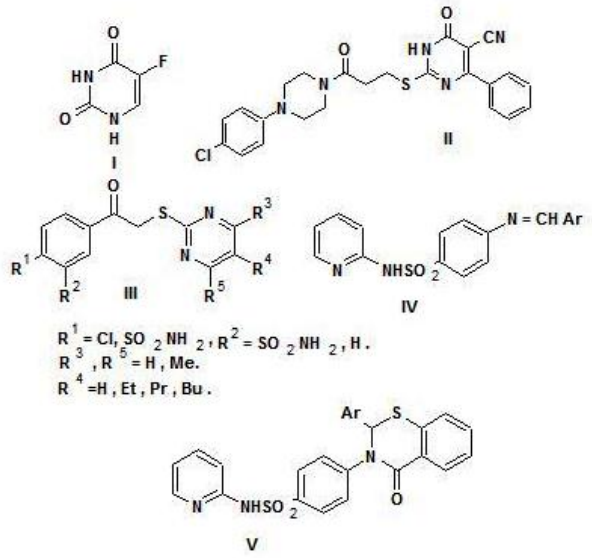
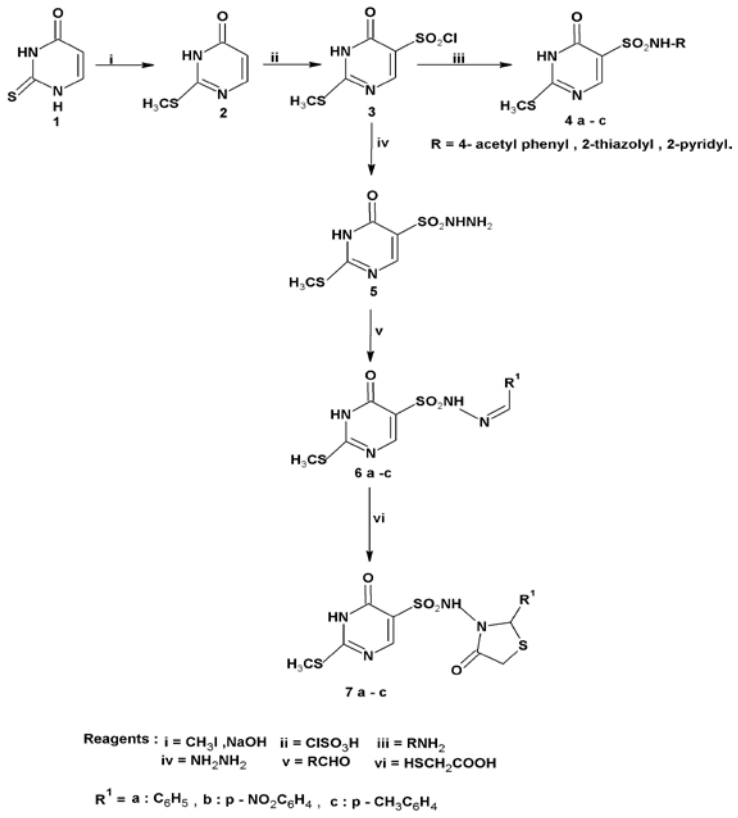


Fig.1. Structure of some active antitumor agents

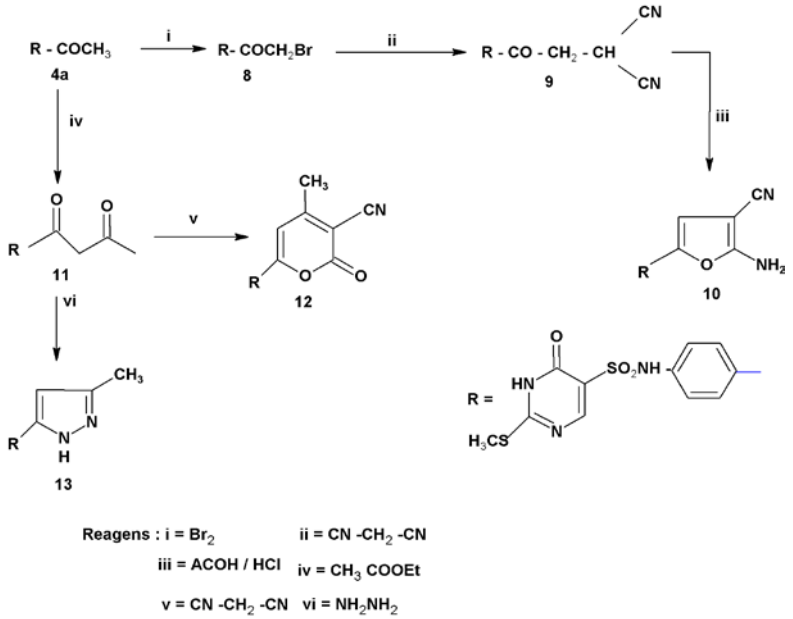
Materials and Methods

Experimental

All melting points were uncorrected and determined using capillary tube on a Boetius melting point microscope. Mass spectra were recorded at 70 eV Finigan SSQ 7000 Mass spectrometer. IR spectra were recorded as potassium bromide pellets on a Beckmann infra-red spectrophotometer PU9712 using KBr discs. ^{1}HNMR spectra were performed on a Joel EX 270 MHz spectrometer against TMS as an internal reference. Microanalyses were performed using Vario, Elmentar apparatus (Shimadzu, Japan), Micro analytical unit, Cairo University. All reactions were checked by TLC. All new compounds yielded spectral data consistent with the proposed structure and microanalysis within ±0.3 % of the theoretical values (Tabe1). Target compounds were synthesized as outlined in Schemes 1 and 2.



Scheme 1: Synthesis of compounds **1-7**



Scheme 2: Synthesis of compounds **8 - 13**

Table 1: Physical and analytical data of newly prepared compound

Comp. No.	Yield%	M. P. °C	Mol. Formula (M.wt.)	*Analysis Calcd./ found (%)		
				C	H	N
3	78	238-9	$C_5H_5ClN_2O_3S_2$ (240.69)	24.95 24.88	2.09 2.16	11.64 11.48
4a	87	287-8	$C_{13}H_{13}N_3O_4S_2$(339.39)	46.01 45.98	3.86 3.75	12.38 12.48
4b	88	297-8	$C_8H_8N_4O_3S_3$(304.37)	31.57 31.67	2.65 2.87	18.41 18.39
4c	77	301-2	$C_{10}H_{10}N_4O_3S_2$(298.34)	40.26 40.35	3.38 3.29	18.78 18.59
5	79	307-8	$C_5H_8N_4O_3S_2$(236.27)	25.42 25.45	3.41 3.39	23.71 23.87
6a	81	289-90	$C_{12}H_{12}N_4O_3S_2$(324.38)	44.43 44.33	3.73 3.63	17.27 17.17
6b	78	307-8	$C_{12}H_{11}N_5O_5S_2$(369.38)	39.02 39.12	3.00 3.21	18.96 18.88
6c	83	286-7	$C_{13}H_{14}N_4O_3S_2$(338.41)	46.14 46. 24	4.17 4.27	16.56 16.66
7a	71	312-3	$C_{14}H_{14}N_4O_4S_3$(398.48)	42.20 42.15	3.54 3.65	14.06 14.19
7b	69	278-9	$C_{14}H_{13}N_5O_6S_3$(443. 48)	37.92 37.89	2.95 2.89	15.79 15.88
7c	65	267-8	$C_{15}H_{16}N_4O_4S_3$(412.51)	43.67 43.78	3.91 3.79	13.58 13.45
8	68	258-9	$C_{13}H_{12}BrN_3O_4S_2$(418.29)	37.33 37.45	2.89 2.95	10.05 10.12
9	77	272-3	$C_{16}H_{13}N_5O_4S_2$(403.44)	47.63 47.48	3.25 3.19	17.36 17.34
10	75	265-6	$C_{16}H_{13}N_5O_4S_2$(403.44)	47.63 47.59	3.25 3.33	17.36 17.45
11	61	291-2	$C_{15}H_{15}N_3O_5S_2$(381.43)	47.23 47.17	3.96 4.01	11.02 11.11
12	64	254-5	$C_{18}H_{14}N_4O_5S_2$(430.46)	50.22 50.34	3.28 3.19	13.02 13.18
13	68	276-7	$C_{15}H_{15}N_5O_3S_2$(377.44)	47.73 47.80	4.01 4.21	18.55 18.45

*C, H and N ARE WITHIN THE LIMIT OF ± 0.3%

Synthesis of S-methyl-2-thiouracil (2)

It was prepared as in literature [29] by the reaction of 2-thiouracil 1 with methyl iodide in sodium hydroxide solution, m.p 323-325 ^{O}C (lit.325^{O}C).

Synthesis of 2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-sulfonyl chloride (3)

A mixture of S-methyl-2-thiouracil **2** (12.5g,0.055 mole) and chlorosulphonic acid (51 mL ,0.055 mole) was heated at 120 ^{o}C for 8 hours, then the reaction mixture was cooled and poured on ice. The precipitate was filtered, dried under suction and used as crude for subsequent work.

IR (KBr) υ (cm^{-1}): 3297(NH), 2980(CH-aliphatic), 1670(C=O), 1460(CH_3-S),1320,1130 (SO_2). MS (EI) m/z:240 ($M^{+\cdot}$17.3%,242 (M+2,5.7%%). ^{1}HNMR (DMSO-d_6) δ (ppm): 2.6(3H,s,H_3C-S),8.2(1H,s,pyrimidine),10 (1H,s,NH,D_2O exchangeable).

General procedure for the synthesis of 4-oxo-2-(methylthio)-1,2,3,4-tetrahydropyrimid-ine-5-sulphonic Acid-N-(substituted) amides (4a-c)

A mixture of **3** (0.005 mole), the appropriate amine (0.005 mole) and pyridine (0.005 mole) in 50 mL absolute ethanol was heated under reflux for 12-17 hours, then cooled, filtered off, dried under suction and recrystallized from DMF / water.

4-Oxo-2-(methylthio)-1,2,3,4-tetrahydropyrimidine-5-sulphonicacid-N-(4-acetylphenyl) amide (4a)

IR (KBr) υ (cm^{-1}): 3288(NH), 2970(CH-aliphatic), 3150 (CH-aromatic),1670, 1718(2C=O), 1469(CH_3-S),1321,1135(SO_2). MS (EI) m/z:339(M^+,34.6%). ^{1}HNMR(DMSO-d_6) δ (ppm): 2.6 (3H,s,H_3C-S),2.5 (3H,s,CH_3-CO),7.1,7.5(4H,dd,aromatic), 8.3(1H,s,pyrimidine),10.1, 10.2(2H,s,NH ,D_2Oexchangeable).

4-Oxo-2-(methylthio)-1,2,3,4-tetrahydropyrimidine-5-sulphonicacid-N-(2-thiazolyl)amide (4b)

IR (KBr) υ (cm^{-1}): 3268(NH), 2977(CH-aliphatic), 1670(C=O), 1467(CH_3-S),1327,1133 (SO_2), MS(EI) m/z: 304(M^+,54.3%). ^{1}HNMR(DMSO-d_6)δ(ppm):2.2(3H,s,H_3C-S),6.9,7.1(2H,s,thiazole),8.3(1H,s,pyrimidine),10.3,10.5(2H,NH,D_2O exchangeable).

4-Oxo-2-(methylthio)-1,2,3,4-tetrahydropyrimidine-5-sulphonicacid-N-(2-pyridyl) amide(4c)

IR (KBr) υ (cm^{-1}): 3298(NH), 2989(CH-aliphatic), 1686(C=O), 1322, 1136 (SO_2), 1469 (CH_3-S). MS(EI) m/z: 298(M^+, 45.7%). ^{1}HNMR(DMSO-d_6) δ (ppm):2.4(3H,s,H_3C-S),6.8-7.2(4H,m,pyridine),8.2(1H,s,pyrimidine),10.1,10.3(2H,s,NH, exchangeable with D_2O).

Synthesis 2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-sulfonohydrazide (5)

A mixture of **3** (0.005 mole), hydrazine hydrate (0.005 mole) and pyridine (0.005 mole) in 50 mL absolute ethanol was heated under reflux for 13 hours, then cooled, filtered off, dried under suction and recrystallized from DMF / water.

IR (KBr) υ (cm^{-1}): 3400-3200(NH,NH_2) ,2956 (CH-aliphatic),1669(C=O),1324,1137(SO_2),1464(CH_3-S). MS (EI) m/z: 236 (M^+,39.8%).^{1}HNMR(DMSO-d_6) δ (ppm):2.5(3H, s,H_3C-S), 3.1,6.1(3H,s,NH,NH_2,D_2Oexchangeable),8.2(1H,s,pyrimidine), 9.8(1H,s, NH, exchangeable with D_2O).

General procedure for the synthesis of 2-(methylthio)-6-oxo-N'-[(Z)-arylmethylidene]-1,6-dihydropyrimidine-5-sulfonohydrazide(6a-c).

A mixture of **5** (0.005mole) and the appropriate aromatic aldehyde (0.005mole) namely benzaldehyde, 4-nitrobenzalaldehyde and 4-methyl benzaldehyde, respectively in ethanol (50 mL) was heated under reflux for 8-12 hrs, then cooled, filtered off and recrystallized from DMF/ water.

2-(Methylthio)- 6-oxo-N'- [(1Z)- phenylmethylene] -1,6-dihydropyrimidine-5-sulfonohydrazide (6a)

IR (KBr) υ (cm^{-1}): 3268(NH), 2984(CH-aliphatic), 3190(CH-aromatic),1666 (C=O),1464(CH_3-S),1320,1137(SO_2). MS (EI) m/z:324(M^+,44.8%). ^{1}HNMR(DMSO-d_6) δ (ppm):2.4(3H,s,H_3C-S),7.0-7.3(5H,m,aromatic),8.2(1H,s,pyrimidine), 8.8(1H,s, N=CH),10.2,10.3 (2H,s, NH,D_2Oexchangeable) .

2-(Methylthio)-N'-[(1Z)-(4-nitrophenyl)methylene]-6-oxo-1,6-dihydropyrimidine-5-sulfonohydrazide (6b)

IR (KBr) υ (cm^{-1}): 3267(NH),2998(CH-aliphatic),3180(CH-aromatic),1687(C=O), 1461 (CH_3-S), 1350,1550(NO_2), 1325,1130(SO_2). MS (EI) m/z: 369 (M^+,62.9%). ^{1}HNMR (DMSO-d_6) δ (ppm): 2.3 (3H,s,H_3C-S), 7.2,7.3 (4H,dd,aromatic), 8.1(1H,s, pyrimidine), 8.8 (1H,s, N=CH), 10.1, 10.3 (2H,s,NH,D_2O exchangeable).

N'-[(1Z)-(4-methylphenyl)methylene]-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-sulfonohydrazide (6c)

IR (KBr) υ (cm^{-1}):3293(NH),2977(CH-aliphatic),3150(CH-aromatic),1678(C=O),1467(CH_3-S),1319,1130(SO_2).MS(EI)m/z: 338(M^+,59.7%). ^{1}HNMR(DMSO-d_6) δ (ppm): 2.3,2.5 (6H,s,2H_3C),7.1,7.4(4H,dd,aromatic),8.1 (1H,s,pyrimidine),8.9 (1H,s,N=CH) ,10.1,10.4 (2H, s, NH, D_2O exchangeable).

General procedure for the synthesis of 2-methylthio-6-oxo-1,6-dihydropyrimidine-5-sulfonic acid (4-oxo-2-substituted-phenyl-thiazolidin-3-yl)-amides (7a-c).

A mixture of **6a-c** (0.005 mole) and mercapto-acetic acid (0.005 mole) was heated under reflux in dry benzene (100 ml) for 8-10 hrs. The volatile solvent was evaporated and the reaction mixture was neutralized with cold dilute sodium bicarbonate solution, the formed product was filtered off and recrystallized from DMF/ water.

2-Methylthio-6-oxo-1,6-dihydropyrimidine-5-sulfonicacid (4-oxo-2-phenyl-thiazolidin-3-yl)-amide (7a)

IR (KBr) υ (cm^{-1}): 3287(NH),2970(CH-aliphatic),3190(CH-aromatic),1674,1688(2C=O),1467(CH_3-S),1321,1136(SO_2).

MS (EI) m/z:398(M^+,52.8%).^{1}HNMR (DMSO-d_6) δ (ppm): 2.3(3H,s,H_3C-S),2.9(2H,s,CH_2),5.6(1H,s,thiazolidine),6.9-7.3(5H,m,aromatic), 8.2(1H,s, pyrimidine),10.1,10.2(2H,s,NH, exchangeable with D_2O).

2-Methylthio-6-oxo-1,6-dihydropyrimidine-5-sulfonic acid (4-oxo-2-(4-nitrophenyl)-thiazolidin-3-yl)-amide (7b)

IR (KBr) υ (cm^{-1}): 3266(NH), 2970(CH-aliphatic), 3150(CH-aromatic), 1672,1681(2C=O),1465(CH_3-S),1360,1540(NO_2), 1325,1137(SO_2). MS(EI)/z:443(M^+,50.4%).^{1}HNMR(DMSO-d_6)δ(ppm):2.5(3H,s,H_3C-S),2.8(2H,s,CH_2),5.5(1H,s,thiazolidine), 7.1,7.3(4H,dd, aromatic), 8.2(1H,s,pyrimidine),10.2,10.3(2H,s,NH exchangeable with D_2O).

2-Methylthio-6-oxo-1,6-dihydropyrimidine-5-sulfonic acid (4-oxo-2-(4-methylphenyl)-thiazolidin-3-yl)-amide (7c)

IR (KBr) υ (cm^{-1}):3267(NH),2956(CH-aliphatic),3180,(CH-aromatic),1670,1685 (2C=O), 1467(CH_3-S),1324,1136(SO_2). MS (EI) m/z: 412(M^+,47.8%). ^{1}HNMR (DMSO-d_6) δ (ppm): 2.3(3H, s,CH_3), 2.5(3H,s,H_3C-S), 2.8(2H,s,CH_2), 5.5(1H,s, thiazolidine),7.1,7.3(4H, dd, aromatic),8.3(1H,s,pyrimidine),10.1,10.3(2H,s,NH exchangeable with D_2O).

Synthesis of N-[4-(bromoacetyl)phenyl]-2-(methylthiol)-6-oxo-1,6-dihydropyrimidine -5-sulfonamide (8)

A mixture of **4a** (1.13g, 0.005 mole) and bromine (0.2 ml, 0.005 mole) in 30 mL glacial acid was stirred at room temperature for 48 hours, the precipitate was filtered off, the filtrate was neutralized with ammonia The precipitate was collected, filtered off, dried under suction and recrystallized from DMF/ water.

IR (KBr) υ (cm^{-1}): 3276(NH),2967(CH-aliphatic), 3160,(CH-aromatic)1680,1720 (2C=O), 1465(CH_3-S),1324,1136(SO_2). MS (EI) m/z: 418(M^+,55%),420(M+2,55.4%). ^{1}HNMR (DMSO-d_6) δ (ppm): 2.5(3H,s ,H_3C-S),3.5(2H,s,CH_2-CO),7.2,7.5 (4H,dd,aromatic),8.2 (1H, s, pyrimidine),10.1,10.2(2H,s,NH exchangeable with D_2O).

Synthesis of N-[4-(3,3-dicyanopropanoyl)phenyl]-2-(methylthio)-4-oxo-1,6-dihydropyrimidine-5-sulfonamide (9)

A solution of equimolar amounts of **8** (0.005 mole) and malononitrile (0.005 mole) in ethanol (50 mL) was treated with sodium hydroxide (2g ,50 mL water) dropwise with constant stirring. After complete addition, the reaction mixture was diluted with water (50 mL) and the solid product, so formed was collected and recrystallized from DMF/ water.

IR (KBr) υ (cm^{-1}): 3285(NH),2980(CH-aliphatic), 3190(CH-aromatic),2222(2CN),1678,1722(2C=O), 1465(CH_3-S),1322, 1130 (SO_2). MS (EI) m/z:403(M^+,56%). ^{1}HNMR(DMSO-d_6) δ (ppm):2.4(3H,s,H_3C-S),2.6(2H,d,CH_2),5.4(1H,t,CH),7.0, 7.3(4H,dd,aromatic),8.2(1H,s, pyrimidine)10.1,10.3(2H,s,NH,exchangeable with D_2O).

Synthesis of N-[4-(5-amino-4-cyanofuran-2-yl) phenyl]-2-(methylthio)-4-oxo-1,6-dihydropyrimidine-5-sulfonamide (10)

A mixture of **9** (0.005 mole) in acetic acid (20mL) and conc. HCl (5 mL) was refluxed for 2 hours. The reaction mixture was cooled and diluted with water. The formed precipitate was filtered off and recrystallized from DMF/ water.

IR (KBr) υ (cm^{-1}): 3288(NH, NH_2), 2969(CH-aliphatic), 3150(CH-aromatic), 2224(CN), 1678,(C=O),1322,1130(SO_2). MS (EI) m/z: 403(M^+, 45.7%). ^{1}HNMR (DMSO-d_6) δ (ppm): 2.3(3H,s, H_3C-S),5.1(2H,s,NH_2,exchangeable with D_2O),7.1-7.3 (5H,m,aromatic),8.1 (1H,s,pyrimidine),10.1,10.3(2H,s,NH, exchangeable with D_2O).

Synthesis of 2-(methylthio)-4-oxo- N-[4-(3-oxobutanoyl)phenyl]-1,6-dihydrop- yrimidine-5-sulfonamide (11)

A solution of **4a** (0.01 mole), in ethyl acetoacetate (50 mL) was slowly added to small pieces of sodium metal (4g). When the initial reaction subsided, the reaction mixture was refluxed for 8 hours then left to cool. 5mL of methanol was added to destroy excess sodium. Then the mixture was poured into water and acidified with acetic acid. The separated oily layer was collected and dried over anhydrous sodium sulphate to give **11** as an oil product.

IR (KBr) υ (cm^{-1}): 3288(NH), 2967 (CH-aliphatic), 3190(CH-aromatic),1678,1712,1730 (3C=O), 1322,1130 (SO_2). MS (EI) m/z:381(M^+,53.9%). ^{1}HNMR (DMSO-d_6) δ (ppm): 2.5(3H,s,CH_3-CO), 2.8 (3H,s,H_3C-S),3.7(2H,s,CH_2), 7.1,7.3 (4H, dd, aromatic), 8.1(1H,s, pyrimidine),10.1,10.4 (2H,s,NH exchangeable with D_2O).

Synthesis of N-[4-(3-cyano-4-methyl-2-oxo-2H-pyran-6-yl)phenyl]-2-(methylthio)-4-oxo-1,6-dihydropyrimidine-5-sulfonamide (12)

A mixture of **11** (0.005 mole), malononitrile (0.003 mole) and ammonium acetate (0.005 mole) in glacial acetic acid (30 mL) was refluxed for 4 hours and the reaction mixture was allowed to cool. The separated solid was filtered off and recrystallized from DMF/ water.

IR (KBr) υ (cm^{-1}):3249(NH,b),2979,CH-aliphatic),3185(CH-aromatic),2225(CN), 1678,1734 (2C=O), 1327,1139 (SO_2). MS (EI) m/z:430(M^+,34.9%). ^{1}HNMR(DMSO-d_6)δ(ppm):2.4(3H,s,H_3C-S),4.7(3H,s,CH_3),7.1-7.5(5H,m,aromatic),8.1(1H, s,pyrimidine), 10.1,10.4(2H,s,NH exchangeable with D_2O).

Synthesis of N-[4-(3-methyl-1H-pyrazol-5-yl)phenyl]-2-(methylthio)-4-oxo-1,6-dihydro pyrimidine-5-sulfonamide (13)

A mixture of **11** (0.005 mole) and 99% hydrazine hydrate (0.02 mole) in acetic acid (50 mL) was refluxed for 7 hours. The reaction mixture was concentrated to 1/4 its volume and left to cool. The obtained precipitate was filtered off and recrystallized from DMF/ water.

IR (KBr) υ (cm^{-1}):3287(NH),2976(CH-aliphatic),3170(CH-aromatic),1678(C=O),1321,137(SO_2). MS(EI) m/z:377(M^+,47.3%). ^{1}HNMR(DMSO-d_6) δ (ppm):2.6(3H,s,H_3C-S),2.8(3H,s,CH_3),7.1-7.3 (5H,m,aromatic),8.1 (1H,s,pyrimidine),9.5,10.1,10.4 (3H,s,NH exchangeable with D_2O).

Anti-Cancer Screening

Experimental

Measurement of Potential Cytotoxicity by (SRB) assay

The cytotoxic activity of the new thiouracil derivatives (3-13) was measured on breast tumor cell line (MCF-7) and colon tumor cell line (CaCo-2) obtained from pharmacology screening unit of the National Cancer Institute (NCI), Cairo University, Egypt, following the Sulfo Rhodamine-B-stain (SRB) assay method [30] in comparison to the known anticancer drugs: 5-flurouracil.

SRB cytotoxic assay

Growing cells were plated in 96-multiwell plate (104cells/well) for 24 hr. Different concentrations of the tested compounds (0, 1, 2.5, 5, 10 µ g/ml) were added to the cell monolayer. Cells were exposed to the tested compounds for 48 hrs at 37°C and in an atmosphere of 5% CO_2 and subsequently fixed, washed and stained with Sulfo-Rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity is measured using an ELISA reader at a wavelength of 570 nm. Results are expressed as means of at least three independent experiments e. The results are expressed as growth inhibition of 50 % (IC_{50}) of cells (Table **2**).

Table2. IC_{50} values [a] (in µg/mL) for cytotoxic activity of the compounds against CaCo-2 and MCF7 cells by SRB assay

Compounds	Cell Lines	
	CaCo-2	MCF7
3	-	-
4a	15.16±1.12	-
4b	13.14±1.51	-
4c	19.27±1.85	-
5	38.55±1.40	28.59± 0.68
6a	6.21±2.05	10.42±1.901
6b	8.28±0.62	16.25±1.95
6c	8.36±0.74	15.18±1.085
7a	4.09±0.65	3.98±1.64
7b	6.02±1.20	7.15±3.40
7c	7.12±1.42	8.18±2.07
8	5.20±2.06	4.05±0.08
9	2.82±2.08	2.92±0.042
10	3.96±3.02	2.95±0.72
11	16.23±3.32	-
12	5.08±1.15	5.17±0.84
13	16.73±2.12	-
5-FU	2.80±0.024	2.6± 0.043

IC_{50} values (in µg/mL), is the concentration required for a 50 % of cell growth inhibition. Results are presented as a mean ± SEM of three independent experiments performed in duplicate.
5-FU: 5- Fluorouracil

Molecular docking study

Molecular structures of all compounds were generated using Spartan′14.28. The generated conformations of each compound were then re-minimized using a higher level of theory (Hartree-Fock, 3-21G). All possible conformations of compounds were saved as mol2 files for use in the docking studies.

Docking

Due to some conformational flexibility exhibited by these molecules, and the close energy differences between the observed conformers, we were interested in examining how a docking simulation would interpret the binding process from a variety of starting points [31]. Accordingly, rather than docking only the lowest energy structures, all the output conformations from the conformer distribution calculations of the compounds were prepared for docking using GOLD [32]. Crystal structures were retrieved from the RCSB protein data bank as PDB files (1T46 for c-kit tyrosine kinase in auto inhibited form [33], 1PKG for c-kit tyrosine kinase in active phosphorylated dimmer form) [34], (Fig. 2). The bound ligands were removed from the structures, and used only as a positional reference. Hydrogen atoms were added to protein residues using the default GOLD settings [32]. All solvent molecules were removed. Active site residues were chosen within a diameter of 15 Å. Gold Score was chosen as the most appropriate target function in the genetic algorithm to balance between computational speed and the reliability of the GOLD predictions for the possible conformations of the different substituted-2-thiouracil binding to c-kit tyrosine kinase. The genetic algorithm parameters were based on previous examples of docking hydrophobic ligands [35]. During the docking process, no limit was placed on the number of binding poses retained, though typically 3-10 solutions were retained by the genetic algorithm.

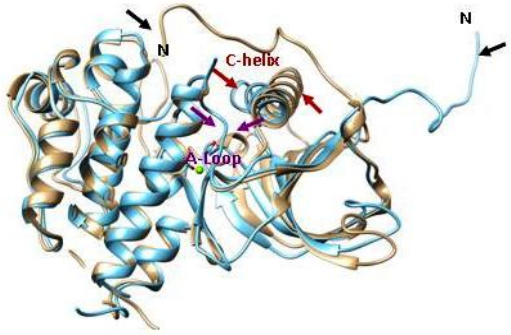


Fig.2. Structural overlay of C-α ribbon diagram c-kit tyrosine kinase holo-structures in both auto inhibited form (brown color) and active form (blue color). The colored arrows show the difference in orientation of the C-helix, A-loop, and amino-terminal juxta membrane domain respectively. The entire juxta membrane region is visible in this structure and inserts between the kinase N- and C-lobes, shifting the position of the C-helix, and sterically blocking the A-loop from attaining its extended active conformation. The auto inhibited A-loop folds back over the kinase C-Lobe and binds as a pseudo substrate. This diagram was produced by Chimera visualizing software.

Results and Discussion

Chemistry

The synthetic strategy to synthesize the target thiouracil sulfonamide derivatives 1- 13 is shown in Schemes 1 and 2. 2-Thiouracil (1) was s-alkylated by CH_3I in 10% NaOH to afford 2-methyl-thiouracil[29] (2) .2-Thiouracil 5-sulphonyl chloride 3 was obtained by refluxing 1 with chlorosulphonic acid at 120^{o}C. Many attempts were done to chloro sulphonate 2-thiouracil in analogous to uracil, but only O. A. Fathalla was the first one that able to prepare 2-thiouracil-5-sulphonyl chloride [36]. Reaction of the latter compound with three different aromatic amines namely 4-aminoacetophenone, 2-aminothiazole and 2-aminopyridine in ethanol containing pyridine as acid scavenger gave sulphonamides [37] 4a-c. Also, reaction of compound 3 with hydrazine hydrate gave sulfonohydrazide 5 which then was reacted with three aldehydes namely, benzaldehyde, p-nitrobenzaldehyde and p-tolaldehyde, respectively yielding sulfonyl hydrazones [20] 6a-c. Cyclocondensation of compounds 6a-c with thioglycolic acid afforded thiazolidinones [38] 7a-c as revealed in Scheme 1. Furthermore, monobromo acetyl derivative 8 was prepared by stirring of 4a with bromine in glacial acetic acid. Reaction of 8 with malononitrile in basic mediun [39] gave dicyano derivative 9. Cyclocondensation of 9 with acetic acid in HCl afforded a furan derivative 10. Also, compound 4a was refluxed with ethyl acetate giving a diketone 11 which was cyclocondensed with malononitrile or hydrazine hydrate giving α-pyrone 12 or a pyrazole derivative 13, respectively as revealed in Scheme 2. The structures of new compounds were confirmed by MS, IR, ^{1}H NMR, as well as elemental analysis. IR spectrum of compound 3 showed C=O band at 1670 cm^{-1} and a stretching NH band at 3297 cm^{-1}. The ^{1}H NMR spectra for 4a-c revealed two signal for NH proton. The IR spectrum of compound 5 showed two absorbance bands at 3400-3200 cm^{-1} corresponding $NHNH_2$. ^{1}H NMR spectrum showed three sing lets of $NHNH_2$ around 3.1 and 6.1 ppm. Also, the IR spectra of 6a-c showed the absence $NHNH_2$ as well as the presence of CH aromatic and ^{1}H NMR showed two signal for NH protons. Also, MS spectra gave their molecular ion peaks. The IR spectra of compounds 7a-c showed the presence of two signals corresponding to C=O of thiouracil and thiazolidine. Compounds 8 and 9 were confirmed by spectral data and the mass spectra studies of these compounds gave additional evidence for the proposed structures. The IR and ^{1}HNMR spectra of compound 10 were compatible with the proposed structure. The IR spectrum of 11 showed the presence of 2C=O group and ^{1}H NMR showed two singlet signals corresponding to two CH_3 group and two singlet corresponding to NH. The IR spectrum of compound 12 showed the presence of CN group. ^{1}H NMR for 13 showed singlet signals for two CH_3 and three NH. Also, MS spectra gave their molecular ion peaks.

Cytotoxic activity

The antitumor activity results indicated that most of the tested compounds exhibited considerable response on both cell lines in comparison to the known anticancer drug: 5 - fluorouracil. Table 2 showed that compounds 4a-c (with sulfonamide moiety in the molecule) (IC_{50}%= 15.16, 13.14 and 19.27 µg/mL, respectively), Compound 11 with (4-oxo-N-[4-(3-oxobutanoyl) phenyl) sulfonamide moiety) (IC_{50}=16.23µg/mL) and compound 13 (with 1H-pyrazol phenyl sulfonamide moiety) (IC_{50}=16.73 µg/mL) showed moderate anticancer activity against CaCo-2 cell line. Compounds 6a-c (with arylmethylidene sulfonohydrazide moiety) and compounds 7b and c showed moderate activity against CaCo-2 and MCF7cell lines. Compound 7a (with 2-phenyl-thiazolidin-4-one sulfonamide moiety) (IC_{50}=4.09 and 3.98 µg/mL, respectively), Compound 8 (with 4-bromoacetyl phenyl sulfonamide moiety) (IC_{50}=5.20 and 4.05 µg/mL, respectively), Compound 10 (with 5-amino-4-cyano furan phenyl sulfonamide moiety) (IC_{50}= 3.96 and 2.95 µg/mL, respectively) and compound 12 (with 3-cyano-α-pyrone phenyl sulfonamide moiety) (IC_{50}= 5.08 and 5.17 µg/mL, respectively) showed

significant cytotoxic activity against CaCo-2 and MCF7 cell lines. Compound 9 (with 3,3-dicyanopropanoyl phenyl sulfonamide moiety) showed high cytotoxic activity against CaCo-2 and MCF7 cell lines (IC_{50}=2.82 and 2.92 µg/mL, respectively). Compound 5 (with sulfonohydrazide moiety in the molecule) showed weak cytotoxic activity against CaCo-2 and MCF7 cell lines (IC_{50}%= 38.55 and 28.59 µg/mL, respectively) (Fig.3).

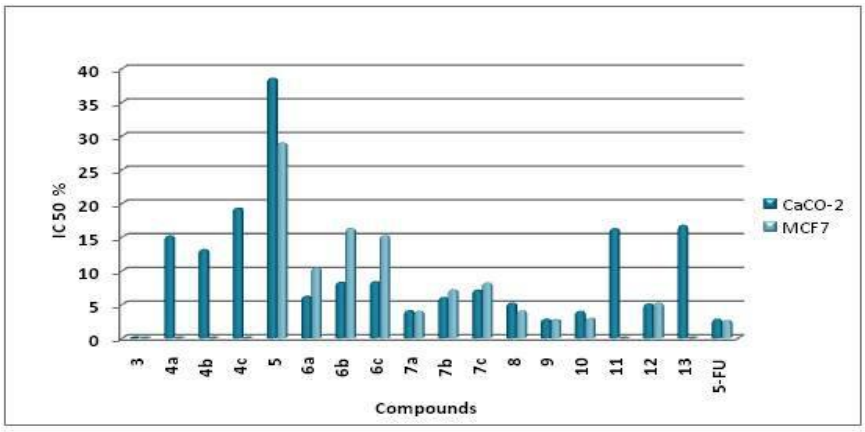


Fig. 3. Screening of anticancer activity of compounds **3-13** by the SRB assay shows that **9** has the highest activity (in comparison with the reference drug (RF). Each value represents a mean ± SEM (n = 3).

Structural-Activity Relationship (SAR).

From these observed results (table 2), we can conclude that the compounds exhibited wide range of variation of IC_{50} being [2.82-38.55] µg/ml. This indicates that SAR of these compounds mainly depends on their main structural feature of: phenyl-2-(methylthio)-4-oxo-1,6-dihydropyrimidine-5-sulfonamide which is considered as the pharmacophoric moiety. Whereas, the attached fragment being thiazolidin-4-one (7a), 4-bromoacetyl (8), 3, 3-dicyanopropanoyl (9), 5-amino- 4-cyano furan (10), 3-cyano- α-pyrone (12) play also a major role in structure activity relationship, generally enhancing group for the antitumor activity.

Molecular docking study

Previous literature shows that sulfonamides mechanism involves tyrosine kinase (TK) inhibition, and this was clearly reported [26, 28]. Depending on the above mentioned idea, herein we investigated the Auto Dock binding affinities of five compounds, containing four significant inhibitors 7a, 8, 10 and 12 and one high inhibitor 9 into c-kit protein tyrosine kinase (PTK). Towards optimization of the selected compounds of the significant antitumor activities, the advanced docking program GOLD [36] was used to evaluate the fitness score (KJ/mol) as potential inhibitors into the target TK macromolecule. Docking scores, amino acids interactions, hydrogen bond distances and short clashes are shown in Table 3. The docking results clearly show that all compounds exhibited proper fitting on the active site of the c-kit tyrosine kinas with fitness score range from 65.13±0.17 to 69.55±0.39 KJ/mol. The most active compound 9 exhibited high fitness score (69.55±0.39 KJ/mol), with hydrogen bonds formed with Cys788, Thr 670, and Lys623 (Fig. 4b). Compound 10 showed amino acid interactions with Glu 640, Asp810 and CYS673 with fitness score 68.64 KJ/mol. Fitness score of 7a, 8 and 12 are 68.40,65.13 and 65.35 (KJ/mol), respectively. Fig.5. illustrates differential binding affinities on docking of compound 9 and comparison of the highest docking poses of compound 9 conformation in both auto inhibited c-kit tyrosine kinase (ligand with orange color) and activated form (ligand with pink color). Compound 9 in active kinase form is forced into an alternative positioning, which sacrifices standard hydrogen bonds with the polar cluster side chains and significantly lose the contact with the main chains in A-loop and c-helix. Compound 9 in active form kinase is positioned closer to the Mg atom in binding pocket which create a steam of short contact with adjacent ligand sulfonyl group. Compound 9 in c-kit tyrosine kinase binding pocket in an auto-inhibited form according to the docking study; show that the majority of the binding pocket is represented in ribbon form that bound compound 9 into the kinase pocket stabilizing the conformational position of A-loop and c-helix (Fig.4a). Key hydrogen bonds are depicted with the stereo view of compound 9 (purple) binding to c-Kit and showing that bonds are formed with the hinge residue Cys788, gate keeper residue Thr670, and residue Lys623(Fig.4b). This binding mode of this compound in an auto-inhibited form may explain the promising activity against CaCo-2 cells and MCF7 cells as cited in biology results. As for the inactive inhibitor 3, compound 3 is a short molecule can't fill the pocket and stabilize the conserved residues in A-loop and c-helix demonstrating loss of binding interactions in both auto inhibited and active tyrosine kinases. Also, the important hydrogen bonds for maintaining the anti-TK activity, are almost absent (Fig. 6). Taken together, the above models reasonably interpreted the biological activity data. Finally, it can be seen from our docking study that the synthesized compounds might possibly act as TK inhibitors, and this may contribute at least in part, to the anticancer activity.

Table 3: Qualitative assessment of the docking calculations, binding interactions and short clashes

Compounds	Fitness score (KJ/mol)	Binding interaction	Short contacts (clashes)
7a	68.40±0.25	S-Glu640(2.9Å), N- Glu640(2.8Å) N- Asp810(2.6Å) CN-CYS673 (2.9Å)	S-Glu640 S-Lys623 S- Asp810 S-CYS809
8	65.13±0.17	S-Lys623(2.9Å, 3.0Å), S-Glu640(2.9Å) S- Asp810(2.9Å) O-Phe811(3.0Å)	S- Asp810 S- Asp810
9	69.55±0.39	CN-CYS788 (2.9Å), S-Lys623(2.9Å, 3.0Å), O-Thr670(2.9)	S- Asp810
10	68.64±0.23	S-Glu640(2.9Å), N- Asp810(2.6Å) N- Glu640(2.8Å) CN-CYS673 (2.9Å)	S-Val645 N- Asp810
12	65.35±0.23	O- Asp810(2.7Å) CN-CYS673 (3.0Å) S-CYS809 (2.7Å)	CN-CYS673 O-Phe811

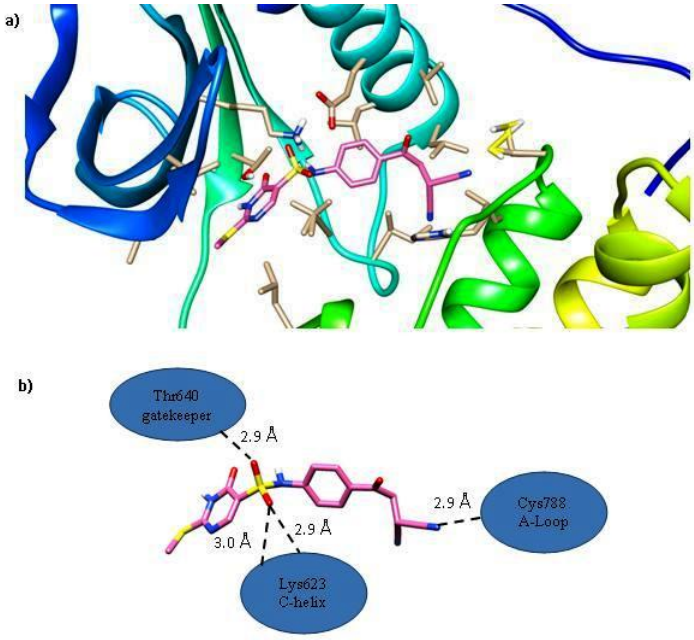


Fig.4. Hydrogen bonding characteristics of compound **9** in c-kit tyrosine kinase binding pocket in an auto-inhibited form according to the docking study

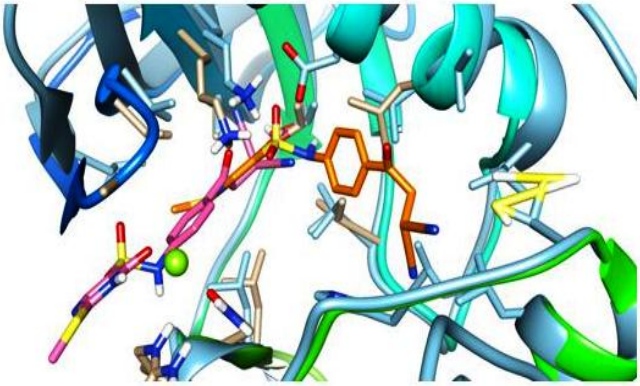


Fig.5: Comparison of the highest score docking poses of compound **9** conformation in both auto inhibited c-kit tyrosine kinase (ligand with orange color) and activated form (ligand with pink color).

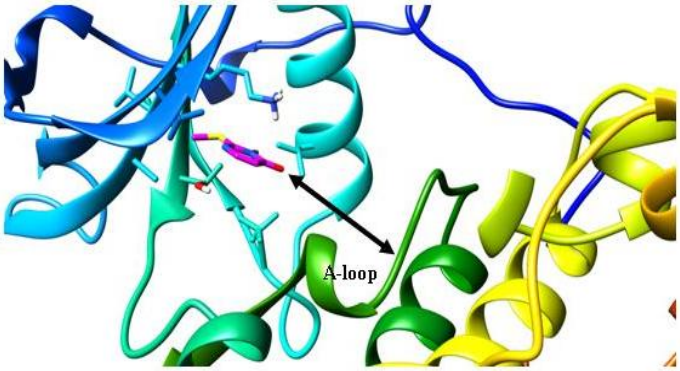


Fig.6. The highest score docking pose of compound **3** docked into binding pocket of the c-kit tyrosine kinase auto inhibited form

Conclusion

In this study, some novel thiouracil sulfonamide derivatives 3-13 were prepared. Most of compounds exhibited considerable response on CaCo-2 and MCF7 in comparison to the known anticancer drug: 5 - fluorouracil. Compound 9 was the most potent against CaCo-2 and MCF7 (IC_{50}=2.82 and 2.92 µg/mL, respectively). Also, the Auto Dock investigation of analogs, 3, 7a, 8, 9, 10 and 12 was carried out for molecular modeling study. Thus, they were docked within c-kit protein tyrosine kinase. The most active compound 9 exhibited high fitness score (69.55±0.39 KJ/mol) and hydrogen bonds formed with Cys788, Thr670, and Lys 623.

Finally, further studies are still needed to identify the SAR of theses derivatives as PTK inhibitors and to improve both the kinase inhibitory activity and the cytotoxic activity of these derivatives.

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