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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 2-THIOURACIL-5-SULFONAMIDE ISOSTERES AS ANTICANCER AGENTS

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ABSTRACT

The present study involved the development of novel 2-thiouracil-5-sulfonamide isosteres. Reaction of 5-hydroxyl methyl -2-thiouracil 1 with thionyl chloride yielded the targeted chloromethyl analogue 2 which was the starting compound for the synthesis of targeted compounds. Compound (2) was reacted with a series of aromatic amines to give amino derivatives 3a-e. The acetyl derivative (3d) was reacted with ethyl cyanoacetate, and a series of aromatic aldehydes in presence of excess ammonium acetate yielded pyridones (4a- e). Also, a series of thiosemicarbazones were obtained via reaction of the compound 3d with alkyl thiosemicarbazides 5a-e. Moreover, the compound 3d was monobrominated with bromine in acetic acid to give a bromo derivative 6 which in turn was reacted with thiosemicarbazones of some aldehydes to give the corresponding thiazole derivatives 7a-e. All new compounds and their chemical isosteres 2-thiouracil-5-sulfonamides were tested for possible anti-cancer activity against MCF-7 and HEPG-2 cell lines in comparison to the reference drug 5-Fluorouracil. Compound 5d was the most active against the breast carcinoma cell line (MCF-7) and the liver carcinoma cell line (HEPG-2) giving promising IC50 values of 0.52 µg/mL and 0.63 µg/mL, respectively, compared with 5-Fluorouracil with IC50 values of 0.67 and 5 µg/mL, respectively. Structure-activity relationship (SAR) was also discussed.

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Introduction

Uracil I is an important constituent of nucleic acids. Drugs such as 5-Flurouracil (5-Fu, II) that contain uracil has been a potent inhibitor of thymidylate synthase [1-3]. Uracil has been preferentially used by tumor tissues for nucleic acid biosynthesis, and a fluorouracil analogue would inhibit tumor cell division by blocking the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine mono-phosphate (thymidylate) [4]. 5-Fu has become one of the most widely used antineoplastic agent after it was invented as an antimetabolite of uracil [5]. It has been used for the treatment of solid tumors, such as colorectal, breast, and gastric cancers [6]. Uracils have been serving as key building blocks for pharmaceutics such as anticancers [7, 8]. Their thio analogs and thiouracils have also been used as potential therapeutic agents for antiviral, anticancer, antibacterial and antifungal activities [9-14]. In particular, its S -acyl and S- alkyl derivatives have been recently reported in potential cytotoxic Agents [15, 16]. Analogs of 5-and 5, 6-substituted 2-thiouracil were reported as anticancer and antimicrobial agents [17,18]. For example, methioprim III, ethioprim IV and benzylthioprim V have been reported for inhibition of tumor growth in mice [19]. Merbarone® VI, a topoisomerase II inhibitor, showed antitumor activity [20] against B16 melanoma, the murine L1210 leukemia and M5076 sarcoma 22. In 2002, 2-thiouracil-5-sulphonyl chloride [21] was successfully prepared after many attempts and used as a starting compound for the synthesis of 2-thiouracil-5-sulfonamide derivatives. Reports from our laboratory [21-23] revealed that these derivatives exerted promising anticancer activity against liver (HepG2), breast (MCF7), colon (CaCo-2 and HCT116) and lung carcinoma A549. A series of novel 2-thiouracils containing benzene sulfonamide moiety have revealed high anticancer activities against most human cancers [24-26]. Moreover, aryl/heteroaryl sulfonamides have

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received great attention as anticancer agents and proven to exhibit substantial activity in vitro and/or in vivo through many mechanisms including their action as carbonic anhydrase inhibitors [27-29], tyrosine kinase (TK) inhibitors [30, 31], PI3K inhibitors [32], apoptosis inducers [33], metallo protienase inhibitors [34], and mi-crotubules assembly disruption [35], for example, the anticancer sulfonamide agents E-7010 VII [36], E7070 VIII and Vemurafenib (PLX4032) IX [37] (Diagram 1). E7070 is a novel sulfonamide antitumor agent, which could affect cell cycle progression and exhibit potent antitumor activity in vitro and in vivo [38]. Prompted by these facts, diverse biological properties of these heterocyclic scaffolds and in continuation to the researchers' efforts to synthesize biologically active compounds against cancer [22, 23] have been considered. Herein, the rationalized design of some novel 2-thiouracil-5-sulfonamide isosteres (compounds 2 - 7a-e) (series I) in position 5 was substituted with various (substituted amino methyl) moieties to act as cytotoxic agents. Comparable 2-thiouracil-5-sulfonamide derivatives (compounds 8-13a-e) (series II), were also prepared (Diagram 2). In both series, different amino methyl and different sulfonamide linkers were used to study the effect of these variations on the cytotoxic activity. All compounds were tested for possible anti-cancer activity on two cell lines (MCF-7 and HEPG-2). Structure-activity relationship (SAR) was also discussed. In this paper, the authors reported the synthesis, anticancer activity, structure-activity relationship (SAR) of novel 2-thiouracil-5-sulfonamide isosteres derivatives and comparable 2-thiouracil-5-sulfonamide derivatives with the hope of finding interesting antitumor activity.

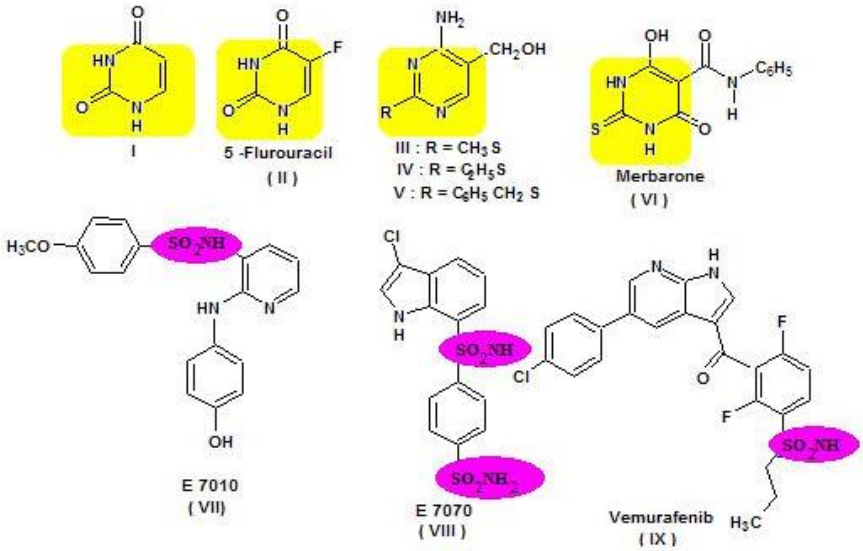


Diagram 1. Biologically active compounds containing uracil, thiouracil and sulfonamide moieties

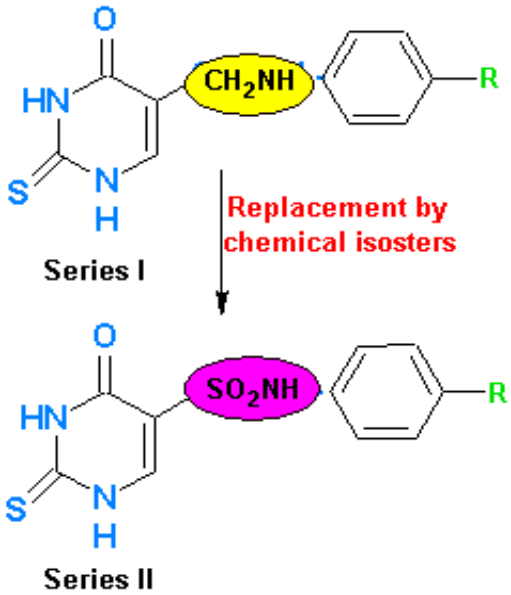
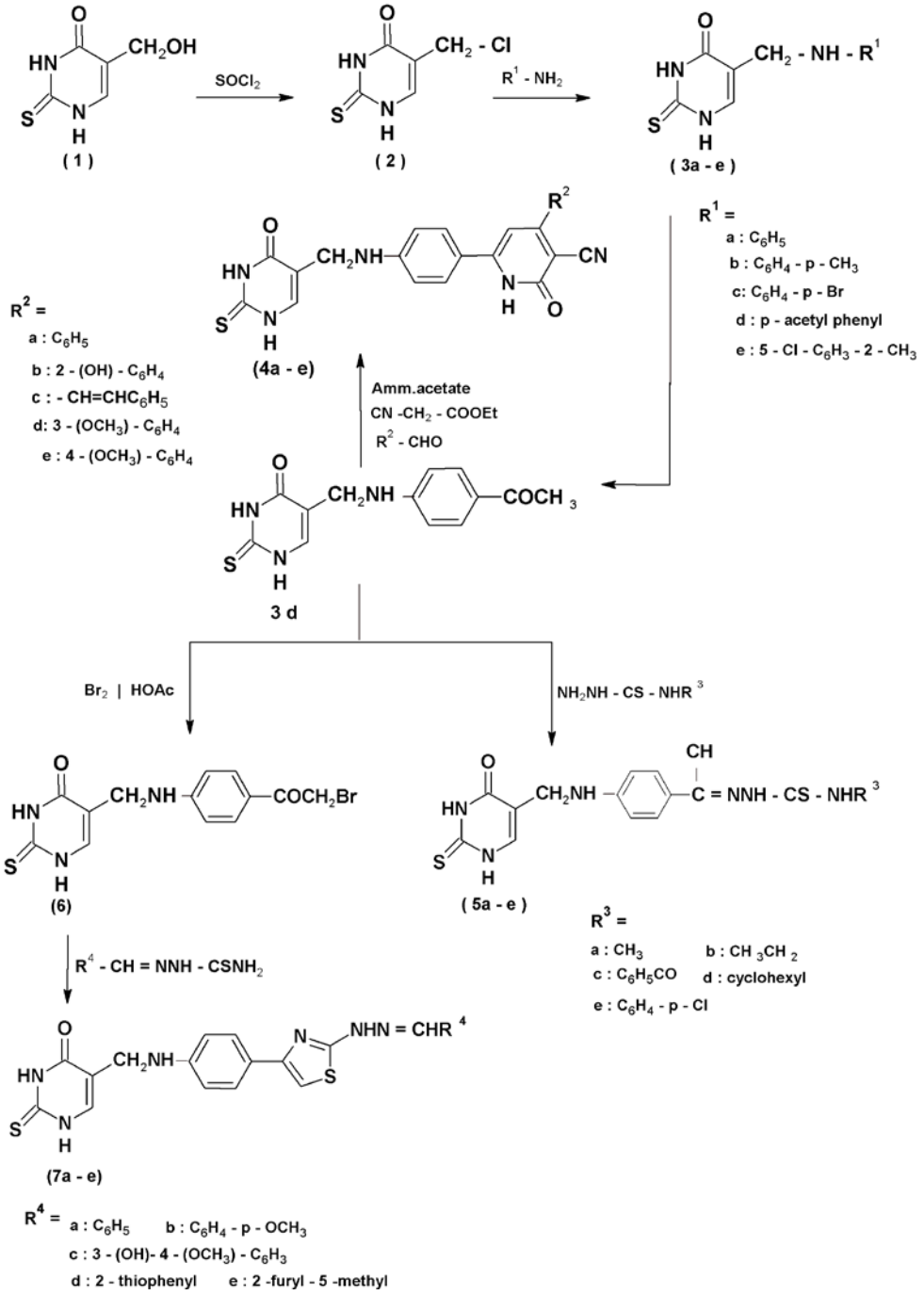


Diagram 2. Genearal structure of synthetic compounds

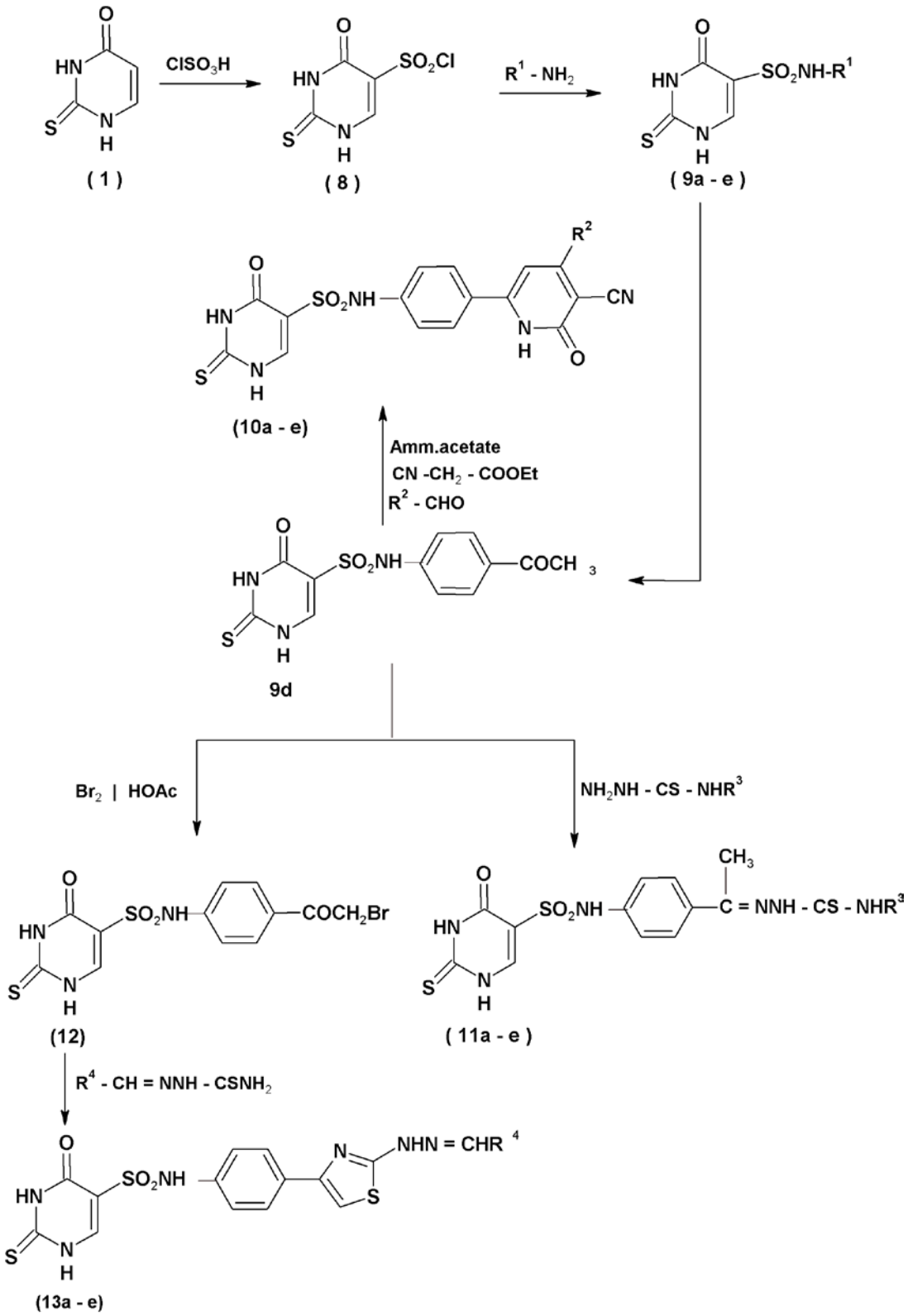
Materials and Methods

Experimental

Melting points have been uncorrected, and they were taken on a Boetius melting point microscope. Microanalyses were performed by the micro analytical unit at Cairo University. IR spectra were recorded on a Beckmann infra spectrophotometer PU9712 using KBr discs. ^{1}HNMR spectra were determined on a Joel EX 270 MHz spectrometer using tetramethyl silane as an internal standard. Mass spectra (MS) were recorded on a Finigan SSQ 7000 Mass spectrometer at 70 ev. All of the reactions were followed and checked by TLC using Chloroform/Methanol (3:1), and the spots were examined under a UV-lamp. All the new compounds gave spectral data consistent with the proposed structure and microanalysis within ±0.3 % of the theoretical values (Table 1 and 2). The 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 compounds

Comp. No.	Yield %	m.p. °C (solvent)	Mol. formula (M.wt.)	*Analysis Calculated/Found C%	H%	N%
2	89	268-9	$C_5H_5ClN_2OS$ (176.62)	34.00 34.08	2.85 2.78	15.86 15.89

3a	86	278-9	$C_{11}H_{11}N_3OS$ (233.29)	56.63 56.77	4.75 4.36	18.01 17.99
3b	85	331-2	$C_{12}H_{13}N_3OS$ (247.32)	58.28 58.37	5.30 5.38	16.99 17.01
3c	89	270-1	$C_{11}H_{10}BrN_3OS$ (312.18)	42.32 42.80	3.23 3.58	13.46 13.64
3d	78	256-2	$C_{13}H_{13}N_3O_2S$ (275.33)	56.71 56.92	4.76 4.44	15.26 15.48
3e	74	278-2	$C_{12}H_{12}ClN_3OS$ (281.76)	51.15 63.21	4.29 4.51	14.91 14.50
4a	83	282-3	$C_{23}H_{17}N_5O_2S$ (427.48)	64.62 61.67	4.01 4.06	16.38 13.44
4b	79	267-8	$C_{23}H_{17}N_5O_3S$ (443.48)	62.29 62.37	3.86 3.90	15.79 15.97
4c	70	276-8	$C_{25}H_{19}N_5O_2S$ (453.52)	66.21 66.35	4.22 4.35	15.44 15.50
4d	72	280-1	$C_{24}H_{19}N_5O_3S$ (457.50)	63.01 63.07	4.19 4.28	15.31 15.42
4e	71	293-5	$C_{24}H_{19}N_5O_3S$ (457.50)	63.01 63.20	4.19 4.39	15.31 15.52
5a	69	306-2	$C_{15}H_{18}N_6OS_2$ (362.47)	49.70 49.86	5.01 5.20	23.19 23.09
5b	69	298-9	$C_{16}H_{20}N_6OS_2$ (376.50)	51.04 51.35	5.35 5.06	22.32 22.12
5c	68	316-7	$C_{21}H_{20}N_6O_2S_2$ (452.55)	55.73 55.80	4.45 4.60	18.57 18.80
5d	66	322-4	$C_{20}H_{26}N_6OS_2$ (430.59)	55.79 55.66	6.09 6.19	19.52 19.50
5e	64	340-1	$C_{20}H_{19}ClN_6OS_2$ (458.99)	52.34 52.25	4.17 4.14	18.31 18.61
6	67	318-9	$C_{13}H_{12}BrN_3O_2S$ (354.22)	44.08 44.28	3.41 3.35	11.86 11.71
7a	77	266-8	$C_{21}H_{18}N_6OS_2$ (434.54)	58.04 58.38	4.18 4.17	19.34 19.39
7b	86	263-5	$C_{22}H_{20}N_6O_2S_2$ (464.56)	56.88 56.78	4.34 4.32	18.09 18.16
7c	67	292-5	$C_{22}H_{20}N_6O_3S_2$ (480.56)	54.98 54,78	4.19 4.25	17.49 17.50
7d	88	278-9	$C_{19}H_{16}N_6OS_3$ (440.56)	51.80 51.81	3.66 3.70	19.08 19.17
7e	87	268-9	$C_{20}H_{18}N_6O_2S_2$ (438.52)	54.78 54.81	4.14 4.15	19.16 19.08

*C, H And N are within the Limit of ± 0.3%

Table 2. Spectral data (IR, M.S, and ^{1}HNMR) for the newly prepared compounds

Comp. No.	IR (KBr) ν (cm^{-1})	M.S, EI m/z	^{1}HNMR (DMSO-d_6) δ (ppm)
2	3170 (NH broad), 2973, (CH-aliphatic), 1707 (C=O), 1683 (C=N).	176 17.1% 178 5.5%%(M+2)	3.5 (2H, s, CH_2Cl), 8.1 (1H, s, pyrimidine), 11,11.5 (2H,s,NH,exchangeable with D_2O).
3a	3176(NH broad), 3150(CH-aromatic, 2980 (CH-aliphatic), 1710 (C=O), 1680 (C=N).	233 15.9%	3.4 (2H, s, CH_2-NH), 7.3-7.8 (5H, m, Ar-H), 8.3 (1H, s, pyrimidine), 4.1,10,10.2 (3H,s,NH, exchangeable with D_2O).
3b	3188(NH broad), 3179(CH-aromatic, 2986 (CH-aliphatic), 1716 (C=O), 1688 (C=N).	247 8.7%	2.5(3H,s,CH_3),3.6 (2H, s, CH_2-NH),7.1, 7.3 (4H, dd, Ar-H), 8.2 (1H, s, pyrimidine), 5.3,11,11.5 (3H,s,NH, exchangeable with D_2O).
3c	3167(NH broad), 3178(CH-aromatic, 2987 (CH-aliphatic), 1717 (C=O), 1686 (C=N).	312 23.4% 314 22.9%(M+2)	3.5 (2H, s, CH_2-NH), 6.9,7.2 (4H, dd, Ar-H), 8.2 (1H, s, pyrimidine), 5.6,11.2,11.5 (3H,s,NH, exchangeable with D_2O).
3d	3177(NH broad), 3173(CH-aromatic, 2989 (CH-aliphatic), 1710,1716 (2C=O), 1668 (C=N).	275 31.2%	2.5(3H,s,CH_3),3.5(2H, s, CH_2-NH), 7.2,7.5 (4H, dd, Ar-H),8.1 (1H, s, pyrimidine), 5.5,10,11(3H,s,NH,exchangeable with D_2O).

3e	3167(NH broad), 3187(CH-aromatic, 2969 (CH-aliphatic), 1710, (C=O), 1669 (C=N).	281 18.5% 283 6.5%(M+2)	2.6(3H, s, CH_3),3.7(2H, s, CH_2-NH), 7.0,7.3, 7.6 (3H, m, Ar-H), 8.1 (1H, s, pyrimidine), 5.6,10.1,10.3 (3H, s, NH, exchangeable with D_2O).
4a	3197(NH broad), 3187(CH-aromatic, 2969 (CH-aliphatic), 2220(CN),1690, 1710(2C=O), 1669 (C=N).	427 12.2%	3.4 (2H,s,CH_2-NH), 6.5-7.9 (9H,m,Ar-H), 7.5 (1H,s,pyridone) ,8.3(1H,s,pyrimidine) , 5.6,11.1,11.3(4H,s,NH, exchangeable with D_2O).
4b	3192(NH, OH, broad), 3177 (CH-aromatic), 2979 (CH-aliphatic), 2225(CN) ,1692, 1712, (2C=O), 1668 (C=N).	443 65.9%	3.6(2H, s, CH_2-NH),7.0-7.6(8H, m, Ar-H),7.9 (1H, s, pyridone),8.1(1H, s, pyrimidine), 5.6, 10.0,10.2,10.5(5H,s,4NH,1OH,exchangea-ble with D_2O).
4c	3198(NH broad), 3177(CH-aromatic, 2969 (CH-aliphatic), 2221(CN),1690, 1710(2C=O), 1668 (C=N).	453 14.7%	3.6(2H, s, CH_2-NH),6.5,6.7(2H, dd, CH=CH) 7.2-7.7 (9H, m, Ar-H), 7.9(1H, s, pyridone, 8.1(1H, s, pyrimidine),5.5,10.2,10,3 (4H, s, NH, exchangeable with D_2O).
4d	3167(NH broad), 3178(CH-aromatic, 2989 (CH-aliphatic), 2226(CN),1695, 1711(2C=O), 1668 (C=N).	457 22.3%	4.1(3H, OCH_3), 3.6 (2H, s, CH_2-NH), 7.1-7.8 (8H, m, Ar-H), 7.9(1H, s, pyridone, 8.2 (1H, s, pyrimidine), 5.6,10.2,10,3 (4H, s, NH, exchangeable with D_2O).
4e	3167(NH broad), 3189(CH-aromatic, 2987(CH-aliphatic), 2224(CN),1688, 1714(2C=O), 1678 (C=N).	457 18.9%	4.2(3H, OCH_3),3.6(2H, s, CH_2-NH), 7.1,7.3 (4H, dd, Ar-H),7.6,7.7(4H, dd, Ar-H),7.9(1H, pyridone,8.2(1H, s, pyrimidine), 5.6,10.2, 10,4 (4H, s, NH, exchangeable with D_2O).
5a	3187(NH broad), 3188(CH-aromatic, 2980(CH-aliphatic), , 1714, (C=O), 1678 (C=N), 1270(C =S).	362 26.5%	2.2, 2.5 (6H, s, CH_3), 3.6(2H, s, CH_2-NH), 7.2-7.7(4H, dd, Ar-H),8.2(1H, s, pyrimidine) ,5.6,11-11.5(5H,s,NH, exchangeable with D_2O).
5b	3184(NH broad), 3178(CH-aromatic, 2967(CH-aliphatic), , 1711, (C=O), 1679 (C=N), 1272(C =S).	376 44.5%	1.2 (3H, t, CH_3), 1.5(2H, q, CH_2), 2.2(3H, s, CH_3),3.6(2H, s, CH_2-NH),7.1-7.3 (4H, dd, Ar-H), 8.1 (1H, s, pyrimidine) ,5.6,10.2-10.6 (5H, s, NH exch-angeable with D_2O).
5c	3187(NH broad), 3198(CH-aromatic, 2977(CH-aliphatic), ,1730, 1710 (2C=O), 1679 (C=N), 1275(C =S).	452 56.4%	2.2(3H,s,CH_3) , 3.6(2H,s,CH_2-NH), 7.1-7.6 (9H, m, Ar-H), 8.1(1H,s,pyrimidine) ,5.6,10.2-10.6 (5H,s, NH, s, exchangeable with D_2O).
5d	3189(NH broad), 3177(CH-aromatic, 2989(CH-aliphatic), , 1715, (C=O), 1677 (C=N), 1270(C =S).	430 5.9%	1.7(10H, t, cyclohexane) 2.1 (3H, s, CH_3), 3.6(2H, s, CH_2-NH),7.1,7.3(4H, ddArH), 8.1 (1H, s, pyrimidine),5.5,10.1-10.7(5H, s, NH, s, exchangeable with D_2O).
5e	3187(NH broad), 3198(CH-aromatic, 2977(CH-aliphatic), , 1710, (C=O), 1679 (C=N), 1275(C =S).	458 18.6% 460 6.6%(M+2)	2.2(3H, s, CH_3) 3.6(2H, s, CH_2-NH), 7.2,7.8 (8H, m, Ar-H), 8.1 (1H, s, pyrimidine), 5.6, 10.2-10.6 (5H, s, NH, exchangeable with D_2O).
6	3178(NH broad), 3178(CH-aromatic, 2980 (CH-aliphatic), 1710,1716 (2C=O), 1669 (C=N).	354 19.8% 356 18.8%	4.5(2H, s, CH_2Br),3.5(2H, s, CH_2NH),7.2 ,7.4 (4H, dd, Ar-H), 5.6,10.2-10.5 (3H, s, NH, exchangeable with D_2O).
7a	3220(NH broad) ,3150(CH-aromatic, 2990(CH-aliphatic, 1610(N = CH), 1710(C =O).	434 11.7%	3.5 (2H, s, CH_2-NH), 7.2-7.9 (9H, m, Ar-H), 7.7(1H, s, thiazole,7.8(1H, s, CH=N),8.5 (1H, s, pyrimidine),5,11-11.5(4H, s, NH, exchan-geable with D_2O).
7b	3225(NH broad), 3160(CH-aromatic, 2970(CH-aliphatic, 1615(N = CH), 1716(C =O).	464 6.9%	4.1 (3H, s, OCH_3),3.6 (2H, s, CH_2-NH), 6.9-7.5(8H, m, Ar-H),7.8(1H, s, thiazole,7.8(1H, s, CH=N), 8.2(1H, s, pyrimidine), 5.6,11-11.7(4H, s, NH, exchangeable with D_2O).
7c	3221 (NH, OH broad), 3176 (CH-aromatic, 2973 (CH-aliphatic,1613(N=CH), 1712 (C =O).	480 23.8%	4.2(3H, s, OCH_3),3.6 (2H, s, CH_2-NH), 6.9-7.5(7H, m, Ar-H),7.8(1H, s, thiazole,7.8(1H, s,CH=N), 8.2 (1H,s,pyrimidine),5.6,9,11-11.5(4H,s,3NH,1OH,s,exchangeable with D_2O).

7d	3219(NH broad), 3179(CH-aromatic, 2979(CH-aliphatic, 1610(N = CH), 1711(C =O).	440 34.9%	3.6 (2H, s, CH_2-NH), 6.8,6.9,7.1 (3H, m, thiophen), 7.2,7,4 (4H, dd, Ar-H),7.1(1H, s, thiazole), 7.8 (1H, s, CH=N), 8.1 (1H, s, pyrimidine), 5.6, 11 -11.7 (4H, s, NH, exchangeable with D_2O).
7e	3230(NH broad), 3189(CH-aromatic, 2979(CH-aliphatic, 1611(N = CH), 1717(C =O).	438 23.7%	2.8(3H, s, CH_3) ,3.6(2H, s, CH_2-NH), 6.8, 7.0(2H, dd, furan), 7.1,7,3 (4H, dd, Ar-H), 7.2(1H,s,thiazole),7.8(1H,s,CH=N),8.1 (1H,s,pyrimidine),5.6,11.11.8(4H,s,NH, exchangeable with D_2O).

5-(Chloromethyl)-2-thioxo-2,3-dihydropyrimidin-4-(1H)-one (2)

A solution of thionyl chloride (3.3 ml, 0.04 mol) in dry chloroform (20ml) was added dropwise into a solution of (1) (6.02g., 0.03 mol) in dry chloroform (60ml) containing 3ml of pyridine, then the mixture was refluxed for 1 hr in anhydrous conditions. The product was separated by extraction with diethyl ether, and the residue was crystallized from DMF/water to give the compound (2).

5-(Arylamino methyl)-2-thioxo-2,3-dihydropyrimidin-4-(1H)-one (3a - e)

A mixture of 2 (1.13 mol) and the proper aromatic amine namely aniline, *p*-toluidine, *p*-bromoaniline, *p*-aminoacetophenone and 5-chloro-2-methyl aniline and pyridine (0.016 mol) in absolute ethanol (50 ml) was refluxed for 12-16 hr, then cooled and filtered off and recrystallized from DMF/ water to give compounds 3 a-e.

2-oxo-6-(4-{[(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl]amino}phenyl)-4-aryl-1,2-dihydropyridine-3-carbonitrile (4 a - e)

A mixture of 3d (1.1 g., 0.003 mol), the appropriate aldehyde (0.003 mol) , ammonium acetate (1.89, 8mol) and ethyl cyanoacetate (0.35g. 0.003mol) in 50 ml of absolute ethanol was refluxed for 6-10 hrs. The reaction mixture was concentrated to its half volume,and filtered , and then the filterate was poured into ice/water, and the precipitate was filtered off, dried and recrystallized from DMF / water.

(2E)-N-methyl-2-[1-(4-{[(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl] amino}phenyl) ethyliden] hydrazinecarbothioamide (5a- e)

A mixture of 3d (1.01g. 0.003 mol) and the appropriate substituted thiosemicarbazides (0.003 mol) in absolute ethanol (30 ml) was refluxed for 12-15 hr. Then cooled , filtered off ,dried and, recrystallized from DMF / water.

5-({[4-(bromoacetyl) phenyl] amino} methyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (6).

A mixture of 3d (1.13g ,0.005 mol) and bromine (0.005 mol) in 30 ml glacial acetic acid was stirred at room temperature for 48 hr., then filtered off, the filtrate was neutralized with ammonia, and the precipitate was collected, dried and recrystallized from DMF/ water.

5-{[(4-{2-[(2E)-2-arylidenehydrazinyl]-1,3-thiazol-5-yl} phenyl) amino] methyl}-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (7a- e)

A mixture of **6** (1.01g. 0.003 mol) and the desired thiosemicarbazone derivatives (0.003 mol) in absolute ethanol (40 ml) were refluxed for 14-18 hr. Then the reaction mixture was cooled, and the formed solid was filtered off, dried and recrystallized from DMF / water.

Compounds 8 - 13 a-e were prepared as in the literature [21].

Biological evaluation

In vitro anticancer activity

Sulforhodamine- B stain (SRB) assay

SRB assay was used to evaluate the in vitro cytotoxicity of all the synthesized compounds against two cell lines of human cancer, namely Breast(MCF7) and Live (HEPG2) cancer obtained from pharmacology screening unit of the National Cancer Institute (NCI), Cairo University, Egypt, applying the method of Skehan et al., [39] in comparison to the known anticancer drugs: 5-Flurouracil. The SRB assay has been one of the most widely used methods , which was developed in 1990, where it depended on the ability of SRB to bind to protein components of the cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). The amount of dye extracted from stained cells has been directly proportional to the cell mass, as the binding of SRB was stoichiometric. The cells were seeded in 96-multiwell plate (10^4cells/well). 24 h later, the cells were treated with different concentrations of the test compounds (0, 1, 2.5, 5, 10 µ g/ml), and Triplicate wells were prepared for each individual dose. The cells were incubated with the test compounds for 48 hrs at 37°C, and in an atmosphere of 5% CO_2. After 48 hr, the cells were fixed with trichloroacetic acid, washed with water, and stained for 30 min with 0.4 % (w t/vol) Sulforhod Amine-B stain dissolved with 1 % acetic acid. Excess stain was removed by four washes with 1 % acetic acid and the attached stain was recovered with Tris EDTA buffer. The color intensity was measured in an ELISA reader. The relation between the surviving fractions and the drug concentrations was plotted to obtain the survival curve of the MCF-7 and HEPGT-2 tumor cell lines after addition of the tested compounds. The parameter used was IC_{50}, which corresponded to the concentration required for 50 % inhibition of the cell viability. IC_{50} was calculated for each test compound. Each concentration was repeated three times. The IC_{50} of the synthesized compounds are shown in Table 3.

Table 3. IC_{50} values [a] (in **µg/mL**) for cytotoxic activity of tested compounds.

Compounds	Cell Lines	
	MCF7	**HEPG2**
2	0.69	1.58
3a	0.72	0.92
3b	0.89	0.86
3c	0.78	0.83
3d	0.93	0.99
3e	0.74	0.82
4a	2.23	2.89
4b	2.78	2.75
4c	2.89	3.67
4d	2.84	5.23
4e	1.67	3.82
5a	0.61	0.65
5b	0.67	0.72
5c	0.72	0.90
5d	0.52	0.63
5e	0.59	0.88
6	0.71	1.78
7a	1.67	1.97
7b	2.75	1.67
7c	3.87	2.29
7d	4.69	1.97
7e	1.76	1.99
8	2.11	2.13
9a	1.95	1.96
9b	2.54	2.23
9c	1.98	1.82
9d	1.23	2.19
9e	1.87	2.67
10a	1.91	3.67
10b	2.25	5.89
10c	1.35	2.84
10d	2.67	2.09
10e	0.68	5.78
11a	0.78	0.78
11b	0.57	0.89
11c	0.68	1.09
11d	0.89	1.97
11e	0.72	1.39
12	0.99	2.45
13a	0.72	4.34
13b	0.69	3.09
13c	0.78	2.98
13d	0.84	3.76
13e	0.73	4.97
5-FU	0.67	5

IC_{50} values [a] (in **µg/mL**), which the concentration required for a 50 % of cell growth inhibition.
[a] The values given are means of three experiments.
5-FU: 5- Fluorouracil.

Results and Discussion

Chemistry

The overview on the literature survey, the importance of 2-thiouracils, sulfonamides and 2- thiouracil-5-sulfonamides in the biological systems prompted us to design and synthesize a new class of 5-substituted-2-thiouracil derivatives as sulfonamide isosteres, aiming at identifying potent anticancer agents. The synthetic route to prepare 2-thiouracil-5-sulfonamide isosteres (2-7a-e) is depicted in Scheme 1. The reaction of 5-hydroxyl methyl -2-thiouracil 1 with thionyl chloride [40] yielded the targeted chloromethyl analogue 2. Further, chloride 2 was introduced in reaction with a series of aromatic amines namely aniline, *p*-toluidine, *p*-bromoaniline, *p*-aminoacetophenone and 5-chloro-2-methyl aniline in absoluteethanol containing pyridine as acid scavenger giving methyl amino derivatives 3 a-e. A series of 3-cyno-pyridin- 2-one derivatives 4a-e was synthesized by one pot reaction of methyl ketone (3d) with ethyl cyanoacetate, and the appropriate aldehydes and excess ammonium acetate. 2-Thiouracil-5-aminomethyl-p-acetophenone 3d was condensed with a series of alkyl thiosemicarbazides namely methyl, ethyl, benzoyl, cyclohexyl and p-chlorophenyl thiosemicarbazides in absolute ethanol to give the corresponding thiosemicarbazones 5a-e, respectively. Moreover, this methyl ketone compound was monobrominated by stirring with bromine in glacial acetic acid giving the bromo derivative 6 which in turn was used as a substrate for preparation of thiazole derivatives 7a-e by its reaction with a series of selected thiosemicarbazones. 2-Thiouracil-5-sulfonamide derivatives 8- 13a-e was prepared as explained in the literature [21] (Scheme2).

The structures of the synthesized compounds (2-7a-e) were confirmed by microanalyses and spectral data (IR, ^{1}HNMR and EI-MS) which showed full agreement with their structures.

Biological evaluation

In vitro anticancer activity

The strategy of this work was based on the synthesis of new anti- cancer agents. Accordingly, the sensitivity of human breast cancer cell lines MCF-7 and human liver cell lines HEPG-2 were evaluated against the new 2-thiouracil-5-sulfonamide isosteres (2,3a-e,4a-e,5a-e,6 and 7a-e) and comparable 2-thiouracil-5-sulfonamide target derivatives (8 ,9a-e,10a-e, 11a-e ,12 and 13a-e) utilizing SRB assay using 5-Flourouracil as the reference drug. The results were expressed as IC_{50} ($\mu g.ml^{-1}$) values which were the average of at least three independent experiments, as outlined in Table 3.

As shown in Table 3, it was found that all the compounds exhibited considerable response on both cell lines. The compound of CH_2Cl atom on the C-5 position (2) showed better anticancer activity((IC_{50}= 0.69 and 1.58μg/mL, respectively) than that of SO_2Cl (8) (IC_{50}= 2.11 and 2.13μg/mL, respectively) against both human breast cell line MCF7 , and liver carcinoma cell line HEPG2, and the same trend also could be observed in compounds 3a-e and 9a-e. Compounds 3a-e (with Arylamino methyl moiety) exhibited significant activity against MCF7 cell line (IC_{50}= 0.72,0.89,0.78,0.93 and 0.74 μg/mL, respectively) and HEPG2 cell line (IC_{50}= 0.92,0.86,0.83,0.99 and 0.82μg/mL, respectively) , while the sulfonamide analogues 9a-e represented less potency against MCF7 cell line as the shown moderate activity, but remained higher than that of 5-FU against HEPG2 cell line. On the other hand, the incorboration of a pyridone ring in the parent acetyl thiouracils 3d or 9d as in the 3-cyno-pyridin-2-one derivatives (4a-e and 10a-d) led to the decreased activity of both series against MCF7 cell line. Compounds 4a-e and 10a-e showed moderate anticancer activity against MCF7 cell line but high activity against HEPG2 cell line. Meanwhile, the reaction of acetyl thiouracil with a series of alkyl thiosemicarbazides affording the thiosemicarbazone derivatives 5a-e and 11a-e was vital for modulating the cytotoxic activity to exceed that of 5-fluorouracil against both cell lines. Compounds 5a-e (IC_{50} = 0.61,0.67,0.72, 0.52 and 0.59 μg/mL; 0.65,0.72, 0.90, 0.63 and 0.88μg/mL, respectively) and 11a-e (IC_{50}=0.78,0.57,0.68,0.89 and 0.72 μg/mL; 0.78,0.89,1.09,1.97and 1.39 μg/mL, respectively) showed high anticancer activity against MCF7 and HEPG2 cell lines. Among these compounds, the most promising compound 5d showed high activity against MCF-7 and HepG2 cancer cell lines, with IC_{50} values of 0.52 and 0.63 μg/mL, respectively, in comparison with the antitumor agent 5-FU (IC_{50} = 0.67 and 5 μg/mL, respectively) as a control. Bromo derivatives 6 and 12 showed significant cytotoxic activity against MCF7 and HepG2 cancer cell lines (IC_{50}= 0.71; 1.78 and 0.99; 2.45 μg/mL, respectively). While the thiazole derivative 13a-e showed more potent activity than that of thiazoles 7a-e towards the human breast cancer cell line MCF7, but lower activity twords liver carcinoma cell line HEPG2. Compounds 7a-e showed a moderate activity against MCF7 cell line ranging from 1.67 to 4.64 μg/mL , and high activity against HEPG2 cell line of IC_{50} ranging from 1.67 to 2.29 μg/mL while, compound 13 a-e showed a siginificant activity against MCF7 cell line of IC_{50} ranging from 0.69 to 0.84 μg/mL, and a high activity against HEPG2 cell line of IC_{50} ranging from 2.98 to 4.97 μg/mL in comparison with 5-FU (IC_{50} = 0.67 and 5 μg/mL , respectively) as a control (Diagram 3).

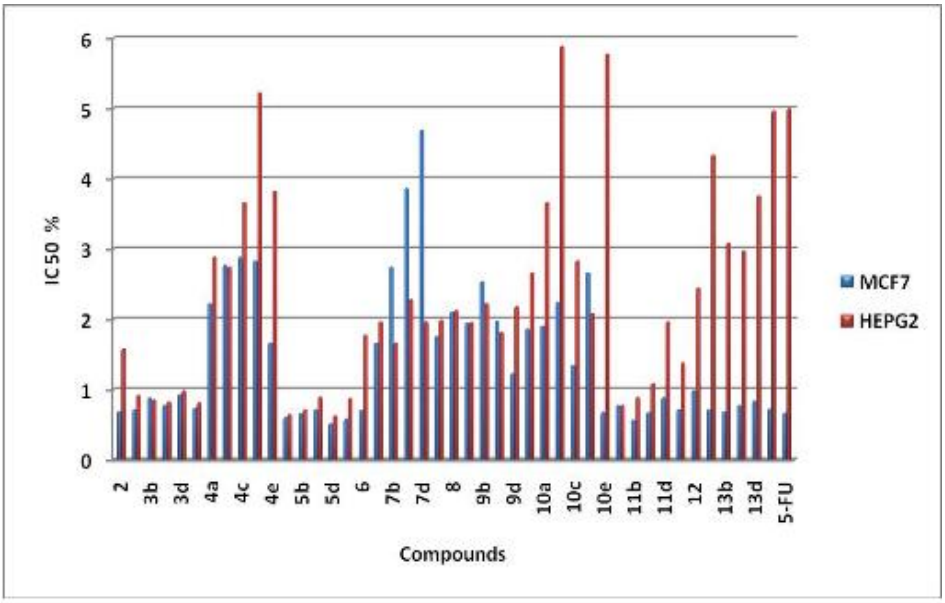


Diagram 3. IC_{50} in **µg/ml** of the synthesized compounds against breast tumor cell line (MCF-7) and liver tumor cell line (HEPG-2)

Structural-Activity Relationship (SAR)

Furthermore, structure-activity relationship (SAR) studies were summarized to determine the effect of type of moiety attached to 2-thiouracil main ring system at position 5 on cytotoxic efficacy of the synthesized compounds (Diagram 4). SAR in these derivatives displayed that compounds with thiosemicarbazides 5a-e and 11a-e showed superior cytotoxic activity against MCF7 and HEPG2 cell lines, which is clearly seen in Table 3.

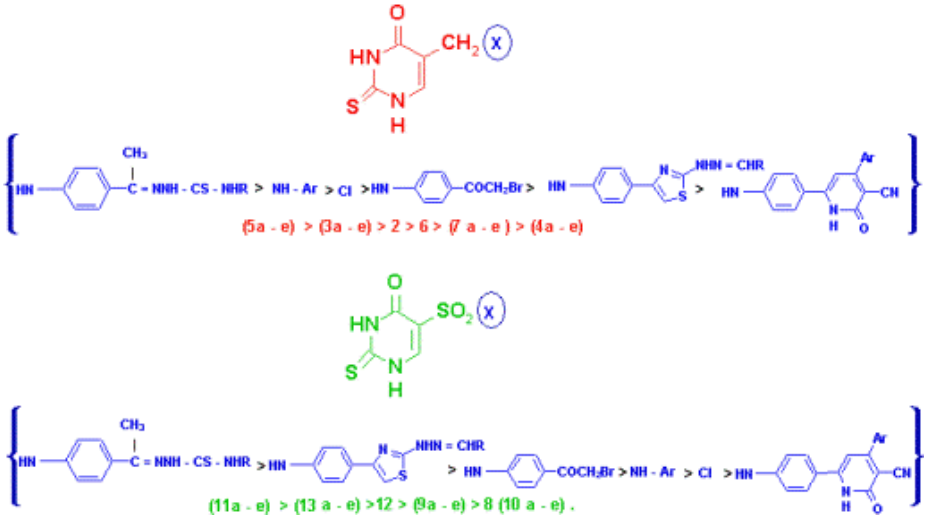


Diagram 4. Structure -activity relationship of synthesized compounds

Conclusion

In summary, two series of 2-thiouracil-5-sulfonamide isosteres and comparable 2-thiouracil-5-sulfonamide derivatives were prepared as cytotoxic agents, and they showed significant in vitro cytotoxic potential on HEPG-2 cell line. They were investigated against cancer cell lines (MCF-7 and HepG-2) determined by SRB assay. The results of the current study revealed that all the synthesized comopounds displayed moderate to good cytotoxic activities against the tested cancer cell lines. Among all the studied synthesized compounds, analogs 5a-e , 11a-e showed high cytotoxicity against MCF7 and HEPG2 cell lines as compared to standard drug 5-Fluorouracil. Compound 5d was the most active against the breast carcinoma cell line (MCF-7), and the liver carcinoma cell line (HEPG-2) gave promising IC_{50} values of 0.52 and 0.63 µg/mL , respectively , compared with the reference drug (5-FU) with IC_{50} values of 0.67 and 5 µg/mL , respectively. Further, more studies are still needed to identify the mechanism of action of theses derivatives and improve their cytotoxic activity .

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