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Synthesis of New 2-Thiouracil-5-Sulphonamide Derivatives with Antibacterial and Antifungal Activity

O. A. Fathalla, S. M. Awad[1], and M. S. Mohamed[1]

Department of Medicinal Chemistry, National Research Centre, Dokki, Cairo, Egypt and [1]Organic Chemistry Department, Helwan University, Faculty of Pharmacy, Helwan, Egypt

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2-Thiouracil-5-sulphonic acid *N*-(4-acetylphenyl) Amide (**1**) was reacted with a series of aromatic aldehydes giving chalcones **2** (Claisen-Schemidt reaction), some of these chalcones were reacted with urea and thiourea giving pyrimidine-2-one and pyrimidine-2 thione derivatives respectively of the type **3a**,**b** and **4a**,**b**. In addition many chalcones were reacted with hydroxylamine hydrochloride giving isoxazoline derivatives **5a**,**b**. They could also reacted with phenylhydrazine to give pyrazoline derivatives **6a**,**b**, chalcones also were reacted withethylcyano acetate and/or malononitryl in pyridine giving pyran derivatives **7a**,**c** and **8a**,**c**. In another pathway chalcones were epoxidised by H_2O_2 giving epoxides **9a**,**c** which in turn were reacted with phenylhydrazine giving 4-hydroxypyrazoline derivatives **10a**,**c**. In another reaction chalcones were reacted with ethylcyanoacetate in presence of amm.acetate giving pyridone derivatives **11a**,**d** which could be prepared also in exellent yield from compound **1** by its reaction with certain aromatic aldehydes and ethylcyanoacetate in presence of ammonium acetate. Finally, compound **1** was reacted with semicarbazide giving semicarbazone intermediate **12** which in turn was reacted with thionyl chloride giving thiadiazole derivative **13**. The biological effects of some of the new synthesized compounds were also investigated.

Key words: 2-Thiouracil-5-sulphonamide-*p*-phenyl derivative, Antibacterial, Anti-fungal activity

INTRODUCTION

In continuation of our work (Fathalla *et al.*, 2002) on the synthesis of some 2-thio uracil-5-sulphonamide derivatives, we reported here the incorporation of 2-thio-uracil into pyrimidines (Fathalla, 1992), Isoxazoleines (Caradonna and Stein, 1960), pyrazolines (Arholdlc *et al.*, 1979), pyranes (Essaway and Wasfy, 1994), pyridones (Sakurai and Midorikawa, 1967), and thiadiazoles (Lalezari *et al.*, 1974). This study was undertaken in view of the fact that pyrimidines, isoxazoline, pyrazolines, pyrans, pyridones, and thiadiazoles have wide range of pharmacological properties. In addition 2-thiouracil itself has certain biological activities as antibacterial (Wyrzykiewicz *et al.*, 1993), antifungal, antiprotozoal and antiviral activity (Lang, 1975), also some 2 thio uracil derivatives have cytotoxic activity (Fathalla, 2002) in addition to antithyroid activity (Dale M. The extrapharmacopeia, 1989). We developed here a program aimed to the synthesis of novel 2-thiouracil derivatives hopping that these compounds might possess certain biological activity.

Correspondence to: O. A. Fathalla, Department of Medicinal Chemistry, National Research Centre, Dokki, Cairo, Egypt
E-mail: omarfathalla@yahoo.com

MATERIALS AND METHODS

All melting points are uncorrected and were determined in capillary tube on Boetius melting point microscope. IR spectra were recorded on a Beckman infrared spectrophotometer PU9712 using KBr discs. ^{1}H-NMR spectra were obtained on JoelEX270MHZ spectrometer using TMS as internal standard. Mass spectra were recorded on SSQ7000 Mass spectrometer at 70 e.v. All reactions were followed and checked by T.L.C. using chloroform/methanol (3:1) and spots were examined by UV lamp.

MATERIALS AND METHODS

2-Thiouracil-5-sulphonic acid *N*-(4-acetylphenyl) amide (1)

It was prepared by the procedure described in literature (Fathalla, 1992).

2-Thiouracil-5-sulphonic acid *N*-(4-(3-substituted-2-propen-1-oxo)phenyl) amide derivatives (2a-d)

A mixture of 1 (1.13 g, 0.005 mole) and the appropriate aromatic aldehydes (0.005 mole) in 50 mL ethanolic sodium hydroxide was stirred at room temperature for 24 h, then refluxed for 1 h, cooled and poured into ice-cold water. The precipitate that appeared after nutralization with dil. HCL was filtered off and recystallized from DMF/water.

2-Thiouracil-5-sulphonic acid *N*-(4-(1,2,5,6-tetrahydro-2-thioxo-6-substituted pyrimidinyl) phenyl) amide derivatives (3a,b)

A mixture of appropriate chalcone (0.001 mol), thiourea (0.11 g, 0.001 mol) and 0.1 g NaOH in 25 mL of 80% dil. ethanol was refluxed for 15 h, then concenterated and cooled, the precipitete was filtered off and recrystallized from DMF/water.

2-Thiouracil-sulphonic acid *N*-(4-(1,2,5,6-tetrahydro-2-oxo-6-substituted pyrimidin-yl) phenyl) amide derivatives (4a,b)

To a solution of (0.01 g, 0.0015 mol) of urea in 20 mL ethanol 5 mL of conc. HCL was added, (0.001 mol) of the appropriate chalcones was added and the mixture was refluxed for 12 h, then concentrated to half its volume and cooled, it was neutralized with ammonium hydroxide solution and the precipitate was recrystallized from DMF/water.

2-Thiouracil-5-sulphonic acid *N*-(4-(5-substituted-3-isoxazolino)phenyl) amide derivatives (5a,b)

A solution of the appropriate chalcone (0.005 mol) and hydroxylamine hydrochloride (0.005 mol) in 20 mL ethanol containing 0.04 g sodium hydroxide was refluxed for 11 h, the product was isolated by concentration of the alchoholic solution, then filtered, dried, and recrystallized from DMF/water.

2-Thiouracil-5-sulphonic acid *N*-(4-(1-phenyl-5-substituted-3-pyrazolino) phenyl) amide derivatives (6a,b)

A mixture of the appropriate chalcone (0.001 mol) and phenylhydrazine (0.001 mol) was heated under reflux for 8-12 h, in 25 mL absolute ethanol then left to cool, the residual material was filtered off and recrystallized from DMF/water.

2-Thiouracil-5-sulphonic acid *N*-(4-(6-amino-5-cyno or carboethoxy-4-substituted pyrano) phenyl) amide derivatives (7a,c) and (8a,c)

A mixture of the appropriate chalcone (0.001 mol), ethylcyanoacetate and/or malononitrile (0.001 mol) and 20 mL pyridine was refluxed for 8 h, cooled and poured into ice/HCL solution, the solid product formed was collected and recrystallized from DMF/water.

Epoxidation of chalcone 9a,c

A solution of the appropriate chalcone (0.001 mol) in 20 mL acetone and 15 mL methyl alcohol was mixed with 8% aqueous sodium hydroxide (12 mL) followed by the addition of hydrogen peroxide (30%, 5 mL), the solution was shaken and heated to the boiling point during 1 h, then allowed to stand overnight at room temperature, water was added and the solution was extracted with ether. The ether layer was evaporated and the residue was recrystallized from petroleum ether to give white powder (m.p. 185°C) in 65% yield.

2-Thiouracil-5-sulphonic acid *N*-(4-(1-phenyl-5-substituted-4-hydroxy pyrazolino) phenyl) amide derivatives (10a,c)

The epoxide (0.001 mol) and phenyl hydrazine (0.001 mol) were refluxed in 25 mL ethanol for 13 h. The solid that separated on cooling was filtered off and recrystallized from DMF/water.

2-Thiouracil-5-sulphonic acid *N*-(4-(5-cyno-6-oxo-4-substituted pyridine-2-yl) phenyl) amide derivatives (11a,d)

A mixture of the appropriate chalcone (0.001 mol), ethycyanoacetate (0.035, 0.001 mol) and excess ammonium acetate (1.89 g, 8 mol) in 50 mL absolute ethanol was refluxed for 12-15 h. The reaction mixture was concentrated till its half volume, filtered and the filtrate was poured into ice/water and the produced precipitate was filtered off, dried under suction and crystallized from DMF/water.

Another procedure

The same pyridone derivatives could be prepared from compound **1** as follow :

A mixture of 1 (1.13 g, 0.003 mol), the appropriate aldehyde (0.003 mol), ethyl cyanoacetate (0 .003 mol), and excess ammonium acetate (1.89 g, 8.0 mol) in 50 mL absolute ethanol was refluxed for 6-10 h. The reaction mixture was concentrated, filtered and the filtrate was poured into ice/water and the produced precipitate was filtered off, dried and crystallized from DMF/water.

Formation of semicarbazone 12

To a solution of 1 (0.001 mol) in 50 mL ethanol was added a solution of semicarbazide hydrochloride (0.001 mol) and sodium acetate (0.002 mol) in 20 mL water. The reaction mixture was refluxed for 4 h, evaporated to half of its volume and then poured into ice-water. The separated solid was filtered off, washed with water, dried and crystallized from DMF/water to give semicarbazone as

pale yellow powder (m.p. >300).

2-Thiouracil-5-sulphonic acid *N*-(4-(1,2,3-thiadiazolo) phenyl) amide derivative (13)

Ten mL thionylchloride was gradually added to the semicarbazone **12** (0.005 mol) and the mixture was gently warmed and then left for 24 h at room temperature. A $NaHCO_3$ ice-cold saturated solution was then added, the product was extracted with ether and the extract was worked up as usual. The residue was crystallized from DMF/water as yellow powder.

The biological effects of some of the new synthesized compounds

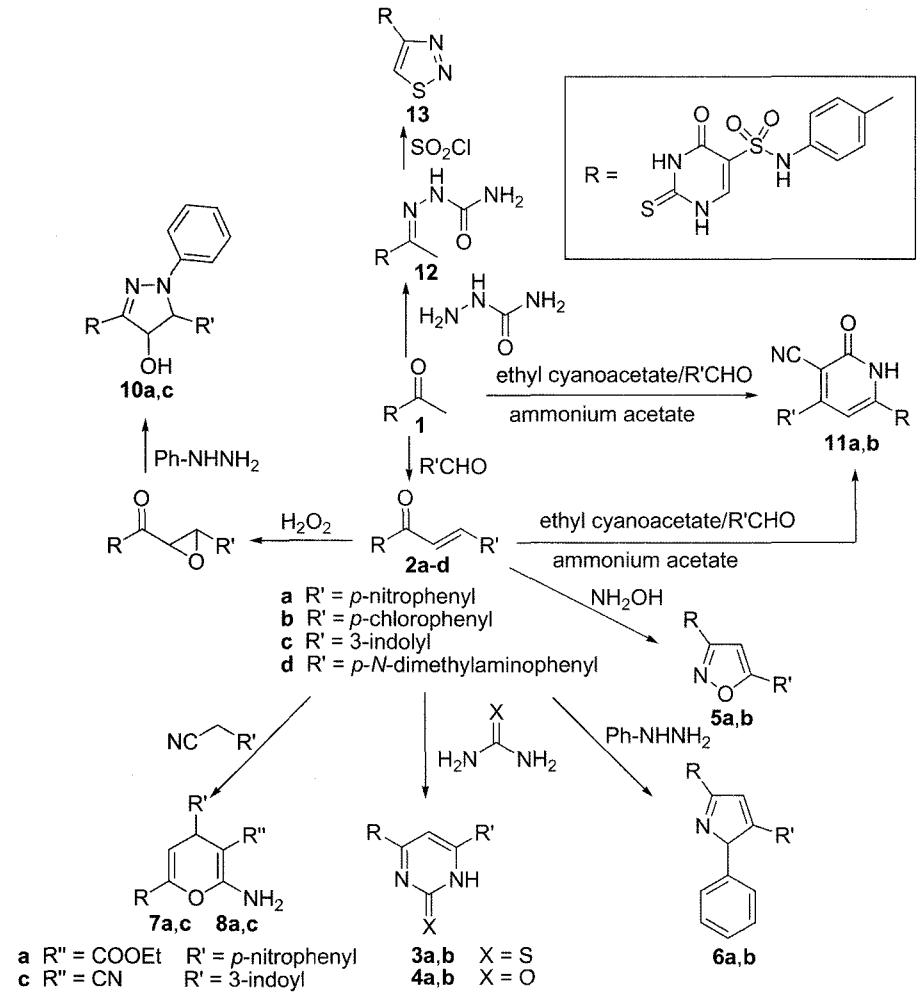
Antibacterial activity

Most compounds were tested.

Bacteria : The following microorganisms were used for determination of bacterostatic and/or bactericidal concentration, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, *Sarcina*, *Pseudomonas aeroginosa*, and *Mycobacterium phlei*. All microorganisms used were obtained from the culture collection of the department of microbiology and immunology, Faculty of Pharmacy, Helwan Univerrisity. Compounds were tested against *Escherichia coli*, *Pseudomonas aeroginosa*, and *Staphylococcus* aureus in nutrient broth, pH 7.0 and against *Bacillus subtilis Sarcina* and *Mycobacterium phlei* in bacto brain hart infusion broth pH 7.0 and against *Candida albicans* in broth containing 1% neo peptone, 2% of dextrose with pH 5.7. A strain of *Eshericia coli* of know antibiotic sensitivity served for control purposes.

Media disk sensitivity tests were nutrient and Muller Henton Agar (MHA) were purchased from Difco. The disk diameter was 5 mm. The compounds with inhibition zone diameter more than 5 mm were active. Compounds were dissolved in sterile DMSO to yield 2.000 μg/mL, passed through 0.2 μm membrane filters (Millipore crop. Bedford. Mass). The filtrates were dissolved as 2 mL samples into sterile, small screwcapped vials, and frozen and kept standed at −15°C. The vials wer refrozen after thawing.

Sensitivity tests : Disc diffusion sensitivity test were done in a manner identical to that of (Bauer *et al.*, 1966),



Scheme 1. Proparation of compounds **1~13**

some compounds with inhibition zone diameter more than 5 mm were subjected to determination of MIC by serial dilution method. Broth dilution tests, utilizing serial log 2 dilurions of the tested compounds over the range of 50 to 0.025 mg/mL were performed by using liquid media and a bacterial inoculum standardized to yield 1.5×10^6 organisms /mL at 0 time. For this purpose organisms in the exponential growth phase (pregrown at 35°C for 6 h in liquid media) were adjusted to Mc farland $BaSO_4$ standard no. 0.5. The turbidity of which corresponds to that of 1.5×10^8 organisms/ mL. The adjusted suspension of organisms was futher diluted 50 fold in the selected liquid medium (corresponding to 3×10^6 organisms/mL). Assay tubes received 1 mL of the respective double strength dilution of antibiotic and 1 mL of bacterial inoculum. Control tubes received 1 mL of MHB and 1 mL of bacterial inoculum. The assay and control tubes were incubated at 35°C for 18 h. The minimal inhibitory concentration (MIC) of tested compounds were defind as the lowest concentration of antibiotic completely inhibiting the growth as judged by visualinspection. The minimal bactericidal concentration (MBC) of the drug was determined through subculture of one 3 mm loopful from clear tubes to quarter sectors of 5% sheep blood agar plates which were incubated at 35°C for 24 h. The MBC was defined as the lowest concentration of gentamicin yielding no growth after subculture to blood agar.

RESULTS AND DISSCUSSION

In view of the above findings it was considered of interest to undertake the synthesis of new 5-substituted-2-thiouracil containing chalcones, pyrimidines, isoxazoles, pyranes, 4-hydroxypyrazolines, pyridones, semicarbazones, and thiadiazoles hopping that these compounds might possess certain anti metabolitic function against living micro-organisms. Synthesis of the described compounds was achieved by firstly preparation of compound **1** (methyl ketone containing compound) by reacting 2-thiouracil-5-sulphonyl chloride with *p*-amino acetophenone in absolute ethanol containing pyridine as acid scavenger, this compound was reacted with aldehydes namely *p*-nitro-benzaldehyde, *p*-chlorobenzaldehyde, indol-3-carboxaldehyde and *N,N*-dimethylaminobenzaldehyde in presence of 10% NaOH solution affording chalcone derivatives **2a~c** (Claisen Schemidt reaction). Chalcone derivatives were condensed with thiourea and urea giving pyrimidines **3a,b** and **4a,b** respectively. A solution of chalcone derivatives in ethanolic KOH on treatment with hydroxylamine hydrochloride gave isoxazoline derivatives **5a,b**.

Condensation reaction of phenylhydrazine with α, β unsaturated ketones was investigated (Abady *et al.*, 1986; Hassaneen *et al.*, 1995). When allowed to react with phenylhydrazine in ethanol in presence of few drops of

Table I. Physical and analytical data of the prepared compounds **1~13**

Comp No	Yieled (%) M.p (°C)	Formula Mol. wt	Analysis (calcd/fownd) %		
			C	H	N
2a	72 255	$C_{19}H_{14}N_4O_6S_2$ (458.47)	49.78 49.52	3.08 3.27	12.22 11.98
2b	70 299	$C_{19}H_{14}N_3O_4S_2Cl$ (447.91)	50.95 50.82	3.15 3.27	9.39 9.19
2c	74 288	$C_{21}H_{16}N_4O_4S_2$ (452.50)	55.74 55.66	3.56 3.42	12.38 12.27
2d	63 315	$C_{21}H_{20}N_4O_4S_2$ (456.53)	55.25 55.32	4.42 4.58	12.27 12.33
3a	64 310	$C_{20}H_{14}N_6O_5S_3$ (514.56)	46.68 45.25	2.74 2.83	16.33 16.23
3b	55 299	$C_{20}H_{14}N_5O_3S_3Cl$ (504.01)	47.66 47.52	2.79 2.72	13.89 13.69
4a	67 320	$C_{20}H_{14}N_6O_6S_2$ (498.40)	48.19 48.32	2.83 2.67	16.86 16.98
4b	61 336	$C_{20}H_{14}N_5O_4S_2Cl$ (487.94)	49.23 49.18	2.89 2.92	14.35 14.19
5a	70 301	$C_{19}H_{13}N_5O_6S_2$ (471.46)	48.40 48.25	2.78 2.99	16.78 16.72
5b	58 317	$C_{19}H_{13}N_4O_4S_2Cl$ (460.91)	49.51 49.35	2.84 2.94	12.16 12.19
6a	59 289	$C_{25}H_{18}N_6O_5S_2$ (546.57)	54.93 54.88	3.32 3.47	15.37 15.09
6b	59 277	$C_{25}H_{18}N_5O_3S_2Cl$ (536.02)	56.01 56.34	3.38 2.99	13.07 13.32
7a	61 355	$C_{24}H_{21}N_5O_8S_2$ (571.64)	50.43 50.60	3.70 3.40	12.25 12.05
7c	70 347	$C_{26}H_{23}N_5O_6S_2$ (565.61)	55.21 55.59	4.09 4.37	12.38 12.05
8a	78 333	$C_{22}H_{16}N_6O_6S_2$ (524.53)	50.37 50.46	3.08 3.05	16.02 16.13
8c	54 323	$C_{24}H_{18}N_6O_4S_2$ (518.62)	55.59 55.38	3.49 3.09	16.21 16.54
9a	54 320	$C_{19}H_{14}N_4O_5S_2$ (474.46)	48.10 48.23	2.97 2.01	11.81 11.95
9c	57 340	$C_{21}H_{16}N_4O_5S_2$ (468.50)	53.83 53.77	3.44 3.64	11.96 11.78
10a	55 360	$C_{25}H_{20}N_6O_6S_2$ (564.59)	53.18 53.07	3.57 3.28	14.89 14.99
10c	70 352	$C_{27}H_{23}N_6O_4S_2$ (559.63)	57.94 57.75	4.14 4.16	15.19 15.25
11a	73 300	$C_{22}H_{13}N_6O_6S_2$ (522.51)	50.57 50.38	2.27 2.92	16.09 16.31
11d	68 317	$C_{24}H_{19}N_6O_4S_2$ (550.64)	52.36 52.28	3.66 3.72	15.26 15.19
12	55 361	$C_{13}H_{14}N_6O_4S_2$ (382.42)	40.82 40.68	3.69 3.82	21.98 21.72
13	57 380	$C_{12}H_9N_5O_3S_3$ (367.43)	39.22 39.52	2.47 2.48	19.06 19.18

Table II. The ^{1}H-NMR, IR, and Mass of some of the prepared compounds

Comp No	^{1}H-NMR (DMSO-d_6) ppm	IR (KBr) cm^{-1}	Mass M$^+$
2a	6.6-7.2 (2H, dd, chalcone), 7.3, 7.6, 7.9, 8.1 (8H, dd, aromatic) 8.2 (1H, s, thiouracil) 11.2-11.4 (3H, s, 3 NH exchangeable with D_2O).	3220 (NH, St., b), 3060 (CH, st., aromatic), 1670 (2 CO), 1630 (C=C), 1350-1550 (NO_2), 1270 (C=S of thio-uracil, 1320, 1140 (SO_2)	458.5
2b	6.3-6.8 (2H, dd, chalcone), 7.5, 7.8, 7.9, 8.3 (4H, dd, of aromatic), 8.6 (1H, s, thiouracil), 11.3, 11.4 (3H, s, 3NH exchangeable with D_2O).	3225 (NH, st., b), 3150 (CH, st, aromatic), 1680 (2CO), 1265 (C=S of thiouracil), 1350, 1160 (SO_2).	447.9
2c	6.4-7.0 (2H, dd, chalcone), 7.2-8.5 (9H, m, aromatic), 8.2 (1H, s, thiouracil), 10, 11.3-11.6 (4H, s, 4NH exchangeable with D_2O).	3310 (NH ,st, b), 3160 (CH, st, aromatic), 1660 (2CO), 1620 (C=C), 1270 (C=S of thio-uracil), 1330-1140 (SO_2).	452.5
2d	3.2 (6H, s, *N*-(CH_3)2), 6.7-7.1 (2H, dd, chalcone), 7.2, 8.1 (8H, dd, aromatic), 8.2 (1H, s, thiouracil), 11.1, 11.3 (3H, s, 3NH exchange-able with D_2O).	3320 (NH, st, b), 3150 (CH, st, aromatic), 2890 (CH, st, aliphatic), 1680 (2CO), 1640 (C=C), 1270 (C=S of thiouracil), 1320, 1140 (SO_2).	456.5
3a	6.9-7.8 (8H, dd, aromatic), 7.5 (1H, s, of thiopyrimidine), 8.4 (1H, s, thiouracil), 11.0, 11.2, 11.3, 11.8 (4H, s, 4NH exchangeable with D_2O).	3350 (NH, St., b), 3150 (CH, st., aromatic), 1670 (CO), 1630 (C=C), 1351-1547 (NO_2), 1271 (C=S of thio-pyrimidine, 1325, 1142 (SO_2).	514.5
3b	6.8-7.9 (8H, dd, aromatic), 7.3 (1H, s, of thiopyrimidine), 8.4 (1H, s, thiouracil), 11.2, 11.3, 11.8, 11.9 (4H, s, 4NH exchangeable with D_2O).	3300 (NH, st., b), 3180 (CH, st, aromatic), 1670 (CO), 1635 (C=C), 1270 (C=S of thio-pyrimidine), 1335, 1148 (SO_2).	504.1
4a	6.8-7.6 (8H, dd, aromatic), 7.4 (1H, s, of pyrimidin-2-one), 8.3 (1H, s, thiouracil), 11.1, 11.2, 11.4, 11.6 (4H, s, 4NH exchangeable with D_2O).	3300 (NH, st, b), 3180 (CH, st, aromatic), 1670, 1680 (2CO), 1630 (C=C), 1350-1550 (NO_2), 1275 (C=S of thiouracil), 1335-1148 (SO_2).	498.5
4b	6.7-7.6 (8H, dd, chalcone), 7.3 (1H, s, of pyrimidin-2-one), 8.3 (1H, s, thiouracil), 11.1, 11,2, 11.7, 11.9 (4H, s, 4NH exchangeable with D_2O).	3320 (NH, b), 3180 (CH aromatic), 1675, 1685 (2C=O), 1640 (C=C), 1270 (C=S oh thiouracil), 1140, 1335 (SO_2).	487.9
5a	6.9, 7.2, 7.8, 7.9 (8H, dd, aromatic), 8.1 (1H, s, of isoxazoline), 8.4 1H, s, thiouracil), 11.2-11.4 (3H, s, 3 NH exchangeable with D_2O).	3370 (NH, b), 3150 (CH aromatic), 1670 (C=O), 1630 (C=C), 1355, 1550 (NO_2), 1270 (C=S of thiouracil), 1140, 1350 (SO_2).	471.5
5b	6.8, 7.2, 7.4, 7.5 (8H, dd, aromatic), 8.0 (1H, s, of isoxazoline), 8.2 (1H, s, thio -uracil), 11.0-11.3 (3H, s, 3NH exchangeable with D_2O).	3360 (NH, b), 3145 (CH aromatic), 1660 (C=O), 1645 (C=C), 1270 (C=S oh thiouracil), 1145, 1345 (SO_2).	460.9
6a	6.8, 6.9, 8.0, 8.1 (13H, dd, aromatic), 7.6 (1H, s, of pyrazoline), 8.7 (1H, s, thiouracil), 11.2-11.4 (3H, s, 3NH exchangeable with D_2O).	3340 (NH, b), 3190 (CH aromatic), 1660 (C=O), 1650 (C=C), 1360, 1540 (NO_2), 1270 (C=S of thiouracil) , 1141, 1340 (SO_2).	546.5
6b	6.9, 7.0, 6.1, 8.2 (13H, dd, aromatic), 7.6 (1H, s, of pyrazoline), 8.7 (1H, s, of thiouracil), 11.2-11.4 (3H, s, 3NH, exchangeable with D_2O).	3351 (NH, b), 3150 (CH aromatic), 1675 (C=O), 1660 (C=C), 1270 (C=S oh thiouracil), 1140, 1352 (SO_2).	536.02
7a	1.7 (3H, t, CH_3), 4.1 (2H, q, CH_2), 7.1 (2H, s, NH_2 exchangeable with D_2O), 7.2 (1H, s of pyran), 7.3-8.5 (9H, dd, aromatic), 11.2-11.8 (3H, s, 3NH exchangeable with D_2O).	3440 (NH, NH_2, b), 3180 (CH aromatic), 1720 (C=O), 1670 (C=O of thiouracil), 1660 (C=C), 1355, 1550 (NO_2), 1270 (C=S of thiouracil), 1135, 1351 (SO_2).	571.5
7c	1.8 3H, t, CH_3), 4.2 (2H, q, CH_2), 7.0 (2H, s, NH_2 exchangeable with D_2O), 7.2 (1H, s of pyran), 7.3-8.4 (9H, m, aromatic), 10, 11.2-11.4 (4H, s, 4NH exchangeable with D_2O).	3400 (NH, NH_2, b), 3190 (CH aromatic), 1725 (C=O), 1665 (C=O of thiouracil), 1660 (C=C), 1270 (C=S of thiouracil), 1140, 1350 (SO_2).	565.5
8a	7.1 (2H, s, NH_2 exchangeable with D_2O) 7.2 (1H, s, of pyran), 7.2-8.4 (8H, dd, aromatic), 11-11.2 (3H, s, 3NH exchangeable with D_2O).	3450 (NH, NH_2, b), 3180 CH aromatic), 2218 (CN), 1680 (C=O), 1665 (C=C), 1350, 1550 (NO_2), 1270 (C=S of thiouracil), 1142, 1350 (SO_2).	524.5
8c	7.2 (2H, s, NH_2 exchangeable with D_2O), 7.2 (1H, s, of pyran), 7.2-8.4 (9 H, m, aromatic), 10 (1H, s, of indole exchangeable with D_2O), 10.9-11.1 (3H, s, 3NH exchangeable with D_2O).	3480 (NH, NH_2, b), 3190 (CH aromatic), 2220 (CN), 1670 (C=O of thiouracil), 1660 (C=C), 1270 (C=S of thiouracil), 1140,1350 (SO_2).	518.43
10a	6.4-7.8 (15H, m, aromatic), 8.4 (1H, s, thiouracil), 11.2, 11.3, 11.4, 11.5 (4H, s, OH, 3NH exchangeable with D_2O).	3420 (NH, OH b), 3180 CH aromatic), 1670 (C=O), 1650 (C=C), 1350, 1550 (NO_2), 1270 (C=S of thiouracil), 1138, 1350 (SO_2).	564.5
10c	6.8-8.4 (16H, m, aromatic), 8.6 (1H, s, thiouracil), 10,11, 11.1-11.4 (5 H, OH, 4NH exchangeable with D_2O).	3410 (NH, OH b), 3150 CH aromatic), 1670 (C=O), 1650 (C=C), 1350, 1270 (C=S of thiouracil), 1138, 1350 (SO_2).	559.63
11a	6.9-7.9 (9H, m, aromatic), 8.2 (1H, s, thiouracil), 10.2-11.4 (4H, s, 4NH exchange-able with D_2O).	3350 (NH, b), 3190 CH aromatic), 2930 (CH, aliphatic), 2215 (CN), 1670 (2C=O) , 1640 (C=C), 1355, 1550 (NO_2), 1270 (C=S of thiouracil), 1142, 1350 (SO_2).	522.5
11d	3.2 (6H, s, *N*-(CH_3)$_2$), 6.8-8 (9H, m, aromatic), 8.1 (1H, s, thiouracil), 11.2-11.4 (4H, s, 4 NH exchangeable with D_2O).	3340 (NH, b), 3180 CH aromatic), 2220 (CN), 1680 (2C=O), 1650 (C=C), 1271 (C=S of thiouracil), 1135, 1360 (SO_2).	550.5
12	2.1 (3H, s, CH_3), 6.1, 6.2 (3H, s, NH, NH_2, exchange-able with D_2O), 7.1, 7.3 (4H, dd, aromatic), 8.1 (1H, s, thiouracil), 11.1-11.2 (3H, s, exchangeable with D_2O).	3250 (NH, NH_2, b), 3160 (CH aromatic), 2940 (CH aliphatic), 1670 (2 C=O), 1640 (C=C), 1270 (C=S of thiouracil), 1140, 1342 (SO_2).	382.5
13	6.9-7.8 (4H, dd, aromatic), 7.2 (1H, s, thiadiazole), 8.2 (1H, s, thiouracil), 11.1-11.4 (3H, s, 3NH exchangeable with D_2O).	3250 (NH, b), 3160 (CH aromatic), 1670 (C=O), 1660 (C=C), 1275 (C=S of thiouracil), 1140, 1361 (SO_2).	367.4

pipridine as a basic catalyst the corresponding pyrazoline derivatives **6a**,**b** were produced.

Analogous to recent report (Essawy and Wasfy, 1994), the base catalyzed condensation of chalcone derivatives with ethylcyanoacetate and/or malononitrile afforded the corresponding pyran derivatives **7a**,**c** and **8a**,**c**. Similar to the behavior of (El-Hashash, 1994) treatment of the alcoholic solution of chalcone derivatives with hydrogen peroxide in alcoholic medium yielded the corresponding α, β epoxy ketones **9a**,**c**. The formation of oxirane nucleus can be theoretically explaned as the oxyien (H-O-O) derived from H_2O_2 attacks the β-carbon atom by the Michael type reaction leading to an intermediate of which the loss of a hydroxide ion producing the desired oxirane. Recently it has been shown that the oxirane ring of α, β-epoxyketones is opened with phenylhydrazine in boiling ethanol to furnish 4-hydroxy pyrazolinederivatives **10a**,**c**. Also as (Fathalla *et al.*, 2002) compound **1** was reacted with ethyl cyanoacetate and the appropriate aldehydes namely p-nitrobenzaldehyde and *N*,*N*-dimethylaminobenzaldehyde in presence of excess ammonium acetate giving the corresponding pyridone derivatives **11a**,**d** which could be prepared by the treatment of chalcone derivatives of the same aldehydes with ethyl cyanoacetate in presence of excess ammonium acetate. In another reaction similar to results fro (Essawy and Wasfy, 1994) compound **1** was easily condensed with semicarbazide in an aqueous ethanolic solution to give the semicarbazone **12**. The purified semicarbazone was then subjected to oxidative cyclization (Hussein *et al.*, 1986) by thionyl chloride to yield the thiadiazol derivative **13**.

Interpretation of microbiological study of the prepared compounds

This work is an attempt to screen the antibacterial and antifungal activity of some novel 2-thiouracil derivatives substituted at 5-position due to their anti metabolite effect (inhibition of nucleic acid synthesis).

Antibacterial activity

According to activity of the known used compounds we can find that 2-thiouracil itself has a potent antibacterial against *B. subtilis*, *E. coli*, *S. aureus*, *P. aeargenosa*, *M. phei*, ant *St. facalis* due to its antimetabolic effect (S1) and may Chlorosulphonation of 2-thiouracil abolishedits activity (S2) but Formation of sulphonamide retained the activity of 2-thiouracil (S3).

In another way incorporation of α, β-unsaturated ketone gave active compounds only against *S. aureus*, *St. faecalis* (**2a**) or *B. subtilis* (**2b** and **2c**), while incorporation

Table III. Antibacterial test of the synthesized compounds with comparison to some known prepared derivatives measured by disc diffusion method a 5 mm (0.5 cm) disk and broth dilution methods

Comp.	*B. subtilis*	*E. coli*	*C. albicans*	*S. aureus*	*Sarcina*	*P. aerogenosa*	*M. phlei*	*St. faecalis*
2a	—	—	—	1.6cm	—	—	—	2.2cm
2b	1 cm	—	—	—	—	—	—	—
2c	0.75 cm	—	—	—	—	—	—	—
3a	2 cm	0.9 cm	—	2 cm	—	—	—	—
3b	0.8 cm	—	—	0.8 cm	—	—	—	—
4b	—	—	—	1 cm	—	—	—	1.1 cm
5a	0.75 cm	—	0.6 cm	0.7 cm	—	0.8 cm	1.1 cm	—
5b	—	—	—	—	—	—	—	—
6a	—	—	—	1.5 cm	—	—	—	2 cm
6b	0.7 cm	—	—	—	—	—	—	—
7a	1.15 cm	—	—	1 cm	—	—	—	—
8a	—	—	—	0.9 cm	—	—	—	1 cm
10c	0.7 cm	—	—	—	—	—	—	—
11a	0.8 cm	—	—	—	—	—	—	—
12	1 cm	—	—	2.5	—	—	—	—
13	—	—	—	1 cm	—	—	—	1.7 cm
S_1	1.0	2.2 cm	—	3 cm	—	1.5 cm	0.5 cm	1.8 cm
S_2	—	—	—	—	—	—	—	—
S_3	1.1	0.8 cm	—	—	0.5 cm	0.7 cm	—	1.3 cm

S_1 = 2-thiouracil, S_2 = 2thiouracil-5-sulphonylchloride, S_3 = 2-thiouracil-5-sulphonic acid *N*-(4-acetylphenyl) amide.
Compounds **4a**, **5b**, **7a**, **8b**, **9a**, **9c**, **10a**, and **11b** have no action on all bacteria.

Table IV. Results of MIC of two compounds **3a** and **5a**

Comp.	MIC μg /L							
	Bsubtilis	*E. coli*	*C. albicans*	*S. aureus*	*Sarcina*	*Paergenus*	*M. phlei*	*St. feacalis*
3a	1.25	412	—	125	—	—	—	—
5a	412	—	1600	412	—	840	412	—

Table V. Results of Ant-fungal activity of 5 selected compounds

Microorganism	Inhibition of zone diametr (mm/mg)				
	2a	**4b**	**6a**	**8a**	**13**
Microsporium canis	25	32	10	23	42
Sporotrichum Schenkii	22	11	18	30	25

of thiopyrimidine ring gave active compounds against *B. subtilis*, *E. coli*, *Saureus* (**3a**) or against *B. subtilis* and *S. aureus* only (**3b**) also incorporation of pyrimidone gave an inactive compounds (**4a**) or a compound with weak activity (**4b**) against *S. aureus* and *St. faecalis*. In addition the incorporation of isoxazoline ring gave an active compound (**5a**) against *B. subtilis*, *C. albicans*, *S. aureus*, *P. aerogenosa* and *M. phlei*. Also incorporation of pyrazoline ring gave compounds active against *S. aureus*, *St. faecalis* (**6a**) or *B. subtilis* (**6b**).

In another pathway the incorporation of pyran ring gave active compounds against *B. subtilis*, Saureus (**7a**) or against *S. aureus* and *St. faecalis* (**8a**). We can observe that compounds (**9a** and **9c**) are microbilogically inactive due to their chemical untability (epoxides). Also the incorporation of 4-hydroxypyrazoline ring abolished the activity or gave a compound **10c** which is only weakly active against *B. subtilis*. In addition incorporation of pyridine ring may abolish the activity (**11b**) or gave a weak compound **11a** against *B. subtilis*.

In another reaction condensation of methyl ketone **1** with semicarbazide gave simicarbazone derivative **12** which was active against *B. subtilis*, and *S. aureus*.

While the incorporation of thiadiazole ring gave compound **13** which was active against *S. aureus* and *St. faecalis*. We can observe also that only one compound of the prepared compounds was active against *C. albicans*.

To sum up generally incorporation of sulphonamido group into 5-position of 2-thiouracil may retain its activity as anti-microbial agent also incorporation of many heterocyclic moieties may retain some of this activity.

Anti-fungal activity

The tested fungi were

I - Microsporium canis : The cause of ring worm infections in man, it is one of the dermatophytes which infect the keratinized surface of the body e.g. hair skin and nails.

II - Sporotrichum Schenkii : the cause of sporotrichosis in man which is a chronic infection of the skin and Subcutaneous tissue. We can observe:

All the five tested compounds had a potent activity as anti-fungal agent especially **2a** which is a chalcone derivative (α, β-unsaturated ketone) and can easily penetrate the cell membrane of the fungus due to its high lipophilicity also compound **13** was highly active (thiadiazol derivatives are of known ant-fungal activity.

Anti-fungal activity *in vitro*

Tested compounds were **2a**, **4b**, **6a**, **8a**, and **13**.

Preliminary *in vitro* anti-fungal testing using Diffusion disk method : A filter paper Sterilized disc saturated with measured quantity of the sample is placed on a plate containing fungal medium (Dox is medium) which has been heavily seeded with the spore suspension of the tested organism. After inoculation, the diameter of the clear zone inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism (Jawetz *et al.*, 1974; Grayer and Harbone, 1994; Muanaza *et al.*, 1994; Irob *et al.*, 1996).

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