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Awad, S. M.,
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Synthesis, Characterization and study of Antimicrobial and Antifungal Activities of some Novel Pyrimidine Derivatives

Awad, S. M. [(1)] ♦♦ and Zohny, Y. M.[(2)]

[(1)] Medicinal Chemistry Department, Faculty of pharmacy, Elmergib University, Al-Khoms, Libya., [(2)] Pharmaceutical Organic Chemistry, Faculty of pharmacy, Helwan, Egypt.

ABSTRACT:

Background: The most widely practiced reaction of diazonium salts is diazocoupling especially with electron rich substrate such as s-alkylmercaptopyrimidines giving azo compounds of certain antimicrobial activity. *Aim:* Synthesis of pyrimidine derivatives bearing the aryl azo group and evaluation their antimicrobial activities. *Material and method:* Thiouracil (1) was methylated with CH_3/I to afford 5-methyl thiouracil (2) which in turn was diazotized at position 5 via the reaction with diazonium salt of *p*-aminoacetophenone giving azo compound (3). This compound could be monobrominated to afford the bromo derivative (4) which is good starting material for the preparation of thiazole derivatives (5a-c) by its reaction with substituted thiosemicarbazones. Also, it could react with some aldehydes yielding chalcones (7a-c). This methyl ketone was also reacted with thiosemicarbazides giving thiosemicarbazone derivatives (8a-c) or reacted with semicarbazide giving semicarbazone (9) which in turn was reacted with SeO or $SOCl_2$ yielding selene diazole or Thiadiazole (10) & (11) respectively. In addition, this methylketone (3) was a good substrate for the preparation of pyridines (12a-c) by its reation with some aldehydes in presence of ethyl acetoacetate and excess ammonium acetate. Finally, it could undergo Mannich reaction with some secondary amines, paraformaldehyde giving Mannich's bases (13a-c). *Results:* The prepared compounds showed a variable activities as antimicrobial agents.Conclusion: Substitution of 2-thiouracil at the 5th position retains the antimicrobial activities.

Keywords: Thiouracil, 5-substituted thiouracil, methylmercaptopyrimidine, antimicrobial pyrimidines.

1. INTRODUCTION

The literature indicated that a compound having pyrimidine nucleus possesses broad range of biological activity like 5-fluorouracil (5-FU) as anticancer; idoxuridine and trifluoridine as antiviral; zidovudine and stavudine as anti-HIV; trimethoprim, sulphamethazine, sulphadiazine as antibacterial; minoxidil and prazosine as antihypertensive; phenobarbitone as sedative hypnotic and anticonvulsant; propylthiouracil as antithyroid; thinozylamine as H_1-antihistaminic and bacimethrine as antibiotics [(1)]. Pyrimidine ring is the building unit of DNA and RNA which explains the fact that pyrimidine derivatives exhibit diverse pharmacological activities. The most pronounced of which are anticancer[(2)], antiviral especially anti-HIC[(3)], antimicrobial[(4)]. anti-inflammatory[(5)] and antioxidant[(6)]. It was reported that some series of pyrimido[4,5-d]pyrimidine-2,5-dione derivatives show antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and antifungal activity against *Candida albicans* and *Aspergillus niger*, as proved by Sharma et al[(7)]. Among the pyrimidine containing heterocycles, thiouracils are potential therapeutics as antiviral, anticancer and antimicrobial agents[(8-10)]. For example, It had been found that solutions of 2-thiouracil in concentrations of 25 and 50 mg/100 ml media completely inhibit the growth of Staphylococcus aureus bacteria. The antibiotic effect of 2-thiouracil was found to be several time greater than that of thiourea [(11)]. In addition, *E. Coli* [(12)], *Lactobacillus arabinosus* [(13)], *L. Leishmani* and *L. Casei* [(14)] were inhibited by 2-thiouracil. On the other hand, several 5-substituted thiouracils possess chemotherapeutic activity against cancer cells as well as antifungal, antiviral and antiparasitic activities [(15,16)]. On the other hand, 5-diazouracils are bacteriostatic, virustatic and cancerostatic. Studies have shown that arylazo groups are active in promoting antibacterial activity by inhibition of folate reductase and in promoting antifungal activity [(17)]. In addition S-alkylation products have been recently reported as novel antibacterial, cytotoxic agents[(18,19)]. Furthermore, incorporation of other heterocyclic rings such as, thiazole, thiadiazole and

corresponding author: Awad, Samirawad2000@yahoo.com

pyridine into pyrimidine nucleus may enhance its biological activities[9].

In the light of the fore mentioned facts, and in continuation for our interest in the synthesis of biologically active heterocyclic compounds, we developed here a synthetic pathway aimed to synthesize novel mercaptopyrimidines diazotized at 5th position to screen them for their antibacterial and/or antifungal activities. The prepared compounds were evaluated for antibacterial activity and antifungal activity using the disc diffusion method.

2. MATERIAL

2.1 Chemicals

2-thiouracil, methyl iodide, *p*-aminoacetophenone, bromine, glacial acetic acid, absolute ethanol, methanol, acetyl semicarbazide, sodium hydroxide, dimethyl formamide, semicarbazide hydrochloride, sodium acetate, selenium oxide, sodium carbonate, anhydrous magnesium sulphate, ether, thionyl chloride, sodium bicarbonate, ammonium acetate, ethylcyanoacetate, paraformaldehyde, morpholine, methyl piperazine, diethyl amine, benzaldehyde and *p*-anisaldehyde.

2.2 Microorganisms

Bacillus subtilis, Escerichia coli, Candida albicans, Staphylococcus aureus, Sarcina, pseudomonas aeroginosa & Mycobacterium phlei are the microorganisms used for the determination of bacteriostatic and/or bactericidal concentration. All microorganisms used were obtained from the culture collection of department of microbiology and immunology, Faculty of Pharmacy, Helwan University. Compounds were tested against Eschericia coli, pseudomonas aeroginosa and Staphylococcus aureus in nutrient bath at PH 7.0, against Bacillus subtilis, Sarcina and Mycobacterium phlei in bactobrain heart infusion bath at pH 7.0 and against Candida albicans in a bath containing 1% neopeptone, 2% dextrose at pH 5.7 while a strain of Eschericia coli of known antibiotic sensitivity was used as a control.

2.3 Media disk sensitivity tests:

nutrient and Muller Henton agar (MHA) were purchased from Diffco. The disk diameter was 5 mm. The compounds with inhibition zone 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 (Millipre corp. Bedford Mass). The filtrates were dissolved as 2 ml samples into sterile, small screw capped vials, frozen and kept standard at -15^{o}C. The vials were refrozen after thawing.

3. Methods

3.1 Chemistry

All melting points are uncorrected and were determined in capillary tube on a Boetius melting point microscope. Microanalyses were performed by the micro analytical unit at Cairo University. IR spectra were recorded as KBr pellets on a Beckmann infra red spectrophotometer PU9712 using KBr discs. ^{1}HNMR spectra were determined on a Joel EX 270 MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Finigan 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 at λmax = 326 n.m.

S-methyl-2-thiouracil (2):

It was prepared as in literature by treating 2-thiouracil with methyl iodide in sodium hydroxide solution[20]. (m.p 325^{0}C).

1-(4-((4-hydroxy-(methylthio)-1,2-dihydropyrimidin-5-yl) diazenyl)phenyl)ethanone (3):

It was prepared as in literature[17]. (m.p 277^{0}C) .

2-Bromo-1- (4-((4-hydroxy-2-(methylthio)pyrimidin-5-yl)diazenyl)phenyl)ethanone (4):

A mixture of **3** (1.13 g, 5 mM) and bromine (5Mm) in glacial acetic acid (30 ml) was stirred at room temperature for 48 hours then filtered. The filtrate was neutralized with ammonia and the resulting precipitate was filtered off, dried and recrystallized from methanol.

5-((E)-(4-(2-(-2-arylidenehydrazinyl)thiazol-4-yl)phenyl)diazenyl)-2-(methylthio)pyrimidin-4-ol (5a-c):

Procedure 1: A mixture of **4** (1.3 g, 3 mM) and the desired thiosemicarbazone derivatives (3 mM) in absolute ethanol (40 ml) was heated under reflux for 15 hours. The reaction mixture was then cooled and the formed solid was filtered off, dried, and recrystallized from methanol.

Procedure 2: A mixture of **4** (1.3 g, 3mM) and acetylsemicarbazide was heated under reflux for 10 hours then cooled. The formed solid was filtered off, dried and recrystallized from methanol to afford **(E)-5-((4-(2-hydrazinylthiazol-4-yl)phenyl)diazenyl)-2-(methylthio)pyrimidin-4-ol (6).** Compound **6** was heated under reflux (1.2 g, 3 mM) with the appropriate aldehyde (3 mM) in ethanol (50 ml). The mixture was then cooled, filtered off, dried and recrystallized from methanol.

(E)-1-(4-((E)-(4-hydroxy-2-(methylthiopyrimidin-5-yl)diazenyl)aryl)-3-phenylprop-2-en-1-ones (7a-c):

A mixture of equimolar amounts of **3** and the appropriate aldehydes in 10% ethanolic sodium hydroxide (50 ml) was shaken at room temperature for 24 hours. The mixture was then heated under reflux for 1 hour then cooled and poured into ice/cold water. The precipitate that formed after neutralization with dilute HCl was filtered off and recrystallized from aqueous DMF.

(E)-2-(1-(4-(4-hydroxy-2(methylthio)pyrimidin-5-yl)diazenyl)phenyly)ethylidene)-N-substitutedhydrazinecarbothioamide (8a-c):

A mixture of **3** (1.1 g, 3 mM) and the appropriate substituted thiosemicarbazide (1.1 g, 3 mM) was heated under reflux in absolute ethanol (30 ml) for 15 hours then cooled. The precipitate was filtered off, dried and recrystallized from aqueous DMF.

(E)-2(1-4-(4-hydroxy-2-(methylthio)pyrimidin-5-yl)diazenyl)phenyl)ethylidene) hydrazinecarboxamide (9):

To a solution of **3** (1 mM) in ethanol (50 ml), a solution of semicarbazide hydrochloride (1 mM) and sodium acetate (2 mM) in water (20 ml) was added. The reaction mixture was heated under reflux for 6 hours then evaporated to half of its volume and then poured onto ice/water. The separated solid was filtered off, washed with water, dried and recrystallized from aqueous DMF to give the semicarbazone as pale yellow powder.

(E)-5-((4-(1,2,3-selenadiazol-4-yl) phenyl)diazenyl)-2-(methylthio)pyrimidin-4-ol (10):

The semicarbazone **9** (1 mM) was dissolved in boiling glacial acetic acid (40 ml) and powdered selenium oxide (0.9 g) was added portion wise with stirring. The reaction mixture was heated under reflux with stirring for 2 hours then cooled and poured into ice/water. The product was extracted with ether and the extract was washed with 10% Na_2CO_3 solution then with water. The product was dried over anhydrous $MgSO_4$. The ether was removed and the residue was recrystallized from ethanol to afford 65% of light brown needles of **10**.

(E)-5-((4-(1,2,3-thiadiazol-4-yl)phenyl)diazenyl)-2-(methylthio)pyrimidin-4-ol (11):

Thionyl chloride (10 ml) was gradually added to the semicarbazone **9** (5 mM) and the mixture was gently warmed and then left for 24 hr at room temperature. An ice- cooled saturated NaHCO3 solution was then added and the product was extracted with ether, and the extract was worked up as usual. The residue was crystallized from aqueous DMF as white crystals.

(E)-6-(4-((4-hydroxy-2-(methylthiopyrimidin-5-yl)diazenyl)phenyl)-2-oxo-4-substituted-1,2-dihydropyridine-3-carbonitrile (12a-c):

A mixture of **3** (1.13g, 3.9 mM), the appropriate aldehyde (3 mM), excess ammonium acetate (1.89, 8.0 mol) and ethylcyanoacetate (0.35 g, 3 mM) in 50 ml absolute ethanol was refluxed for about 8 – 10 hours, the reaction mixture was concentrated to its half volume, filtered and the filtrate was poured into ice / water and the produced precipitate was filtered off, dried and recrystallized from aq. DMF.

(E)-1-(4-((4-hydroxy-2-(methylthio)pyrimidin-5-yl)diazenyl)phenyl)-3-morpholinopropan-1-one (13a), (E)-1-(4-((4-hydroxy-2-(methylthio)pyrimidin-5-yl)diazenyl)phenyl)-3-(4-methylpiperazin-1-yl)propan-1-one (13b) and (E)-3-(diethylamino)-1-(4-((4-hydroxy-2-(methylthio)pyrimidin-5-yl)diazenyl)phenyl)propan-1-one (13c):

A mixture of 1.8 gm (60 mM) of paraformaldehyde, and (5 mM) of the appropriate amine in absolute ethanol (25 ml) was heated till complete solubility of paraformaldehyde, (2.5 g ; 8.7 mM) of **3** dissolved in ethanol (10 ml) was then added and the mixture was heated under reflux for 5 hours, cooled and filtered. The produced solid was dried and recrystallized from methanol.

3.2 ANTIBACTERIAL ACTIVITY:

Sensitivity tests:

Disc diffusion sensitivity tests were done in a manner identical to that of Bauer et al 1966[21]. Some compounds with inhibition zone diameter more than 5 mm were subjected to determination of minimal inhibitory concentration (MIC) by Serial Dilution Method. Broth dilution tests,utilizing serial log2 dilutions of the tested compounds over the range of 50 to 0.025 µg/ml, were performed by using liquid media and a bacterial inoculum standardized to yield $1.5x10^6$ organisms/ml 0 time.For this purpose, organisms in the exponential growth phase (pre grown for 6hr at 35^0 C in liquid media) were adjusted to McFarland BaSO4 standard no.o.5, the turbidity of which corresponds to that of 1.5×10^8 organisms/ml. The adjusted suspention of organisms was further was diluted 50 fold in the selected liquid medium (corresponding to 3 $x10^6$ organisms / ml). Assay tubes received 1ml of the respective double strength dilution of antibiotic and 1ml of bacterial inoculum. Control tubes received 1ml of MHB and 1ml of bacterial inoculum. The assay and control tubes were incubated at 35^0C for 18 hr. The minimal inhibitory concentration (MIC) of tested compounds were defined as the lowest concentration of antibiotic completely inhibiting growth as judged by visual inspection. 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 35^0C for 24 hr. The MBC was defined as the lowest concentration of gentamicin yielding no growth after subculture to blood agar.

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%*
4	95	267-9 (Methanol)	$C_{12}H_9BrN_4O_2S$ (353.19)	40.81 40.88	2.57 2.28	15.86 15.89
5a	90	288-9 (Methanol)	$C_{21}H_{17}N_7OS_2$ (447.54)	56.36 56.41	3.83 3.45	21.91 21.92
5b	88	311-2 (Methanol)	$C_{22}H_{19}N_7O_2S_2$ (477.56)	55.33 55.37	4.01 4.38	20.53 20.21
5c	86	260-1 (Methanol)	$C_{20}H_{17}N_7O_2S_2$ (451.52)	53.21 53.80	3.79 3.88	21.71 21.94
6	80	266-2 (Methanol)	$C_{14}H_{13}N_7OS_2$ (359.43)	46.78 46.92	3.65 3.44	27.28 27.48
7a	81	288-2 (aqueous DMF)	$C_{20}H_{16}N_4O_2S$ (376.43)	63.82 63.71	4.28 4.55	14.88 14.54
7b	85	292-3 (aqueous DMF)	$C_{21}H_{18}N_4O_3S$ (406.46)	62.05 61.97	4.46 4.66	13.78 13.84
7c	79	287-8 (aqueous DMF)	$C_{19}H_{16}N_4O_3S$ (380.42)	59.99 59.87	4.24 4.50	14.73 14.97
8a	70	286-8 (aqueous DMF)	$C_{15}H_{17}N_7OS_2$ (375.47)	47.98 47.55	4.56 4.75	26.11 26.20
8b	72	260-1 (aqueous DMF)	$C_{16}H_{19}N_7OS_2$ (389.50)	49.34 48.91	4.92 4.58	25.17 25.22
8c	71	223-5 (aqueous DMF)	$C_{20}H_{18}ClN_7OS_2$ (471.99)	50.89 50.52	3.84 3.99	20.77 20.39
9	69	201-2 (aqueous DMF)	$C_{14}H_{15}N_7O_2S$ (345.38)	48.69 48.75	4.38 4.60	28.39 28.49
10	69	195-7 (Ethanol)	$C_{13}H_{10}N_6OSSe$ (377.28)	41.39 41.35	2.67 3.06	22.28 22.10
11	68	205-7 (aqueous DMF)	$C_{13}H_{10}N_6OS_2$ (330.39)	47.26 47.64	3.05 3.10	25.44 25.60
12a	66	178-9 (aqueous DMF)	$C_{23}H_{16}N_6O_2S$ (440.48)	62.72 62.66	3.66 3.99	19.08 19.30
12b	64	140-1 (aqueous DMF)	$C_{24}H_{18}N_6O_3S$ (470.50)	61.27 61.25	3.86 4.14	17.86 17.61
12c	67	>300 (Ethanol)	$C_{22}H_{16}N_6O_3S$ (444.47)	59.45 58.88	3.63 3.35	18.91 18.71
13a	63	266-8 (Ethanol)	$C_{18}H_{21}N_5O_3S$ (387.46)	55.80 55.88	5.46 5.77	18.08 17.89
13b	64	263-5 (Ethanol)	$C_{19}H_{24}N_6O_2S$ (400.50)	56.98 56.58	6.04 6.32	20.98 21.16
13c	61	208-9 (Ethanol)	$C_{18}H_{23}N_5O_2S$ (373.47)	57.89 58.11	6.21 6.44	18.75 18.98

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)*
4	3370 (OH-Hydrogen bonded), 2980 (CH-aliphatic), 1730 (C=O), 1617 (N=N), 1460 (H_3C-S)	353.2 15.1%	3.1 (2H, s, H_3C-S), 2.6 (2H, s,CH_2Br), 7-7.8 (4H, dd, Ar-H), 8.1 (1H, s, pyrimidine), 10.5 (1H, s, OH exchangeable with D_2O).
5a	3380 (OH), 3320 (NH), 2981 (CH-aliphatic), 1620 (N=N), 1462 (CH_3-S)	447.2 54.3%	2.5 (3H, s, CH_3-S), 6.5 (1H, s, N=CH), 7.1-7.9 (10H, m, Ar-H), 8.2 (1H, s, pyrimidine), 9.1 (1H, s, OH exchangeable with D_2O), 10.5 (1H, s, NH exchangeable with D_2O)
5b	3370 (OH), 3310 (NH), 2970 (CH-aliphatic), 1617 (N=N), 1461 (CH_3-S)	477.5 8.5%	2.5 (3H, s, CH_3-S), 4.1 (1H, s, OCH_3), 6.4 (1H, s, N=CH), 6.9-7.6 (4H, d,d, Ar-H), 7.7-7.8 (4H, d,d, Ar-H), 7.9 (1H, s, thiazole), 9 (1H, s, OH exchangeable with D_2O), 10.6 (1H, s, NH exchangeable with D_2O)
5c	3375 (OH), 3315 (NH), 2980 (CH-	451.6	2.3 (3H, s, CH_3), 6.3 (1H, s, N=CH), 7.2-8 (8H, m, aromatic),

Comp. No.	*IR (KBr) ν (cm^{-1})*	*M.S, EI m/z*	*^{1}HNMR (DMSO-d_6) δ (ppm)*
	aliphatic), 1619 (N=N), 1460 (CH_3-S)	73.4%	8.2 (1H, s, pyrimidine), 9.1 (1H, s, OH exchangeable with D_2O), 10.5 (1H, s, NH exchangeable with D_2O)
6	3400 (OH), 3350, 3310 (NH-NH_2), 2982 (CH-aliphatic), 1620 (N=N), 1460 (CH_3-S)	359.4 43.2%	2.5, 3.2 (3H, NH-NH_2 exchangeable with D_2O), 2.4 (3H, s, CH_3), 7.1-7.4 (4H, d,d, Ar-H), 7.9 (1H, s, thiazole), 8.2 (1H, s, pyrimidine), 10.4 (1H, s, OH exchangeable with D_2O)
7a	3370 (OH), 2971 (CH-aliphatic), 1690 (C=O), 1620 (N=N), 1450 (CH_3-S)	376.4 12.5%	2.4 (3H, s, CH_3), 6.4-6.7 (2H, dd, Ar-H), 7.1-7.8 (9H, m, Ar-H), 8.1 (1H, s, pyrimidine), 10.5 (1H, s, OH exchangeable with D_2O)
7b	3366 (OH), 2970 (CH-aliphatic), 1690 (C=O), 1625 (N=N), 1460 (CH_3-S)	406.3	2.4 (3H, s, CH_3), 6.5-6.7 (2H, dd, Ar-H), 7.1-7.9 (8H, dd, Ar-H), 8.2 (1H, s, pyrimidine), 10.4 (1H, s, OH exchangeable with D_2O)
7c	3370 (OH), 2966 (CH-aliphatic), 1685 (C=O), 1625 (N=N), 1467 (CH_3-S)	380.4 65.9%	2.2 (3H, s, CH_3), 2.3 (3H, s, CH_3), 6.4-6.6 (2H, dd, Ar-H), 7.1-7.8 (6H, m, Ar-H), 8.1 (1H, s, pyrimidine), 10.6 (1H, s, OH exchangeable with D_2O)
8a	3360 (OH-Hydrogen bonded), 3310 (NH), 2980 (CH-aliphatic), 1630 (N=N), 1460 (CH_3-S)	375.4 13.7%	2.1(3H, s, CH_3), 2.2 (3H, s, CH_3), 2.3 (3H, s, CH_3), 6.1 (1H, s, NH exchangeable with D_2O), 6.3 (1H, s, NH exchangeable with D_2O), 7.2-7.7 (2H, dd, Ar-H), 8.1 (1H, s, pyrimidine), 10.5 (1H, s, OH exchangeable with D_2O)
8b	3360 (OH-Hydrogen bonded), 3330 (NH), 2980 (CH-aliphatic), 1630 (N=N), 1460 (CH_3-S)	389.1 37.3%	2.0 (3H, s, CH_3), 2.9 (2H, q, ???), 2.2 (3H, s, CH_3), 2.3 (3H, s, CH_3), 6.1 (1H, s, NH exchangeable with D_2O), 6.3 (1H, s, NH exchangeable with D_2O), 7.3-7.8 (4H, dd, Ar-H), 8.1 (1H, s, pyrimidine), 10.4 (1H, s, OH exchangeable with D_2O)
8c	3350 (OH-Hydrogen bonded), 3330 (NH), 3100 (CH-aromtic), 2970 (CH-aliphatic), 1630 (N=N), 1460 (CH_3-S)	472.0 100%	2.1 (3H, s, CH_3), 2.2 (3H, s, CH_3), 6.1 (1H, s, NH exchangeable with D_2O), 6.4 (1H, s, NH exchangeable with D_2O), 7.2-7.3 (4H, dd, Ar-H), 7.5-7.7 (4H, dd, Ar-H), 8.2 (1H, s, pyrimidine), 10.4 (1H, s, OH exchangeable with D_2O)
9	3360 (OH-Hydrogen bonded), 3350 NH_2, 3335 (NH) 3120 (CH-aromtic), 2980 (CH-aliphatic), 1637 (N=N), 1660 (C=O), 1460 (CH_3-S)	345.4 32.5%	2.2 (3H, s, CH_3), 2.3 (3H, s, CH_3), 6.2 (1H, s, NH exchangeable with D_2O), 6.3 (1H, s, NH exchangeable with D_2O), 7.2-7.5 (4H, dd, Ar-H), 8.2 (1H, s, pyrimidine), 10.5 (1H, s, OH exchangeable with D_2O)
10	3360 (OH-Hydrogen bonded), 3350 NH_2, 3335 (NH) 3120 (CH-aromtic), 2980 (CH-aliphatic), 1637 (N=N), 1660 (C=O), 1460 (CH_3-S)	377.3 33.5%	2.2 (3H, s, CH_3), 7.1-8 (5H, m, Ar-H), 8.2 (1H, s, pyrimidine), 10.5 (1H, s, OH exchangeable with D_2O)
11	3500 (OH-Hydrogen bonded), 3150 (CH-aromtic), 2980 (CH-aliphatic), 1630 (N=N), 1450 (CH_3-S)	330.4 76.4%	2.3 (3H, s, CH_3), 7.2-8.5 (5H, m, Ar-H), 8.1 (1H, s, pyrimidine), 10.4 (1H, s, OH exchangeable with D_2O)
12a	3370(OH-Hydrogen bonded),3125(CH-aromatic), 2970 (CH-aliphatic),2222 (CN) 1680(C=O) 1630 (N=N), 1450 (CH_3-S)	440.4 5.9%	2.3 (3H, s, CH_3), 7.1-82(9H,m),8.2(1H,s,pyrimidine),8.3(1H,s,pyridine),10.5,11(2H,s,OH,NH exchangeable with D2O)
12b	3340(OH-Hydrogen bonded),3155(CH-aromatic), 2975 (CH-aliphatic),2232 (CN) 1670(C=O) 1630 (N=N), 1456 (CH_3-S)	470.5 54.6%	2.3 (3H, s, CH_3), 4.4(3H,S,OCH3),7.2-8.1(8H,dd),8.3(1H,s,pyrimidine),8.3(1H,s,pyridine),10.6,11(2H,s,OH,NH exchangeable with D2O)
12c	3347 (OH-Hydrogen bonded),3158 (CH-aromatic), 2985 (CH-aliphatic),2220 (CN) 1676(C=O) 1637 (N=N), 1459 (CH_3-S)	444.4 19.8%	2.3 (3H, s, CH_3),2.4 (3H,CH3,furan),),7.0-8.2 (6H,m),8.3(1H,s,pyrimidine),8.3(1H,s,pyridine),10.4,11.(2H,s, OH,NH exchangeable with D2O)
13a	3349(OH-Hydrogen bonded),3170 (CH-aromatic), 2975 (CH-aliphatic), 1702(C=O) 1637 (N=N), 1459 (CH_3-S)	387.4 96%	2.1 (3H, s, CH_3),2.3,2.4(8H of morpholine),2.5(4H,CH2CH2), 7.3,7,9 (4H,dd,aromatic)8.3(1H,s,pyrimidine), 10.4 (1H, s, OH exchangeable with D_2O).
13b	3356(OH-Hydrogen bonded),3167 (CH-aromatic), 2985 (CH-aliphatic), 1710(C=O) 1639 (N=N), 1465 (CH_3-S)	400.2 6.9%	2.2 (6H, s,2 CH_3), 2.3,2.4(8H of piperazine),2.5(4H,CH2CH2), 7.1,7,9 (4H,dd,aromatic), 8.3(1H,s,pyrimidine), 10.4 (1H, s, OH exchangeable with D_2O).
13c	3376(OH-Hydrogen bonded),3180 (CH-aromatic), 2965 (CH-aliphatic),	373.3 23.2%	2.2 (3H, s, CH_3),1.9(6H,t),2.8 (4H,q), 2.5(4H,CH2CH2),7.2,7,9 (4H,dd,aromatic), 8.3(1H,s,pyrimidine), 10.3 (1H, s, OH

Comp. No.	***IR (KBr) ν (cm^{-1})***	***M.S, EI m/z***	***^{1}HNMR (DMSO-d_6) δ (ppm)***
	1712(C=O) 1647 (N=N), 1498 (CH_3-S)		exchangeable with D_2O).

Table 3: Antibacterial test of the of the synthesized compounds with comparison to some known prepared derivatives measured by disc diffusion method a 5mm (0.5 cm)disk and broth dilution methods.

Comp.	***B.subtilis***	***E.coli***	***C.albicans***	***S.aureus***	**Sarcina**	***P.aeroginosa***	***M.phlei***	***St.faecalis***
4	….	….	….	….	1.9cm	….	….	….
5a	….	1.1cm	1.5cm	....	….	1.0cm	….	0.9cm
5b	…..	1.7cm	2.3 cm	….	1.6cm	….	….	. …
5c	0.7cm	1.3cm	…..	1.1cm	1.9cm	....	….	….
6		0.8cm	1.2cm		1.2cm			0.8cm
7a	….	….	….	….	….	….	….	….
7b	….	….	….	….	….	….	….	….
7c	….	….	….	….	….	….	….	….
8a	1.9cm	0.7cm	…..	….	1.8cm	….	….	1.9cm
8b	0.9cm	1.9cm	2.6cm	…..	1.8cm	….	…..	1.1cm
8c	1.9cm	1.5cm	2.8cm	…..	0.9cm	….	….	2.8cm
9	….	….	….	1.2cm	….	….	….	….
10	0.9cm	1.8cm	1.3cm	….	0.7cm	1.9cm	1.5cm	0.8cm
11	….	0.9cm	1.9cm	….	0.7cm	2.6cm	0.9cm	1.4cm
12a	0.7cm	….	….	0.9cm	0.7cm	0.8cm	….	….
12b	….	0.7cm	….	….	0.9cm	…..	1.2cm	…..
12c	1.0cm	0.9cm	….	….	….	….	….	….
13a	2.3cm	0.8cm	2.9cm	….	….	….	1.6cm	2.7cm
13b	…..	1.4cm	…….	….	1.9cm	…..	…..	….
13c	….	….	1.5cm	….	….	1.4cm	….	…..
S1	….	….	1.6cm	….	1.0 cm	….	1.5cm	….
S2	….	….	1.4cm	….	1.0 cm	….	1.3cm	….
S3	….	….	….	….	1.8cm	….	….	….

S1= 2-Thiouracil S2 = 2-Methyl-2-thiouracil

S3 = (E)-1-(4-((4-hydroxy-(methylthio)-1,2-dihydropyrimidin-5-yl)diazenyl)phenyl)ethanone (3)

Table4: Results of MIC, µg/L of some potents compounds:

Comp.	***B.subtilis***	***E.coli***	***C.albicans***	***S.aureus***	**Sarcina**	***P.aeroginosa***	***M.phlei***	***St.faecalis***
8a	128	412	….	….	756	….	….	456
8b	125	453	1600	….	165	….	….	653
8c	812	435	1236	….	635	….	….	432
13a	492	367	1435	….	….	….	764	423

4. RESULTS AND DISCUSSION

As it was mentioned earlier, studies have shown that aryl azo groups and alkyl mercaptopyrimidines are active in promoting antimicrobial activity [17]. Recently, the synthesis of a number of pyrimidine derivatives bearing the aryl azo group has been reported from these laboratories. Thiouracil itself could not be diazotized at position 5, but S-coupling occurs due to the high nucleophilicity of sulphur atom at position-2,therefore S-methylation of 2-thiouracil by methyl iodide in sodium hydroxide solution was carried out to diazotize it successfully at position-5[17], thus, here we developed a program to incorporate many nuclei of known antimicrobial activities such as thiazole, pyridine, thiadiazole to the aryl azo groups. Synthesis of the targeted compounds was achieved by methylation of 2-thiouracil **(1)** giving 2-methylthiouracil **(2)** which was successfully diazotized by diazonium salt of *p*-amino acetophenone to afford the methyl ketone derivative **3**. (Scheme I).

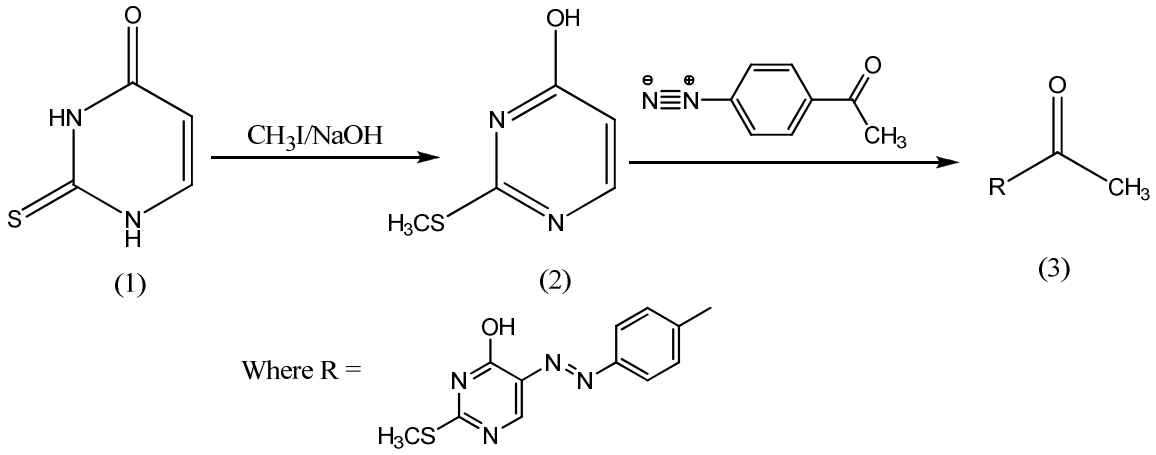
The key intermediate **3** could be brominated to afford the bromo derivative 4, which then was used as starting material for the synthesis of thiazolo derivatives **5a-c**.

The chalcone analogues **7a-c** were synthesized by reacting **3** with three different aldehydes in presence of ethanolic sodium hydroxide.

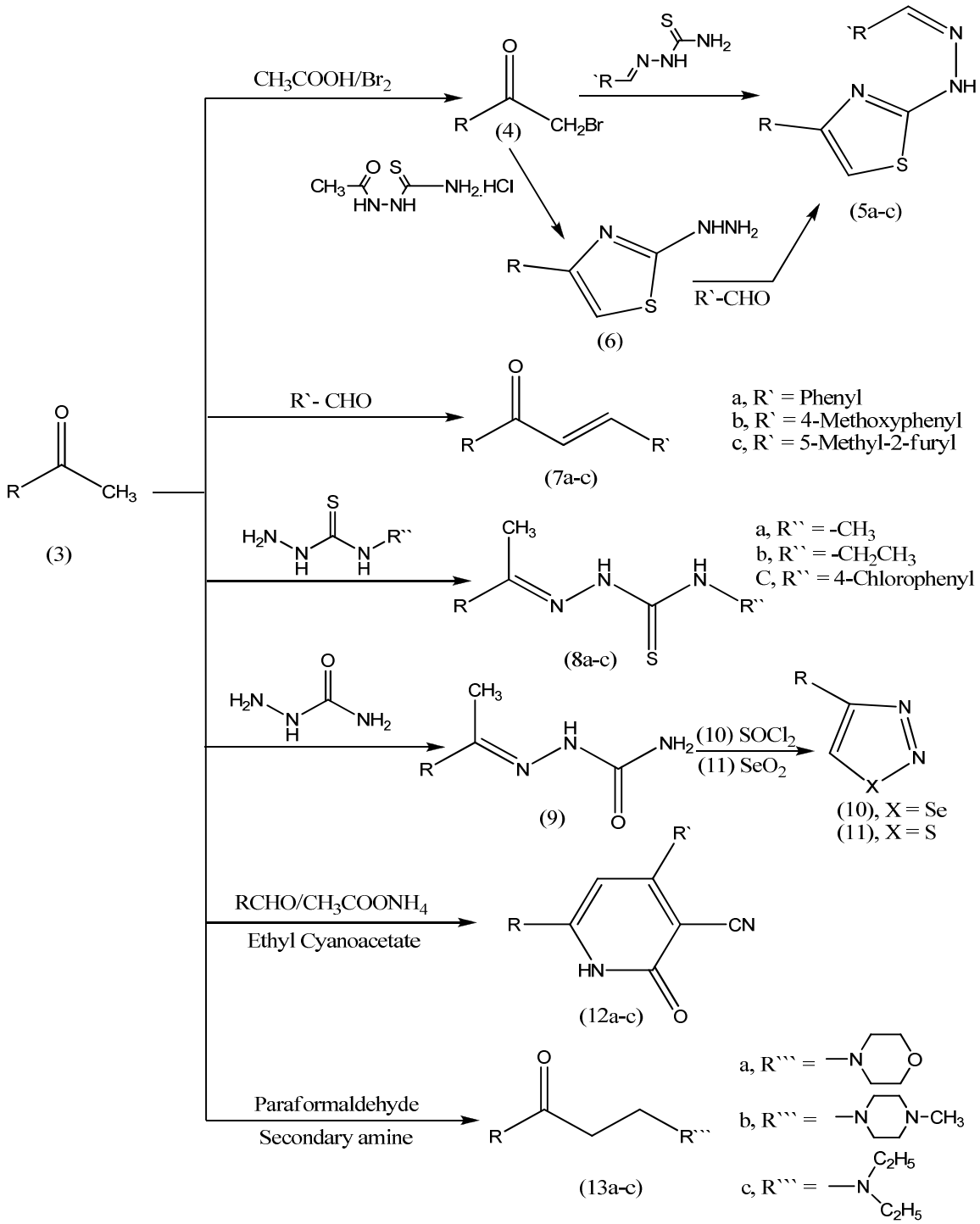
On the other hand, the methyl ketone **3** could be reacted with some substituted thiosemicarbazides to give thiosemicarbazones **8a-c** and with semicarbazide to yield the semicarbazone derivative **9** which was cyclized by $SOCl_2$ and SeO_2 affording the Selenadiazole and thiodiazole derivatives **10** & **11** respectively.

Moreover, some pyridine derivatives **12a-c** were prepared *via* a one pot reaction starting with compound **3** with the appropriate aldehydes, ethyl cyanoacetate in the presence of excessive ammonium acetate.

Finally, compound **3** could undergo Mannich's reaction with paraformaldehyde and secondary amines such as morpholine, methyl piperazine and diethyl amine to afford derivatives **13a-c** respectively. (Scheme II).



Scheme (1)



Scheme (2)

5. CONCLUSION

This work is an attempt to screen the antibacterial and antifungal activity of some novel pyrimidines substituted at 5- position due to their antimetabolite effect (inhibition of nucleic acid synthesis). All tested compounds had a variable activity except chalcones 7a-c which were devoid of any antimicrobial activity. The methyl ketone 3 and its bromo derivative 4 had a weak activity, but incorboration of thiazole, thiadiazole, selenadiazole and pyridine moieties increased the antimicrobial activity

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