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Original Research Paper

SYNTHESIS, CHARACTERIZATION AND STUDY OF ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES OF SOME NOVEL PYRIMIDINE DERIVATIVES

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ABSTRACT

Synthesis of pyrimidine derivatives bearing the aryl azo group and evaluation of their antimicrobial activities was achieved using Thiouracil **(1)** as a starting material. The starting compound was methylated with CH_3I to afford 5-methylthiouracil **(2)** which in turn was diazotized at position 5 via the reaction with diazonium salt of *p*-aminoacetophenone giving the azo compound **(3)**. This compound was monobrominated to afford the bromo derivative **(4)** which was used for the preparation of thiazole derivatives **(5a-c)** by its reaction with substituted thiosemicarbazones. Also, it was reacted with some aldehydes yielding chalcones **(7a-c)**. The methyl ketone **(3)** was also reacted with thiosemicarbazides giving thiosemicarbazone derivatives **(8a-c)**. On the other hand, reacting **(3)** with semicarbazide afforded the corresponding semicarbazone **(9)** which in turn was reacted with SeO or $SOCl_2$ yielding selene diazole or Thiadiazole **(10)** & **(11)** respectively. In addition, the methyl ketone **(3)** was a good substrate for the preparation of pyridines **(12a-c)** by its reaction with some aldehydes in presence of ethyl acetoacetate and excess ammonium acetate. Finally, Mannich reaction was carried out by reacting the key intermediate **(3)** with some secondary amines, paraformaldehyde to afford Mannich's bases **(13a-c)**. The prepared compounds showed variable activities as antimicrobial agents and indicated that substitution of 2-thiouracil at the 5^{th} position retained the antimicrobial activity.

Keywords: Thiouracil, 5-Substituted thiouracil, Methylmercaptopyrimidine, Antimicrobial pyrimidines.

INTRODUCTION

The literature indicated that a compound having pyrimidine nucleus possesses broad range of biological activities such as 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] It is known that the Pyrimidine ring is a building unit in both nucleic acids 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 between 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 times 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 pyridine into pyrimidine nucleus may enhance its biological activities.[9] In the light of the aforementioned 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.

MATERIALS AND METHODS

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.

Experimental

S-methyl-2-thiouracil (2)

It was prepared as in literature by treating 2-thiouracil with methyl iodide in sodium hydroxide solution [20]. mp 323–325 °C (lit. 325 °C).

1-{4-[(4-hydroxy-(methylthio)-1,2-dihydropyrimidin-5-yl)diazenyl]phenyl}ethanone (3)

It was prepared as in literature [17]. mp 276–278 °C (lit. 277 °C).

2-Bromo-1-{4-[(4-hydroxy-2-(methylthio)pyrimidin-5-yl)diazenyl]phenyl}ethanone (4)

A mixture of **3** (1.13 g, 0.005 mole) and bromine (0.005 mole) 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-{[4-(2-(2-arylidenehydrazinyl)thiazol-4-yl)phenyl]diazenyl}-2-(methylthio)pyrimidin-4-ol (5a-c)

Procedure 1

A mixture of **4** (1.1 g, 0.003 mole) and the desired thiosemicarbazone derivatives (0.003 mole) 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** (1.1 g, 0.003 mole) and acetylsemicarbazide was heated under reflux for 10 hours then cooled. The formed solid was filtered off, dried and recrystallized from methanol to afford *5-{[4-(2-hydrazinylthiazol-4-yl) phenyl] diazenyl}-2-(methylthio) pyrimidin-4-ol (6)*. Compound **6** was heated under reflux (1.2 g, 0.003 mole) with the appropriate aldehyde (0.003 mole) in ethanol (50 ml). The mixture was then cooled, filtered off, dried and recrystallized from methanol.

1-{4-[(4-hydroxy-2-(methylthio)pyrimidin-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.

2-{1-[4-(4-hydroxy-2(methylthio)pyrimidin-5-yl)diazenyl]phenyl}ethylidene-N-substituted hydrazine carbothioamide (8a-c)

A mixture of **3** (1.1 g, 0.003 mole) and the appropriate substituted thiosemicarbazide (0.003 mole) 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.

2-{1-[4-(4-hydroxy-2-(methylthio)pyrimidin-5-yl)diazenyl]phenyl}ethylidene hydrazinecarboxamide (9)

To a solution of **3** (1.1 g, 0.003 mole) in ethanol (50 ml), a solution of semicarbazide hydrochloride (0.001 mole) and sodium acetate (0.002 mole) 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.

5-{[4-(1,2,3-selenadiazol-4-yl)phenyl]diazenyl}-2-(methylthio)pyrimidin-4-ol (10)

The semicarbazone **9** (0.53 mole) 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**.

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** (0.005 mole) and the mixture was gently warmed and then left for 24 hr at room temperature. An ice- cooled saturated $NaHCO_3$ 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.

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, 0.003 mole), the appropriate aldehyde (0.003 mole), excess ammonium acetate (1.89g, 8.0 mole) and ethylcyanoacetate (0.35 g, 0.003 mole) in 50 ml absolute ethanol was heated under reflux 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 aqueous DMF.

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

A mixture of (1.8g, 0.005 mole) of paraformaldehyde, and (0.005 mole) of the appropriate amine in absolute ethanol (25 ml) was heated till complete solubility of paraformaldehyde, (2.5g, 0.005 mole) of compound **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.

BIOLOGICAL RESULTS

Antibacterial Activity

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.

Media disk sensitivity tests were 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.

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 at 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 x 10^{8} organisms/ml. The adjusted suspension of organisms was further was diluted 50 folds 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^{0}C 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^{0}C 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. ^{o}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	$C_{20}H_{16}N_4O_2S$	63.82	4.28	14.88

		(aqueous DMF)	(376.43)	63.71	4.55	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)	$C_{19}H_{24}N_6\ O_2S$ (400.50)	56.98 56.58	6.04 6.32	20.98 21.16
13c	61	208-9 (Ethanol)	$C_{18}H_{23}N_5\ O_2S$ (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 (270 MHz, 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, CH_2Br), 2.6 (3H, s, H_3C-S), 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 (3H, s, OCH_3), 6.4 (1H, s, N=CH), 6.9-7.6 (4H, dd, Ar-H), 7.7-7.8 (4H, dd, Ar-H), 7.9 (1H, s, thiazole), 8.2 (1H, s, pyrimidine) 9 (1H, s, OH exchangeable with D_2O), 10.6 (1H, s, NH exchangeable with D_2O)
5c	3375 (OH), 3315 (NH), 2980 (CH-aliphatic), 1619 (N=N), 1460 (CH_3-S)	451.6 73.4%	2.3 (3H, s, CH_3), 6.3 (1H, s, N=CH), 7.2-8 (7H, m, aromatic), 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-S), 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-S), 6.4-6.7 (2H, d,d, -CH=CH-), 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-S), 3.9 (3H, s, CH_3-O), 6.5-6.7 (2H, d,d, -CH=CH-), 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), 2.4 (3H, s, CH_3-S), 6.4-6.6 (2H, d,d, -CH=CH-), 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 (4H, dd, Ar-H), 8.1 (1H, s, pyrimidine), 10.5 (1H, s, OH exchangeable with D_2O)
8b	3360 (OH-Hydrogen bonded), 3330	389.1	2.0 (3H, t, CH_3), 2.9 (2H, q, CH_2), 2.2 (3H, s,

	(NH), 2980 (CH-aliphatic), 1630 (N=N), 1460 (CH_3-S)	37.3%	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-aromatic), 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-aromatic), 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 (2H, s, NH_2 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-aromatic), 2980 (CH-aliphatic), 1637 (N=N), 1660 (C=O), 1460 (CH_3-S)	377.3 33.5%	2.2 (3H, s, CH_3-S), 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-aromatic), 2980 (CH-aliphatic) , 1630 (N=N), 1450 (CH_3-S)	330.4 76.4%	2.3 (3H, s, CH_3-S), 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-S), 7.1-8.0(9H,m, Ar-H), 8.2(1H,s,pyrimidine),8.3(1H,s,pyridine),10.5, 11(2H,s,OH,NH exchangeable with D_2O)
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-S), 4.4(3H,S, CH_3-O), 7.2-8.1(8H,dd, Ar-H), 8.3(1H,s,pyrimidine),8.3(1H,s,pyridine),10.6, 11(2H,s,OH,NH exchangeable with D_2O)
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-S),2.4 (3H, CH_3,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 D_2O)
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-S),2.3,2.4(8H, m, morpholine),2.5(4H, t, t, CH_2CH_2), 7.3,7,9 (4H, dd, Ar-H), 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 (3H, s, CH_3), 2.4 (3H, s, CH_3), 2.3,2.4(8H, m, piperazine),2.5(4H, t, t, CH_2CH_2), 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), 1712(C=O) 1647 (N=N), 1498 (CH_3-S)	373.3 23.2%	1.9(6H,t-N,N-diethylamino), 2.2 (3H, s, CH_3), ,2.8 (4H,q, N,N-diethylamino), 2.5(4H, t, t, CH_2CH_2),7.2,7,9 (4H,dd, Ar-H), 8.3(1H,s,pyrimidine), 10.3 (1H, s, OH 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)

Table 4: Results of MIC, µg/L of some potent compounds

Comp.	*S.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

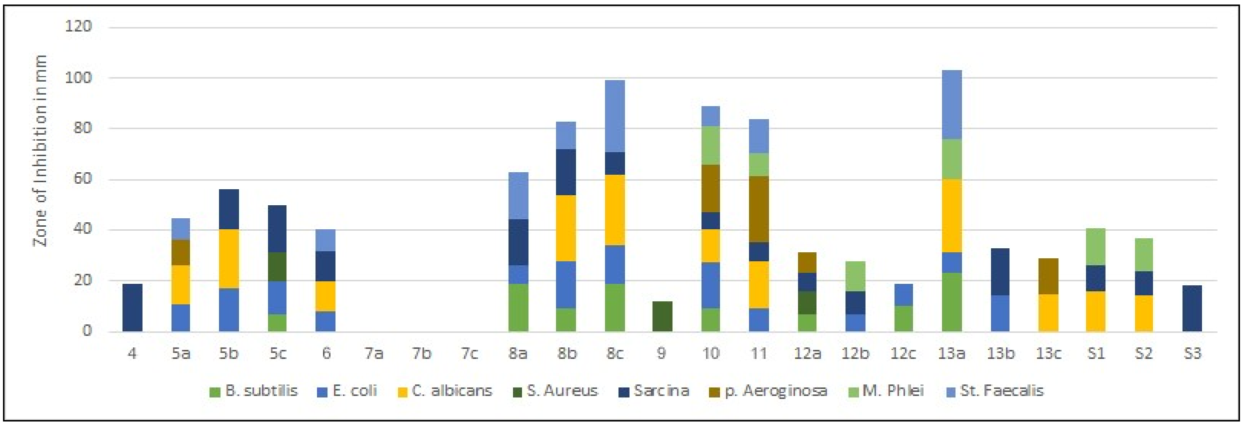


Figure 1: Antimicrobial activity (Gram +ve, -ve, Fungi) of synthesized compounds

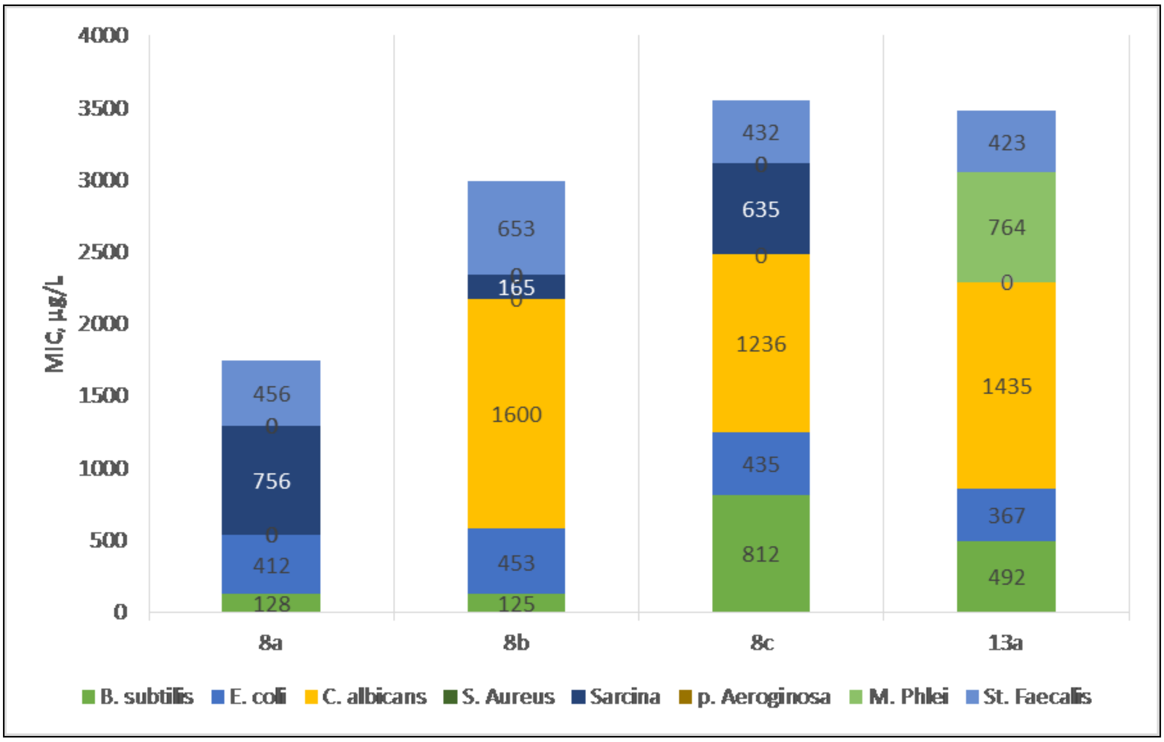


Figure 2: Results of MIC, µg/L of some potent compounds

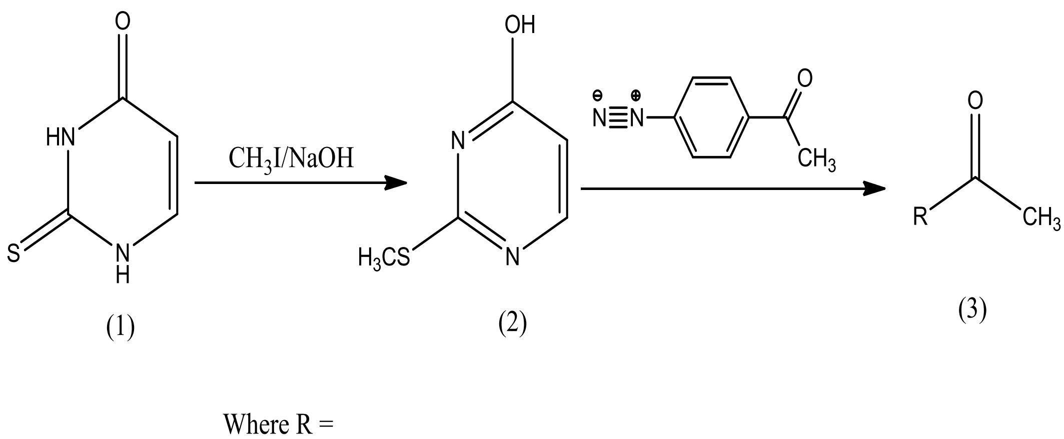
RESULTS AND DISCUSSION

As it was mentioned earlier, studies have shown that aryl azo groups and alkyl mercaptopyrimidines are active in promoting antimicrobial activity. The synthesis of a number of pyrimidine derivatives bearing the aryl azo group has been reported. 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] Moreover, 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).

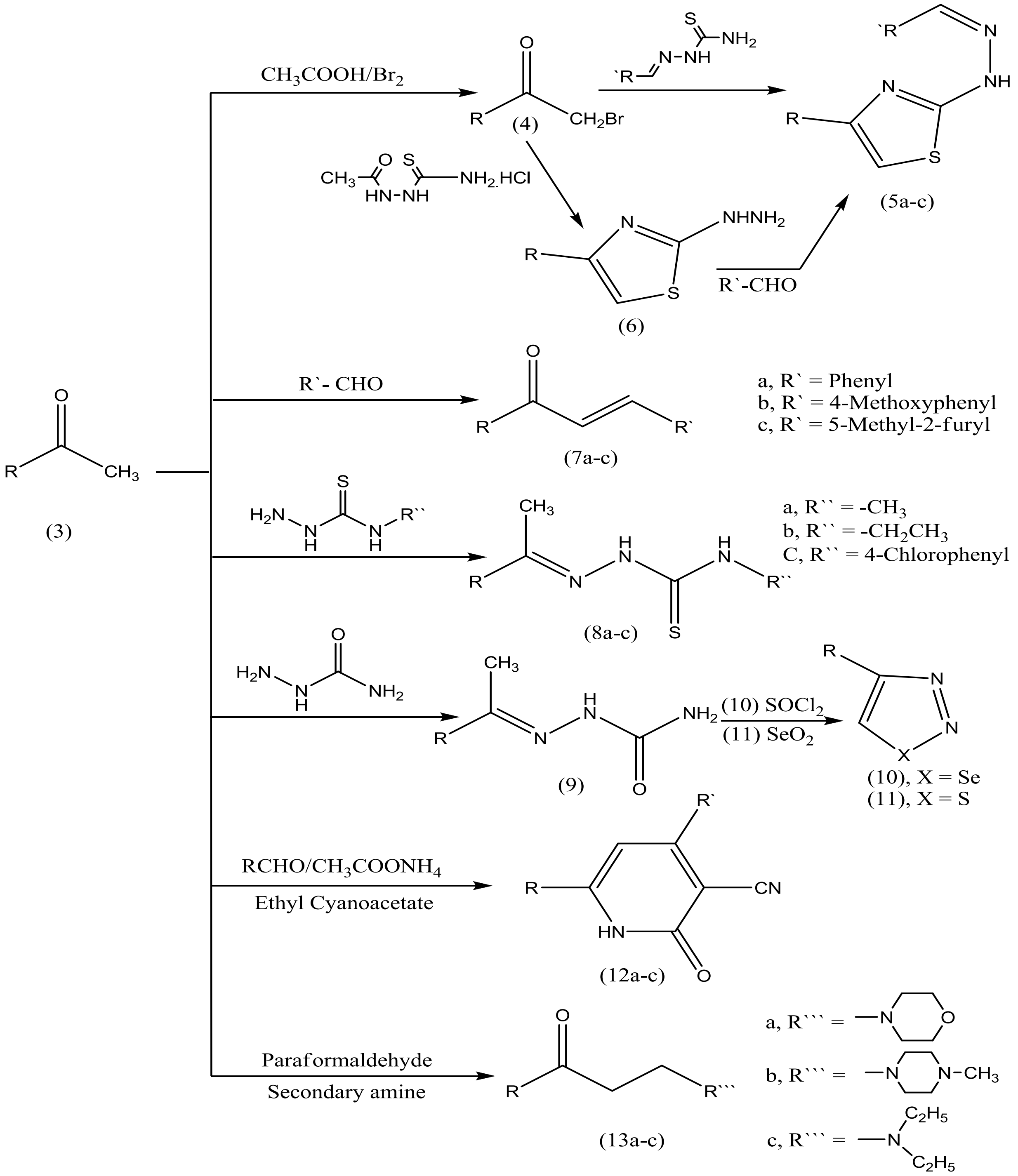
The key intermediate **3** was brominated to afford the bromo derivative **4**, which then was used as starting material for the synthesis of thiazolo derivatives **5a-c**. The chalcone analogues **7a-c** were synthesized by adopting the same procedure that was reported befor[22,23] by reacting **3** 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 technique[22-24] 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).

CONCLUSION

This work described herein 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 the incorporation of thiazole, thiadiazole, selenadiazole and pyridine moieties promoted the antimicrobial activity.



Scheme (1)



Scheme (2)

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