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Synthesis and Antimicrobial Evaluation of Some New Dihydropyrimidine Derivatives

Ashraf M. Hamouda and Khaled O. Mohamed

Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo, P.O. Box, 11562 Egypt

ABSTRACT

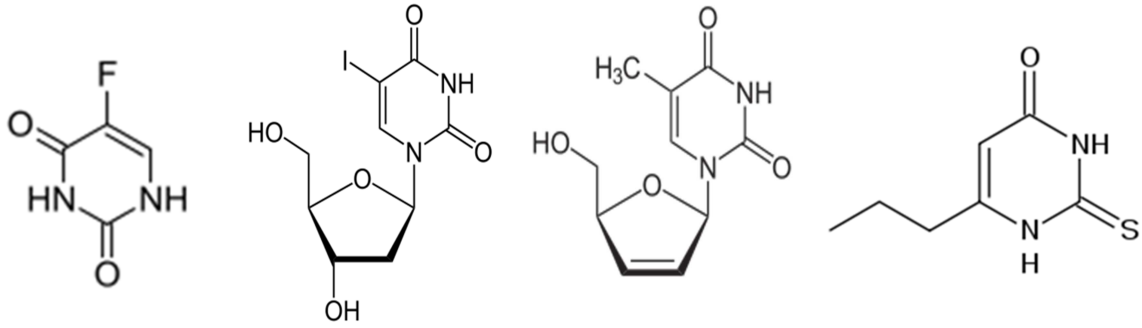
By the application of Beginili conditions, a new series of 1-substituted and 1-unsubstituted of 5-cyano-2-thiouracil ***1(a-h)*** *derivatives were synthesized and evaluated for their antimicrobial activities. Reacting our main tautomeric target compound 6-(benzo[b]thiophen-2-yl)-4-oxo-2-thioxo-1,2,3,4- tetrahydropyrimidine -5-carbonitrile* ***(1a)*** *with methyl iodide gave the dimethyl derivative* ***2*** *. Hydrazinolysis of compound* ***2*** *yielded the hydrazino compound* ***3****, which was converted to the corresponding pyrazole derivatives* ***4 (a,b)*** *by reacting with1-(4-bromophenyl)-3-(4-bromo or 4-fluorophenyl) prop-2-en-1-one. A series of azomethine compounds* ***6 (a-d)*** *were obtained by reacting the hydrazino compound* ***5*** *with different aromatic aldehydes, which was obtained by hydrazinolysis of compound* ***1a*** *with hydrazine hydrate. All the compounds were characterized by physical and spectral data. The compounds were screened for anti-microbial activity.*

Keywords: tetrahydropyrimidines, hydrazino, pyrazole, azomethine, antimicrobial , activity.

INTRODUCTION

The literature reported that Pyrimidine compounds have a wide range of applications in medicine due to their pronounced biological activity like1-β-D-arabinosylcytocine (Ara-C) and 5-flourouracil (5-FU) as anticancer; Idoxuridine and Triflouridine as antiviral; Zidovudine and Stavudine as anti-HIV; Trimethoprim, Sulphamethiazine and Sulphadiazine as antibacterial, Phenobarbitone as sedative, hypnotic and anticonvulsant; Propylthiouracil as antithyroid; Thonzylamine as H1-antihistaminics and Bacimethrine as antibiotics Fig 1 [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-HIV [3], antimicrobial [4], anti-inflammatory [5] and antioxidant [6]. Furthermore dihydropyrimidine derivatives also show the different pharmacological activities like antitumor [7], analgesic [8], antineoplastic [9], cardiovascular [10], antiallergic [11] Recently in the last year, It was reported that some series of 5- cyano-2-thiouracil derivatives show antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and antifungal activity against *Candida albicans* and *Aspergillus niger*, [12,13].Furthermore,2-hydrazinyl-4-morpholinotheino [3,2-d]pyrimidine derivatives were reported to possess a potent antitumor activity [14]. Among the pyrimidine containing heterocycles, thiouracils are potential therapeutics as antiviral, anticancer and antimicrobial agents [15-17]. For example, *S*-alkylation and N-alkylation products have been recently reported as novel antibacterial, cytotoxic agents [18,19] and unique HIV reverse transcriptase inhibitors [20]. Moreover, 6-aryl-5-cyano-2-thiouracil derivatives and their condensed heterocycles exerted promising chemotherapeutic activity as antimicrobial and anticancer agents [21-24]. Reports from our laboratory revealed that several hydrazino pyrimidine derivatives show significant biological activities [25-27]. This study was under taken in view of the fact that hyrazone moiety [28, 29] has been reported to possess significant chemotherapeutic activities.

On the other hand, Many pyrazole derivatives are reported to have a broad spectrum of biological activities, such as anti-inflammatory [30,31], antifungal [32], antiviral [33], cytotoxic [34], A3 adenosine receptor antagonists [35], antihypertensive [36], tranquilizing, muscle relaxant, psychoanaleptic, hypnotic, ulcerogenic, antidepressant, antibacterial and analgesic effects [37].. In the light of the aforementioned facts, and in continuation for our interest in the synthesis of biologically active heterocyclic compounds, we report here in the synthesis of novel 5-cyano-2-thiouracil derivatives **(schemes1 and 2)** incorporated with different biologically active heterocycles to investigate whether the resulting compounds have better biological activity as antimicrobial agents. All compounds were evaluated for antibacterial activity and antifungal activity.



5-flourouracil (5-FU) **Idoxuridine** **Stavudine (Zerit)** **Propyl thiouracil**

Figure . 1. Structures of some drugs containing dihydropyrimidine moiety

MATERIALS AND METHODS

Chemistry

General remarks; Melting point are uncorrected and determined in one end capillary tube using Gallen Kamp melting point apparatus MFP-595-010M (Gallen Kamp). Microanalysis was carried out at The Regional Center for Mycology and Biotechnology Al-Azhar University, Analysis indicated were within ± 0.5% of the theoretical value. Infrared spectra were recorded on Schimadzue FT-IR 8400S spectrophotometer (Schimadzue), and expressed in wave number (cm^{-1}) using potassium bromide disc. The NMR spectra were recorded on Bruker Hight Performance Digital FT-NMR Spectrophotometer Avance III 400 MHz,respectively, Faculty of Pharmacy, Cairo University, Cairo, Egypt,, ^{1}H spectra were run at 400 MHZ and ^{13}C at100.6 MHZ in dimethyl sulphoxide (DMSO-d6) using TMS as an internal standard. Chemical shift were quoted at δ and were related to that of the solvents .Mass spectra were performed as EI at 70ev on Hewlett Packard Varian (Varian,Polo,USA) and Shimadzu Gas Chromatograph Mass spectrometer-Qp 1000 EX, In The Regional Center for Mycology and Biotechnology Al-Azhar University. TLC was carried out using Art. DC-Plastikfolien, Kieselgel 60F254 sheets (Merck), the developing system were benzene acetone (4:1) and the spot were visualized at 366, 254 nm by UV Vilbre Lourmat 77202(Vilber).

General procedure for the preparation of 4-oxo-1,6-disubstituted-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1a-h)

A mixture of thiourea or substituted thiourea (0.1 mole), ethyl cyanoacetate (0.1 mole) and the appropriate aldehydes (0.1 mole) was stirred and refluxed in ethanolic solution of potassium hydroxide (0.1 mole in 20 ml) for 6 h and then the reaction mixtures were poured onto ice-water, then acidify with acetic acid. The precipitate formed was filtered off, dried then crystallized from DMF/H_2O to give compounds (**1a-h)**.

6-(Benzo[b]thiophen-2-yl) -4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1a).

M.p. >300°C, yield (52%).IR (KBr,cm^{-1}):3246,3220 (NH),3088,3037(CH aromatic),2220 (CN),1670 (C=O), 1635 (C=C),1213 (C=S). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm: 7.47-8.27 (m,4H, CH aromatic), δ ppm: 8.33(s,1H,NH-CS exchangeable by D_2O), δ ppm: 8.65(s,1H,C_3 of thiophene), *δ ppm:* 12.84 (s,1H,NH-CO exchangeable by D_2O). ^{13}C-NMR (100 MHz, DMSO-*d6*) *δ ppm:* 102.31(1) ,114.18(1) ,116.37(1) ,123.14(1) ,125.86(1) ,126.25(1) ,127.48(1) ,135.88(1) ,138.20(1) ,1141.28(1) ,159.79(1) ,163.67(1) ,178.12(1). Anal. Calcd. for $C_{13}H_7N_3OS_2$ (285): C, 54.70; H, 2.45; N, 14.73. Found: C, 54.91; H, 2.43; N, 14.76.

6-(Benzo[b]thiophen-7-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1b).

M.p. 295-297°C, yield (48%). IR (KBr, cm^{-1}): 3288, 3219 (NH), 3076,3037 (CH aromatic),2231 (CN), 1674(C=O) , 1625 (C=C), 1228 (C=S). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm: 7.57-8.14 (m, 5H, CH aromatic),δ ppm: 13.32.13.57 (2s,2H, NH-CS , NH-CO exchangeable by D_2O). MS (m/z) %: 287 (M^++2) 1.76%, 286 (M^++1) 3.33 %,

285 (M^+) 18.1 %. Anal. Calcd. for $C_{13}H_7N_3OS_2$ (285): C , 54.70 ; H, 2.45 ; N, 14.73. Found: C, 54.69; H, 2.40; N, 14.71.

4-(5-cyano-4-oxo-6-(thiophen-2-yl)-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl) benzenesulfonamide(1c).
M.p. 195-197°C, yield (58%). IR (KBr, cm^{-1}): 3394, 3313, 3224 (NH), 3076 , 3062 (CH aromatic), 2240 (CN), 1650 (C=O), 1597 (C=C), 1325 (S=O), 1236 (C=S). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm: 7.27(s,2H,SO_2NH_2 exchangeable by D_2O), δ ppm:7.67-8.17 (m,7H, CH aromatic), δ ppm: 10.16 (s,1H,NH exchangeable by D_2O). MS (m/z) %: 390 (M+) 1.77%. Anal. Calcd. for $C_{15}H_{10}N_4O_3S_3$ (390): C, 46.15; H, 2.56; N, 14.35. Found: C, 46.22; H, 2.67; N, 14.78.

4-(5-cyano-4-oxo-6-(2-flourophenyl)-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl) benzenesulfonamide (1d).
M.p. 180-182°C, yield (52%). IR (KBr, cm^{-1}): 3394, 3313, 3226 (NH), 3101, 3064 (CH aromatic), 2220 (CN), 1660(C=O), 1605 (C=C),, 1325(S=O), 1236 (C=S). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm: 7.27(s,2H,SO_2NH_2 exchangeable by D_2O), *δ ppm:* 7.67-8.17 (m,8H, CH aromatic),), δ ppm: 10.16 (s,1H,NH exchangeable by D_2O). MS (m/z) %: 402 (M+) 0.13%. Anal. Calcd. for $C_{17}H_{11}FN_4O_3S_2$ (402): C, 50.74; H, 2.73; N, 13.93. Found: C, 50.65; H, 2.79; N, 13.81.

4-(5-cyano-4-oxo-6-(3-flourophenyl)-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl) benzenesulfonamide (1e).
M.p.225-227°C, yield (68%).IR :(KBr, cm^{-1}) 3394, 3313,3226 (NH), 3072,3043 (CH aromatic), 2222 (CN), 1680 (C=O), 1616 (C=C),),(S=O)1325, 1244 (C=S). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm: 7.27(s, 2H, SO_2NH_2 exchangeable by D_2O) , δ ppm: 7.40-8.21 (m, 8H, CH aromatic), δ ppm: 10.44(s, 1H, NH exchangeable by D_2O).^{13}C-NMR(100 MHz,DMSO-*d6*) *δ ppm:* 109.83(1), 116.85(1), 117.45(1), 119.40(1), 122.19(2), 126.62(1) ,126.75(2) ,131.64(1) ,134.98(1), 139.07(1) ,143.29(1) ,150.50(1) ,161.28(1) ,163.71(1) ,181.84(1). Anal. Calcd. for $C_{17}H_{11}FN_4O_3S_2$ (402) C, 50.74; H, 2.73; N, 13.93. Found: C, 50.62; H, 2.82; N, 13.87.

4-(5-cyano-4-oxo-6-(4-flourophenyl)-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl) benzenesulfonamide (1f) .
M.p.209-211°C,yield(75%).IR:(KBr,cm^{-1})3394,3313,3224(NH),3085,3062 (CH aromatic), 2208 (CN), 1660(C=O),1620(C=C),1325 (S=O),1236 (C=S).^{1}H-NMR (400 MHz,DMSO-*d6*) *δ* ppm: 7.26(s,2H,SO_2NH_2 exchangeable by D_2O), *δ ppm* : 7.37-8.13(m,8H, CH aromatic),), *δ ppm* : 10.43(s,1H,NH exchangeable by D_2O). MS (m/z) %: 402 (M+) 1.48%. Anal. Calcd. for $C_{17}H_{11}FN_4O_3S_2$ (402): C, 50.74; H, 2.73; N, 13.93. Found: C, 50.52; H, 2.61; N, 13.80.

4-(5-cyano-4-oxo-6-(4-methoxyphenyl)-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)benzenesulfonamide (1g).
M.p. 190-192°C, yield (66%). IR:(KBr,cm^{-1}) 3394, 3313,3224 (NH), 3066,3049 (CH aromatic), 2981(CH aliphatic),2223 (CN), 1676(C=O) , 1587 (C=C),), 1313(S=O), 1263 (C=S). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm : 3.86(s,3H,OCH_3), δ ppm : 7.27(s,2H,SO_2NH_2 exchangeable by D_2O), *δ ppm :* 7.11-8.16 (m,8H, CH aromatic),), δ ppm : 10.27(s,1H,NH exchangeable by D_2O). MS (m/z) %: 414 (M+) 0.74%. Anal. Calcd. for $C_{18}H_{14}N_4O_4S_2$ (414): C, 52.17; H, 3.38; N, 13.52. Found: C, 52.35; H, 3.64; N, 13.22.

4-(5-cyano-4-oxo-6-(4-cholorophenyl)-2-thioxo-3,4-dihydropyrimidin-1(2H)-yl)benzenesulfonamide (1h).
M.p. 233-235°C , yield (58%). IR:(KBr,cm^{-1}) 3394, 3315,3232 (NH), 3065,3043 (CH aromatic), 2210 (CN), 1660(C=O) , 1625 (C=C),), 1323 (S=O), 1236 (C=S). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm : 7.26(s,2H,SO_2NH_2 exchangeable by D_2O), δ ppm : 7.59-8.06(m,8H, CH aromatic),), δ ppm : 10.51(s,1H,NH exchangeable by D_2O). MS (m/z) %: 418.08 (M+) 0.13%. Anal. Calcd. for $C_{17}H_{11}ClN_4O_3S_2$ (418.5): C, 48.74; H, 2.62; N, 13.38. Found: C, 48.65; H, 2.69; N, 13.50.

4-(benzo[b]thiophen-2-yl)-1-methyl-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5- carbonitrile (2).
To a solution of 5-cyano-2-mercapto-6-–(benzo[b]thiophene-2-yl)-3,4-dihydropyrimidin-4-one 1a (0.01 mole) in DMF (20ml), potassium carbonate (0.02 mole) and methyl iodide (0.02 mole) were added and the mixture was stirred for 3 h at room temperature,. The contents were poured into water, the formed precipitate was filtered and crystallized from DMF.
M.p. 296-298°C, yield (88%). IR:(KBr,cm^{-1}) 3041 (CH aromatic), 2955,2860 (CH aliphatic), 2218 (CN), 1670(C=O),1620(C=C),1585(C=N). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm : 2.66 (s,3H, SCH_3), δ ppm : 3.46 (s,3H,NCH_3) δ ppm :7.44-8.35 (m,4H,CH aromatic) δ ppm : 8.60(s,1H,H of thiophene).^{13}C-NMR(100 MHz, DMSO-*d6*) *δppm:*15.68(1),31.33(1),97.82(1),112.83(1), 114.28(1), 122.46(1), 123.18(1), 125.83(1), 126.39(1), 137.82(1), 139.21(1), 142.81(1), 160.38(1), 161.18(1), 175.41(1).Anal.Calcd.for $C_{15}H_{11}N_3OS_2$ (313): C, 57.51; H, 3.51; N, 13.41. Found: C, 57.32; H, 3.31; N, 13.65.

4-(benzo[b]thiophen-2-yl)-2-hydrazinyl-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (3).
A mixture of 5-cyano-3-N-methyl-2-methylthio-6-(benzo [b] thiophene-2-yl)-3,4-dihydropyrimidin

-4-one **2** (0.01 mole) and hydrazine hydrate (0.05 mole,99%) in absolute ethanol was refluxed for 12 h in an oil bath and poured onto crushed ice, the formed precipitate was filtered and crystallized from ethanol.
M.p. >300°C, yield (70%). IR :(KBr,cm^{-1}) 3329, 3250 (NH), 3055, 3041 (CH aromatic), 2924(CH aliphatic), 2208 (CN), 1670 (C=O) ,1622 (C=C), 1585 (C=N). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm : 3.29 (s,3H,NCH_3) *δ ppm* :3.45 (s,2H,NH_2,exchangeable by D_2O), δ ppm :7.33-8.37 (m,4H,CH aromatic,s,1H,NH, exchangeable by D_2O), δ ppm :8.49(s,1H,H of thiophene). ^{13}C-NMR (100 MHz, DMSO-*d6*) *δ ppm: 28.23*(1), 97.90(1), 113.86(1), 122.77(1),123.26(1), 123.99(1), 124.86(1),125.75(1), 134.75(1), 138.77(1), 140.16(1), 155.71(1), 158.43(1), 164.81(1). Anal.Calcd. For $C_{14}H_{11}N_5OS$ (297): C, 56.57; H, 3.70; N, 23.56. Found: C, 56.31; H, 3.60; N, 23.70.

General procedure for the preparation of 4-(aryl)-2-(5-(2-bromophenyl)-3-(4-bromophenyl)-1H-pyrazol-1-yl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (4a,b).
A mixture of compound 3 (0.04 mole), the appropriate 1-propenone (0.04 mole) and sodium hydroxide (0.2g, 0.05 mole) in absolute ethanol (30 ml) was refluxed for 72 h. The reaction mixture was poured on water, neutralized with 2N hydrochloric acid and the residue was filtered off. The crude product obtained was crystallized from isopropanol.

4-(benzo[b]thiophen-2-yl)-2-(3-(4-bromophenyl)-5-(4-fluorophenyl)-1H-pyrazol-1-yl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (4a).
M.p. 205-208°C, yield (55%).IR :(KBr,cm^{-1}),3055 (CH aromatic), 2924 (CH aliphatic), 2214 (CN), 1676(C=O) ,1602(C=C) 1583 (C=N). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm :3.45 (s, 3H, N-CH_3), *δ ppm :* 6.73 (s, 1H, C4 pyrazole), δ ppm :6.93-8.12 (m, 12H, aromatic CH), δ ppm :8.49(s.1H,H of thiophene).^{13}C-NMR(100 MHz,DMSO-*d6*) *δppm:*29.59(1),45.00(1),75.03(1), 115.38(1), 116.48(2), 117.65(1), 123.01(2),123.55(2),125.74(1),127.83(2),128.69(2)132.6(2),132.21(1),140.03(2) ,141.27 (1) , 141.68(1) , 147.92(1) , 148.92(1) , 152.58(1) , 161.38(1) , 162.63(1), 167.08(1). Anal.Calcd.for $C_{29}H_{17}BrFN_5OS$ (582): C, 59.79; H, 2.92; N, 12.02. Found: C, 59.43; H, 3.21; N, 11.87.

4-(benzo[b]thiophen-2-yl)-2-(3-(4-bromophenyl)-5-(2-bromophenyl)-1H-pyrazol-1-yl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (4b).
M.p. 170-173°C, yield (65%). IR:(KBr,cm^{-1}) 3055 (CH aromatic), 2953(CH aliphatic), 2214 (CN), 1662(C=O) ,1616(C=C) 1583 (C=N). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm :3.44 (s, 3H, N-CH_3), *δ ppm :* 6.90 (s, 1H, C4 pyrazole), δ ppm :7.41-7.98 (m, 12H, aromatic CH), δ ppm :8.64(s.1H,H of thiophene). MS (m/z) %: 645 (M^++2) 0.18%. Anal. Calcd. for $C_{29}H_{17}BrFN_5OS$ (643): C, 54.12; H, 2.64; N, 10.88. Found: C, 54.72; H, 2.91; N, 10.98.

4-(benzo[b]thiophen-2-yl)-2-hydrazinyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (5).
A mixture of 1a (0.005 mole) and hydrazine hydrate (0.005mole, 99%) in 30 ml absolute ethanol was refluxed for 30 hrs ,then cooled and poured on ice-water. The produced precipitate was filtered off, dried and crystallized from ethanol.
M.p. 298-300°C, yield (45%). IR:(KBr,cm^{-1}) 3309,3273,3213 (NH), 3053,3032 (CH aromatic), 2204 (CN), 1670(C=O) ,1647(C=C) 1589 (C=N).). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm :3.43 (s,1H,NH, exchangeable by D_2O), *δ ppm* :6.21(s,2H,NH_2,exchangeable by D_2O), δ ppm :7.4-8.23 (m,4H,CH aromatic), δ ppm :8.45 (s,1H,H of thiophene), δ ppm :10.81 (s,1H, NH=CO, exchangeable by D_2O). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ ppm: 97.90(1), 119.28(1), 122.87(1), 125.34(1), 125.69(1), 126.21(1), 126.75(1), 139.97(1), 141.00(1),142,75(1),154.81(1),161.43(1), 164.81(1). Anal. Calcd. for $C_{13}H_9N_5OS$ (283): C, 55.12; H, 3.18; N, 24.73. Found: C, 55.15; H, 3.20; N, 24.71.

General procedure for the preparation of (E)-4-(benzo[b]thiophen-2-yl)-2-(2-arylidenehydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6a-d).
A mixture of 5 (0.001 mole) and appropriate aromatic aldehydes (0.001 mole) in 15 DMF containing few drops of glacial acetic acid was heated under reflux for 6-8 hrs. , then cooled and poured on ice-water. The produced solid was filtered off, dried and crystallized from ethanol.

(E)-4-(benzo[b]thiophen-2-yl)-2-(2-benzylidenehydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile(6a).
M.p. 200-210°C, yield (75%).IR:(KBr,cm^{-1})3235 (NH), 3053,3035 (CH aromatic), 2210 (CN), 1693(C=O),1616 (C=C),1593(C=N). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm : δ ppm :3.44 (s,1H,NH, exchangeable by D_2O), 7.33-8.03 (m,9H,CH aromatic), *δ ppm* :8.59 (s,1H,H of thiophene), δ ppm : 9.32 (s,1H,N=CH), *δ ppm* :12.56 (1H, NH, exchangeable by D_2O). MS (m/z) %: 371 (M^+) 0.10%. Anal. Calcd. for $C_{20}H_{13}N_5OS$ (371): C, 64.69; H, 3.50; N, 18.87. Found: C, 49.15; H, 1.02; N, 13.70

(E)-4-(benzo[b]thiophen-2-yl)-2-(2-(4-fluorobenzylidene)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6b).

M.p. 295-297°C, yield (72%).IR:(KBr,cm^{-1})3235 (NH), 3066 (CH aromatic), 2214 (CN), 1689(C=O),1616(C=C),1589 (C=N). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm :3.55 (s,1H,NH,D_2O exchangeable), δ ppm :7.34-8.07 (m,8H,CH aromatic), δ ppm :8.68(s,1H,H of thiophene), δ ppm :9.32 (s,1H,N=CH), *δ ppm :*12.52 (2H,2NH, D_2O exchangeable). MS (m/z) %: 389 (M^+) 10.52%. Anal. Calcd. for $C_{20}H_{12}FN_5OS$ (405.5): C, 61.69; H, 3.08; N, 17.99. Found: C, 61.52; H, 3.12; N, 17.88

(E)-4-(benzo[b]thiophen-2-yl)-2-(2-(4-chlorobenzylidene)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6c) .

M.p. > 300°C , yield (55%).IR:(KBr,cm^{-1}) 3385,3207 (NH), 3078,3049 (CH aromatic), 2212 (CN), 1660(C=O),1635(C=C)1604 (C=N). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm :3.36(s,1H,NH,D_2O exchangeable), δ ppm :7.44-8.08 (m,8H,CH aromatic), *δ* :8.52(s,1H,H of thiophene), δ ppm :8.68 (s,1H,N=CH), *δ ppm :*12.59 (1H, NH, D_2O exchangeable). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ ppm :115.1981(1), 117.71(1), 122.99(1), 125.59(1), 126.05(1), 128.06(1), 129.52(2), 130.25(1), 130.46(2), 135.45(1), 136.48(1), 139.15(1), 141.34(1), 146.57(1), 153.57(1), 161.04(1),162.07(1),162.19(1).Anal.Calcd.for $C_{20}H_{12}ClN_5OS$ (405.5): C, 59.19; H, 2.96; N, 17.26. Found: C, 59.2; H, 2.65; N, 17.19.

(E)-4-(benzo[b]thiophen-2-yl)-2-(2-(4-nitrobenzylidene)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6d)

M.p. 282-284°C, yield (65%).IR:(KBr,cm^{-1}) 3300,3219 (NH), 3055,3014 (CH aromatic), 2214 (CN), 1699(C=O),1606(C=C),1590(C=N). ^{1}H-NMR (400 MHz, DMSO-*d6*) δ ppm :3.73(s,1H,NH,D_2O exchangeable), δ ppm :7.43-8.01 (m,8H,CH aromatic), δ ppm :8.58 (s,1H,H of thiophene), δ ppm :9.33(s,1H,N=CH), *δ ppm :* 12.69 (1H, NH, exchangeable by D_2O) MS (m/z) %: 416 (M^+) 0.14%.. Anal. Calcd. for $C_{20}H_{12}N_6O_3S$ (416): C, 57.69; H, 2.88; N, 20.19. Found: C, 57.45; H, 2.78; N, 20.21.

Antimicrobial activity screening

The newly synthesized compounds were evaluated for their in vitro antibacterial activity against *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC CC33, *Escherichia coli* ATCC 5087 and *Pseudomonas aeruginosa* ATCC 9027, as well as for their antifungal activity against *Candida albicans* ATCC 60193 *and Aspergillums Niger* ATCC 1718109 using the micro broth dilution method. [38]. The Gram-positive antibacterial agent, amoxicillin, the Gram-negative antibacterial agent, gentamycin, and the anti-fungal agent, amphotericin B, were used as controls. In addition to, MICs (minimum inhibitory concentration, MBCs (minimum bactericidal concentration and IC_{50} (the concentration which inhibits 50% of microorganisms) of all compounds were determined according to reported method.[38,39] .The in vitro antimicrobial properties against a number of Gram-negative and Gram-positive bacteria,and yeasts are presented in Tables 1.

Determination of the Minimum Inhibitory Concentration (MIC)

The preliminary MICs were firstly determined by the micro broth dilution method .[38]Briefly, 100 µL of double strength DMSO (Sigma-Aldrich, Germany) were placed in each well of a 96-well micro titer plate. Aliquot of 100 µL of the solutions to be tested were added to the first column, then two fold dilutions were carried out from one well to the next up to final well in each row for each tested compound. MICs were then determined using agar streaking technique as per Clinical Laboratory Standard Institute guidelines.[38] A total of 15 mL molten (45°C) Nutrient agar (Sigma-Aldrich, Germany) were supplemented with the required concentration then were added into sterilized Petri dishes, allowed to solidify. Then 10 µL of each bacterial or fungal suspension (105 CFU mL-1) were streaked onto the surface. Finally all plates were incubated at 37 °C for 24 hours for bacterial strains and 25 °C for 48 hours for fungal strains under aerobic conditions. MIC was determined as the average between the last plate had growth and the first plate with no growth.

Determination of the MBC and IC50

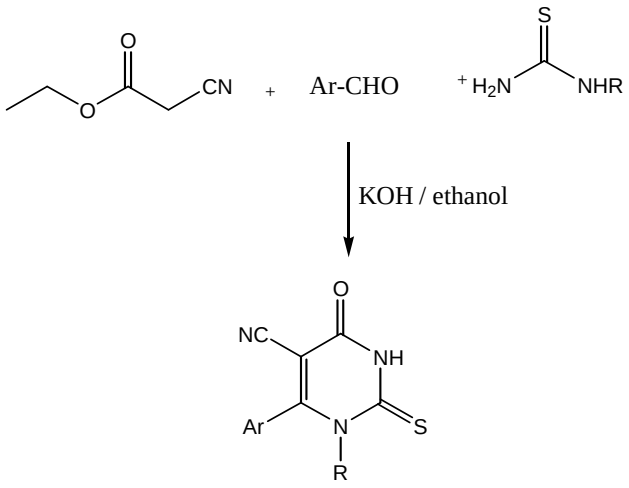
MBC and IC_{50} were determined in 96 well micro titer plate where a 100 µL of trypcase soya broth (Oxoid, USA) for bacterial isolates or sabaroud's dextrose broth for fungal strains were placed in each well. A proper amount of the stock solution of the tested compounds was added to reach the desired concentration. All columns were then inoculated with 20 µL of bacterial suspension (106 CFU mL-1) and incubated for 5-6 hours. An aliquot of 100 µL from each well was transferred into another pre-supplemented with 100 µL of Dey- engly broth medium (Fluka, USA) and allowed to stand for 10-20 minutes to neutralized any antimicrobial activities. Then these neutralized solutions were subjected to proper dilutions and streaked onto trypcase soya agar or sabaroud's dextrose agar plates to determine the viable count. [39] Controls were done for sterility and growth and subjected to the same regimen of treatment. MBC was determined as the lowest concentration which decreased the number of viable bacteria by 3 log units. IC_{50} was determined as the lowest concentration reduced the viable count by about 50%.

RESULTS AND DISCUSSION

Chemistry:

The synthetic pathways used for the preparation of the requried new compounds are illustrated in (schemes **1** and **2)** . The starting 5-cyano-2-thiouracil derivatives **1 a-h** were synthesized in one pot three components reaction from thiourea or 4-sulphamoylphenyl thiourea, ethyl cyanoacetate and different aromatic aldehydes in presence of potassium hydroxide in refluxing ethanol, the reaction proceed by Knoevenagal condensation and the condensed product react with thiourea or 4-sulphamoylphenylthiourea to form an intermediate, which is subsequently cyclized by nucleophilic attack of nitrogen on carbonyl carbon, The IR spectrum showed peacks in the regions 3394-3219 cm^{-1} (NH stretching), 2240-2208 cm^{-1} (CN) and 1670-1637 cm^{-1} (C=O) amide as diagnostic absorbtion, and ^{1}H-NMR spectra showed signals at δ =7.3-8.2 ppm(aromatic protons) and δ = 10.1-13.5 ppm (NH). Reaction of compound 1a with double amount of methyl iodide yielded compound 2 (scheme 2) where its structure was confirmed by spectra and analytical data. Disappearance of bands at 3394-3219 cm^{-1} corresponding to NH and as well as apperance of band at 2955,2860 cm^{-1} due to CH aliphatic.In addition, presence of a 2 singlet signals at δ = 2.76 ,3.46 ppm of two methyl groups with disapperance of NH signals in ^{1}H-NMR .Subjecting compound 2 to reaction with hydrazine hydrate yielded 3 . IR spectra showed appearance of bands at 3329-3250 cm^{-1} corresponding to NH, and in ^{1}H-NMR spectra showed two singlets signals at δ = 3.57 and 8.3 ppm exchanged with D_2O corresponding to hydrazine moiety,the disapperance of singlet signal at δ = 3.46 ppm due to S-CH_3 group. Consequently cyclocondensation of compound 3 with chalcone derivatives in refluxing ethanol gave pyrazoles 4a,bthe structure were confirmed by spectral and analytical data. Disappearance of bands at 3329-3250 cm^{-1} due to NH groups in IR spectra and increased number of aromatic protones in ^{1}H-NMR spectra confirmed reaction of hydrazine group,in addition appearance of one proton of CH-(pyrazole) appeared as singlets at δ= 6.73,6.90 ppm (4a,b) respectively, which confirm the structure of pyrazole ring. Reaction of compound 1a with hydrazine hydrate afforded compound 5,where it structure confirmed by IR spectra which showed bands at 3309-3213 cm^{-1} corresponding to NH groups , while in ^{1}H-NMR spectra showed signals at δ =3.4,6,21 and 10,8 ppm exchanged with D_2O. Schiffes bases 6a-d were achieved upon reaction of compound 5 with different aromatic aldehydes , the structure of compound 6a-d illucidate by IR specrta, showed peak of imine at 1604-1616 $cm^{-1}$and in addition ,signals at δ = 8.6-9.3 ppm in ^{1}H-NMR spectra.

Scheme 1



(1a-h)

1a) Ar = Benzo[b]thiophen-2-yl , R= H

1b) Ar = Benzo[b]thiophen-7-yl , R= H

1c) Ar = 2-Thienyl , R= 4-Sulfamoylphenyl

1d) Ar = 2-Flourophenyl , R= 4-Sulfamoylphenyl

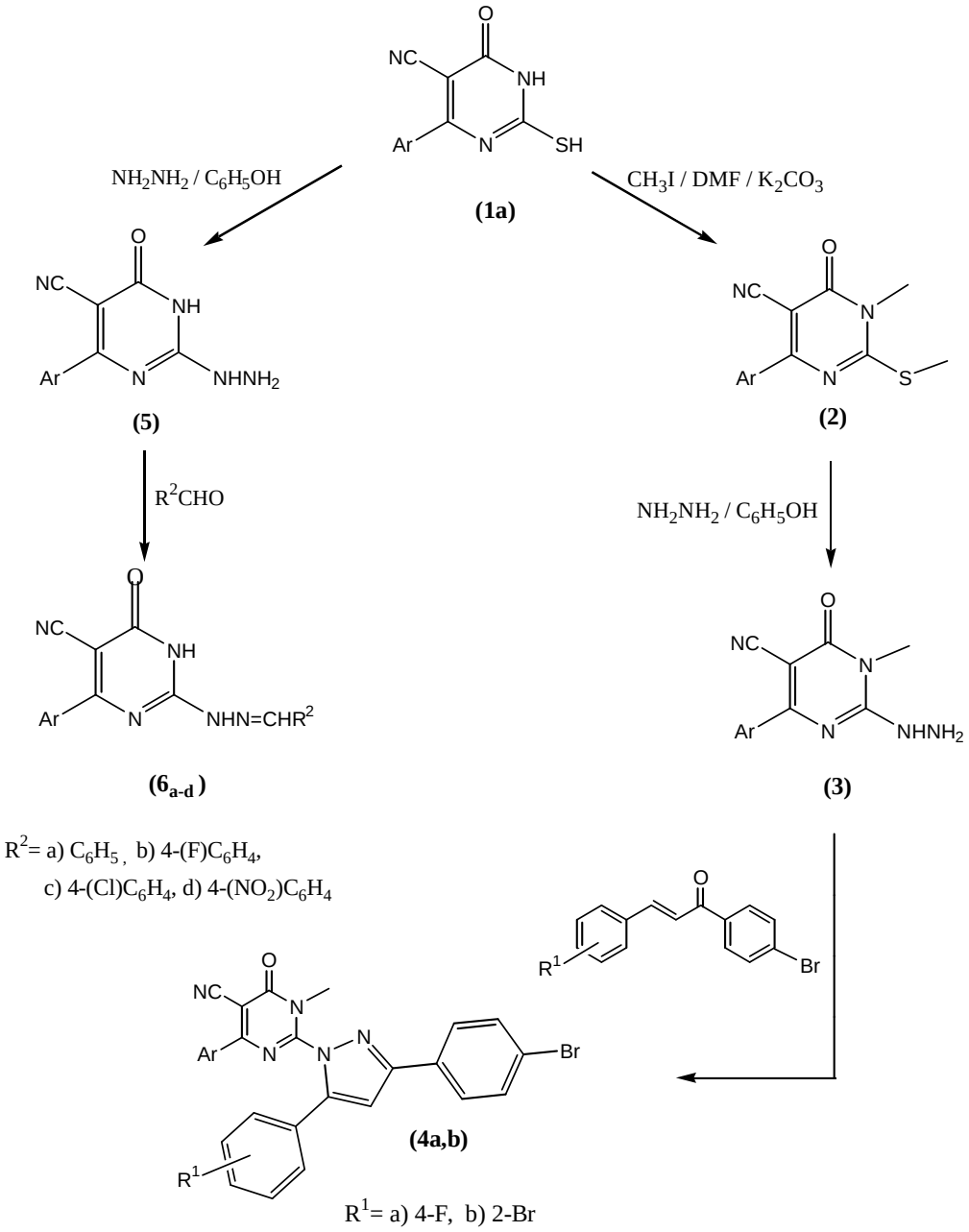
1e)Ar = 3-Flourophenyl , R= 4-Sulfamoylphenyl

1f) Ar = 4-Flourophenyl , R= 4-Sulfamoylphenyl

1g) Ar =4-Methoxyphenyl, R= 4-Sulfamoylphenyl

1h) Ar =4-Chlorophenyl , R= 4-Sulfamoylphenyl

Scheme 2



Antimicrobial activity

The newly synthesized compounds were subjected for evaluation of their antimicrobial activities using micro broth dilution method [39]. The data presented in table 1 which revealed that compounds **1d,1e,1f** showed broad spectrum antibacterial and antifungal activities, compounds **1c**,**1h,** showed antibacterial activity against Gram positive and Gram negative bacteria, while compounds **1g,4a** showed antibacterial activity against Gram positive bacteria. Compounds **4b,** showed antifungal activity. The remaining compounds **had** no significant activity against any of the tested strains at concentration up to 50 µg / mL.

Table 1 . Antimicrobial activity of the synthesized compounds expressed as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and concentration that inhibit 50% of microorganisms (IC_{50}) in µg /against the pathological strains based on two fold serial dilution technique.

Compound no		*Gram positive bacteria*		*Gram negative bacteria*		*Fungi*	
		S. aureus	*B. subtlis*	*E. coli*	*P.aeruginosa*	*C.albicans*	*A. niger*
1a	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
1b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
1c	MIC	18.75	18.75	18.75	18.75	>50	>50
	MBC	18.75	18.75	18.75	18.75	>50	>50
	IC50	12.5	12.5	9.3	12.5	>50	>50
1d	MIC	18.75	18.75	18.75	18.75	18.75	37.5
	MBC	18.75	18.75	18.75	18.75	18.75	37.5
	IC_{50}	12.5	12.5	9.3	12.5	12.5	12.5
1e	MIC	18.75	18.75	18.75	18.75	18.75	37.5
	MBC	18.75	18.75	18.75	18.75	18.75	37.5
	IC_{50}	12.5	12.5	9.3	12.5	12.5	12.5
1f	MIC	18.75	18.75	18.75	18.75	18.75	37.5
	MBC	18.75	18.75	18.75	18.75	18.75	37.5
	IC_{50}	12.5	12.5	9.3	12.5	12.5	12.5
1g	MIC	37.5	37.5	>50	>50	>50	>50
	MBC	37.5	37.5	>50	>50	>50	>50
	IC_{50}	25	25	>50	>50	>50	>50
1h	MIC	18.75	18.75	18.75	18.75	>50	>50
	MBC	18.75	18.75	18.75	18.75	>50	>50
	IC_{50}	12.5	12.5	12.5	12.5	>50	>50
2	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC50	>50	>50	>50	>50	>50	>50
3	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
4a	MIC	4.7	4.7	>50	>50	>50	>50
	MBC	4.7	4.7	>50	>50	>50	>50
	IC_{50}	2.3	2.3	>50	>50	>50	>50
4b	MIC	>50	>50	>50	>50	18.75	18.75
	MBC	>50	>50	>50	>50	18.75	18.75
	IC_{50}	>50	>50	>50	>50	12	9.3
5	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
6a	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
6b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
6c	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
6d	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC_{50}	>50	>50	>50	>50	>50	>50
Amoxicillin	MIC	10	100	NO Action	NO Action	NO Action	NO Action
Gentamycin	MIC	NO Action	NO Action	10	25	NO Action	NO Action
Amphotricin B	MIC	NO Action	NO Action	NO Action	NO Action	10	15

CONCLUSION

Present study describes the synthesis of derivative of pyrimidines. The compounds were characterized by spectral techniques such as IR and ^{1}H- NMR,^{13}C-NMR spectra,Mass spectra and elemental analysis. All the title compounds were screened for their antibacterial activity against *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC CC33, *Escherichia coli* ATCC 5087 and *Pseudomonas aeruginosa* ATCC 9027, as well as for their antifungal activity against *Candida albicans* ATCC 60193 *and Aspergillus niger* ATCC 1718109. In addition their MICs

(minimum inhibitory concentration, MBCs (minimum bactericidal concentration and IC_{50} (the concentration which inhibits 50% of microorganisms) were determined. The results of antibacterial activity showed that the sulfamoylphenyl moiety is essential for antimicrobial activity and potentiated by the presence of electron withdrawing group in the benzene ring that present in position 6.

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