SYNTHESIS OF NEW PYRIMIDINE DERIVATIVES AND EVALUATION OF THEIR ANTICANCER AND ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Objectives: The objective of this work is to synthesize new pyrimidine derivatives starting from ethyl 2,4-dioxo-4-(thiophen-2-yl)butanoate. Several oxadiazole, triazole, and thiadiazole moieties were incorporated into the pyrimidine backbone. The structure of the novel compounds was characterized by elemental analysis and spectroscopic methods.

Methods: Synthesis of the target compounds was materialized starting from 2-oxo-6-(thiophen-2-yl)-2,3-dihydropyrimidine-4-carbohydrazide (4) which was prepared from the appropriate ethyl 2-oxo-6-(thiophen-2-yl)-2,3-dihydropyrimidine-4-carboxylate (2). Several synthetic pathways were used for the preparation of the targets. Some of the newly synthesized compounds were subjected to *in vitro* cytotoxic screening against breast carcinoma and colon carcinoma cell lines. On the other hand, the antimicrobial activity evaluation of some newly prepared compounds was performed using cup plate diffusion method.

Results: It was observed that the oxadiazole derivative 7b was the most potent compound against breast carcinoma cell line (IC$_{50}$=7.6 µg/ml). However, pyrimidine carrying substituted 1,2,4-triazole-2-thione moiety at position 6, 11 showed the highest cytotoxic activity against colon carcinoma cell line (IC$_{50}$=4.7 µg/ml). On the other hand, compound 5c was the most active broad spectrum antimicrobial agent against the chosen microbial strains.

Conclusion: From the observed results, further investigations recommended for the synthesis of heterocycles incorporated to pyrimidine backbone as cytotoxic as well as broad spectrum antimicrobial agents.

Keywords: Pyrimidine, Oxadiazole, Triazole, Thiadiazole, *In vitro* anticancer study, Antimicrobial study.

INTRODUCTION

The pyrimidine moiety is a versatile lead molecule in pharmaceutical development because of their diverse chemical reactivity, accessibility and a wide range of biological activities. In the past few years, the therapeutic interest of pyrimidine derivatives in pharmaceutical and medical fields has been given a great attention to the medicinal chemists. Literature survey revealed that pyrimidine derivatives are well-known to have antimicrobial [1-3], anti-malarial [4], anti-tubercular [5], anti-inflammatory [6], analgesic [7], anticonvulsant [8], and anticancer [9-13] activities.

Furthermore, it is evident from literature that thiophene derivatives are known to be associated with broad spectrum biological activity, including antimicrobial [14-16] and cytotoxic activities [17-20].

In addition, heterocycles bearing symmetrical triazole moiety were reported to show a wide range of biological activities [21-25]. Moreover, antimicrobial [26], antifungal [27], anticancer [28,29] as well as antioxidant [30] activities were reviewed. Moreover, 1,3,4-oxadiazole nucleus exhibited interesting biological properties such as antimicrobial [31], antifungal [32], and antioxidant [33] activities. Likewise, different substituted 1,3,4-thiadiazoles were reported to have anticancer [34] and antimicrobial [35] activities.

In the brightness of the previous literature survey, and in concurrence with our objective, our attempts were aimed to synthesize a novel series of pyrimidine derivatives incorporated with an assortment of ring systems such as thiophene, triazole, oxadiazole, and thiadiazole. The synthesized products were evaluated for their cytotoxicity and antimicrobial activity, hoping to obtain potent anticancer and antimicrobial effects.

METHODS

Melting points were determined on a Griffin apparatus and are uncorrected. Infrared (IR) spectra were determined as KBr discs on Shimadzu Fourier transform IR 8000 spectrophotometer (Japan). ^{1}HNMR, ^{13}CNMR spectra were carried out on Varian Gemini 300 MHz and 75 MHz spectrophotometer (Switzerland) using TMS as internal standard and DMSO-d$_6$ as a solvent. Mass spectra were run on Hewlett Packard 5988 spectrometer Microanalytical Center, Cairo University, Egypt. Elemental analysis was carried out using VARIO EL ELEMENTAR Apparatus at the Regional Center for Mycology and Biotechnology, Al-Azhar University Campus, Nasr City, Cairo, Egypt. The progress of the reaction was monitored by thin layer chromatography (TLC) using TLC sheets precoated with UV fluorescent silica gel Merk 60 F254 and spots were visualized by iodine vapor or irradiation with UV lamp. MCF-7 breast cancer and HCT-116 colon cancer cell lines were obtained from the Regional Center for Mycology and Biotechnology, Al-Azhar University Campus, Nasr City, Cairo, Egypt. The used bacterial and fungal cultures were obtained from Microbiology Department, Faculty of Pharmacy, Zagazig University, Egypt. Compound 1 was synthesized according to the reported method [36].

Synthesis of ethyl 2-hydroxy-6-(thiophen-2-yl)pyrimidine-4-carboxylate (2)

Compound 1 (0.45 g, 2 mmol) and urea (0.12 g, 2 mmol) were dissolved in ethanol (15 ml) and conc. HCl (2 ml) was added. The reaction mixture was heated under reflux for 72 hrs. After cooling, the solid product was collected and crystallized from ethanol to give greenish white crystals. Yield 84%; m.p. 215-217°C; IR (KBr; cm^{-1}): 3451 (OH), 3100 (CH aromatic), 2992, 2919 (CH aliphatic), 1739 (C=O), 1622 (C=N), 1523 (C=C); ^{1}HNMR (DMSO-d$_6$, 300 MHz) d: 1.36 (t, J=7.2 Hz, 3H, CH$_3$), 4.33-4.40 (q, J=7.2 Hz, 2H, CH$_2$), 6.70-8.19 (m, 4H, Ar-H), 7.24-8.19 (m,

3H, Ar-H), 12.01 (s, 1H, OH exchangeable with D_2O); m/z: 250 (M⁺), 208 (23.61), 178 (100); Elemental analysis (%): Calcd. for $C_{11}H_{10}N_2O_3S$ (250.04): C, 52.79; H, 4.03; N, 11.19, found: C, 52.91; H, 4.11; N, 11.37.

General procedure for the synthesis of compounds (3a, b)
Compound 2 (0.5 g, 2 mmol), appropriate alkyl halide (2 mmol) in dimethyl formamide (DMF) (4 ml) containing K_2CO_3 (0.276 g, 2 mmol) were stirred at room temperature 24 hrs. The solution was quenched with ice, filtered. The separated solid was crystallized from ethanol as yellow crystals.

Synthesis of ethyl 3-methyl-2-oxo-6-(thiophen-2-yl)-2,3-dihydropyrimidine-4-carboxylate (3a)
Yield 69%; m.p. 240-242°C; IR (KBr, cm⁻¹): 3093 (CH aromatic), 2995, 2958 (CH aliphatic), 1737 (C=O, ester), 1652 (C=O, pyrimidine), 1607 (C=N), 1580 (C=C); ¹HNMR (DMSO-d_6, 300 MHz) δ: 1.35 (t, J=7.2 Hz, 3H, CH_3), 3.32 (s, 3H, N-CH_3), 4.38-4.40 (q, J=7.2 Hz, 2H, O-CH_2), 6.76 (s, 1H, pyrimidine-H), 7.27-8.24 (m, 3H, thiophene-H); elemental analysis (%): Calcd. for $C_{12}H_{12}N_2O_3S$ (264.30): C, 54.53; H, 4.58; N, 10.60, found: C, 54.67; H, 4.66; N, 10.74.

Synthesis of ethyl 3-butyl-2-oxo-6-(thiophen-2-yl)-2,3-dihydropyrimidine-4-carboxylate (3b)
Yield 65%, m.p. 130-132°C; IR (KBr, cm⁻¹): 3083 (CH aromatic), 2962, 2932, 2870 (CH aliphatic), 1745 (C=O, ester), 1655 (C=O, pyrimidine), 1575 (C=N), 1543 (C=C); ¹HNMR (DMSO-d_6) δ: 0.97 (t, 3H, CH_3, butyl), 1.35 (t, 3H, CH_3, ester), 1.42-1.77 (m, 4H, $2CH_2$, butyl), 3.31 (t, 2H, N-CH_2), 4.38 (q, 2H, O-CH_2), 7.26-8.23 (m, 4H, Ar-H); m/z (%): 306.10 (20.02) (M⁺), 232.05 (82.48), 134.05 (100). Elemental analysis (%): Calcd. for $C_{15}H_{18}N_2O_3S$ (306.38): C, 58.80; H, 5.92; N, 9.14, Found: C, 58.93; H, 5.98; N, 9.21.

Synthesis of 2-hydroxy-6-(thiophen-2-yl)pyrimidine-4-carbohydrazide (4)
A mixture of compound 2 (0.5 g, 2 mmol) and hydrazine hydrate (98%) (0.1 g, 2 mmol) in ethanol (10 ml) was heated under reflux for 5 hrs. Excess ethanol was evaporated then the separated solid was filtered, washed with ethanol and crystallized from acetic acid to give yellow crystals.

Yield 55%; m.p.281-283°C; IR (KBr, cm⁻¹): 3421 (OH), 3312, 3302 (NH, NH_2), 3097 (CH aromatic), 1660 (C=O), 1607 (C=N), 1532 (C=C); ¹HNMR (DMSO-d_6, 300 MHz) δ: 4.35 (s, 2H, NH_2 exchangeable with D_2O), 7.27-8.09 (m, 4H, Ar-H), 9.90 (s, 1H, NH exchangeable with D_2O), 12.25 (s, 1H, OH exchangeable with D_2O); ¹³CNMR (DMSO-75 MHz): 101, 129, 03, 130.45, 132.61, 140.87, 160.5; m/z (%): 236 (85.05) (M⁺), 178 (100), 134 (75.88); elemental analysis (%): Calcd. for $C_9H_8N_4O_2S$ (236.25): C, 45.75; H, 3.41; N, 23.72, found: C, 45.88; H, 3.39; N, 23.98.

General procedure for the synthesis of compounds 5a-g
A mixture of compound 4 (0.47 g, 2 mmol) and the appropriate aromatic aldehyde (2 mmol) in glacial acetic acid (15 ml) was heated under reflux for 6 hrs. After cooling and dilution with water, the solid product was collected and crystallized from acetic acid.

Synthesis of N′-benzylidene-2-hydroxy-6-(thiophen-2-yl) pyrimidine-4-carbohydrazide (5a)
Yield (53%); m.p. 290-292°C; IR (KBr, cm⁻¹): 3444 (OH), 3261 (NH), 3085 (CH aromatic), 1655 (C=O), 1639(C=N), 1603(C=C); ¹HNMR (DMSO-d_6, 300 MHz) δ: 7.27-8.19 (m, 9H, Ar-H), 8.59 (s, 1H, N=CH), 12.04 (s, 1H, NH exchangeable with D_2O), 12.31 (s, 1H, OH exchangeable with D_2O); m/z (%): 323.95 (2.54) (M⁺), 220.9 (65.57), 178 (100); Elemental analysis (%): Calcd. for $C_{16}H_{12}N_4O_2S$ (324.07): C, 59.25; H, 3.73; N, 17.27, Found: C, 59.43; H, 3.76; N, 17.35.

Synthesis of 2-hydroxy-N′-(4-methoxybenzylidene)-6-(thiophen-2-yl) pyrimidine-4-carbohydrazide (5b)
Yield 78%; m.p. 280-282°C; IR (KBr, cm⁻¹): 3486 (OH), 3363(NH), 3085, 3033 (CH aromatic), 1698 (C=O), 1595 (C=N), 1554 (C=C);

¹HNMR (DMSO-d_6) δ: 3.81 (s, 3H, OCH_3), 7.01-8.17 (m, 8H, Ar-H), 8.51 (s, 1H, N=CH), 11.89 (s, 1H, NH exchangeable with D_2O), 12.25 (s, 1H, OH exchangeable with D_2O); m/z (%): 354 (12.54) (M⁺), 221 (96.05), 178 (100); Elemental analysis (%): Calcd. for $C_{17}H_{14}N_4O_3S$ (354.38): C, 57.62; H, 3.98; N, 15.81, Found: C, 57.81; H, 4.03; N, 15.92.

Synthesis of 2-hydroxy-N′-(4-hydroxybenzylidene)-6-(thiophen-2-yl)pyrimidine-4-carbohydrazide (5c)
Yield 70%; m.p. 315-317°C; IR (KBr, cm⁻¹): 3439 (2OH), 3221 (NH), 3090 (CH aromatic), 1656 (C=O), 1597 (C=N), 1511 (C=C); ¹HNMR (DMSO-d_6, 300 MHz) δ: 6.87-8.17 (m, 8H, Ar-H), 8.46 (s, 1H, N=CH), 9.99 (s, 1H, p-OH exchangeable with D_2O), 11.83 (s, 1H, NH, exchangeable with D_2O), 12.25 (s, 1H, OH pyrimidine, exchangeable with D_2O); m/z (%): 340 (8.41) (M⁺), 221 (61.81), 178 (100); Elemental analysis (%): Calcd. for $C_{16}H_{12}N_4O_3S$ (340.06): C, 56.46; H, 3.55; N, 16.46, found: C, 56.55; H, 3.59; N, 16.58.

Synthesis of 2-hydroxy-N′-(4-nitrobenzylidene)-6-(thiophen-2-yl) pyrimidine-4-carbohydrazide (5d)
Yield 85%, m.p. 300-302°C, IR (KBr, cm⁻¹): 3434 (OH), 3191(NH), 3027 (CH aromatic), 1663 (C=O), 1621 (C=N), 1567 (C=C), 1515,1339 (NO_2); ¹HNMR (DMSO-d6, 300 MHz) δ: 7.27-8.34 (m, 8H, Ar-H), 8.85 (s, 1H, N=CH), 11.83 (s, 1H, NH exchangeable with D_2O), 12.35 (s, 1H, OH exchangeable with D_2O); m/z (%): 369 (1.62) (M⁺), 221 (44.99), 178 (100); Elemental analysis (%): Calcd. for $C_{16}H_{11}N_5O_4S$ (369.35): C, 52.03; H, 3.00; N, 18.96, Found: C, 52.14; H, 3.01 N, 19.17.

Synthesis of 2-hydroxy-N′-(4-methylbenzylidene)-6-(thiophen-2-yl) pyrimidine-4-carbohydrazide (5e)
Yield 78%; m.p. 275-277°C; IR (KBr, cm⁻¹): 3482 (OH), 3242 (NH), 3086 (CH aromatic), 2921, 2807 (CH aliphatic), 1685 (C=O), 1594 (C=N), 1558 (C=C); ¹HNMR (DMSO-d_6, 300 MHz) δ: 2.27 (s, 3H, CH_3), 7.20-8.18 (m, 8H, Ar-H), 8.54 (s, 1H, N=CH), 11.96 (s, 1H, NH exchangeable with D_2O), 12.23 (s, 1H, OH exchangeable with D_2O); m/z (%): 338 (6.37) (M⁺), 221 (84.54), 178 (100). Elemental analysis (%): Calcd for $C_{17}H_{14}N_4O_2S$ (338.38): C, 60.34; H, 4.17; N, 16.56, found: C, 60.49; H, 4.24; N, 16.74.

Synthesis of N′-(4-chlorobenzylidene)-2-oxo-6-(thiophen-2-yl)-2,3-dihydro-pyrimidine-4-carbohydrazide (5f)
Yield 82%; m.p. 267-269ºC; IR (KBr, cm⁻¹): 3384 (NH), 3212 (NH), 3081 (CH aromatic), 1677 (C=O), 1645 (C=O), 1597 (C=N), 1559 (C=C).¹HNMR (DMSO-d6, 300 MHz) δ: 7.25-8.59 (m, 8H, Ar-H), 8.70 (s, 1H, N=CH), 10.47 (s, 1H, NH exchangeable with D_2O), 12.39 (s, 1H, NH exchangeable with D_2O); m/z (%): 360 (0.31) (M⁺+2), 358 (0.61) (M⁺), 278 (33.58), 236 (68.07), 178 (100). Elemental analysis (%): Calcd. for $C_{16}H_{11}ClN_4O_2S$ (358.80): C, 53.56; H, 3.09; N, 15.61, found: C, 53.72; H, 3.16; N, 15.79.

Synthesis of 2-hydroxy-6-(thiophen-2-yl)-N′-(3,4,5-trimethoxybenzylidene) pyrimidine-4-carbohydrazide (5g)
Yield 79%; m.p. 270-272°C; IR (KBr, cm⁻¹): 3434 (OH), 3278 (NH), 3086 (CH aromatic), 2985, 2936, 2837 (CH aliphatic), 1690 (C=O), 1599 (C=N), 1575 (C=C); ¹HNMR (DMSO-d6, 300 MHz) d: 3.72 (s, 3H, p-OCH_3), 3.84 (s, 6H, 2 m-OCH_3), 7.03-8.18 (m, 6H, Ar-H), 8.49 (s, 1H, N=CH), 12.02 (s, 1H, NH exchangeable with D_2O), 12.31 (s, 1H, OH exchangeable with D_2O); ¹³CNMR (DMSO-75 MHz): 55.90, 56.00, 60.16, 104.62, 105.61, 115.22, 119.00, 129.11, 129.46, 130.45, 132.48, 139.62, 140.22, 150.22, 153.22, 160.76, 161.23, 167.88, 173.73; m/z (%): 414 (45.96) (M⁺), 220 (32.24), 178 (100); Elemental analysis (%): Calcd. for $C_{19}H_{18}N_4O_5S$ (414.44): C, 55.06; H, 4.38; N, 13.52, Found: C, 55.13; H, 4.45; N, 13.61.

General procedure for the synthesis of (6a-c)
The appropriate acid chloride (2 mmol) and compound 4 (0.47 g, 2 mmol) were heated under reflux in pyridine (5 ml) for 2 hrs. After cooling, the reaction mixture was then quenched with ice, acidified to litmus paper with glacial acetic acid and filtered. The product was crystallized from ethanol as yellow crystals.

Synthesis of *N′*-benzoyl-2-hydroxy-6-(thiophen-2-yl)-pyrimidine-4-carbo-hydrazide (6a)

Yield 84%; m.p. 215-217°C; IR (KBr; cm⁻¹): 3447 (OH), 3371 (NH), 3109 (NH), 3031 (CH aromatic), 1659 (2C=O), 1601 (C=N), 1551 (C=C); ¹HNMR (DMSO-d₆, 300 MHz) δ: 7.27-8.16 (m, 9H, Ar-H), 10.80 (s, 1H, NH exchangeable with D₂O), 10.82 (s, 1H, NH, exchangeable with D₂O), 12.36 (s, 1H, OH exchangeable with D₂O); m/z (%): 340.10 (13.76) (M⁺), 128 (5.09), 105.05 (100); Elemental analysis (%): Calcd. for $C_{16}H_{12}N_4O_3S$ (340.36): C, 56.46; H, 3.55; N, 16.46. found: C, 56.62; H, 3.59; N, 16.63.

Synthesis of 2-hydroxy-*N′*-(4-methoxybenzoyl)-6-(thiophen-2-yl) pyrimidine-4-carbohydrazide (6b)

Yield 80%; m.p. 200-202°C; IR (KBr; cm⁻¹): 3450 (OH), 3370 (NH), 3287 (NH), 3091 (CH aromatic), 1670 (C=O), 1660 (C=O), 1599 (C=N), 1560 (C=C); ¹HNMR (DMSO-d₆, 300 MHz) δ: 3.82 (s, 3H, OCH₃), 7.27-8.51 (m, 8H, Ar-H), 11.89 (s, 1H, NH, exchangeable with D₂O), 12.28 (s, 1H, NH exchangeable with D₂O), 12.30 (s, br, 1H, OH exchangeable with D₂O); m/z (%): 370.1 (0.21) (M⁺), 221 (90.81), 178 (100); Elemental analysis (%): Calcd. for $C_{17}H_{14}N_4O_4S$ (370.38): C, 55.13; H, 3.81; N, 15.13, Found C, 55.26; H, 3.87; N, 15.26.

Synthesis of *N′*-(4-chlorobenzoyl)-2-hydroxy-6-(thiophen-2-yl)-pyrimidine-4-carbohydrazide (6c)

Yield 82%; m.p. 308-310°C, IR (KBr; cm⁻¹): 3449 (OH), 3365 (NH), 3199 (NH), 3035 (CH aromatic), 1652 (2 C=O), 1601 (C=N), 1555 (C=C);¹HNMR (DMSO-d₆, 300 MHz) δ: 7.27-8.16 (m, 8H, Ar-H), 10.60 (s, 1H, NH exchangeable with D₂O.), 10.83 (s, 1H, NH exchangeable with D₂O), 12.30 (s, 1H, OH exchangeable with D₂O); Elemental analysis (%): Calcd for $C_{16}H_{11}ClN_4O_3S$ (374.80): C, 51.27; H, 2.96; N, 14.95, Found C, 51.41; H, 2.94; N, 15.09.

General procedure for synthesis of (7 a-f)

Phosphorus oxychloride (5 ml) was added to the appropriate aromatic acid derivatives (2 mmol) and compound 4 (0.47 g, 2 mmol); the mixture was heated under reflux for 6 hrs. Excess phosphorus oxychloride was distilled under reduced pressure then the residue was quenched in ice. Sodium carbonate was added until neutral to litmus paper, and the separated solid was filtered and crystallized from DMF as brown crystals.

Synthesis of 4-(5-phenyl-1,3,4-oxadiazol-2-yl)-6-(thiophen-2-yl) pyrimidin-2-ol (7a)

Yield 86%; m.p. 225-227°C; IR (KBr; cm⁻¹): 3447 (OH), 3095 (CH aromatic), 1620 (C=N), 1587 (C=C);¹HNMR (DMSO-d₆, 300 MHz) δ: 7.29-8.45 (m, 9H, Ar-H), 11.11 (s, 1H, OH exchangeable with D₂O); m/z (%): 322.05 (2.11) (M⁺), 254 (9.62), 134.05 (100); Elemental analysis (%): Calcd For $C_{16}H_{10}N_4O_2S$ (322.05): C, 59.62; H, 3.13; N, 17.38, Found C, 59.87; H, 3.19; N, 17.56.

Synthesis of 4-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-6-(thiophen-2-yl) pyrimidin-2-ol (7b)

Yield 85%; m.p. 250-252°C; IR (KBr; cm⁻¹): 3447 (OH), 3006 (CH aromatic), 2962, 2932, 2837 (CH aliphatic), 1637 (C=N), 1510 (C=C); ¹HNMR (DMSO-d₆) δ: 3.86 (s, 3H, OCH₃), 7.06-8.62 (m, 8H, Ar-H), 11.87 (s, 1H, OH exchangeable with D₂O); Elemental analysis (%): Calcd. for $C_{17}H_{12}N_4O_3S$ (352.06): C, 57.95; H, 3.43; N, 15.90, Found C, 58.13; H, 3.41; N, 16.18.

Synthesis of 4-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-6-(thiophen-2yl) pyrimidin-2-ol. (7c)

Yield 81%; m.p. 230-232°C; IR (KBr; cm⁻¹): 3447 (OH), 3095 (CH aromatic), 1595 (C=N), 1543 (C=C);¹HNMR (DMSO-d₆, 300 MHz) δ: 7.22-8.18 (m, 8H, Ar-H), 11.04 (s, 1H, OH exchangeable with D₂O); Elemental analysis (%): Calcd for $C_{16}H_9ClN_4O_2S$ (356.79): C, 53.86; H, 2.54; N, 15.70, Found C, 53.98; H, 2.58; N, 15.87.

Synthesis of 4-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)-6-(thiophen-2-yl)pyrimidin-2-ol (7d)

Yield 86%; m.p. 220-222°C, IR (KBr; cm⁻¹): 3423 (OH), 3099 (CH aromatic), 1610 (C=N) 1588 (C=C), 1523, 1345 (NO₂);¹HNMR (DMSO-d₆, 300 MHz) δ: 7.28-8.50 (m, 8H, Ar-H), 11.04 (s, 1H, OH, exchangeable with D₂O); elemental analysis (%): Calcd. for $C_{16}H_9N_5O_4S$ (367.34): C, 52.31; H, 2.47; N, 19.07, found: C, 52.49; H, 2.44; N, 19.29.

Synthesis of 4-(5-(pyridin-3-yl)-1,3,4-oxadiazol-2-yl)-6-(thiophen-2-yl)pyrimidin-2-ol (7e)

Yield 83%; m.p. 294-296°C; IR (KBr; cm⁻¹): 3419 (OH), 3095 (CH aromatic), 1580 (C=N), 1530 (C=C);¹HNMR (DMSO-d₆, 300 MHz) δ: 7.29-8.85 (m, 7H, Ar-H), 9.39 (s, 1H, CH=N pyridine), 11.33 (s, 1H, OH exchangeable with D₂O); Elemental analysis (%): Calcd. for $C_{15}H_9N_5O_2S$ (323.33): C, 55.72; H, 2.81; N, 21.66, Found: C, 55.79; H, 2.79; N, 21.92.

Synthesis of 4-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)-6-(thiophen-2-yl)pyrimidin-2-ol (7f)

Yield (80%); m.p. 288-290°C; IR (KBr; cm⁻¹): 3448 (OH), 3095 (CH aromatic), 1636 (C=N), 1586 (C=C); ¹HNMR (DMSO-d₆, 300 MHz) δ: 7.29-8.82 (m, 8H, Ar-H), 10.57 (s, 1H, OH exchangeable with D₂O); m/z (%): 323.30 (0.17), 264 (10.48), 63.95 (100); elemental analysis (%): Calcd. for $C_{15}H_9N_5O_2S$ (323.33): C, 55.72; H, 2.81; N, 21.66, Found: C, 55.86; H, 2.80; N, 21.78.

Synthesis of *N*-(4-bromophenyl)-2-(2-hydroxy-6-(thiophen-2-yl) pyrimidine-4-carbonyl) hydrazinecarbothioamide (8d)

A mixture of compound 4 (0.94 g, 4 mmol) and *p*-bromophenyl isothiocyanate (0.9 g, 4 mmol) in glacial acetic acid (15 ml) was heated under reflux for 7 hrs. The reaction mixture was diluted with ice cold water. The separated solid was filtered and crystallized from DMF as shiny yellow crystals.

Yield (82%); m.p. 225-227°C; IR (KBr; cm⁻¹): 3448 (OH), 3314 (NH), 3195 (NH), 3097 (CH Aromatic), 1668 (C=O) 1641 (C=N), 1594 (C=C), 1236 (C=S); ¹HNMR (DMSO-d₆, 300 MHz) δ: 7.27-8.15 (m, 8H, Ar-H), 9.96 (s, 1H, NH exchangeable with D₂O), 9.97 (s, 1H, NH exchangeable with D₂O), 10.81 (s, 1H, NH exchangeable with D₂O), 12.40 (s, 1H, OH exchangeable with D₂O); Elemental analysis (%): Calcd. for $C_{16}H_{12}BrN_5O_2S_2$ (449.33): C, 42.73; H, 2.67; N, 15.58, Found C, 42.74; H, 2.65; N, 15.72.

General method for synthesis of compounds (9a-c)

A mixture of compound 4 (0.94 g, 4 mmol) and substituted isothiocyanate (4 mmol) in glacial acetic acid (15 ml) was heated under reflux for 7 hrs. After cooling, the reaction mixture was quenched with ice. The separated solid was filtered and crystallized from DMF as yellow crystals.

Synthesis of 3-[2-hydroxy-6-(thiophen-2-yl)pyrimidin-4-yl]-4-phenyl-1H-1,2,4-triazole-5(4H)-thione (9a)

Yield (91%); m.p. 255-257°C; IR (KBr; cm⁻¹): 3447 (OH), 3250 (NH), 3096 (CH aromatic), 1631 (C=N), 1526 (C=C), 1268 (C=S); ¹HNMR (DMSO-d₆, 300 MHz) δ: 7.21-8.18 (m, 9H, Ar-H), 12.17 (s, 1H, OH exchangeable with D₂O), 14.46 (s, 1H, NH, exchangeable with D₂O); m/z (%): 353.05 (43.83), 134 (86.42), 77 (100); Elemental analysis (%): Calcd. for $C_{16}H_{11}N_5OS_2$ (353.42): C,54.37; H, 3.14; N, 19.82, Found: C, 54.49; H, 3.19; N, 20.01.

Synthesis of 3-[2-hydroxy-6-(thiophen-2-yl)pyrimidin-4-yl]-4-(4-methoxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (9b)

Yield (87%); m.p. 320-322°C; IR (KBr; cm⁻¹): 3438 (OH), 3271 (NH), 3062 (CH aromatic), 2958, 2931, 2833 (CH aliphatic), 1626 (C=N), 1536 (C=C), 1248 (C=S); ¹HNMR (DMSO-d₆, 300 MHz) δ: 3.80 (s, 3H, OCH₃), 7.02-7.88 (m, 8H, Ar-H), 12.15 (s, 1H, OH exchangeable with D₂O), 14.22 (s, 1H, NH exchangeable with D₂O); ¹³CNMR (DMSO-75 MHz): 55.42, 104.54, 114.23, 126.76, 129.01, 129.54, 129.96, 132.56, 140.25, 146.98, 159.57, 161.52, 169.66, 172.01; m/z (%): 383 (100%); Elemental analysis (%): Calcd. for $C_{17}H_{13}N_5O_2S_2$ (383.45): C,53.25; H, 3.42; N, 18.26, Found: C, 53.48; H, 3.46; N, 18.40.

Synthesis of 4-allyl-3-[2-hydroxy-6-(thiophen-2-yl)pyrimidin-4-yl]-1H-1,2,4-triazole-5(4H)-thoine (9c)

Yield (89%); m.p. 260-262°C; IR (KBr; cm⁻¹): 3447 (OH), 3320 (NH), 3093 (CH aromatic), 2921 (CH aliphatic), 1603 (C=N), 1560 (C=C),

1247(C=S);^{1}HNMR (DMSO-d_6, 300 MHz) δ: 4.99-5.04 (m, 2H, NCH_2), 5.10-5.22 (m, 2H, $=CH_2$), 5.88-5.99 (m, 1H, =CH), 7.24-8.15 (m, 4H, Ar-H), 12.30 (s, 1H, OH, exchangeable with D_2O), 14.39 (s, 1H, NH exchangeable with D_2O); Elemental analysis (%): Calcd. for $C_{13}H_{11}N_5OS_2$ (317.39): C, 49.19; H, 3.49; N, 22.07, Found: C, 49.42; H, 3.57; N, 22.18.

Synthesis of -[(4-(4-methoxyphenyl)-1-methyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl]-1-methyl-4-(thiophen-2-yl) pyrimidin-2(1H)-one(10)

Methyl iodide (1 ml) was added to a mixture of compound 9b (0.38 g, 1 mmol) and K_2CO_3 (0.3 g, 2 mmol) in DMF (10 ml). The reaction mixture was stirred at room temperature for 8 hrs then diluted with water, filtered and crystallized from DMF as pale yellow crystals.

Yield (89%); m.p. 260-262°C; IR (KBr; cm⁻¹): 3076 (CH aromatic), 2996, 2928, 2838 (CH aliphatic), 1666 (C=O), 1597 (C=N), 1541 (C=C), 1243 (C=S);^{1}HNMR (DMSO-d_6, 300 MHz) δ: 2.66 (s, 3H, N-CH_3 triazole), 3.81 (s, 3H, N-CH_3 pyrimidine), 3.97 (s, 3H, OCH_3), 6,87-8.14 (m, 8H, Ar-H); m/z (%): 411.30 (9.19), 395.00 (9.80), 134.00 (100); Elemental analysis (%): Calcd. for $C_{19}H_{17}N_5O_2S_2$ (411.50): C, 55.46; H, 4.16; N, 17.02, Found: C, 55.79; H, 4.19; N, 17.14.

Synthesis of 4-(4-bromophenyl)-[2-hydroxy-6-(thiophen-2-yl) pyrimidin-4-yl]-1H-1,2,4-triazole-5(4H)-thione (11)

Compound 8d (0.45 g, 1 mmol.) was refluxed for 8 hrs in 2N sodium hydroxide (10 ml). The reaction mixture was then quenched with ice, acidified to litmus paper with glacial acetic acid, filtered. The separated product was crystallized from DMF as brown crystals.

Yield (82%); m.p. 233-235°C; IR (KBr; cm⁻¹): 3453 (OH), 3294 (NH-triazole), 3092 (CH aromatic), 1628(C=N), 1559 (C=C), 1345 (C=S);^{1}HNMR (DMSO-d_6, 300 MHz) δ: 7.23-9.11 (m, 8H, Ar-H), 11.12 (s, 1H, OH exchangeable with D_2O), 14.5 (s, 1H, NH exchangeable with D_2O); m/z (%): 433 (M+2) (11.65), 431 (M⁺) (11.70), 134.05 (100); Elemental analysis (%): Calcd. for $C_{16}H_{10}BrN_5OS_2$ (431.32): C, 44.54; H, 2.32; N, 16.24. Found C, 44.53; H, 2.30, N, 16.42.

Synthesis of [5-(4-bromophenylamino)-1,3,4-thiadiazol-2-yl]-4-(thiophen-2-yl) pyrimidin-2(1H)-one (12)

To compound 8 d (0.45 g, 1 mmol), ice cold concentrated sulfuric acid (6 ml) was added drop wise while stirring. The reaction mixture was left for 24 hrs, quenched with ice and treated with concentrated ammonia solution until neutral to litmus paper. The separated solid was washed with water, filtered, dried and crystallized from ethanol as brown crystals

Yield (85%); m.p. 315-317°C; IR (KBr; cm⁻¹): 3169 (br, 2NH), 3033 (CH aromatic), 2935 (CH aliphatic), 1660 (C=O), 1634 (C=N), 1597 (C=C); ^{1}HNMR (DMSO-d_6, 300 MHz) δ: 7.26-8.16 (m, 8H, Ar-H), 10.03 (br, s, 2H, 2 NH exchangeable with D_2O); m/z (%): 433 (M+2) (9.72), 431 (M⁺) (9.72), 46 (100); elemental analysis (%): Calcd. for $C_{16}H_{10}BrN_5OS_2$ (431.32): C, 44.54; H, 2.32; N, 16.24. Found: C, 44.71; H, 2.31; N, 16.39.

In vitro cytotoxic activity

Potential cytotoxicity of the newly obtained derivatives was tested using the method of Skehan *et al.* [37]. Cells were plated in the 96-multiwell plate (5 × 10³cells/well) for 24 hrs before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of the tested compounds (0, 5, 12.5, 25 and 50 μg/ml) were added to the cell monolayer triplicate wells prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 hrs at 37°C under a 5% CO_2 atmosphere. The culture was fixed with cold trichloroacetic acid and stained with 0.4% Sulforhodamine-B stain dissolved in 1% acetic acid. Excess stain was washed with acetic acid and attached stain was recovered with tris ethylenediaminetetraacetic acid buffer and the color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each

tumor cell line of the specified compound. IC_{50} values were calculated from the calibration curve and illustrated in Table 1.

Antimicrobial activity

Antibacterial activity

The newly synthesized compounds were tested for their *in vitro* antibacterial activity, in comparison to Cefotaxime and sulfamethoxazole as reference drugs using the standard agar cup diffusion method [38-42]. Bacterial strains were individually cultured for 48 hrs in 100 ml conical flasks containing 30 ml nutrient agar. The assay was done in 10 cm sterile petri dishes into which a bacterial suspension (1 ml) in nutrient agar (15 ml) was poured. Plates were shaken gently to homogenize the contents. After solidification of the media, 5 mm cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. The test compounds and the reference drugs were dissolved in DMF (100 μmol/ml) and were pipette in the cavities. In addition, other cavities were pipette with DMF and served as a negative control. The seeded plates were incubated at 28±2°C for 48 hrs. The radii of inhibition zones (in mm) of triplicate sets were measured and the results are cited in Table 2.

Antifungal activity

The test compounds were evaluated for their antifungal activity *in vitro*, in comparison to Nystatin as a reference drug using the agar cup diffusion method [38-42] against two fungal strains: *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 10231). Spore suspensions in sterile distilled water were prepared from 7-day-old culture of the test fungi growing on Sabouraud's dextrose broth (30 ml) media in 100 ml conical flasks. The final spore concentration was nearly 5 × 10⁴ spores/ml. About 15 ml of the growth medium was introduced on sterilized Petri dishes of 10 cm diameter and inoculated with 1 ml of spore suspension. Plates were shaken gently to homogenize the inocula. After solidification of the media, 5 mm cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer and was filled with the solutions of the test compounds and Nystatin (100 μmol/ml in DMF). In addition, other cavities were impregnated with solvent (DMF) and served as a negative control. The seeded plates were incubated at 28±2°C for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured at successive intervals during the incubation period, and the results are cited in Table 2.

RESULTS AND DISCUSSION

Chemistry

The novel pyrimidine derivatives were prepared as outlined in schemes 1-4.

The intermediate 1 was prepared as previously reported method [36] and refluxed with urea in ethanol containing conc. HCl to afford ethyl 2-oxo-6-(thiophen-2-yl)-2,3dihydropyrimidine-4-carboxylate (2).

Table 1: IC_{50}^{a} (μg/ml)

Compound no	Breast carcinoma (MCF7) cell line	Colon carcinoma (HCT116) cell line
4	33.7±0.23	NA[b]
5b	38.7±0.24	46.4±0.20
5c	37±0.23	NA[b]
5f	41.2±0.22	41.8±0.22
6b	30.3±0.26	24±0.28
7b	7.6±0.27	12.1±0.29
8d	NA[b]	NA[b]
9b	NA[b]	49.1±0.19
11	10.6±0.25	4.7±0.30
12	NA[b]	NA[b]
Doxorubicin	1.17±0.07	7.7±1.78

a=IC_{50} is a dose required to inhibit the cell growth by 50% (values are the mean±SD of three separate experiments), NA[b]: No activity (i.e., IC_{50} values are higher than 50 μg/ml), SD: Standard deviation

Table 2: The antimicrobial activity

Sample no.	Diameter (mm) of inhibition zones against the corresponding standard strains of different microorganisms								
	Gram-positive bacteria			Gram-negative bacteria				Fungi	
	Staphylococcus aureus ATCC 6538	*Staphylococcus epidermidis* ATCC 12228	*Micrococcus* spp. ATCC 10240	*Pseudomonas aeruginosa* ATCC 9027	*Klebsiella pneumonia* ATCC 27736	*Salmonella typhimurium* ATCC 14028	*Escherichia coli* ATCC 10536	*Aspragillus niger* ATCC 16404	*Candida albicans* ATCC 10231
2	40	41	40	13	11	12	12	41	42
3a	30	29	31	-	-	-	-	-	-
3b	-	-	-	-	-	-	-	36	35
4	39	40	40	13	14	13	13	35	34
5a	20	21	20	15	14	13	14	26	25
5b	22	23	21	16	17	15	16	24	23
5c	42	41	43	32	33	31	32	43	42
5d	20	21	20	22	23	21	22	31	32
5e	39	40	39	21	22	21	21	-	-
5f	23	22	23	-	-	-	-	-	-
5g	22	21	23	21	20	21	20	13	12
6a	15	14	16	25	26	24	25	24	23
6b	-	-	-	29	28	28	28	-	-
6c	20	21	20	25	26	24	25	23	22
7a	20	19	21	21	22	21	21	29	28
7b	12	13	12	20	21	19	20	31	32
7c	16	17	16	23	24	22	23	26	25
7d	15	16	15	25	26	24	25	24	23
7e	21	22	20	-	-	-	-	24	23
7f	23	24	22	-	-	-	-	40	39
8d	34	35	34	26	27	25	26	40	39
9a	36	37	36	25	26	24	25	30	28
9b	29	30	28	25	24	23	24	34	33
9c	32	31	33	20	19	21	20	30	29
11	29	30	29	25	25	26	25	26	25
12	38	39	38	26	27	27	26	-	-
Cefotaxime (control)	30	28	35	30	32	33	35	-	-
Sulphame-thoxazole (control)	25	23	30	25	27	28	30	-	-
Nystatin (control)	-	-	-	-	-	-	-	25	20
DMF (control)	-	-	-	-	-	-	-	-	-

The method used is agar cup diffusion method, each cup is filled with 100 ml from each sample, Conc. of each sample is 5 mg/ml, Conc. of antimicrobial agents is 5 mg/ml, DMF: Dimethyl formamide

This new compound was stirred with methyl or butyl iodide in DMF containing K_2CO_3 at room temperature to give compounds 3a,b. On the other hand, refluxing compound 2 with hydrazine hydrate in ethanol for 5 hrs afforded 2-oxo-6-(thiophen-2-yl)-2,3-dihydropyrimidine-4-carbohydrazide (4).

Moreover, new hydrazone derivatives 5a-g were synthesized in moderate to high yields through condensation of the acid hydrazide 4 with different aromatic aldehydes in acetic acid under reflux (Scheme 1).

In an attempt to synthesize the oxadiazole derivatives 7a-c using benzoyl chloride or substituted benzoyl chloride (*p*-Cl, *p*-OCH₃) and the hydrazide 4 in pyridine under reflux, only the N-benzoylated compounds 6a-c were achieved. The structure of compounds 6a-c was characterized using spectroscopic and elemental analysis. IR spectroscopy showed the presence of two NH bands at 3371-3109 cm⁻¹ and two carbonyl bands at 1660-1652 cm⁻¹ range indicating open chain structure. Furthermore, ¹HNMR spectra verified the structure by the appearance of two exchangeable NH signals at 10.60-12.30 ppm range. On the other hand, the desired oxadiazole derivatives 7a-f were obtained through the reaction of the acid hydrazide 4 with aromatic acid derivatives in phosphorous oxychloride at reflux temperature. The structure of the oxadiazoles 7a-f was verified using elemental analysis and spectroscopic data. Structure elucidation of the oxadiazole 7b can be

done chemically through the cyclodehydration of compound 6b using phosphorous oxychloride under reflux for only 4 hrs (Scheme 2).

It was reported that reaction of the hydrazide derivatives with different substituted isothiocyanates lead to N-substituted thiosmicarbazides [43]. In our work, substituted isothiocyanates reacted with the hydrazide 4 differently. When phenyl, *p*-methoxy phenyl or allyl isothicyanate was used, the unexpected 3-[2-hydroxy-6-(thiophen-2-yl)pyrimidin-4-yl]-4-substituted-1H-1,2,4-triazole-5(4H)-thione (9a-c) were obtained instead of the desired thiosemicarbazide derivatives (8a-c). The structure of the obtained products was confirmed using elemental analysis, spectroscopic data as well as chemical reaction. IR spectroscopy provided only two NH bands at 3447-3421 cm⁻¹ and 3344-3250 cm⁻¹ ranges. Moreover, C=S band appeared at 1268-1247 cm⁻¹ range and only one carbonyl band appeared. In addition, ¹HNMR showed two NH signals at position 12.24 and 14.03 ppm. Structure elucidation of compounds 9a-c was carried out using methylation reaction. Compound 9b was reacted with excess methyl iodide in DMF containing potassium carbonate anhydrous at room temperature to give dimethylated compound 10 indicating the cyclic structure of compounds 9a-c (Scheme 3).

Furthermore, the target thiosemicarbazide derivative 8d was obtained only when the hydrazide 4 reacted with *p*-bromophenyl isothiocyanate in refluxing acetic acid for 7 hrs. The structure of compound 8d
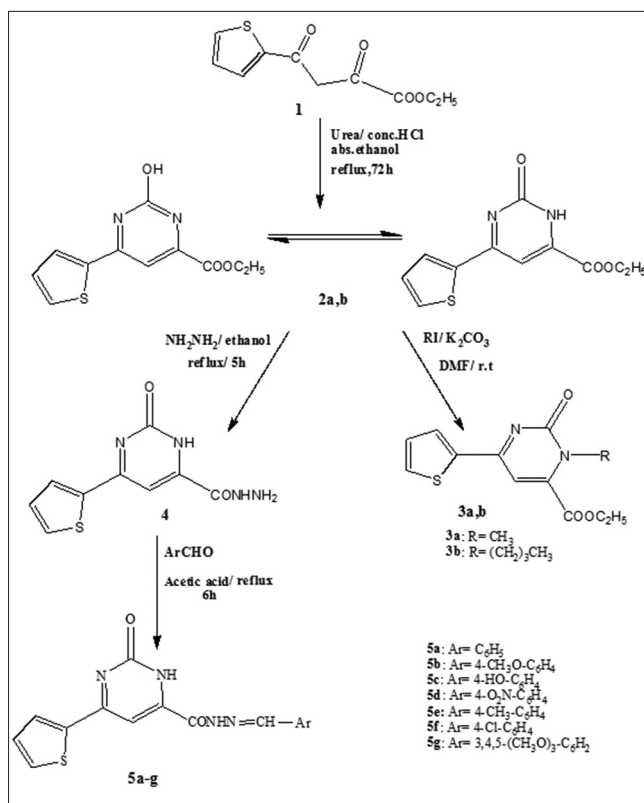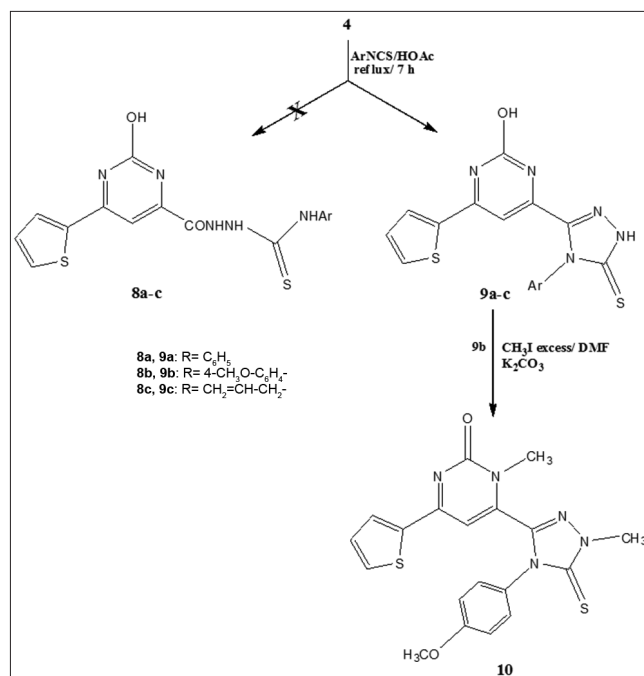
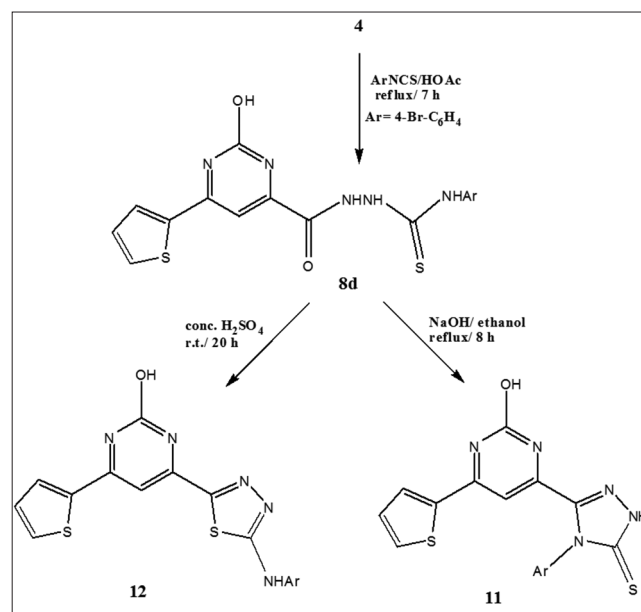**Scheme 1: Synthesis of target compounds 1-5**
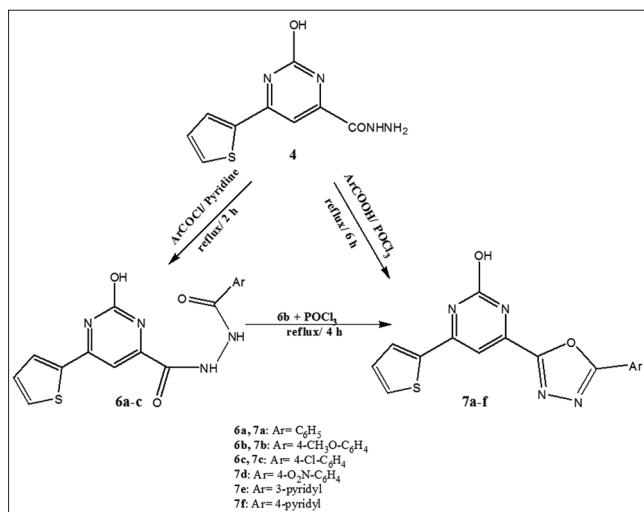
Scheme 2: Synthesis of target compounds 6, 7

Scheme 3: Synthesis of target compounds 9, 10

Scheme 4: Synthesis of target compounds 8d, 11 and 12

was proved using spectroscopic data and elemental analysis. IR spectroscopy showed the presence of 3NH bands at 3448, 3314 and 3195 cm^{-1}. Furthermore, the presence of carbonyl band at 1668 cm^{-1} indicated the open chain structure. On the other hand, ^{1}HNMR showed four exchangeable signals appeared at 9.96, 9.97, 10.81 ppm referred to three NH of the open chain structure and 12.40 ppm for one OH of the pyrimidine ring. The structure of compound 8d was also evidenced by its reaction with either 2N NaOH or cold conc. sulfuric acid to give 4-(4-bromophenyl)-3-(2-hydroxy-6-(thiophen-2-yl)pyrimidin-4-yl)-1H-1,2,4-triazole-5(4H)-thione (11) and 6-(5-(4-bromophenylamino)-1,3,4-thiadiazol-2-yl)4-(thiophen-2-yl)pyrimidin-2-(1H)-one (12) respectively. The new heterocyclic compounds 11 and 12 were subjected to spectroscopic and elemental analysis to verify their structures (Scheme 4).

***In vitro* cytotoxic activity**

From the newly synthesized compounds, only ten compounds 4, 5b, 5c, 5f, 6b, 7b, 8d, 9b, 11 and 12 were selected to be evaluated for their cytotoxicity against human breast carcinoma (MCF7) cell line and colon carcinoma (HCT116) cell line using Skehan *et al.* method [37]. The inhibitory activities were presented as micromolar concentrations of the compound that cause 50% inhibition per unit of enzyme (IC$_{50}$) under the assay condition. Results of cancer cellular assays are shown in Table 1. By investigating the variation in selectivity of the tested compounds over the cell lines, it was noticed that, acid hydrazide 4 has moderate activity against breast carcinoma cell line (IC$_{50}$=33.7 µg/ml) but it is devoid of cytotoxic activity against colon carcinoma cell line. By its condensation with different aromatic aldehydes to give hydrazone derivatives, their cytotoxic activity were reduced against breast carcinoma cell line and showed certain activities against colon

carcinoma cell line for compounds 5b and 5f. However, compound 5c showed only activity against breast cancer cell line.

Acylation of acid hydrazide 4 using *p*-methoxybenzoyl chloride afforded compound 6b which has moderate activity against colon carcinoma cell line (IC_{50}=24 μg/ml) and breast carcinoma cell line (IC_{50}=30.3 μg/ml).

Cyclization of compound 6b using phosphorous oxychloride afforded the most active oxadiazole derivative 7b against breast carcinoma cell lines (IC_{50} =7.6 μg/ml). On the other hand, the [1,2,4]-triazole-5-thione derivative 9b which was obtained from direct cyclization of the acid hydrazide 4 using *p*-methoxyphenyl isothiocyanate, showed weak activity against colon carcinoma cell line and no activity against breast carcinoma cell line. However, the [1,2,4]-triazole-5-thione derivative 11 which was obtained from cyclization of the totally inactive thiosmicarbazide 8d was the most active cytotoxic compound against colon cancer cells, even more powerful than the standard drug. Finally, cyclization of compound 8d to 1,3,4–thiadiazole derivative 12 did not change cytotoxic activity against both cell lines.

Preliminary antimicrobial screening
All of the newly synthesized compounds were screened for their antimicrobial activity using cup plate diffusion method [38-42].

Staphylococcus aureus (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228) and *Micrococcus* spp. (ATCC 10240) were be used as Gram-positive bacterial strains. However, *Pseudomonas aeruginosa (*ATCC 9027), *Klebsiella pneumonia* (ATCC 27736), *Salmonella typhimurium* (ATCC 14028), and *Escherichia coli* (ATCC 10536) were be used as Gram-negative bacterial strains. In addition, two fungi strains, including *A. niger* (ATCC 16404) and *C. albicans* (ATCC 10231) were be used in this evaluation. The results were reported as zone of inhibition compared to standard Cefotaxime and Sulphamethoxazole as antibacterial drugs and Nystatin as antifungal drug. The results illustrated in Table 2 revealed that many of the tested compounds were found to exhibit good to excellent antimicrobial activity. It was observed that, the most active compound against both bacterial and fungal strains was the hydrazone derivative 5c. However, compounds 2 and 4 showed high activity against Gram-positive strains, fungal strains and weak activity against Gram-negative strains. Alkylation of compound 2 to give compound 3a and 3b, lead to only anti-Gram-positive activity for compound 3a and antifungal activity for compound 3b indicating the importance of NH in pyrimidine ring for broad spectrum activity. On the other hand, the hydrazone derivatives 5a-g, except compound 5c and 5f, showed moderate antimicrobial activity. N-benzylation of the hydrazide 4 declined both anti-Gram-positive and antifungal activities; however, anti-Gram-negative activity enhanced. Compounds 7 a-f exhibited variable antimicrobial activity. The phenylsubstituted oxadiazoles 7 a-d, showed moderate antimicrobial activity. However, the pyridylsubstituted oxadiazoles 7 e, f showed only anti-Gram-positive and antifungal activities. The thiosemithiocarbazide 8d represented potent antimicrobial compound. Cyclization of compound 8d gave the less potent [1,2,4]triazole derivative 11 as anti-Gram-positive and antifungal compound. However, 1,3,4-thiadiazole 12 devoid of antifungal activity. On the other hand, [1,2,4]triazoles 9a-c showed high potency against Gram-positive and fungal strains in comparison with the [1,2,4] triazole derivative 11.

CONCLUSION

In the present work, a new class of pyrimidine derivatives was obtained starting from the hydrazide 4. This hydrazide was utilized in the synthesis of several hydrazones, oxadiazoles, triazoles, and a thiadiazole derivative. The newly synthesized heterocycles were evaluated for their anticancer and antimicrobial properties. The most potent cytotoxic compounds were oxadiazole 7b and the [1,2,4]triazole 11. Accordingly, we can conclude that incorporation of five-member heterocyclic rings with the pyrimidine moiety is important for cytotoxic activity. Considering antimicrobial activity, we observed that most of the targets exhibited broad spectrum activity. However, compounds 2,

4, 5c, and 8d represented the most potent ones. These results suggested that pyrimidine moiety linked to several chains and other heterocycles is interesting molecules for further synthesis and biological evaluation.

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