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Antimicrobial Activity of New 4,6-Disubstituted Pyrimidine, Pyrazoline, and Pyran Derivatives

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A number of new 2,6-didisubstituted pyrimidine, pyrazoline, and pyran derivatives were synthesized starting from their chalcone derivative. The synthesized compounds displayed different degrees of antimicrobial activity against *Bscillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* species (Actinomycetes).

Key words: Pyrimidines, Pyrazolines, Pyrans, Antimicrobial activity

INTRODUCTION

The rapid development of resistance to existing antimicrobial drugs poses a major threat to public health. Consequently, there is a pressing need to develop new antimicrobial agents with potent activity against the resistant micro-organisms. Electron-rich nitrogen heterocycles play an important role in diverse biological activities. Pyrimidines are an important class of compounds and have widespread applications from pharmaceuticals to materials (Brown, 1996), with activities such as Tie-2 kinase inhibitors (Matloobi and Kappe, 2007), HIV-1 inhibitor (Gadhachanda et al., 2007), antimalarial (Agarwal et al., 2005), adenosine A1 receptor antagonism (Chang et al., 2004), anticancer (Capdeville et al., 2002), analgesic (Zaki et al., 2006), cardiovascular (Atwal, 1987), and antiallergic (Ozeki et al., 1989) activities. A number of synthetic pharmacophores with antibacterial (Hegab et al., 2007), antifungal (Gholap et al., 2008), and antimycotic activities (Keutzberger and Gillessen, 1985) are based on the pyrimidyl motif. 2-pyrazoline derivatives have antimicrobial (Nauduri and Reddy, 1998; Grant et al., 1998; Turan-Zitouni et al., 2005a, 2005b), anti-inflammatory (Nasr and Said, 2003), and antihypertensive (Turan-Zitouni et al., 2000) activities. Introducing a pyrazolidinone ring (Jungheim et al., 1987a, 1987b) in place of the β-lactam ring (in penicillins and cephalosporins; Boyd, 1982) enhances activity. A second nitrogen in the five-membered ring also influences the antibacterial or pharmacokinetic properties (Jungheim et al., 1988a, 1988b; Ternansky and Draheim, 1990). Pyran derivatives have attracted a great deal of interest because of their antimicrobial activity (Bedair et al., 2000; El-Agrody et al., 2000, 2001), inhibition of influenza, virus sialidases (Taylor et al., 1998), mutagenic activity (Hirmoto et al., 1997), activity as antiviral (Martinez and Marco, 1997) and antiproliferation agents (Dell and Smith, 1993), sexpheromones (Bianchi and Tava, 1987), as well as antitumor (Eiden and Denk, 1991) and anti-inflammatory agents (Shishoo et al., 1981). As part of the continuation of our program of identifying new, potent, selective, and less toxic antimicrobial agents (Abdel-Rahman et al., 2008; El-Sayed et al., 2008, 2009) we report here the synthesis and antimicrobial activity of new disubstituted pyrimidine, pyrazoline, and pyran derivatives.

MATERIALS AND METHODS

Melting points were determined using a Büchi apparatus. IR spectra (KBr) were recorded with a Bruker-Vector22 instrument (Bruker). ^{1}H NMR spectra were recorded with a Varian Gemini spectrometer at 300

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MHz and 200 MHz with TMS as the internal standard. Chemical shifts were reported on a δ scale (ppm) relative to TMS as a standard, and the coupling constants (*J* values) are given in Hz. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F_{245}. EI-mass spectra were recorded with a HP D5988 A 1000 MHz instrument (Hewlett-Packard). Elemental analyses were performed at the Microanalytical Data Centre at the Faculty of Science, Cairo University, Egypt. Antimicrobial activity was tested at the botany department, Faculty of Science, Menoufia University, Egypt.

3-(4-Substitutedphenylphenyl)-1-(thiophen-2-yl) prop-2-en-1-one (2a,b)

To a solution of 2-acetylthiophene (1.26 g, 10 mmol) in ethanol (50 mL) and potassium hydroxide (0.25 g), water (5 mL) was added over a period of 30 min at 0°C. Then the corresponding aldehyde (10 mmol) was slowly added to the reaction mixture with stirring at room temperature for 12 h. Then the reaction mixture was cooled and poured into water. The solid that formed was filtered, dried, and recrystallized from ethanol to afford compound **2** in 90% yield.

3-(4-Chlorophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (2a)

Yellow solid (2.23 g, 90%), m.p. 225-227°C; IR (KBr) ν 1708 cm^{-1} (C=O); ^1H NMR (DMSO-d_6) δ 7.20 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.55 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.68 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.82 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.95 (m, 2H, Ar-H), 8.37 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 248 (M^+, 8). Anal. Calcd. for $C_{13}H_9ClOS$: C, 62.78; H, 3.65. Found: C, 62.50; H, 3.40%.

3-(4-Bromophenyl)-1-(thiophen-2-yl)prop-2-en-1-one (2b)

Yellow solid (2.69 g, 92%), m.p. 233-234°C; IR (KBr) ν 1710 cm^{-1} (C=O); ^1H NMR (DMSO-d_6) δ 7.21 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.55 (d, 2H, *J* = 8.2 Hz, Ar-H), 7.69 (d, 1H, *J* = 8.5 Hz, Ar-H), 7.84 (d, 1H, *J* = 8.2 Hz, Ar-H), 7.95 (m, 2H, Ar-H), 8.39 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 293 (M^+, 12). Anal. Calcd. for $C_{13}H_9BrOS$: C, 53.26; H, 3.09. Found: C, 53.10; H, 3.01%.

4-(4-Substitutedphenyl)-6-(thiophen-2-yl)pyrimidin-2-amine (3a,b)

To a solution of guanidine hydrochloride (10 mmol) in ethanol (50 mL), sodium metal (0.6 g) was added. The reaction mixture was refluxed for 2 h and then compound **2a,b** (10 mmol) was added to it and refluxed for 8 h. The reaction mixture was cooled and poured into water. The solid that formed was filtered, dried, and recrystallized from dioxane to afford **3a,b** in 73-78% yields.

4-(4-Chlorophenyl)-6-(thiophen-2-yl)pyrimidin-2-amine (3a)

Yellow solid (2.03 g, 73%), m.p. 276-278°C; IR (KBr) ν 3389 (NH_2), 1608 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 6.56 (brs, 2H, NH_2), 7.23 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.57 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.65 (s, 1H, Ar-H), 7.96 (m, 2H, Ar-H), 8.33 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 287 (M^+, 14). Anal. Calcd. for $C_{14}H_{10}ClN_3S$: C, 58.43; H, 3.50; N, 14.60. Found: C, 58.12; H, 3.32; N, 14.50%.

4-(4-Bromophenyl)-6-(thiophen-2-yl)pyrimidin-2-amine (3b)

Yellow solid (2.57 g, 78%), m.p. 280-281°C; IR (KBr) ν 3389 (NH_2), 1608 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 6.59 (brs, 2H, NH_2), 7.23 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.57 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.65 (s, 1H, Ar-H), 7.96 (m, 2H, Ar-H), 8.34 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 330 (M^+, 18). Anal. Calcd. for $C_{14}H_{10}ClN_3S$: C, 50.61; H, 3.03; N, 12.65. Found: C, 50.31; H, 3.10; N, 12.51%.

5-(4-chlorophenyl)-1-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole (4a,b)

A mixture of **2a,b** (10 mmol) and phenylhydrazine (1.08 g, 10 mmol) was heated under reflux for 10 h in ethanol (20 mL). Then the reaction mixture was cooled, filtered, dried, and recrystallized from ethanol to give **4a,b** in 77-80% yields.

5-(4-Chlorophenyl)-1-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole (4a)

Yellow solid (2.60 g, 77%), m.p. 269-270°C; IR (KBr) ν 1605 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 3.42 (s, 2H, CH_2), 4.42 (s, 1H, CH), 6.76 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.42 (m, 3H, Ar-H), 7.87 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.96 (m, 4H, Ar-H), 8.29 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 338 (M^+, 10). Anal. Calcd. for $C_{19}H_{15}ClN_2S$: C, 67.35; H, 4.46; N, 8.27. Found: C, 67.10; H, 4.31; N, 8.30%.

5-(4-Bromophenyl)-1-phenyl-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole (4b)

Yellow solid (3.07 g, 80%), m.p. 266-267°C; IR (KBr) ν 1605 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 3.44 (s, 2H, CH_2), 4.44 (s, 1H, CH), 6.76 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.42 (m, 3H, Ar-H), 7.87 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.96 (m, 4H, Ar-H), 8.29 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 384 (M^+, 15). Anal. Calcd. for $C_{19}H_{15}BrN_2S$: C, 59.54; H, 3.94; N, 7.31. Found: C, 59.31; H, 4.10; N,

7.30%.

2-Amino-4-(4-substitutedphenyl)-6-(thiophen-2-yl)-4*H*-pyran-3-carbonitrile (5a,b)

A solution of **2a,b** (10 mmol) and malononitrile (0.66 g, 10 mmol) in dry pyridine (15 mL) was refluxed for 10 h. The solution was cooled and poured onto ice/HCl and the solid that formed was filtered, washed several times with water, dried, and recrystallized from ethanol to give **5a,b** in 72-74% yields.

2-Amino-4-(4-chlorophenyl)-6-(thiophen-2-yl)-4*H*-pyran-3-carbonitrile (5a)

Yellow solid (2.26 g, 72%), m.p. 255-256°C; IR (KBr) ν 3430 (NH_2), 2215 cm^{-1} (CN); ^1H NMR (DMSO-d_6) δ 6.72 (brs, 2H, NH_2), 7.15 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.69 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.83 (s, 1H, Ar-H), 7.97 (m, 2H, Ar-H), 8.20 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 314 (M^+, 5). Anal. Calcd. for $C_{16}H_{11}ClN_2OS$: C, 61.05; H, 3.52; N, 8.90. Found: C, 60.90; H, 3.30; N, 8.70%.

2-Amino-4-(4-bromophenyl)-6-(thiophen-2-yl)-4*H*-pyran-3-carbonitrile (5b)

Yellow solid (2.64 g, 74%), m.p. 259-260°C; IR (KBr) ν 3438 (NH_2), 2225 cm^{-1} (CN); ^1H NMR (DMSO-d_6) δ 6.75 (brs, 2H, NH_2), 7.18 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.70 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.85 (s, 1H, Ar-H), 7.94 (m, 2H, Ar-H), 8.22 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 359 (M^+, 15). Anal. Calcd. for $C_{16}H_{11}BrN_2OS$: C, 53.49; H, 3.09; N, 7.80. Found: C, 53.31; H, 3.00; N, 7.60%.

5-(4-Substitutedphenyl)-3-(thiophen-2-yl)-4,5-dihydroisoxazole (6a,b)

A solution of **2a,b** (10 mmol) and hydroxylamine hydrochloride (0.69 g, 10 mmol) in ethanol (15 mL) containing 0.4 g sodium hydroxide was refluxed for 8 h. The reaction mixture was evaporated under reduced pressure and the residue was recrystallized from ethanol to give compounds **6a,b** in 76-79% yield.

5-(4-Chlorophenyl)-3-(thiophen-2-yl)-4,5-dihydroisoxazole (6a)

Yellow solid (1.99 g, 76%), m.p. 260-261°C; IR (KBr) ν 1605 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 3.44 (s, 2H, CH_2), 4.43 (s, 1H, CH), 6.96 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.43 (m, 1H, Ar-H), 7.88 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.95 (m, 1H, Ar-H), 8.20 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 263 (M^+, 10). Anal. Calcd. for $C_{13}H_{10}ClNOS$: C, 59.20; H, 3.82; N, 5.31. Found: C, 59.01; H, 3.60; N, 5.20%.

5-(4-Bromophenyl)-3-(thiophen-2-yl)-4,5-dihydroisoxazole (6b)

Yellow solid (2.43 g, 79%), m.p. 269-270°C; IR (KBr) ν 1610 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 3.47 (s, 2H, CH_2), 4.46 (s, 1H, CH), 6.98 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.43 (m, 1H, Ar-H), 7.88 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.96 (m, 1H, Ar-H), 8.21 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 308 (M^+, 10). Anal. Calcd. for $C_{13}H_{10}BrNOS$: C, 50.66; H, 3.27; N, 4.53. Found: C, 50.50; H, 3.10; N, 4.31%.

6-(4-Substitutedphenyl)-4-(thiophen-2-yl)-5,6-dihydropyrimidin-2(1*H*)-ones and thiopyrimidinones (7a,b and 8a,b)

General procedure: A mixture of **2a,b** (10 mmol), urea or thiourea (10 mmol), and NaOH (1.12 g, 25 mmol) in ethanol (30 mL) was refluxed for 6 h. The reaction mixture was concentrated, cooled, and filtered. The precipitate was recrystallized from ethanol to give **7a,b** and **8a,b** in 75-82% yields.

6-(4-Chlorophenyl)-4-(thiophen-2-yl)-5,6-dihydropyrimidin-2(1*H*)-ones (7a)

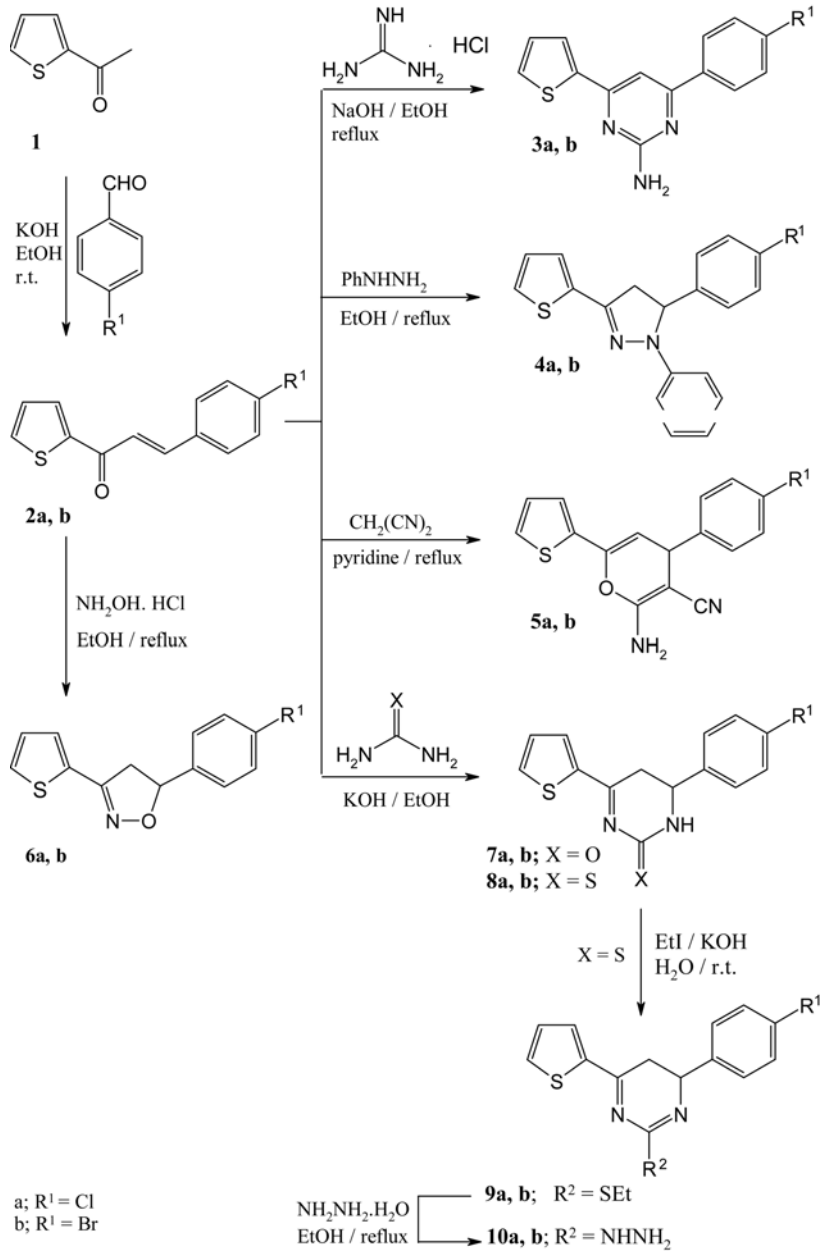
White solid (2.17 g, 75%), m.p. 277-278°C; IR (KBr) ν 3321 (NH), 1670 (C=O), 1612 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 3.41 (s, 2H, CH_2), 4.47 (s, 1H, CH), 7.24 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.43 (m, 1H, Ar-H), 7.87 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.92 (m, 1H, Ar-H), 8.04 (d, 1H, *J* = 7.8 Hz, Ar-H), 8.30 (brs, 1H, NH); MS *m/z* (%) 290 (M^+, 4). Anal. Calcd. for $C_{14}H_{11}ClN_2OS$: C, 57.83; H, 3.81; N, 9.63. Found: C, 57.70; H, 3.60; N, 9.50%.

6-(4-Bromophenyl)-4-(thiophen-2-yl)-5,6-dihydropyrimidin-2(1*H*)-ones (7b)

White solid (2.60 g, 78%), m.p. 282-283°C; IR (KBr) ν 3331 (NH), 1672 (C=O), 1610 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 3.43 (s, 2H, CH_2), 4.47 (s, 1H, CH), 7.24 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.44 (m, 1H, Ar-H), 7.87 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.93 (m, 1H, Ar-H), 8.06 (d, 1H, *J* = 7.8 Hz, Ar-H), 8.36 (brs, 1H, NH); MS *m/z* (%) 334 (M^+, 12). Anal. Calcd. for $C_{14}H_{11}BrN_2OS$: C, 50.16; H, 3.31; N, 8.36. Found: C, 50.21; H, 3.21; N, 8.30%.

6-(4-Chlorophenyl)-4-(thiophen-2-yl)-5,6-dihydropyrimidin-2(1*H*)-thione (8a)

White solid (2.45 g, 80%), m.p. 230-231°C; IR (KBr) ν 3335 (NH), 1608 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 3.42 (s, 2H, CH_2), 4.48 (s, 1H, CH), 7.25 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.43 (m, 1H, Ar-H), 7.88 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.92 (m, 1H, Ar-H), 8.10 (d, 1H, *J* = 7.8 Hz, Ar-H), 12.15 (brs, 1H, NH); MS *m/z* (%) 306 (M^+, 4). Anal. Calcd. for $C_{14}H_{11}ClN_2OS$: C, 54.80; H, 3.61; N, 9.13. Found: C, 54.71; H, 3.60; N, 8.90%.



Scheme 1. Synthesis of substituted pyrimidine, pyrazole, and pyran derivatives

6-(4-Bromophenyl)-4-(thiophen-2-yl)-5,6-dihydropyrimidin-2(1*H*)-thione (8b)

White solid (2.89 g, 82%), m.p. 225-226°C; IR (KBr) ν 3331 (NH), 1610 cm^{-1} (C=N); ^{1}H NMR (DMSO-d_6) δ 3.44 (s, 2H, CH_2), 4.50 (s, 1H, CH), 7.24 (d, 2H, J = 8.5 Hz, Ar-H), 7.45 (m, 1H, Ar-H), 7.88 (d, 2H, J = 8.5 Hz, Ar-H), 7.93 (m, 1H, Ar-H), 8.11 (d, 1H, J = 7.8 Hz, Ar-H), 11.98 (brs, 1H, NH); MS *m/z* (%) 352 (M^+, 8). Anal. Calcd. for $C_{14}H_{11}BrN_2S_2$: C, 47.87; H, 3.16; N, 7.97. Found: C, 47.61; H, 3.10; N, 7.81%.

4-(4-Substitutedphenyl)-2-(ethylthio)-6-(thiophen-2-yl)-4,5-dihydropyrimidine (9a,b)

To a well stirred solution of **8a,b** (10 mmol) and KOH (1.12 g, 10 mmol) in ethanol (8 mL), water (16 mL) was added ethyl iodide (1.56 g, 10 mmol), and the resulting mixture was stirred at 60°C for 2 h. The reaction mixture was allowed to cool at room temperature and the precipitated solid was filtered, washed with water, and recrystallized from ethanol to afford **9a,b** in 80-82% yields.

4-(4-Clorophenyl)-2-(ethylthio)-6-(thiophen-2-yl)-4,5-dihydro-pyrimidine (9a)

Yellow solid (2.67 g, 80%), m.p. 160-161°C; IR (KBr) ν 3332 (NH), 1612 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 1.11 (t, 3H, *J* = 5.2 Hz, CH_3), 3.45 (s, 2H, CH_2), 3.90 (q, 2H, *J* = 5.2 Hz, CH_2), 4.49 (s, 1H, CH), 7.22 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.44 (m, 1H, Ar-H), 7.87 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.92 (m, 1H, Ar-H), 8.12 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 334 (M^+, 5). Anal. Calcd. for $C_{16}H_{15}ClN_2S_2$: C, 57.38; H, 4.51; N, 8.37. Found: C, 57.10; H, 4.41; N, 8.20%.

4-(4-Bromophenyl)-2-(ethylthio)-6-(thiophen-2-yl)-4,5-dihydro-pyrimidine (9b)

Yellow solid (3.12 g, 82%), m.p. 165-166°C; IR (KBr) ν 3328 (NH), 1614 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 1.18 (t, 3H, *J* = 5.2 Hz, CH_3), 3.46 (s, 2H, CH_2), 3.98 (q, 2H, *J* = 5.2 Hz, CH_2), 4.48 (s, 1H, CH), 7.24 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.45 (m, 1H, Ar-H), 7.88 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.90 (m, 1H, Ar-H), 8.11 (d, 1H, *J* = 7.8 Hz, Ar-H); MS *m/z* (%) 380 (M^+, 5). Anal. Calcd. for $C_{16}H_{15}BrN_2S_2$: C, 50.66; H, 3.99; N, 7.38. Found: C, 50.50; H, 3.81; N, 7.21%.

4-(4-Substitutedphenyl)-2-hydrazinyl-6-(thiophen-2-yl)-4,5-dihydropyrimidine (10a,b)

A solution of **9a,b** (10 mmol) and $NH_2NH_2.H_2O$ (0.5 g, 10 mmol) in ethanol (10 mL) was heated under reflux with stirring for 4 h. It was allowed to cool at room temperature and the precipitated solid was filtered, washed with water, and recrystallized from ethanol to afford **10a,b** in 85-87% yields.

4-(4-Clorophenyl)-2-hydrazinyl-6-(thiophen-2-yl)-4,5-dihydro-pyrimidine (10a)

White solid (2.58 g, 85%), m.p. 221-222°C; IR (KBr) ν 3432 (NH_2), 3330 (NH), 1612 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 3.45 (s, 2H, CH_2), 4.49 (s, 1H, CH), 5.12 (s, 2H, NH_2), 7.10 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.32 (m, 1H, Ar-H), 7.70 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.89 (m, 1H, Ar-H), 8.08 (d, 1H, *J* = 7.8 Hz, Ar-H), 9.22 (brs, 1H, NH); MS *m/z* (%) 304 (M^+, 5). Anal. Calcd. for $C_{14}H_{13}ClN_4S$: C, 55.17; H, 4.30; N, 18.38. Found: C, 55.10; H, 4.21; N, 18.20%.

4-(4-Clorophenyl)-2-hydrazinyl-6-(thiophen-2-yl)-4,5-dihydro-pyrimidine (10b)

White solid (3.04 g, 87%), m.p. 224-225°C; IR (KBr) ν 3435 (NH_2), 3325 (NH), 1612 cm^{-1} (C=N); ^1H NMR (DMSO-d_6) δ 3.41 (s, 2H, CH_2), 4.47 (s, 1H, CH), 5.18 (brs, 2H, NH_2), 7.12 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.33 (m, 1H, Ar-H), 7.70 (d, 2H, *J* = 8.5 Hz, Ar-H), 7.89 (m, 1H, Ar-H), 8.11 (d, 1H, *J* = 7.8 Hz, Ar-H), 9.31 (brs, 1H, NH); MS *m/z* (%) 350 (M^+, 5). Anal. Calcd. for $C_{14}H_{13}BrN_4S$: C, 48.15; H, 3.75; N, 16.04. Found: C, 48.10; H, 3.61; N, 15.80%.

Antimicrobial activity

The synthesized compounds were tested for their antimicrobial activity against three microorganisms, and the minimal inhibitory concentrations (MICs) of the tested compounds were determined by the dilution method.

Sample preparation

Each of the test compounds and standards were dissolved in 12.5% DMSO at concentrations of 500 μg/mL. Further dilutions of the compounds and standards were made in test medium.

Culture of microorganisms

Bacterial strains were supplied from the Botany Department, Faculty of Science, Menoufia University, Egypt, namely *Bacillus subtilis* (ATCC 6633) (Gram-positive), *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative) and *Streptomyces* species (Actinomycetes). The bacterial strains were maintained on MHA (Mueller - Hinton agar) medium (Oxoid, Chemical Co.) for 24 h at 37°C. The medium was molten on a water bath, inoculated with 0.5 mL of the culture of the specific microorganism, and poured into sterile Petri dishes to form a layer of about 3-4 mm. The layer was allowed to cool and harden. With the aid of cork-borer, cups of about 10 mm diameter were produced (Jorgensen et al., 1999).

Agar diffusion technique

Antibacterial activities were tested against *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative) and *Streptomyces* species (Actinomycetes) using MH medium (17.5 g casein hydrolysate, 1.5 g soluble starch, 1000 mL beef extract). A stock solution of each synthesized compound (500 μg/mL) in DMSO was prepared and incorporated in sterilized liquid MH medium. Different concentrations of the test compounds in DMF were placed separately in cups in the agar medium. All plates were incubated at 37°C overnight. The inhibition zones were measured after 24 h. The minimum inhibitory concentration (MIC) was defined as the intercept of the grave of logarithm concentrations *versus* diameter of the inhibition zones (Janssen et al., 1987; Greenwood, 2000).

RESULTS AND DISCUSSION

2-acetylthiophen (**1**) was reacted with 4-chlor or 4-

bromobanzladehyde in ethanol in the presence potassium hydroxide to afford the corresponding 3-(4-substitutedaryl)-1-(thiophen-2-yl)prop-2-en-1-one derivatives **2a,b**. The ^{1}H NMR spectra of **2a,b** showed aromatic protons at δ 7.20-8.39 ppm. In addition, the mass spectra showed signals of the molecular ion peaks corresponding to the molecular formulas of **2a,b**.

The reaction of **2a,b** with guanidine hydrochloride in ethanol in and sodium hydroxide afforded the corresponding 4-(4-substitutedaryl)-6-(thiophen-2-yl) pyrimidin-2-amine **3a,b** in 77-80% yields. The IR spectra of **3a,b** showed a characteristic absorption band at 3489 cm^{-1} corresponding to the NH_2 but no absorption band of a carbonyl group. ^{1}H NMR spectra showed an NH_2 group signal as a singlet at δ 6.56-6.59 ppm in addition to the signals of the aromatic protons at δ 7.23-8.34 ppm.

When **2a,b** were allowed to react with phenyl hydrazine in ethanol at reflux temperature, the corresponding 1,3,5-trisubstituted pyrazoline derivatives **4a,b** were obtained in 80-84% yields. The ^{1}H NMR spectra of **4a,b** showed a CH_2 signal at δ 3.42-3.44 ppm, H-5 as a triplet at δ 4.42-4.44 ppm, and aromatic protons at δ 6.76-8.30 ppm.

The reaction of **2a,b** with malononitrile in pyridine at reflux temperature afforded the substituted pyran derivatives **5a,b** in 72-74% yields. The IR spectra of **5a,b** showed characteristic absorption bands at 3430-3438 and 2215-2225 cm^{-1}, corresponding to the NH_2 and CN groups, respectively. The ^{1}H NMR spectra showed an NH_2 signal at δ 6.72-6.75 ppm in addition to the aromatic protons signals at δ 7.15-8.22 ppm.

The reaction of **2a,b** with hydroxylamine hydrochloride in ethanol at reflux temperature gave the corresponding oxazole derivatives **6a,b** in 76-79% yields. The ^{1}H NMR spectra of **6a,b** showed a CH_2 signal at δ 3.44-3.47 ppm, H-5 at δ 4.43-4.46 ppm, and the aromatic protons at δ 6.96-8.21 ppm. When the enone derivatives **2a,b** were allowed to react with urea or thiourea in the presence of potassium hydroxide in ethanol at reflux temperature, the corresponding pyrimidine and thiopyrimidine derivatives, **7a,b** and **8a,b**, respectively, were obtained in 80-84% yields. The structures of **6a, b** and **8a,b** were confirmed by IR, ^{1}H NMR and mass spectra. The ^{1}H NMR spectrum of **7b** showed the CH_2 at δ 3.43 ppm and H-6 at δ 3.47 ppm, in addition to the aromatic protons at δ 7.24-8.06 ppm and NH at δ 8.36 pm.

Alkylation of **8a,b** with ethyl iodide in the presence of potassium hydroxide in a mixture of ethanol and water (1:2) at 60°C afforded the corresponding *S*-ethyl derivatives **9a,b** in 78-80% yields. Hydrazinolysis of **9a,b** by hydrazine hydrate in ethanol at reflux temperature gave the corresponding hydrazine derivatives **10a,b**, respectively, in 80-83% yields. The IR spectra of **10a,b** showed absorption bands at 3319-3327 cm^{-1} corresponding to the NH_2. In addition, the mass spectra of **10a,b** showed a molecular ion peak corresponding to their molecular formulas and the ^{1}H NMR spectra agreed with the assigned structures.

Table I. Minimum inhibitory concentration (MIC in μg/mL)

Compound	*Bacillus subtilis* (Gram-positive)	*Pseudomonas aeruginosa* (Gram-negative)	*Streptomyces species* (Actinomycetes)
2a	250	500	500
2b	500	-[a]	-[a]
3a	100	125	125
3b	250	500	250
4a	125	250	125
4b	250	500	250
5a	75	75	250
5b	500	500	-[a]
6a	250	250	500
6b	500	-[a]	500
7a	100	125	75
7b	125	250	100
8a	75	100	75
8b	100	100	100
9a	125	125	250
9b	125	250	500
10a	75	75	100
10b	125	100	100
Penicillin	31	46	33

The negative control, DMSO, showed no activity.
[a]Totally inactive (MIC > 500 μg/mL).

Antimicrobial activity

The antimicrobial activity of the synthesized compounds was evaluated against three microorganisms: *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* species (Actinomycetes). **5a**, **8a**, and **10a** showed the highest activity against *B. subtilis*, with MIC values of 75 μg/mL, followed by compounds **3a**, **7a**, and **8b** (Table I). Compounds **5a** and **10a** showed the highest inhibition of *P. aeruginosa*, whereas **7a** and **8a** were the most active against *Streptomyces* species, with MIC values of 75 μg/mL. Some compounds showed little or no activity against the microorganisms (Table I).

The 2,6-disubstituted pyrimidine derivatives with a free thiol-thione group showed the highest activity against both *B. subtilis* and *Streptomyces* species. In addition, the pyran derivative with the amino and

nitrile functionalities **5a** as well as the substituted 2-hydrazinyl dihydropyrimidine derivative **10a** exhibited the highest activity against *B. subtilis* and *P. aeruginosa*.

We also tested the influence of functionality such as alkylthio, hydrazine, amino-cyano, para substituted chlorophenyl, and para substituted bromopheny on this activity. Chlorine compounds were more effective than bromine derivatives with the same structural skeleton that differed only in substitution at the *para* position in the phenyl attached to the heterocyclic ring. Even compound **10a**, in which activity could be enhanced by the presence of the 2-hydrazinyl pyrimidine moiety, was more active than **10b** with the *p*-bromophenyl ring.

Substituted thiopyrimidine derivatives **8a** and **8b** had higher activity than their corresponding oxo-analogous **7a** and **7b** against *B. subtilis* and *P. aeruginosa*. On the other hand, the substituted pyrazoline derivatives showed higher activity than the corresponding oxazoline analogues.

CONCLUSION

New disubstituted pyrimidine, pyrazoline, and pyran derivatives were synthesized and showed moderate to high antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Streptomyces* species (Actinomycetes). The presence of free thiol-thione group in the pyrimidine ring, in addition to amino and nitrile functionalities in the pyran moiety, increase the antimicrobial activity of the tested compounds. Derivatives containing *p*-substituted chlorine showed higher activity than their corresponding bromine analogues.

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