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Synthesis and antimicrobial activity of thiazolyl pyrazoles and isoxazoles

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Abstract Thiazolyl pyrazoles and isoxazoles were prepared and their antimicrobial activity studied. Chloro- and nitro-substituted thiazolyl isoxazoles were potential antibacterial agents against *Staphylococcus aureus*, and nitro-substituted thiazolyl pyrazole was a potential antifungal agent against *Aspergillus niger*.

Keywords Pyrazoles · Isoxazoles · Antibacterial activity · Antifungal activity

Introduction

Among the diverse aza-heterocyclic ring systems considered as potent pharmacological agents, the pyrazole and isoxazole core structures constitute the most promising five-membered heterocyclic units [1, 2]. Pyrazole derivatives exhibit a wide range of biological activities such as antimicrobial [3, 4], insecticidal [5], fungicidal [5], antiviral [6], anti-inflammatory [7], antiobesity [8], and anticancer [9]. Celecoxib, a pyrazole derivative, is widely used in the market as an anti-inflammatory drug [10]. The popular methods for synthesis of pyrazolines include 1,3-dipolar cycloaddition of dipolar reagents to unsaturated compounds and [2 + 3] cyclocondensation of hydrazine derivatives with α,β-unsaturated carbonyl compounds [11]. Isoxazoles and their derivatives are important scaffolds for synthesis of various natural compounds and their congeners [12]. The therapeutic potential of isoxazoline derivatives is evident from their antimicrobial [13, 14], cyclooxygenase

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(COX)-2 inhibitory [15], antioxidant [16], anticancer [17], and antitumor activities [18]. Among the known methods of isoxazole synthesis, [2 + 3] cycloaddition of 1,3-dipoles to alkynes and reaction of hydroxylamine with 1,3-diketones or α,β-unsaturated ketones are the most important [19]. Thiazoles and their derivatives exhibit antibacterial [20], antifungal [21], and anti-inflammatory [22] activities. The thiazole nucleus is also an integral part of all penicillins, which have revolutionized therapy of bacterial diseases [23]. Study of structure–activity relationships revealed that change of substituents resulted in change of bioactivity. Thus, the combination of different azole motifs may alter the biopotency to accommodate multiple biological targets. In continuation of our efforts to develop simple and novel molecules of biological importance, the present work addresses the synthesis and antimicrobial activity of thiazolyl pyrazoles and isoxazoles.

Experimental

Apparatus and analysis

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The purity of the compounds was checked by thin-layer chromatography (TLC, silica gel H, BDH, ethyl acetate/hexane, 1:3). Infrared (IR) spectra were recorded on a Thermo Nicolet IR 200 Fourier-transform (FT)-IR spectrometer as KBr pellets, and wavenumbers are given in cm^{-1}. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded in dimethyl sulfoxide (DMSO)-d_6 on a Bruker-400 spectrometer (400 MHz). ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker spectrometer operating at 100 MHz. All chemical shifts are reported in δ (ppm) using tetramethylsilane (TMS) as internal standard. High-resolution mass spectra were recorded on a Micromass Q-TOF spectrometer using electrospray ionization. Ultrasonication was performed in a Bandelin Sonorex RK 102H ultrasonic bath operating at frequency of 35 kHz. Microanalyses were performed on a PerkinElmer 240C elemental analyzer. Reaction progress was monitored by TLC using silica gel plates (silica gel 60 F254 0.25 mm), and components were visualized by observation under ultraviolet (UV) light (254 and 365 nm). The heteroaryl chalcones, 3-(2,4-dichlorothiazol-5-yl)-1-arylprop-2-en-1-ones (**3**) were prepared as per literature procedure [24].

General procedure for synthesis of 5-(2,4-dichlorothiazol-5-yl)-4,5-dihydro-3-aryl-1*H*-pyrazole (4a–f)

A mixture of compounds **3a–f** (1 mmol), hydrazine hydrate (1.02 g, 1.5 mmol), and ethanol (10 mL) was sonicated at laboratory temperature for 30–45 min. The contents of the flask was poured into water, extracted with ethyl acetate, and dried. The solvent was removed under vacuum, and the resultant solid was recrystallized from 2-propanol.

*Spectral data for compounds **4a–f***

5-(2,4-Dichlorothiazol-5-yl)-4,5-dihydro-3-phenyl-1H-pyrazole (***4a***) Yield 72 %, m.p. 120–122 °C. IR (KBr) (cm^{-1}): 3256 (NH), 1618 (C=C), 1570 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.78 (dd, 1H, H_X, $J_{AX} = 5.1$ Hz, $J_{MX} = 11.1$ Hz), 3.45 (dd, 1H, H_M, $J_{AM} = 12.6$ Hz), 4.52 (dd, 1H, H_A), 7.25–7.75 (m, 5H, Ar–H), 7.85 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.5 (C-5), 41.3 (C-4), 134.8 (C-5′), 136.7 (C-4′), 149.4 (C-2′), 152.1 (C-3), 124.0, 128.7, 131.4, 135.0 ppm (aromatic carbons). HRMS: (*m/z*) 321.1811 [M + Na]. Anal. Calcd. for $C_{12}H_9Cl_2N_3S$: C, 48.33; H, 3.04; N, 14.09 %. Found: C, 48.41; H, 3.06; N, 14.20 %.

5-(2,4-Dichlorothiazol-5-yl)-4,5-dihydro-3-p-tolyl-1H-pyrazole (***4b***) Yield 68 %, m.p. 115–117 °C. IR (KBr) (cm^{-1}): 3254 (NH), 1616 (C=C), 1567 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H, Ar–CH_3), 2.75 (dd, 1H, H_X, $J_{AX} = 5.0$ Hz, $J_{MX} = 11.0$ Hz), 3.42 (dd, 1H, H_M, $J_{AM} = 12.4$ Hz), 4.51 (dd, 1H, H_A), 7.15–7.64 (m, 4H, Ar–H), 7.84 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.7 (Ar–CH_3), 37.2 (C-5), 41.1 (C-4), 134.6 (C-5′), 136.6 (C-4′), 148.8 (C-2′), 152.0 (C-3), 123.7, 128.4, 131.2, 134.9 ppm (aromatic carbons). HRMS: (*m/z*) 335.2077 [M + Na]. Anal. Calcd. for $C_{13}H_{11}Cl_2N_3S$: C, 50.00; H, 3.55; N, 13.45 %. Found: C, 50.1; H, 3.59; N, 13.59 %.

3-(4-Chlorophenyl)-5-(2,4-dichlorothiazol-5-yl)-4,5-dihydro-1H-pyrazole (***4c***) Yield 70 %, m.p. 157–159 °C. IR (KBr) (cm^{-1}): 3261 (NH), 1624 (C=C), 1578 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.84 (dd, 1H, H_X, $J_{AX} = 5.4$ Hz, $J_{MX} = 11.3$ - Hz), 3.49 (dd, 1H, H_M, $J_{AM} = 12.9$ Hz), 4.58 (dd, 1H, H_A), 7.43–7.78 (m, 4H, Ar–H), 7.87 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.8 (C-5), 41.7 (C-4), 136.4 (C-5′), 137.2 (C-4′), 153.4 (C-2′), 153.7 (C-3), 124.6, 131.2, 135.8, 137.2 ppm (aromatic carbons). HRMS: (*m/z*) 355.6263 [M + Na]. Anal. Calcd. for $C_{12}H_8Cl_3N_3S$: C, 43.33; H, 2.42; N, 12.63 %. Found: C, 43.42; H, 2.47; N, 12.8 %.

3-(4-Bromophenyl)-5-(2,4-dichlorothiazol-5-yl)-4,5-dihydro-1H-pyrazole (***4d***) Yield 69 %, m.p. 144–146 °C. IR (KBr) (cm^{-1}): 3257 (NH), 1620 (C=C), 1575 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.82 (dd, 1H, H_X, $J_{AX} = 5.2$ Hz, $J_{MX} = 11.2$ - Hz), 3.47 (dd, 1H, H_M, $J_{AM} = 12.7$ Hz), 4.54 (dd, 1H, H_A), 7.42–7.76 (m, 4H, Ar–H), 7.86 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.5 (C-5), 41.5 (C-4), 136.3 (C-5′), 137.0 (C-4′), 151.8 (C-2′), 152.8 (C-3), 124.2, 130.9, 132.8, 135.2 ppm (aromatic carbons). HRMS: (*m/z*) 400.0761 [M + Na]. Anal. Calcd. for $C_{12}H_8BrCl_2N_3S$: C, 38.22; H, 2.14; N, 11.14 %. Found: C, 38.33; H, 2.18; N, 11.29 %.

5-(2,4-Dichlorothiazol-5-yl)-4,5-dihydro-3-(4-methoxyphenyl)-1H-pyrazole (***4e***) Yield 66 %, m.p. 118–120 °C. IR (KBr) (cm^{-1}): 3252 (NH), 1613 (C=C), 1563 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.72 (dd, 1H, H_X, $J_{AX} = 4.8$ Hz, $J_{MX} = 10.9$ - Hz), 3.38 (dd, 1H, H_M, $J_{AM} = 12.2$ Hz), 3.76 (s, 3H, OCH_3), 4.50 (dd, 1H, H_A), 7.22–7.55 (m, 4H, Ar–H), 7.82 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.1 (C-5), 40.9 (C-4), 56.1 (OCH_3), 132.4 (C-5′), 134.6 (C-4′), 148.5 (C-2′), 151.9 (C-3), 115.8, 126.7, 130.6, 164.2 ppm (aromatic carbons). HRMS: (*m/*

z) 351.2062 [M + Na]. Anal. Calcd. for $C_{13}H_{11}Cl_2N_3OS$: C, 47.57; H, 3.38; N, 12.80 %. Found: C, 47.69; H, 3.36; N, 12.93 %.

5-(2,4-Dichlorothiazol-5-yl)-4,5-dihydro-3-(4-nitrophenyl)-1H-pyrazole (***4f***) Yield 74 %, m.p. 164–166 °C. IR (KBr) (cm^{-1}): 3264 (NH), 1626 (C=C), 1579 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.86 (dd, 1H, H_X, $J_{AX} = 6.0$ Hz, $J_{MX} = 11.5$ - Hz), 3.52 (dd, 1H, H_M, $J_{AM} = 13.0$ Hz), 4.59 (dd, 1H, H_A), 7.89 (bs, 1H, NH), 7.72–8.15 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 38.9 (C-5), 41.9 (C-4), 137.2 (C-5′), 137.9 (C-4′), 151.9 (C-2′), 154.1 (C-3), 125.7, 131.5, 141.4, 152.7 ppm (aromatic carbons). HRMS: (*m/z*) 366.1779 [M + Na]. Anal. Calcd. for $C_{12}H_8Cl_2N_4O_2S$: C, 42.00; H, 2.35; N, 16.33 %. Found: C, 42.08; H, 2.38; N, 16.45 %.

General procedure for synthesis of 5-(2,4-dichlorothiazol-5-yl)-4,5-dihydro-3-arylisoxazole (5a–f)

Compounds **3a–f** (1 mmol), hydroxylamine hydrochloride (1.38 g, 1.5 mmol), and ethanol (10 mL) were sonicated at room temperature for 25–40 min. The contents of the flask was poured into water and extracted with ethyl acetate. The organic layer was dried and solvent was removed in vacuo. The resultant solid was recrystallized from 2-propanol.

*Spectral data for compounds **5a–f***

5-(2,4-Dichlorothiazol-5-yl)-4,5-dihydro-3-phenylisoxazole (***5a***) Yield 78 %, m.p. 114–116 °C. IR (KBr) (cm^{-1}): 1621 (C=C), 1572 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.80 (dd, 1H, H_X, $J_{AX} = 5.8$ Hz, $J_{MX} = 11.4$ Hz), 4.15 (dd, 1H, H_M, $J_{AM} = 12.9$ Hz), 4.82 (dd, 1H, H_A), 7.25–7.78 (m, 5H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.3 (C-4), 72.4 (C-5), 136.2 (C-5′), 136.9 (C-4′), 151.4 (C-2′), 156.8 (C-3), 124.5, 129.2, 131.4, 135.0 ppm (aromatic carbons). HRMS: (*m/z*) 322.1658 [M + Na]. Anal. Calcd. for $C_{12}H_8Cl_2N_2OS$: C, 48.18; H, 2.69; N, 9.36 %. Found: C, 48.29; H, 2.71; N, 9.26 %.

5-(2,4-Dichlorothiazol-5-yl)-4,5-dihydro-3-p-tolylisoxazole (***5b***) Yield 75 %, m.p. 119–121 °C. IR (KBr) (cm^{-1}): 1618 (C=C), 1569 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.78 (dd, 1H, H_X, $J_{AX} = 5.6$ Hz, $J_{MX} = 11.2$ Hz), 2.79 (S, 3H, CH_3), 4.12 (dd, 1H, H_M, $J_{AM} = 12.8$ Hz), 4.80 (dd, 1H, H_A), 7.20–7.68 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.9 (CH_3), 43.7 (C-4), 72.2 (C-5), 135.5 (C-5′), 136.7 (C-4′), 150.9 (C-2′), 155.4 (C-3), 123.7, 128.9, 131.4, 134.9 ppm (aromatic carbons). HRMS: (*m/z*) 336.1929 [M + Na]. Anal. Calcd. for $C_{13}H_{10}Cl_2N_2OS$: C, 49.85; H, 3.22; N, 8.94 %. Found: C, 49.94; H, 3.18; N, 9.05 %.

3-(4-Chlorophenyl)-5-(2,4-dichlorothiazol-5-yl)-4,5-dihydroisoxazole (***5c***) Yield 70 %, m.p. 145–147 °C. IR (KBr) (cm^{-1}): 1626 (C=C), 1579 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.83 (dd, 1H, H_X, $J_{AX} = 6.0$ Hz, $J_{MX} = 11.6$ Hz), 4.18 (dd, 1H, H_M, $J_{AM} = 13.1$ Hz), 4.88 (dd, 1H, H_A), 7.45–7.80 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 43.7 (C-4), 72.8 (C-5), 136.8 (C-5′), 137.2 (C-

4′), 151.9 (C-2′), 157.2 (C-3), 129.4, 130.5, 133.4, 137.6 ppm (aromatic carbons). HRMS: (*m/z*) 356.6117 [M + Na]. Anal. Calcd. for $C_{12}H_7Cl_3N_2OS$: C, 43.20; H, 2.11; N, 8.40 %. Found: C, 43.3; H, 2.16; N, 8.55 %.

3-(4-Bromophenyl)-5-(2,4-dichlorothiazol-5-yl)-4,5-dihydroisoxazole (***5d***) Yield 66 %, m.p. 132–134 °C. IR (KBr) (cm^{-1}): 1623 (C=C), 1577 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.81 (dd, 1H, H_X, $J_{AX} = 5.9$ Hz, $J_{MX} = 11.5$ Hz), 4.16 (dd, 1H, H_M, $J_{AM} = 13.0$ Hz), 4.84 (dd, 1H, H_A), 7.44–7.78 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.2 (C-4), 72.5 (C-5), 136.6 (C-5′), 137.0 (C-4′), 151.6 (C-2′), 157.1 (C-3), 122.9, 128.6, 130.9, 134.3 ppm (aromatic carbons). HRMS: (*m/z*) 401.0620 [M + Na]. Anal. Calcd. for $C_{12}H_7BrCl_2N_2OS$: C, 38.12; H, 1.87; N, 7.41 %. Found: C, 38.04; H, 1.90; N, 7.58 %.

5-(2,4-Dichlorothiazol-5-yl)-4,5-dihydro-3-(4-methoxyphenyl)isoxazole (***5e***) Yield 68 %, m.p. 110–112 °C. IR (KBr) (cm^{-1}): 1616 (C=C), 1565 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.74 (dd, 1H, H_X, $J_{AX} = 5.5$ Hz, $J_{MX} = 11.0$ Hz), 3.76 (s,3H, OCH_3), 4.10 (dd, 1H, H_M, $J_{AM} = 12.7$ Hz), 4.82 (dd, 1H, H_A), 7.42–7.73 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 44.1 (C-4), 56.3 (OCH_3), 70.9 (C-5), 133.9 (C-5′), 135.5 (C-4′), 150.7 (C-2′), 155.2 (C-3), 120.6, 126.8, 130.5, 160.5 ppm (aromatic carbons). HRMS: (*m/z*) 352.1921 [M + Na]. Anal. Calcd. for $C_{13}H_{10}Cl_2N_2O_2S$: C, 47.43; H, 3.06; N, 8.51 %. Found: C, 47.44; H, 3.08; N, 8.39 %.

5-(2,4-Dichlorothiazol-5-yl)-4,5-dihydro-3-(4-nitrophenyl)isoxazole (***5f***) Yield 72 %, m.p. 155–157 °C. IR (KBr) (cm^{-1}): 1629 (C=C), 1581 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.88 (dd, 1H, H_X, $J_{AX} = 6.2$ Hz, $J_{MX} = 11.7$ Hz), 4.20 (dd, 1H, H_M, $J_{AM} = 13.2$ Hz), 4.89 (dd, 1H, H_A), 7.75–8.18 (m, 4H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 40.9 (C-4), 72.9 (C-5), 136.9 (C-5′), 137.4 (C-4′), 152.6 (C-2′), 157.8 (C-3), 129.5, 130.7, 140.4, 154.6 ppm (aromatic carbons). HRMS: (*m/z*) 367.1640 [M + Na]. Anal. Calcd. for $C_{12}H_7Cl_2N_3O_3S$: C, 41.88; H, 2.05; N, 12.21 %. Found: C, 41.76; H, 2.10; N, 12.08 %.

General procedure for synthesis of -5-(2,4-dichlorothiazol-5-yl)-3-aryl-1*H*-pyrazole (6a–f)/-5-(2,4-dichlorothiazol-5-yl)-3-arylisoxazole (7a–f)

A solution of **4a–f/5a–f** (1 mmol) and chloranil (1.2 mmol) in xylene (10 mL) was sonicated at 60 °C for 35–45 min, then treated with 5 % NaOH solution. The organic layer was separated, repeatedly washed with water, and dried. The solvent was removed in vacuo. The solid obtained was recrystallized from 2-propanol.

*Spectral data for compounds **6a–f***

5-(2,4-Dichlorothiazol-5-yl)-3-phenyl-1H-pyrazole (***6a***) Yield 70 %, m.p. 160–162 °C. IR (KBr) (cm^{-1}): 3258 (NH), 1622 (C=C), 1574 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.75–7.55 (m, 6H, Ar–H & C_4–H), 8.12 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 104.7 (C-4), 120.3 (C-5′), 136.8 (C-4′), 137.4 (C-5), 148.0 (C-3), 151.5 (C-2′), 123.6, 128.5, 130.2, 133.1 ppm (aromatic

carbons). HRMS: (*m/z*) 319.1657 [M + Na]. Anal. Calcd. for $C_{12}H_7Cl_2N_3S$: C, 48.66; H, 2.38; N, 14.19 %. Found: C, 48.75; H, 2.42; N, 14.33 %.

5-(2,4-Dichlorothiazol-5-yl)-3-p-tolyl-1H-pyrazole (***6b***) Yield 71 %, m.p. 145–147 °C. IR (KBr) (cm^{-1}): 3255 (NH), 1620 (C=C), 1571 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.35 (s, 3H, CH_3), 6.72–7.48 (m, 5H, Ar–H & C_4-H), 8.10 (bs,1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.4 (CH_3), 104.4 (C-4), 120.1 (C-5′), 136.2 (C-4′), 137.8 (C-5), 147.6 (C-3), 151.8 (C-2′), 123.4, 127.6, 129.8, 132.9 ppm (aromatic carbons). HRMS: (*m/z*) 333.1919 [M + Na]. Anal. Calcd. for $C_{13}H_9Cl_2N_3S$: C, 50.33; H, 2.92; N, 13.55 %. Found: C, 50.43; H, 2.90; N, 13.67 %.

3-(4-Chlorophenyl)-5-(2,4-dichlorothiazol-5-yl)-1H-pyrazole (***6c***) Yield 65 %, m.p. 185–187 °C. IR (KBr) (cm^{-1}): 3262 (NH), 1628 (C=C), 1582 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.78–7.58 (m, 5H, Ar–H & C_4–H), 8.15 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 106.3 (C-4), 121.8 (C-5′), 137.4 (C-4′), 138.3 (C-5), 148.3 (C-3), 152.3 (C-2′), 128.0, 129.4, 131.8, 148.1 ppm (aromatic carbons). HRMS: (*m/z*) 353.6106 [M + Na]. Anal. Calcd. for $C_{12}H_6Cl_3N_3S$: C, 43.59; H, 1.83; N, 12.71 %. Found: C, 43.67; H, 1.78; N, 12.82 %.

*3-(4-Bromophenyl)-5-(2,4-dichlorothiazol-5-yl)-1H-*pyrazole (***6d***) Yield 67 %, m.p. 173–175 °C. IR (KBr) (cm^{-1}): 3259 (NH), 1626 (C=C), 1578 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.76–7.56 (m, 5H, Ar–H & C_4–H), 8.14 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 105.4 (C-4), 120.0 (C-5′), 137.2 (C-4′), 137.6 (C-5), 148.2 (C-3), 152.1 (C-2′), 124.2, 128.6, 131.6, 134.6 ppm (aromatic carbons). HRMS: (*m/z*) 398.0612 [M + Na]. Anal. Calcd. for $C_{12}H_6BrCl_2N_3S$: C, 38.43; H, 1.61; N, 11.20 %. Found: C, 38.31; H, 1.64; N, 11.35 %.

5-(2,4-Dichlorothiazol-5-yl)-3-(4-methoxyphenyl)-1H-pyrazole (***6e***) Yield 68 %, m.p. 154–156 °C. IR (KBr) (cm^{-1}): 3254 (NH), 1619 (C=C), 1568 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.85 (s, 3H, OCH_3), 6.72–7.47 (m, 5H, Ar–H & C_4–H), 8.09 (bs,1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.6 (OCH_3), 103.3 (C-4), 121.0 (C-5′), 137.1 (C-4′), 135.2 (C-5), 145.7 (C-3), 152.2 (C-2′), 123.1, 127.5, 129.6, 158.5 ppm (aromatic carbons). HRMS: (*m/z*) 349.1911 [M + Na]. Anal. Calcd. for $C_{13}H_9Cl_2N_3OS$: C, 47.87; H, 2.78; N, 12.88 %. Found: C, 47.96; H, 2.80; N, 13.04 %.

5-(2,4-Dichlorothiazol-5-yl)-3-(4-nitrophenyl)-1H-pyrazole (***6f***) Yield 66 %, m.p. 197–199 °C. IR (KBr) (cm^{-1}): 3265 (NH), 1631 (C=C), 1583 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.82–7.65 (m, 5H, Ar–H & C_4–H), 8.20 (bs,1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 106.9 (C-4), 121.9 (C-5′), 137.8 (C-4′), 138.8 (C-5), 148.7 (C-3), 152.9 (C-2′), 128.2, 129.6, 139.8, 148.4 ppm (aromatic carbons). HRMS: (*m/z*) 364.1630 [M + Na]. Anal. Calcd. for $C_{12}H_6Cl_2N_4O_2S$: C, 42.25; H, 1.77; N, 16.42 %. Found: C, 42.32; H, 1.82; N, 16.30 %.

*Spectral data for compounds **7a–f***

5-(2,4-Dichlorothiazol-5-yl)-3-phenylisoxazole (***7a***) Yield 65 %, m.p. 152–154 °C. IR (KBr) (cm^{-1}): 1624 (C=C), 1578 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.85–7.56 (m, 6H, Ar–H & C_4–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 104.9 (C-4), 120.8 (C-5′), 137.8 (C-4′), 152.9 (C-2′), 158.7 (C-5), 162.4 (C-3), 127.8, 128.9, 131.4, 133.5 ppm (aromatic carbons). HRMS: (*m/z*) 320.1499 [M + Na]. Anal. Calcd. for $C_{12}H_6Cl_2N_2OS$: C, 48.50; H, 2.04; N, 9.43 %. Found: C, 48.6; H, 2.08; N, 9.6 %.

5-(2,4-Dichlorothiazol-5-yl)-3-p-tolylisoxazole (***7b***) Yield 70 %, m.p. 143–145 °C. IR (KBr) (cm^{-1}): 1622 (C=C), 1573 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, CH_3), 6.85–7.54 (m, 5H, Ar–H & C_4–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.6 (CH_3), 103.4 (C-4), 121.5 (C-5′), 137.2 (C-4′), 151.7 (C-2′), 158.8 (C-5), 162.6 (C-3), 127.4, 129.5, 132.1, 134.8 ppm (aromatic carbons). HRMS: (*m/z*) 334.1768 [M + Na]. Anal. Calcd. for $C_{13}H_8Cl_2N_2OS$: C, 50.18; H, 2.59; N, 9.00 %. Found: C, 50.26; H, 2.62; N, 9.12 %.

3-(4-Chlorophenyl)-5-(2,4-dichlorothiazol-5-yl)isoxazole (***7c***) Yield 66 %, m.p. 167–169 °C. IR (KBr) (cm^{-1}): 1630 (C=C), 1584 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.89–7.75 (m, 5H, Ar–H & C_4–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 108.3 (C-4), 121.8 (C-5′), 137.6 (C-4′), 152.8 (C-2′), 158.3 (C-5), 162.8 (C-3), 128.7, 131.5, 137.9, 147.9 ppm (aromatic carbons). HRMS: (*m/z*) 354.5951 [M + Na]. Anal. Calcd. for $C_{12}H_5Cl_3N_2OS$: C, 43.46; H, 1.52; N, 8.45 %. Found: C, 43.36; H, 1.50; N, 8.30 %.

3-(4-Bromophenyl)-5-(2,4-dichlorothiazol-5-yl)isoxazole (***7d***) Yield 68 %, m.p. 171–173 °C. IR (KBr) (cm^{-1}): 1627 (C=C), 1581 (C=C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.86–7.72 (m, 5H, Ar–H & C_4–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 106.9 (C-4), 120.9 (C-5′), 137.5 (C-4′), 152.6 (C-2′), 158.2 (C-5), 162.3 (C-3), 128.6, 131.4, 134.7, 135.6 ppm (aromatic carbons). HRMS: (*m/z*) 399.0462 [M + Na]. Anal. Calcd. for $C_{12}H_5BrCl_2N_2OS$: C, 38.33; H, 1.34; N, 7.45 %. Found: C, 38.42; H, 1.38; N, 7.58 %.

5-(2,4-Dichlorothiazol-5-yl)-3-(4-methoxyphenyl)isoxazole (**7e**) Yield 65 %, m.p. 133–135 °C. IR (KBr) (cm^{-1}): 1621 (C=C), 1571 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.86 (s, 3H, OCH_3), 6.75–7.46 (m, 5H, Ar–H & C_4–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.8 (OCH_3), 103.4 (C-4), 121.2 (C-5′), 137.0 (C-4′), 152.4 (C-2′), 158.2 (C-5), 162.1 (C-3), 123.1, 128.9, 130.8, 160.5 ppm (aromatic carbons). HRMS: (*m/z*) 350.1761 [M + Na]. Anal. Calcd. for $C_{13}H_8Cl_2N_2O_2S$: C, 47.72; H, 2.46; N, 8.56 %. Found: C, 47.84; H, 2.49; N, 8.70 %.

5-(2,4-Dichlorothiazol-5-yl)-3-(4-nitrophenyl)isoxazole (***7f***) Yield 69 %, m.p. 190–192 °C. IR (KBr) (cm^{-1}): 1632 (C=C), 1587 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.95–8.34 (m, 5H, Ar–H & C_4–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 109.4 (C-4), 121.8 (C-5′), 137.8 (C-4′), 153.2 (C-2′), 158.8 (C-5), 163.7 (C-3), 128.9, 131.6, 139.8, 148.6 ppm (aromatic carbons). HRMS: (*m/*

z) 365.1479 [M + Na]. Anal. Calcd. for $C_{12}H_5Cl_2N_3O_3S$: C, 42.12; H, 1.47; N, 12.28 %. Found: C, 42.19; H, 1.52; N, 12.39 %.

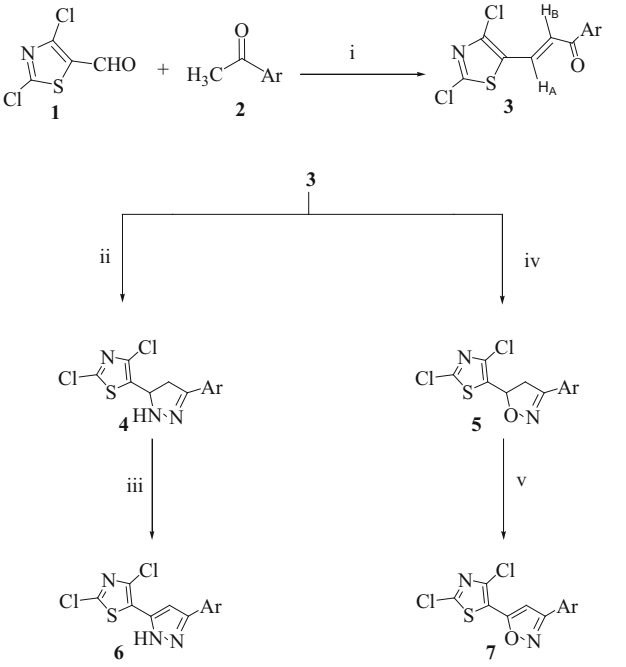
Experimental procedure for antimicrobial activity

In vitro antimicrobial studies were carried out by agar well diffusion method against test organisms [25, 26]. Nutrient broth (NB) plates were swabbed with 24-h-old broth culture (100 µL) of test bacteria. Using a sterile cork borer, wells (6 mm) were made into each Petri plate. The compounds were dissolved in DMSO at 5 mg/mL, and from this 2.5, 5, 10, and 20 µL (12.5, 25, 50, 100 µg/well) were added to the wells using sterile pipettes. Simultaneously, standard antibiotics (chloramphenicol for antibacterial activity, ketoconazole for antifungal activity) were tested as positive control against the pathogens. The samples were dissolved in DMSO, which showed no zone of inhibition and acts as negative control. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. After appropriate incubation, the diameter of zone of inhibition of each well was measured. Triplicates were maintained, and average values were calculated for eventual antibacterial activity. The broth dilution test was used to determine the minimum inhibitory concentration (MIC) of the above-mentioned samples [27, 28]. Freshly prepared nutrient broth was used as diluent. The 24-h-old culture of the test bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* and the test fungi *Aspergillus niger* and *Penicillium chrysogenum* were diluted 100-fold in nutrient broth (100 µL bacterial cultures in 10 mL NB). Stock solution of synthesized compounds was prepared in DMSO by dissolving 5 mg of compound in 1 mL of DMSO. Increasing concentrations of test samples (1.25, 2.5, 5, 10, 20, and 40 µL of stock solution contains 6.25, 12.5, 25, 50, 100, and 200 µg of the compounds) were added to test tubes containing the bacterial and fungal cultures. All tubes were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The tubes were examined for visible turbidity and using NB as control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as the MIC. To determine the minimum bactericidal concentration (MBC) [29] and minimum fungicidal concentration (MFC) [30] for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient broth (for bacteria) and potato dextrose agar (PDA, for fungi) by streaking. Plates inoculated with bacteria and fungi were incubated at 37 °C for 24 h and at 28 °C for 48 h, respectively. After incubation, the lowest concentration at which no visible growth was observed was noted as the MBC (for bacteria) or MFC (for fungi).

Results and discussion

Claisen–Schmidt reaction of 2,4-dichlorothiazole-5-aldehyde with various aryl ketones under acidic conditions led to heteroaryl chalcones (*E*)-3-(2,4-dichlorothiazol-5-yl)-1-phenylprop-2-en-1-one (**3**). The ^{1}H NMR spectrum of **3a** exhibited two doublets at δ

7.04 and 7.72 ppm due to olefin protons, H_A and H_B. The downfield shift was assigned to H_A. The coupling constant value $J = 14.7$ Hz indicated *trans* geometry. Cyclocondensation of **3** with hydrazine hydrate in ethanol resulted in directly linked bis heterocyclic compounds 5-(2,4-dichlorothiazol-5-yl)-4,5-dihydro-3-aryl-1*H*-pyrazoles (**4**). Similar reaction of **3** with hydroxylamine hydrochloride gave 5-(2,4-dichlorothiazol-5-yl)-4,5-dihydro-3-arylisoxazoles (**5**). The ^{1}H NMR spectra of **4a** and **5a** displayed an AMX splitting pattern for pyrazoline and isoxazoline ring protons. The double doublets observed at δ 2.78, 3.45, and 4.52 in **4a** and 2.80, 4.15, and 4.82 ppm in **5a** were assigned to H_X, H_M, and H_A. The coupling constant values $J_{AM} \approx 12.7$, $J_{AX} \approx 5.5$, and $J_{MX} \approx 11.2$ Hz indicated that H_A, H_M are *trans*, H_A, H_X are *cis*, and H_M, H_X are *geminal*. Besides, compound **4a** exhibited a broad singlet at δ 7.85 ppm due to NH of pyrazoline, which disappeared on deuteration. Oxidation of compounds **4** and **5** with chloranil in xylene gave the aromatized products 5-(2,4-dichlorothiazol-5-yl)-3-aryl-1*H*-pyrazole (**6**) and 5-(2,4-dichlorothiazol-5-yl)-3-arylisoxazole (**7**). Absence of the AMX splitting pattern in the ^{1}H NMR spectra of **6** and **7** confirmed that aromatization occurred. The C_4–H of pyrazole and isoxazole displayed signal in a much more



Scheme 1 Synthesis of thiazolyl pyrazoles and isoxazoles

downfield region and merged with aromatic protons. The structures of all compounds were further established by ^{13}C NMR, HRMS, and elemental analysis (Scheme 1).

Biological activity

Compounds **4**–**7** were dissolved in DMSO at concentrations of 12.5, 25, 50, and 100 μg/well. Bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* and fungi *Aspergillus niger* and *Penicillium chrysogenum* were obtained from Department of Microbiology, S.V. University, Tirupati.

Antibacterial activity

Compounds **4**–**7** were evaluated for antibacterial activity at four different concentrations: 12.5, 25, 50, and 100 μg/well. The results of antibacterial activity presented in Table 1 indicated that Gram-positive bacteria are more susceptible to the tested compounds than Gram-negative bacteria. The aromatized compounds thiazolyl pyrazoles (**6**) and thiazolyl isoxazoles (**7**) exhibited greater activity when compared with their dihydro derivatives, thiazolyl pyrazolines (**4**) and thiazolyl isoxazolines (**5**). In fact, compound **7f** showed slightly higher activity against *Staphylococcus aureus* than the standard drug chloramphenicol, whereas compound **7c** exhibited activity equal to the standard drug at the tested concentrations. Further, it was observed that there was not much difference in activity between the compounds having pyrazole and isoxazole rings. It was also noticed that the presence of electron-withdrawing substituents increases the activity. In fact, with increasing electronegativity, the activity increases [31–33]. Compounds **6c**, **d**, **f** and **7c**, **d**, **f** having chloro, bromo, and nitro substituents displayed greater activity than compounds **6b**, **e** and **7b**, **e** having electron-donating substituents. This was substantiated by the fact that the compounds with methoxy substituents **6e** and **7e** exhibited least activity, which may be due to positive mesomeric effect (Fig. 1).

Antifungal activity

Amongst compounds **4**–**7**, the aromatized compounds **6** and **7** inhibited spore germination of the tested fungi. The dihydro derivatives **4** and **5** were not active except **4f** and **5f**, which showed least activity. Compounds **6** and **7** exhibited more activity towards *Aspergillus niger* than *Penicillium chrysogenum*. It was also observed that thiazolyl pyrazoles (**6**) showed slightly higher activity than thiazolyl isoxazoles (**7**). The nitro-substituted thiazolyl pyrazole (**6f**) exhibited greater activity than the standard drug ketoconazole at all tested concentrations. The compounds having electron-withdrawing substituents exhibited greater activity than those having electron-donating ones (Table 2; Fig. 2).

Table 1 In vitro antibacterial activity of compounds **4–7**

Compound	Zone of inhibition (mm)							
	Gram-positive bacteria							
	Staphylococcus aureus				*Bacillus subtilis*			
	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well
4a	–	–	–	–	–	–	–	–
4b	–	–	–	–	–	–	–	–
4c	–	–	–	–	–	–	–	–
4d	–	–	–	–	–	–	–	–
4e	–	–	–	–	–	–	–	–
4f	–	9 ± 3	11 ± 1	15 ± 4	–	–	9 ± 1	11 ± 4
5a	–	–	–	–	–	–	–	–
5b	–	–	–	–	–	–	–	–
5c	–	–	–	–	–	–	–	–
5d	–	–	–	–	–	–	–	–
5e	–	–	–	–	–	–	–	–
5f	8 ± 3	10 ± 4	12 ± 2	16 ± 1	–	9 ± 4	11 ± 2	13 ± 1
6a	20 ± 2	21 ± 3	25 ± 4	27 ± 2	14 ± 3	16 ± 2	17 ± 2	20 ± 1
6b	12 ± 3	15 ± 4	17 ± 2	20 ± 1	12 ± 2	14 ± 3	15 ± 1	17 ± 1
6c	24 ± 3	27 ± 2	30 ± 4	31 ± 1	19 ± 2	21 ± 1	22 ± 2	24 ± 4
6d	22 ± 2	24 ± 4	27 ± 3	29 ± 2	16 ± 1	18 ± 2	19 ± 4	22 ± 1
6e	9 ± 1	11 ± 2	13 ± 4	17 ± 3	9 ± 1	11 ± 2	13 ± 3	15 ± 2
6f	26 ± 2	29 ± 1	31 ± 3	32 ± 2	20 ± 4	22 ± 1	24 ± 3	25 ± 2
7a	21 ± 1	23 ± 3	26 ± 2	28 ± 4	15 ± 2	17 ± 1	18 ± 3	21 ± 4
7b	14 ± 4	17 ± 2	19 ± 1	21 ± 3	13 ± 4	15 ± 1	16 ± 3	18 ± 1

Table 1 continued

Compound	Zone of inhibition (mm)							
	Gram-positive bacteria							
	Staphylococcus aureus				*Bacillus subtilis*			
	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well
7c	28 ± 1	30 ± 2	32 ± 3	33 ± 4	21 ± 2	23 ± 3	25 ± 4	26 ± 2
7d	23 ± 4	25 ± 3	28 ± 1	30 ± 2	17 ± 3	19 ± 1	20 ± 2	23 ± 4
7e	10 ± 1	12 ± 3	14 ± 2	19 ± 4	11 ± 3	13 ± 1	14 ± 2	16 ± 1
7f	30 ± 1	31 ± 4	35 ± 3	36 ± 1	22 ± 2	25 ± 4	27 ± 1	28 ± 4
Chloramphenicol	28 ± 1	30 ± 4	33 ± 1	35 ± 2	30 ± 1	32 ± 3	34 ± 4	38 ± 1
Control (DMSO)	–	–	–	–	–	–	–	–
Compound	Zone of inhibition (mm)							
	Gram-negative bacteria							
	Pseudomonas aeruginosa				*Klebsiella pneumoniae*			
	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well
4a	–	–	–	–	–	–	–	–
4b	–	–	–	–	–	–	–	–
4c	–	–	–	–	–	–	–	–
4d	–	–	–	–	–	–	–	–
4e	–	–	–	–	–	–	–	–
4f	–	–	8 ± 1	11 ± 2	–	–	–	–
5a	–	–	–	–	–	–	–	–
5b	–	–	–	–	–	–	–	–

Table 1 continued

Compound	Zone of inhibition (mm)							
	Gram-negative bacteria							
	Pseudomonas aeruginosa				*Klebsiella pneumoniae*			
	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well
5c	–	–	–	–	–	–	–	–
5d	–	–	–	–	–	–	–	–
5e	–	–	–	–	–	–	–	–
5f	–	–	9 ± 1	12 ± 2	–	–	–	8 ± 2
6a	11 ± 2	14 ± 3	15 ± 1	19 ± 2	8 ± 4	10 ± 1	11 ± 3	13 ± 1
6b	9 ± 3	11 ± 2	13 ± 1	15 ± 2	–	–	10 ± 2	11 ± 3
6c	15 ± 3	18 ± 2	21 ± 1	24 ± 4	12 ± 2	13 ± 1	16 ± 3	20 ± 1
6d	13 ± 2	16 ± 3	18 ± 1	21 ± 2	10 ± 4	12 ± 3	13 ± 1	15 ± 2
6e	–	–	10 ± 3	13 ± 2	–	–	–	9 ± 1
6f	18 ± 3	20 ± 4	22 ± 3	25 ± 1	13 ± 2	14 ± 1	17 ± 2	19 ± 4
7a	12 ± 1	15 ± 3	17 ± 2	20 ± 1	9 ± 3	11 ± 1	12 ± 4	14 ± 2
7b	10 ± 2	13 ± 4	14 ± 1	17 ± 2	–	9 ± 3	10 ± 2	12 ± 1
7c	19 ± 1	21 ± 2	23 ± 3	26 ± 1	16 ± 2	17 ± 3	19 ± 2	22 ± 4
7d	14 ± 3	17 ± 2	19 ± 3	22 ± 2	11 ± 4	12 ± 2	14 ± 3	16 ± 4
7e	–	8 ± 1	11 ± 2	14 ± 3	–	–	8 ± 3	10 ± 2
7f	20 ± 3	22 ± 1	24 ± 3	27 ± 2	17 ± 3	20 ± 2	23 ± 1	25 ± 3
Chloramphenicol	23 ± 1	25 ± 5	27 ± 6	30 ± 2	36 ± 6	38 ± 1	40 ± 2	42 ± 3
Control (DMSO)	–	–	–	–	–	–	–	–

(–) No activity. Values are mean ± standard deviation (SD) of three replicates

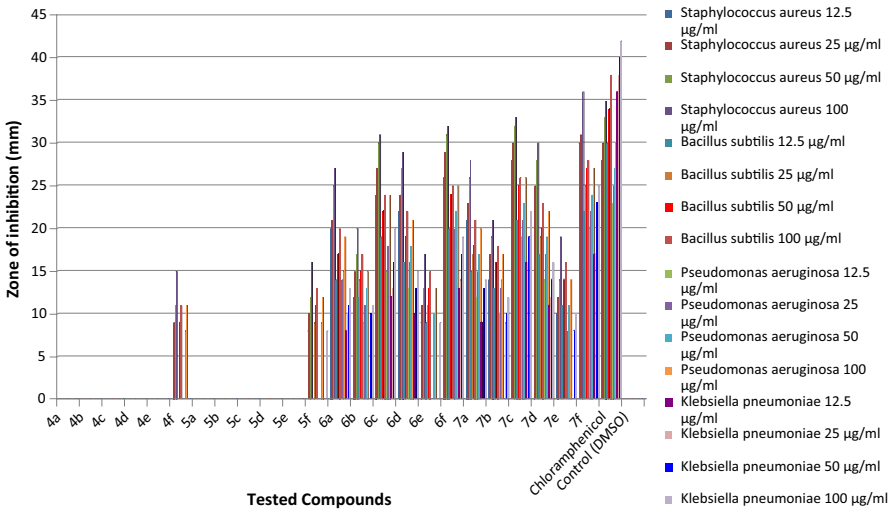


Fig. 1 In vitro antibacterial activity of compounds **4–7**

$$s = \sqrt{\frac{\sum (x - \bar{x})}{N - 1}},$$

where s is the standard deviation, x is each value in the sample, $\bar{x}$ is the mean of the values, and N is the number of values (the sample size).

MIC, MBC, and MFC of compounds 6c, f and 7c, f

The compounds which showed greatest antibacterial and antifungal activities were further assayed for minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC); the values are listed in Table 3. The MIC is the lowest concentration of an antimicrobial that inhibits visible growth of a microorganism (although it is not certain that the microorganism is completely killed). The MBC/MFC is the lowest concentration of antibiotic required to kill a particular bacterium/fungus. The MBC/MFC involves an additional set of steps performed once the minimum inhibitory concentration (MIC) is determined. Antimicrobials are usually regarded as bactericidal/fungicidal if the MBC/MFC is not greater than four times the MIC [25]. Compounds **7c** and **7f** displayed low MIC values when compared with **6c** and **6f**. In fact, the MIC value of **7c** and **7f** against *Staphylococcus aureus* is equal to the standard drug chloramphenicol and the MBC value is 2 × MIC. On the other hand, compound **6f** displayed lower MIC value for *Aspergillus niger* than **6c**, **7c**, and **7f**, equal to the standard ketoconazole, and the MFC value is 2 × MIC. This suggested that chloro- and nitro-substituted thiazolyl isoxazoles **7c** and **7f** are potential antibacterial agents

Table 2 In vitro antifungal activity of compounds **4–7**

Compound	Zone of inhibition (mm)							
	Aspergillus niger				*Penicillium chrysogenum*			
	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well
4a	–	–	–	–	–	–	–	–
4b	–	–	–	–	–	–	–	–
4c	–	–	–	–	–	–	–	–
4d	–	–	–	–	–	–	–	–
4e	–	–	–	–	–	–	–	–
4f	–	9 ± 2	11 ± 1	12 ± 1	–	–	8 ± 2	10 ± 1
5a	–	–	–	–	–	–	–	–
5b	–	–	–	–	–	–	–	–
5c	–	–	–	–	–	–	–	–
5d	–	–	–	–	–	–	–	–
5e	–	–	–	–	–	–	–	–
5f	–	–	10 ± 2	11 ± 2	–	–	–	9 ± 3
6a	17 ± 4	20 ± 1	22 ± 4	25 ± 1	11 ± 4	13 ± 1	14 ± 3	17 ± 4
6b	15 ± 1	17 ± 2	20 ± 1	23 ± 4	9 ± 1	11 ± 2	12 ± 1	14 ± 2
6c	27 ± 3	30 ± 4	31 ± 2	34 ± 2	17 ± 5	19 ± 3	21 ± 2	24 ± 4
6d	21 ± 1	24 ± 3	25 ± 1	27 ± 3	13 ± 1	15 ± 4	17 ± 5	19 ± 3
6e	13 ± 2	15 ± 4	18 ± 2	20 ± 1	–	10 ± 5	11 ± 2	12 ± 1
6f	30 ± 3	33 ± 2	34 ± 1	37 ± 3	18 ± 3	21 ± 2	23 ± 4	26 ± 3
7a	16 ± 2	18 ± 4	21 ± 3	24 ± 2	10 ± 3	12 ± 2	13 ± 2	15 ± 3
7b	14 ± 4	16 ± 1	19 ± 2	21 ± 1	–	11 ± 3	12 ± 4	13 ± 2
7c	22 ± 2	25 ± 3	27 ± 4	29 ± 2	14 ± 4	16 ± 4	18 ± 1	22 ± 2
7d	18 ± 1	22 ± 2	24 ± 1	26 ± 1	12 ± 2	14 ± 2	16 ± 2	18 ± 4
7e	9 ± 5	11 ± 4	14 ± 3	16 ± 4	–	–	10 ± 3	11 ± 1
7f	24 ± 2	27 ± 5	29 ± 2	31 ± 2	16 ± 3	18 ± 5	20 ± 1	23 ± 2
Ketoconazole	29 ± 1	31 ± 6	33 ± 4	36 ± 3	33 ± 3	35 ± 1	36 ± 2	38 ± 3
Control (DMSO)	–	–	–	–	–	–	–	–

(–) No activity. Values are mean ± SD of three replicates

against *Staphylococcus aureus* and nitro-substituted thiazolyl pyrazole **6f** is a potential antifungal agent against *Aspergillus niger*.

Conclusions

A new class of directly linked bis heterocycles, thiazolyl pyrazoles and isoxazoles, were prepared and their antimicrobial activity studied. The chloro- and nitro-substituted thiazolyl isoxazoles (**7c**, **f**) are potential antibacterial agents against

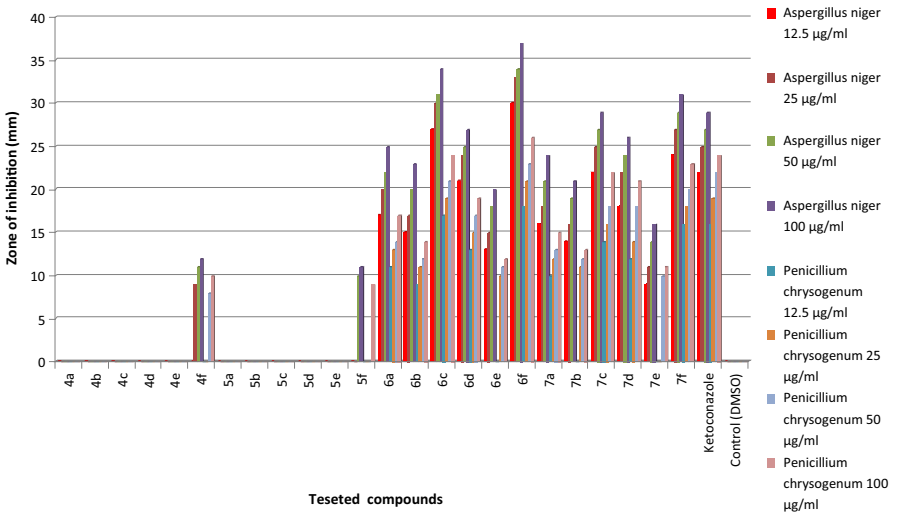


Fig. 2 In vitro antifungal activity of compounds **4**–**7**

Table 3 MIC, MBC, and MFC of compounds **6c**, **f** and **7c**, **f**

Compound	Minimum inhibitory concentration, MIC (MBC/MFC) µg/well					
	Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Aspergillus niger*	*Penicillium chrysogenum*
6c	25 (100)	50 (200)	100 (>200)	200 (–)	12.5 (50)	100 (>200)
6f	25 (100)	50 (200)	50 (200)	200 (–)	6.25 (12.5)	100 (>200)
7c	6.25 (12.5)	50 (200)	100 (>200)	200 (–)	50 (200)	200 (–)
7f	6.25 (12.5)	50 (200)	50 (200)	200 (–)	50 (200)	100 (>200)
Chloramphenicol	6.25	6.25	6.25	12.5	–	–
Ketoconazole	–	–	–	–	6.25	12.5

(–) No activity

Staphylococcus aureus, and nitro-substituted thiazolyl pyrazole (**6f**) is a potential antifungal agent against *Aspergillus niger*.

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