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Synthesis and Antimicrobial Activity of Some New 3,5-Disubstituted Pyrazoles and Isoxazoles

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Abstract

A new class of 3,5-disubstituted pyrazoles and isoxazoles were prepared from the Michael acceptors 1-furanyl / thiophenyl / pyridinyl-3-indole-prop-2-en-1-ones under ultrasonication and evaluated for antimicrobial activity. Amongst all the tested compounds fluoro substituted thiophene linked compounds **12b** and **18b** displayed promising antibacterial activity particularly against *Bacillus subtilis* and antifungal activity against *Aspergillus niger*. Furthermore, compounds with more number of electron withdrawing groups showed higher antimicrobial activity. This result indicates that compounds **12b** and **18b** can be used as lead compounds in the future studies.

Keywords: Pyrazoles; Isoxazoles; Antimicrobial activity

Introduction

The five membered heterocyclic compounds with two heteroatoms particularly pyrazoles and isoxazoles have considerable interest in various fields because of their wide range of pharmacological and physical applications. A number of pyrazole and isoxazole derivatives possess antimicrobial [1-6], anti-inflammatory [7,8], anticancer [9,10], analgesic [11,12], anticonvulsant [13,14], anthelmintic and antioxidant [15,16] activities. Besides, pyrazole containing drugs celecoxib demonstrates anti-inflammation effect and inhibits COX-2 [17] rimonabant functions as cannabinoid receptor and is utilized in obesity treatment [18] fomepizole inhibits alcohol dehydrogenase and sildenafil inhibits phosphodiesterase [19]. Isoxazole motif is ubiquitous in many natural products such as ibotenic acid, muscimol, isoxazole-4-carboxylic acid and drugs like valdecoxib, leflunomide, cloxacillin, oxacillin [20-24], dicloxacillin [25], isocarboxazide [26] and sulfisoxazole [27]. Among the various methods for the synthesis of pyrazoles, 1,3-dipolar cycloaddition and [2+3] cyclocondensation reactions are the prominent ones [28]. Among the different methods of isoxazole synthesis, [2+3] cycloaddition of 1,3-dipoles to alkynes and the reaction of hydroxylamine with 1,3-diketone or an α,β-unsaturated ketones have gained importance [29]. Moreover, the activated olefins are valuable intermediates in a variety of synthetic transformations and useful as building blocks in the synthesis of carbocyclic and heterocyclic compounds. In fact, we have exploited various activated olefins to develop pyrazoles and isoxazoles and studied their biological properties [30-33]. Recently, ultrasound method has been successfully employed as non-conventional method, to promote 1,3-dipolar cycloadditions [34]. Hence, development of pharmacologically active heterocycles adopting simple and efficient methodologies is one of the major challenges for organic chemists. With this background and in continuation of our studies in this direction, the present work synthesis and antimicrobial activity of 3,5-disubstituted pyrazoles and isoxazoles under ultrasonication has been taken up.

Experimental Protocols

All the chemicals were purchased from commercial sources and used without further purification. Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The homogeneity of the compounds was checked by TLC (silica gel H, BDH, hexane/ethyl acetate, 3:1). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. The ^1H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM λ-400 MHz spectrometer. The ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM spectrometer operating at λ-100 MHz. High-resolution mass spectra were recorded on Micromass Q-TOF micromass spectrometer using electrospray ionization. All chemical shifts were reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The temperature was measured by flexible probe throughout the reaction. Ultrasonication was performed in a Bandelin Sonorex RK 102H ultrasonic bath operating at frequency of 35 KHz.

General procedure for the synthesis of (E)-1-(furan-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one (5a, b) / (E)-1-(5-bromothiophen-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one (6a, b) / (E)-3-(1H-indol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (7a, b)

To a solution of substituted indole-3-carboxaldehyde (1) (1.0 mmol) in anhydrous methanol (4 mL), 2-acetyl furan (2) / 2-acetyl-5-bromothiophene (3)/4-acetyl pyridine (4) (1.0 mmol) were added followed by diisopropylethylamine (DIPEA) (0.33 mmol) and subjected to ultrasonication at a frequency of 35 KHz at room temperature for 60-80 min. After completion of reaction (monitored by TLC), the contents of the flask were allowed to cool and poured into ice water. It was neutralized with 10% acetic acid. The separated solid was filtered, dried and recrystallized from 2-propanol.

(E)-1-(Furan-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one (5a): M.p. 190-191°C, yield 87%; IR (KBr) (cm^{-1}): 3329 (NH), 1683 (C=O), 1632 (C=C); 1H NMR (400 MHz, DMSO-d_6): δ 6.93 (d, 1H, HB, J=15.4 Hz), 7.54-7.90 (m, 8H, Ar-H) 8.02 (d, 1H, HA, J=15.4 Hz), 11.74 (s, 1H, NH) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 133.9 (C-HB), 137.1 (C-HA), 176.4 (C=O), 111.3, 112.9, 113.1, 113.8, 115.3, 118.6, 120.8, 125.2, 125.6, 134.4, 147.8, 152.4 ppm (aromatic carbons). HRMS (m/z): 260.0687 [M+Na]; Anal. calcd. for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90%. Found: C, 76.05; H, 4.70; N, 6.02%.

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(E)-3-(5-Fluoro-1H-indol-3-yl)-1-(furan-2-yl)prop-2-en-1-one (5b): M.p. 163-165°C, yield 89%; IR (KBr) (cm^{-1}): 3322 (NH), 1675 (C=O), 1634 (C=C); 1H NMR (400 MHz, DMSO-d_6): δ 6.76 (d, 1H, HB, J=15.2 Hz), 7.63-7.65 (m, 7H, Ar-H) 8.13 (d, 1H, HA, J=15.2 Hz), 11.98 (s, 1H, NH) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 134.0 (C-HB), 137.5 (C-HA), 176.9 (C=O), 110.6, 112.7, 113.5, 113.4, 115.4, 117.7, 125.3, 125.4, 134.6, 147.3, 153.3, 157.0 ppm (aromatic carbons). HRMS (m/z): 278.0593 [M+Na]; Anal. calcd. for $C_{15}H_{10}FNO_2$: C, 70.58; H, 3.95; N, 5.49%. Found: C, 70.66; H, 3.97; N, 5.64%.

(E)-1-(5-Bromothiophen-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one (6a): M.p. 202-204°C, yield 90%; IR (KBr) (cm^{-1}): 3326 (NH), 1672 (C=O), 1628 (C=C); 1H NMR (400 MHz, DMSO-d6): δ 7.21 (d, 1H, HB, J=15.6 Hz), 7.65-7.88 (m, 7H, Ar-H) 8.15 (d, 1H, HA, J=15.6 Hz), 11.94 (s, 1H, NH) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 125.0 (C-HB), 138.8 (C-HA), 180.4 (C=O), 112.4, 112.6, 113.8, 120.6, 121.2, 122.6, 122.7, 132.2, 132.6, 133.8, 137.5, 148.1 ppm (aromatic carbons). HRMS (m/z): 353.9569 [M+Na]; Anal. calcd. for $C_{15}H_{10}BrNOS$: C, 54.23; H, 3.03; N, 4.22%. Found: C, 54.33; H, 3.08; N, 4.39%.

(E)-1-(5-Bromothiophen-2-yl)-3-(5-fluoro-1H-indol-3-yl)prop-2-en-1-one (6b): M.p. 177-179°C, yield 92%; IR (KBr) (cm^{-1}): 3318 (NH), 1676 (C=O), 1631 (C=C); 1H NMR (400 MHz, DMSO-d_6): δ 7.04 (d, 1H, HB, J=15.8 Hz), 7.52-7.83 (m, 6H, Ar-H) 8.02 (d, 1H, HA, J=15.8 Hz), 12.02 (s, 1H, NH) ppm; 13C NMR (100 MHz, DMSO-d6): δ 126.3 (C-HB), 138.4 (C-HA), 180.7 (C=O), 112.7, 113.3, 114.0, 121.8, 122.3, 122.9, 124.1, 132.7, 137.3, 140.1, 148.3, 157.6 ppm (aromatic carbons). HRMS (m/z): 371.9470 [M+Na]; Anal. calcd. for $C_{15}H_9BrFNOS$: C, 51.45; H, 2.59; N, 4.00%. Found: C, 51.57; H, 2.63; N, 4.16%.

(E)-3-(1H-Indol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (7a): M.p. 209-211°C, yield 88%; IR (KBr) (cm^{-1}): 3336 (NH), 1688 (C=O), 1638 (C=C); 1H NMR (400 MHz, DMSO-d_6): δ 7.23 (d, 1H, HB, J=15.9 Hz), 7.61-7.92 (m, 9H, Ar-H) 8.17 (d, 1H, HA, J=15.9 Hz), 12.01 (s, 1H, NH) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 137.6 (C-HB), 144.2 (C-HA), 188.2 (C=O), 110.2, 112.5, 112.8, 114.6, 120.6, 121.4, 122.9, 125.0, 134.5, 140.9, 151.6 ppm (aromatic carbons). HRMS (m/z): 271.0847 [M+Na]; Anal. calcd. for $C_{16}H_{12}N_2O$: C, 77.40; H, 4.87; N, 11.28%. Found: C, 77.49; H, 4.89; N, 11.39%.

(E)-3-(5-Fluoro-1H-indol-3-yl)-1-(pyridin-4-yl)prop-2-en-1-one (7b): M.p. 186-188°C, yield 91%; IR (KBr) (cm^{-1}): 3330 (NH), 1686 (C=O), 1633 (C=C); 1H NMR (400 MHz, DMSO-d_6): δ 7.08 (d, 1H, HB, J=15.7 Hz), 7.59-7.86 (m, 8H, Ar-H) 8.23 (d, 1H, HA, J=15.7 Hz), 12.09 (s, 1H, NH) ppm; 13C NMR (100 MHz, DMSO-d6): δ 134.1 (C-HB), 144.5 (C-HA), 188.4 (C=O), 110.8, 112.9, 114.5, 114.8, 121.5, 125.4, 125.5, 135.8, 140.3, 150.6, 157.2 ppm (aromatic carbons). HRMS (m/z): 289.0753 [M+Na]; Anal. calcd. for $C_{16}H_{11}FN_2O$: C, 72.17; H, 4.16; N, 10.52%. Found: C, 72.30; H, 4.20; N, 10.66%.

General procedure for the synthesis of 3-(furan-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (8a, b) / 3-(5-bromothiophen-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (9a, b) / 3-(pyridin-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (10a, b)

A mixture of 5/6/7 (1 mmol), hydrazine hydrate (1.5 mmol) and ethanol (6 mL) was subjected to ultrasonication at a frequency of 35 KHz at room temperature for 70-90 min. After completion of the reaction (monitored by TLC), the contents of the flask were poured onto crushed ice. The separated residue was extracted with dichloromethane. The organic layer was washed with water, brine and dried (an. Na_2SO_4). The solvent was removed under vacuum. The resultant residue was purified by column chromatography (silica gel, 60-120 mesh) using hexane/ethyl acetate (4:1) as eluent.

3-(Furan-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (8a): M.p. 208-210°C, yield 80%; IR (KBr) (cm-1): 3338 (NH), 1640 (C=C), 1578 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.24 (dd, 1H, HX, JAX=6.5 Hz, JMX=10.7 Hz), 4.28 (dd, 1H, HM, JAM=12.5 Hz, JMX=10.9 Hz), 4.43 (dd, 1H, HA, JAM=12.5 Hz, JAX=6.5 Hz), 6.69-7.73 (m, 8H, Ar-H), 7.85 (bs, 1H, NH-pyrazoline), 10.21 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 41.6 (C-4'), 44.9 (C-5'), 143.6 (C-3'), 110.2, 110.6, 113.1, 118.3, 120.5, 122.8, 126.4, 129.6, 131.0, 136.2, 142.4, 150.7 (aromatic carbons). HRMS (m/z): 274.0956 [M+Na]; Anal. calcd. for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72%. Found: C, 71.83; H, 5.24; N, 16.92%.

3-(Furan-2-yl)-5-(5-fluoro-5-1H-indol-3-yl)-2-pyrazoline (8b): M.p. 178-180°C, yield 82%; IR (KBr) (cm^{-1}): 3320 (NH), 1636 (C=C), 1577 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.32 (dd, 1H, HX, JAX=6.8 Hz, JMX=10.9 Hz), 4.13 (dd, 1H, HM, JAM=12.8 Hz, JMX=10.9 Hz), 4.46 (dd, 1H, HA, JAM=12.8 Hz, JAX=6.8 Hz), 6.73-7.78 (m, 7H, Ar-H), 7.88 (bs, 1H, NH-pyrazoline), 10.32 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 41.4 (C-4'), 44.6 (C-5'), 143.9 (C-3'), 113.2, 113.8, 117.4, 119.3, 123.6, 127.1, 129.2, 131.6, 136.8, 143.4, 150.6, 151.3 (aromatic carbons); HRMS (m/z): 292.0862 [M+Na]; Anal. calcd. for $C_{15}H_{12}FN_3O$: C66.91; H, 4.49; N, 15.60%. Found: C, 67.02; H, 4.50; N, 15.78%.

3-(5-Bromothiophen-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (9a): M.p. 212-214°C, yield 84%; IR (KBr) (cm^{-1}): 3328 (NH), 1639 (C=C), 1584 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.34 (dd, 1H, HX, JAX=6.3 Hz, JMX=10.2 Hz), 4.19 (dd, 1H, HM, JAM=12.4 Hz, JMX=10.2 Hz), 4.66 (dd, 1H, HA, JAM=12.4 Hz, JAX=6.3 Hz), 6.92-7.68 (m, 7H, Ar-H), 7.82 (bs, 1H, NH-pyrazoline), 10.56 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 41.7 (C-4'), 45.1 (C-5'), 139.4 (C-3'), 111.3, 112.5, 118.4, 118.7, 120.7, 121.9, 125.3, 127.2, 128.7, 132.3, 134.1, 151.8 (aromatic carbons); HRMS (m/z): 367.9842 [M+Na]; Anal. calcd. for $C_{15}H_{12}BrN_3S$: C, 52.03; H, 3.49; N, 12.14%. Found: C, 52.13; H, 3.50; N, 12.36%.

3-(5-Bromothiophen-2-yl)-5-(5-fluoro-1H-indol-3-yl)-2-pyrazoline (9b): M.p. 181-183°C, yield 82%; IR (KBr) (cm^{-1}): 3324 (NH), 1642 (C=C), 1577 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.38 (dd, 1H, HX, JAX=6.5 Hz, JMX=10.3 Hz), 4.23 (dd, 1H, HM, JAM=12.3 Hz, JMX=10.3 Hz), 4.68 (dd, 1H, HA, JAM=12.3 Hz, JAX=6.5 Hz), 7.01-7.82 (m, 6H, Ar-H), 7.89 (bs, 1H, NH-pyrazoline), 10.62 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 42.1 (C-4'), 45.4 (C-5'), 138.9 (C-3'), 117.6, 120.4, 111.8, 113.2, 125.4, 126.7, 128.3, 132.1, 136.4, 140.8, 151.2, 152.4 (aromatic carbons); HRMS (m/z): 385.9739 [M+Na]; Anal. calcd. for $C_{15}H_{11}BrFN_3S$: C, 49.46; H, 3.04; N, 11.54%. Found: C, 49.58; H, 3.06; N, 11.79%.

3-(Pyridin-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (10a): M.p. 227-229°C, yield 83%; IR (KBr) (cm^{-1}): 3327 (NH), 1646 (C=C), 1589 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.43 (dd, 1H, HX, JAX=5.5 Hz, JMX=10.7 Hz), 4.10 (dd, 1H, HM, JAM=11.8 Hz, JMX=10.7 Hz), 4.64 (dd, 1H, HA, JAM=11.8 Hz, JAX=5.5 Hz), 7.14-7.88 (m, 9H, Ar-H), 7.91 (bs, 1H, NH-pyrazoline), 11.63 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 42.6 (C-4'), 45.6 (C-5'), 142.9 (C-3'), 121.2, 137.5, 113.1, 117.3, 123.5, 124.7, 125.1, 127.3, 128.6, 130.4, 151.6 (aromatic carbons); HRMS (m/z): 285.1116 [M+Na]; Anal. calcd. for $C_{16}H_{14}N_4$: C, 73.26; H, 5.38; N, 21.36%. Found: C, 73.20; H, 5.36; N, 21.28%.

3-(Pyridin-2-yl)-5-(5-fluoro-1H-indol-3-yl)-2-pyrazoline (10b): M.p. 201-203°C, yield 79%; IR (KBr) (cm^{-1}): 3341 (NH), 1643 (C=C), 1596 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.45 (dd, 1H, HX, JAX=5.7 Hz, JMX=10.2 Hz), 4.18 (dd, 1H, HM, JAM=11.6 Hz,

JMX=10.2 Hz), 4.67 (dd, 1H, HA, JAM=11.6 Hz, JAX=5.7 Hz), 7.23-7.90 (m, 8H, Ar-H), 7.94 (bs, 1H, NH-pyrazoline), 11.76 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 41.5 (C-4'), 45.2 (C-5'), 142.3 (C-3'), 121.4, 138.7, 113.4, 117.8, 121.4, 123.7, 127.5, 128.2, 129.9, 151.3, 152.7 (aromatic carbons); HRMS (m/z): 303.1022 [M+Na]; Anal. calcd. for $C_{16}H_{13}FN_4$: C, 68.56; H, 4.67; N, 19.99%. Found: C, 68.66; H, 4.70; N, 20.18%.

General procedure for the synthesis of 3-(furan-2-yl)-5-(1H-indol-3-yl)-2-isoxazoline (14a, b) / 3-(5-bromothiophen-2-yl)-5-(1H-indol-3-yl)-2-isoxazoline (15a, b) / 3-(pyridin-2-yl)-5-(1H-indol-3-yl)-2-isoxazoline (16a, b)

A solution of 5/6/7 (1 mmol), hydroxylamine hydrochloride (1.1 mmol) in ethanol (6 mL) was kept under ultrasonication at room temperature for 60-70 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the contents of the flask were poured onto crushed ice. It was extracted with dichloromethane. The organic layer was washed with water, brine and dried (an. Na_2SO_4). The solvent was removed under reduced pressure. The resultant residue was purified by column chromatography (silica gel, 60-120 mesh) using hexane/ethyl acetate (4:1) as eluent.

3-(Furan-2-yl)-5-(1H-indol-3-yl)-2-isoxazoline (14a): M.p. 168-170°C, Yield 79%; IR (KBr) (cm-1): 3335 (NH), 1644 (C=C), 1588 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.26 (dd, 1H, HX, JAX=6.6 Hz, JMX=10.5 Hz), 4.52 (dd, 1H, HM, JAM=12.6 Hz, JMX=10.5 Hz), 4.98 (dd, 1H, HA, JAM=12.6 Hz, JAX=6.8 Hz), 6.88-7.87 (m, 8H, Ar-H), 10.48 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 42.5 (C-4'), 45.8 (C-5'), 141.2 (C-3'), 113.8, 117.2, 119.7, 120.8, 122.8, 127.9, 129.6, 134.5, 138.1, 140.3, 151.6, 156.4 (aromatic carbons); HRMS (m/z): 275.0796 [M+Na]; Anal. calcd. for $C_{15}H_{12}N_2O_2$: C, 71.42; H, 4.79; N, 11.10%. Found: C, 71.51; H, 4.78; N, 11.26%.

3-(Furan-2-yl)-5-(5-fluoro-1H-indol-3-yl)-2-isoxazoline (14b): M.p. 182-184°C, yield 81%; IR (KBr) (cm-1): 3343 (NH), 1635 (C=C), 1579 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.30 (dd, 1H, HX, JAX=6.7 Hz, JMX=10.8 Hz), 4.56 (dd, 1H, HM, JAM=12.7 Hz, JMX=10.8 Hz), 5.08 (dd, 1H, HA, JAM=12.7 Hz, JAX=6.7 Hz), 6.91-7.89 (m, 7H, Ar-H), 10.53 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 43.2 (C-4'), 45.3 (C-5'), 141.6 (C-3'), 113.9, 114.2, 119.4, 127.7, 129.3, 134.5, 138.4, 138.8, 151.6, 140.6, 151.5, 158.1 (aromatic carbons); HRMS (m/z): 293.0708 [M+Na]; Anal. calcd. for $C_{15}H_{11}FN_2O$: C, 66.66; H, 4.10; N, 10.37%. Found: C, 66.61; H, 4.12; N, 10.40%.

3-(5-Bromothiophen-2-yl)-5-(1H-indol-3-yl)-2-isoxazoline (15a): M.p. 217-218°C, yield 78%; IR (KBr) (cm-1): 3332 (NH), 1637 (C=C), 1587 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.41 (dd, 1H, HX, JAX=5.8 Hz, JMX=10.6 Hz), 4.57 (dd, 1H, HM, JAM=12.4 Hz, JMX=10.6 Hz), 5.12 (dd, 1H, HA, JAM=12.4 Hz, JAX=5.8 Hz), 7.12-8.09 (m, 7H, Ar-H), 10.71 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 41.8 (C-4'), 45.7 (C-5'), 140.2 (C-3'), 114.2, 114.6, 117.3, 120.2, 121.8, 123.5, 125.7, 127.0, 128.5, 130.6, 139.3, 151.8 (aromatic carbons); HRMS (m/z): 368.9681 [M+Na]; Anal. calcd. for $C_{15}H_{11}BrN_2OS$: C, 51.89; H, 3.19; N, 8.07%. Found: C, 51.97; H, 3.18; N, 8.25%.

3-(5-Bromothiophen-2-yl)-5-(5-fluoro-1H-indol-3-yl)-2-isoxazoline (15b): M.p. 187-189°C, yield 80%; IR (KBr) (cm-1): 3336 (NH), 1632 (C=C), 1584 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.47 (dd, 1H, HX, JAX=5.8 Hz, JMX=10.6 Hz), 4.57 (dd, 1H, HM, JAM=12.4 Hz, JMX=10.6 Hz), 5.12 (dd, 1H, HA, JAM=12.4 Hz, JAX=5.8 Hz), 7.12-8.09 (m, 6H, Ar-H), 10.78 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 41.4 (C-4'), 45.9 (C-5'), 140.7 (C-3'), 117.6, 117.9, 117.8, 118.3, 120.9, 123.8, 128.0, 129.1, 136.6, 139.5, 152.0, 152.3 (aromatic carbons); HRMS (m/z): 386.9579 [M+Na]; Anal. calcd. for $C_{15}H_{10}BrFN_2OS$: C, 49.33; H, 2.76; N, 7.67%. Found: C, 49.44; H, 2.79; N, 7.90%.

3-(Pyridin-2-yl)-5-(1H-indol-3-yl)-2-isoxazoline (16a): M.p. 216-218°C, yield 86%; IR (KBr) (cm-1): 3338 (NH), 1631 (C=C), 1585 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.52 (dd, 1H, HX, JAX=6.2 Hz, JMX=10.8 Hz), 4.62 (dd, 1H, HM, JAM=12.2 Hz, JMX=10.8 Hz), 5.20 (dd, 1H, HA, JAM=12.2 Hz, JAX=6.2 Hz), 7.26-8.24 (m, 9H, Ar-H), 11.96 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 42.4 (C-4'), 45.8 (C-5'), 140.1 (C-3'), 123.3, 136.9, 113.7, 117.1, 121.5, 124.9, 126.4, 127.7, 129.0, 140.3, 151.5 (aromatic carbons); HRMS (m/z): 286.0956 [M+Na]; Anal. calcd. for $C_{16}H_{13}N_3O$: C, 72.99; H, 4.98; N, 15.96%. Found: C, 73.11; H, 4.96; N, 16.22%.

3-(Pyridin-2-yl)-5-(5-fluoro-1H-indol-3-yl)-2-isoxazoline (16b): M.p. 186-188°C, yield 82%; IR (KBr) (cm-1): 3335 (NH), 1633 (C=C), 1582 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 3.56 (dd, 1H, HX, JAX=6.4 Hz, JMX=10.5 Hz), 4.65 (dd, 1H, HM, JAM=12.2 Hz, JMX=10.5 Hz), 5.23 (dd, 1H, HA, JAM=12.2 Hz, JAX=6.4 Hz), 7.31-8.27 (m, 8H, Ar-H), 11.98 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 42.7 (C-4'), 45.5 (C-5'), 141.6 (C-3'), 123.9, 138.5, 117.7, 121.0, 124.7, 126.8, 127.6, 128.8, 129.2, 151.9, 152.3 (aromatic carbons); HRMS (m/z): 304.0862 [M+Na]; Anal. calcd. for $C_{16}H_{12}FN_3O$: C, 68.32; H, 4.30; N, 14.94%. Found: C, 68.41; H, 4.32; N, 15.14%.

General procedure for the synthesis of 3,5-diaryl pyrazoles (11 a, b / 12 a, b / 13 a, b) and 3,5-diaryl isoxazoles (17 a, b / 18 a, b / 19 a, b)

A solution of 8 / 9 / 10 / 14 / 15/ 16 (1 mmol) in xylene (7 mL) and chloranil (1.2 mmol) were subjected to ultrasonication at reflux temperature for 1-2 hrs. Then, it was treated with 5% NaOH solution. The organic layer was separated and repeatedly washed with water and dried (an. Na_2SO_4). The solvent was removed in vaccuo. The solid obtained was purified by recrystallization from 2-propanol.

3-(Furan-2-yl)-5-(1H-indol-3-yl)pyrazole (11a): M.p. 212-214°C, yield 73%; IR (KBr) (cm^{-1}): 3342 (NH), 1638 (C=C), 1575 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 6.83-7.72 (m, 9H, Ar-H), 7.63 (bs, 1H, NH-pyrazole), 10.46 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 108.3 (C-4'), 131.0 (C-5'), 132.5 (C-3'), 109.8, 110.6, 111.4, 112.8, 113.2, 118.7, 120.9, 125.3, 125.7, 134.6, 141.1, 152.5 (aromatic carbons); HRMS (m/z): 272.0800 [M+Na]; Anal. calcd. for $C_{15}H_{11}N_3O$: C, 72.28; H, 4.45; N, 16.86%. Found: C, 72.22; H, 4.48; N, 17.02%.

3-(Furan-2-yl)-5-(5-fluoro-1H-indol-3-yl)pyrazole (11b): M.p. 183-185°C, yield 75%; IR (KBr) (cm^{-1}): 3337 (NH), 1648 (C=C), 1598 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 6.94-7.67 (m, 8H, Ar-H), 7.68 (bs, 1H, NH-pyrazole), 10.53 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 108.1 (C-4'), 131.3 (C-5'), 132.7 (C-3'), 110.4, 110.7, 110.9, 113.3, 113.6, 117.8, 124.3, 125.5, 134.8, 141.6, 153.1, 157.4 (aromatic carbons); HRMS (m/z): 290.0706 [M+Na]; Anal. calcd. for $C_{15}H_{10}FN_3O$: C, 67.41; H, 3.77; N, 15.72%. Found: C, 67.49; H, 3.78; N, 15.91%.

3-(5-Bromothiophen-2-yl)-5-(1H-indol-3-yl)pyrazole (12a): M.p. 217-219°C, yield 73%; IR (KBr) (cm^{-1}): 3342 (NH), 1647 (C=C), 1594 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 7.32-7.71 (m, 8H, Ar-H), 7.84 (bs, 1H, NH-pyrazole), 10.84 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 109.1 (C-4'), 130.6 (C-5'), 133.5 (C-3'), 111.0, 112.2, 112.8, 114.2, 120.5, 121.9, 122.5, 124.3, 132.4, 136.9, 142.4,

153.4 (aromatic carbons); HRMS (m/z): 365.9681 [M+Na]; Anal. calcd. for $C_{15}H_{10}BrN_3S$: C, 52.34; H, 2.93; N, 12.21%. Found: C, 52.44; H, 2.92; N, 12.45%.

3-(5-Bromothiophen-2-yl)-5-(5-fluoro-1H-indol-3-yl)pyrazole (12b): M.p. 184-186°C, yield 70%; IR (KBr) (cm^{-1}): 3346 (NH), 1640 (C=C), 1583 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 7.41-7.78 (m, 7H, Ar-H), 7.79 (bs, 1H, NH-pyrazole), 10.86 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 108.6 (C-4'), 131.8 (C-5'), 133.3 (C-3'), 110.2, 111.7, 112.4, 113.8, 114.6, 122.2, 123.1, 124.5, 135.3, 142.7, 153.8, 157.6 (aromatic carbons); HRMS (m/z): 383.9582 [M+Na]; Anal. calcd. for $C_{15}H_9BrFN_3S$: C, 49.74; H, 2.50; N, 11.60%. Found: C, 49.82; H, 2.52; N, 11.79%.

3-(Pyridin-2-yl)-5-(1H-indol-3-yl)pyrazole (13a): M.p. 220-222°C, yield 76%; IR (KBr) (cm^{-1}): 3344 (NH), 1649 (C=C), 1596 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 7.20-8.03 (m, 10H, Ar-H), 7.84 (bs, 1H, NH-pyrazole), 11.21 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 109.2 (C-4'), 131.4 (C-5'), 133.2 (C-3'), 110.3, 112.7, 111.6, 114.3, 120.1, 121.4, 122.2, 125.1, 134.6, 139.1, 148.4 (aromatic carbons); HRMS (m/z): 283.0960 [M+Na]; Anal. calcd. for $C_{16}H_{12}N_4$: C, 73.83; H, 4.65; N, 21.52%. Found: C, 73.92; H, 4.68; N, 21.73%.

3-(Pyridin-2-yl)-5-(5-fluoro-1H-indol-3-yl)pyrazole (13b): M.p. 208-210°C; yield 73%; IR (KBr) (cm-1): 3348 (NH), 1656 (C=C), 1591 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 7.25-8.09 (m, 9H, Ar-H), 7.87 (bs, 1H, NH-pyrazole), 11.28 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 109.5 (C-4'), 131.9 (C-5'), 133.5 (C-3'), 110.9, 112.4, 114.6, 120.6, 122.7, 125.3, 125.5, 135.7, 139.4, 148.9, 157.3 (aromatic carbons); HRMS (m/z): 301.0865 [M+Na]; Anal. calcd. for $C_{16}H_{11}FN_4$: C, 69.06; H, 3.98; N, 20.13%. Found: C, 69.17; H, 4.01; N, 20.36%.

3-(Furan-2-yl)-5-(1H-indol-3-yl)isoxazole (17a): M.p. 174-176°C, yield 79%; IR (KBr) (cm^{-1}): 3334 (NH), 1644 (C=C), 1588 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 6.91-7.69 (m, 9H, Ar-H), 10.72 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 108.5 (C-4'), 131.6 (C-5'), 132.3 (C-3'), 110.2, 111.4, 112.5, 112.7, 113.9, 120.5, 120.3, 121.1, 132.4, 133.9, 141.2, 152.7, (aromatic carbons); HRMS (m/z): 273.0649 [M+Na]; Anal. calcd. for $C_{15}H_{10}N_2O_2$: C, 71.99; H, 4.03; N, 11.19%. Found: C, 72.12; H, 4.08; N, 11.47%.

3-(Furan-2-yl)-5-(5-fluoro-1H-indol-3-yl)isoxazole (17b): M.p. 187-188°C, yield 77%; IR (KBr) (cm^{-1}): 3338 (NH), 1639 (C=C), 1585 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 7.01-7.72 (m, 8H, Ar-H), 10.78 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 108.9 (C-4'), 132.0 (C-5'), 132.9 (C-3'), 109.8, 110.5, 111.7, 113.4, 113.9, 118.1, 125.6, 126.4, 135.2, 141.9, 153.5, 157.2 (aromatic carbons); HRMS (m/z): 291.0546 [M+Na]; Anal. calcd. for $C_{15}H_9FN_2O_2$: C, 67.16; H, 3.38; N, 10.44%. Found: C, 67.27; H, 3.41; N, 10.66%.

3-(5-Bromothiophen-2-yl)-5-(1H-indol-3-yl)isoxazole (18a): M.p. 221-223°C, yield 74%; IR (KBr) (cm-1): 3341 (NH), 1655 (C=C), 1592 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 7.21-7.85 (m, 8H, Ar-H), 10.89 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 108.7 (C-4'), 132.1 (C-5'), 133.4 (C-3'), 110.5, 111.9, 112.3, 113.8, 118.4, 121.1, 122.8, 126.3, 132.4, 136.0, 143.2, 154.0 (aromatic carbons); HRMS (m/z): 366.9517 [M+Na]; Anal. calcd. for $C_{15}H_9BrN_2OS$: C, 52.19; H, 2.63; N, 8.11%. Found: C, 52.31; H, 2.67; N, 8.36%.

3-(5-Bromothiophen-2-yl)-5-(5-fluoro-1H-indol-3-yl)isoxazole (18b): M.p. 204-206°C, yield 78%; IR (KBr) (cm^{-1}): 3339 (NH), 1651 (C=C), 1589 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 7.29-7.87 (m, 7H, Ar-H), 10.94 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 109.2 (C-4'), 132.7 (C-5'), 133.8 (C-3'), 111.2, 112.0, 112.7, 114.2, 118.7, 121.8, 123.1, 127.3, 136.3, 143.6, 154.5, 157.9 (aromatic carbons); HRMS (m/z): 384.9422 [M+Na]; Anal. calcd. for $C_{15}H_8BrFN_2OS$: C, 49.60; H, 2.22; N, 7.71%. Found: C, 49.53; H, 2.23; N, 7.86%.

3-(Pyridin-2-yl)-5-(1H-indol-3-yl)isoxazole (19a): M.p. 224-226°C; yield 71%; IR (KBr) (cm^{-1}): 3343 (NH), 1657 (C=C), 1595 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 7.32-8.14 (m, 10H, Ar-H), 11.34 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 109.6 (C-4'), 132.3 (C-5'), 133.6 (C-3'), 110.4, 111.6, 111.9, 114.5, 120.7, 121.8, 122.5, 125.3, 134.8, 139.2, 149.4 (aromatic carbons); HRMS (m/z): 284.0800 [M+Na]; Anal. calcd. for $C_{16}H_{11}N_3O$: C, 73.55; H, 4.24; N, 16.08%. Found: C, 73.64; H, 4.26; N, 16.26%.

3-(Pyridin-2-yl)-5-(5-fluoro-1H-indol-3-yl)isoxazole (19b): M.p. 198-200°C, yield 69%; IR (KBr) (cm-1): 3345 (NH), 1653 (C=C), 1598 (C=N); 1H NMR (400 MHz, DMSO-d_6): δ 7.36-8.17 (m, 9H, Ar-H), 11.37 (bs, 1H, NH-indole) ppm; 13C NMR (100 MHz, DMSO-d_6): δ 109.8 (C-4'), 132.5 (C-5'), 133.9 (C-3'), 111.2, 113.1, 114.8, 121.4, 122.9, 125.8, 126.0, 136.3, 139.8, 149.2, 157.6 (aromatic carbons); HRMS (m/z): 302.0706 [M+Na]; Anal. calcd. for $C_{16}H_{10}FN_3O$: C, 68.81; H, 3.61; N, 15.05%. Found: C, 68.74; H, 3.60; N, 15.21%.

Antimicrobial Testing

The compounds **8-19** were dissolved in DMSO at different concentrations of 50 and 100 µg/well.

Cells

Bacterial strains *S. aureus*, *B. subtilis*, *P. aeruginosa*, *K. pneumoniae* and fungi *A. niger*, *P. chrysogenum* were obtained from Department of Applied Microbiology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati.

Antibacterial and antifungal assays

The *in vitro* antimicrobial studies were carried out by agar well diffusion method against test organisms [35,36]. Nutrient broth (NB) plates were swabbed with 24 h old broth culture (100 µl) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each petriplate. Various concentrations of DMSO dissolved compounds (50, 100 µg/well) were added into the wells by using sterile pipettes. Simultaneously the standard antibiotics, Chloramphenicol for antibacterial activity and Ketoconazole for antifungal activity (as positive control) were tested against the pathogens. The samples were dissolved in DMSO which showed no zone of inhibition 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. Duplicates were maintained and the average values were calculated for eventual antibacterial activity.

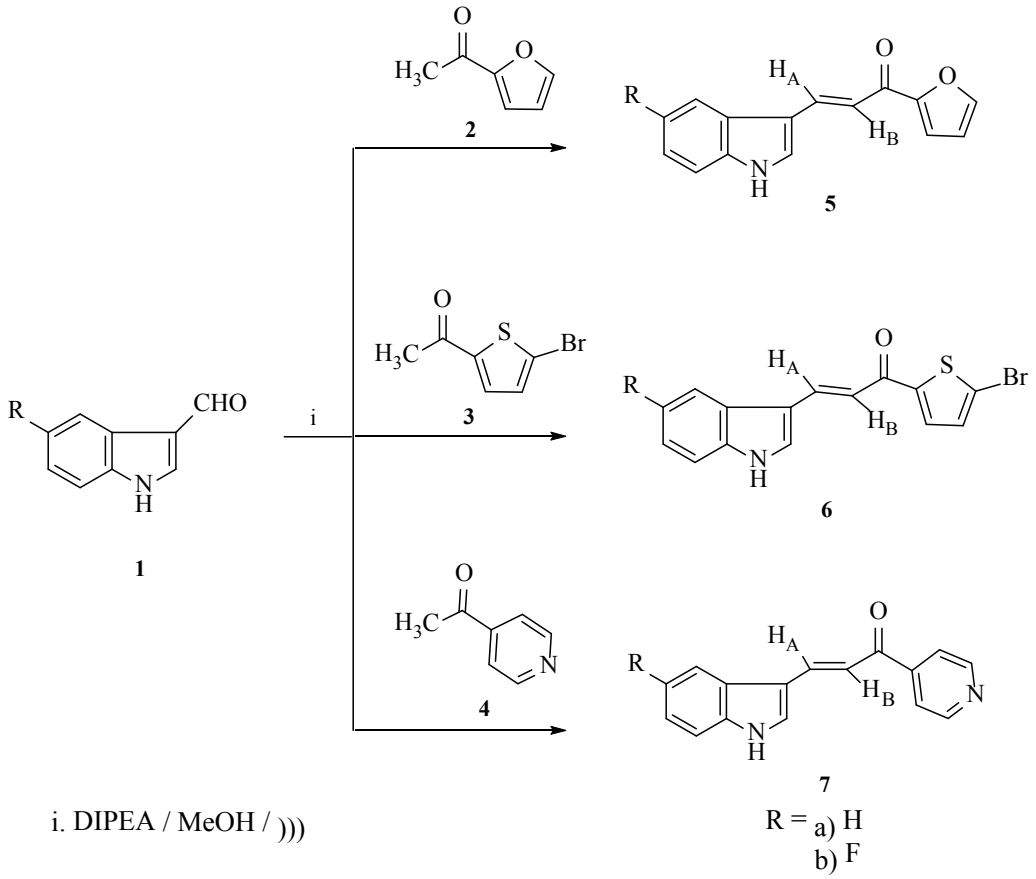
Broth dilution test was used to determine minimum inhibitory concentration (MIC) of the above mentioned samples [37,38]. Freshly prepared nutrient broth was used as diluents. The 24 h old culture of the test bacteria *S. aureus*, *B. subtilis*, *P. aeruginosa*, *K. pneumoniae* and fungi *A. niger*, *P. chrysogenum* were diluted 100 folds in nutrient broth (100 µl bacterial cultures in 10 ml NB). Increasing concentrations of the test samples (1.25, 2.5, 5, 10, 20, 40 µl of stock solution contains 6.25, 12.5, 25, 50, 100, 200 µg/well of the compounds) were added to the test tubes containing the bacterial and fungal cultures. All the 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 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 MIC. To determine the minimum bactericidal concentration (MBC) [39] and minimum fungicidal concentration (MFC) [40] 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 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 was noted as MBC (for bacteria) or MFC (for fungi) at which no visible growth was observed.

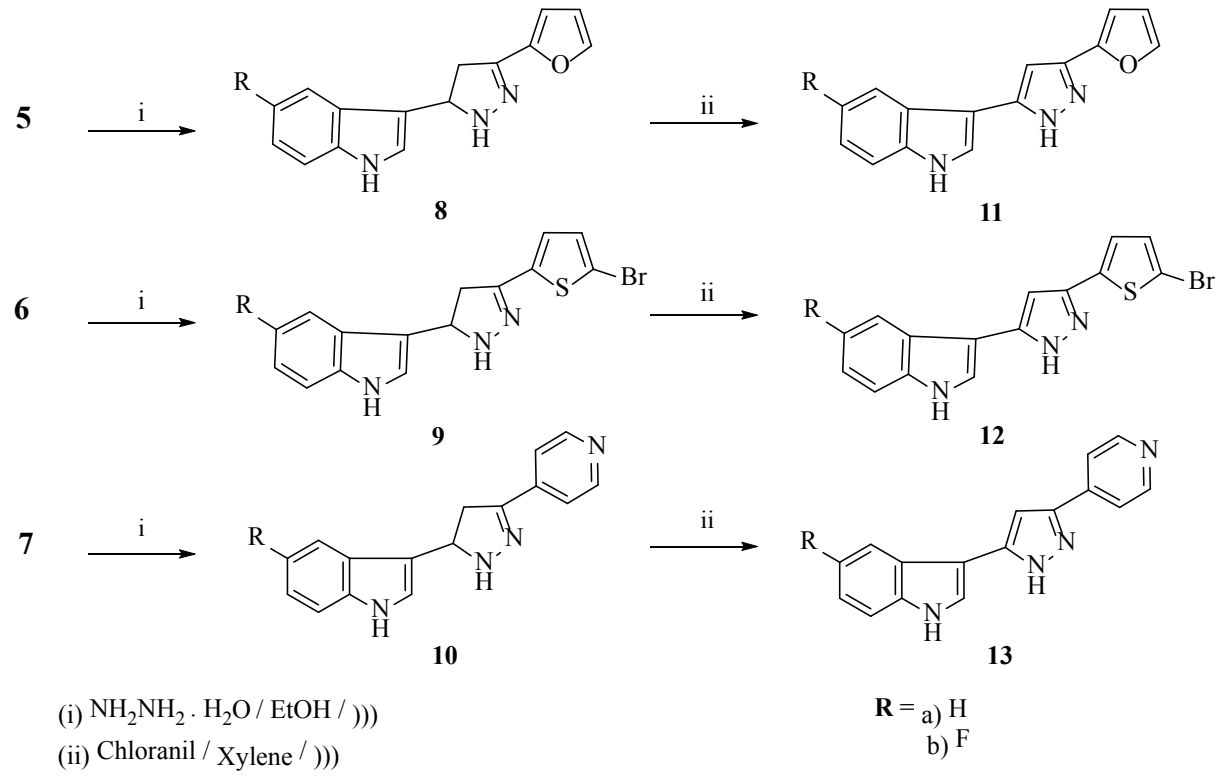
Results and Discussion

Chemistry

The Michael acceptors (E)-1-(furan-2-yl)-3-(1H-indol-3-yl) prop-2-en-1-one (**5**), (E)-1-(5-bromothiophen-2-yl)-3-(1H-indol-3-yl)prop-2-en-1-one (**6**) and (E)-3-(1H-indol-3-yl)-1-(pyridin-4-yl) prop-2-en-1-one (**7**) were utilized as synthons to synthesize a new class of 3,5-disubstituted pyrazoles and isoxazoles. The synthetic intermediates **5**, **6** and **7** were in turn obtained by the Claisen-Schimdt reaction of indole-3-carboxaldehyde (**1**) with 2-acetylfuran (**2**) / 2-acetyl-5-bromothiophene (**3**) / 4-acetylpyridine (**4**) in the presence of diisopropylethylamine (DIPEA) in methanol under ultrasonication (Scheme 1). It was observed that these compounds were obtained in shorter reaction times with high yield in ultrasonication method when compared with the conventional method. The ^{1}H NMR spectra of compounds **5a**, **6a** and **7a** exhibited two doublets were observed at 6.93, 7.21, 7.23 and 8.02, 8.15, 8.17 which were accounted to olefin protons H_A and H_B. The coupling constant values J_{AB}=15.4, 15.6 and 15.9 Hz revealed that they possess trans geometry. In addition to these, a broad singlet was observed at 11.74, 11.94 and 12.01 ppm was assigned to NH which disappeared on deuteration. The enone moiety present in **5** / **6** / **7** was exploited to develop pyrazoline and isoxazoline rings adopting [2+3] cyclocondensation. Thus, the cyclocondensation reaction of compounds **5**, **6** and **7** with hydrazine hydrate under ultrasonication furnished 3-(furan-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (**8**), 3-(5-bromothiophen-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (**9**) and 3-(pyridin-2-yl)-5-(1H-indol-3-yl)-2-pyrazoline (**10**) respectively (Scheme 2). The ^{1}H NMR spectra of compounds **8a**, **9a** and **10a** displayed an AMX splitting pattern due to methine and methylene protons of pyrazoline ring. Thus the three double doublets present at δ 4.43, 4.28, 3.24 in **8a**; at 4.66, 4.19, 3.34 in **9a** and at 4.64, 4.10, 3.43 ppm in **10a** were accounted to H_A, H_M and H_X, respectively. Moreover, two broad singlets appeared at δ 7.85, 7.82 and 7.91 and at 10.21, 10.56 and 11.63 ppm were assigned to NH of pyrazoline and indole rings in addition to signals due to aromatic protons. The signals due to NH disappeared on deuteration. The oxidation of compounds **8**, **9** and **10** with chloranil in xylene provided 3-(furan-2-yl)-5-(1H-indol-3-yl) pyrazole **(11)**, 3-(5-bromothiophen-2-yl)-5-(1H-indol-3-yl)pyrazole **(12)** and 3-(pyridin-2-yl)-5-(1H-indol-3-yl)pyrazole **(13)**, respectively. The absence of an AMX splitting pattern due to pyrazoline ring protons in the ^{1}H NMR spectra of compounds **11a**, **12a** and **13a** indicated that aromatization occurred. The singlets corresponding to $C_{4'}$-H of pyrazole appeared at downfield region and merged with aromatic protons. Apart



Scheme 1: Synthesis of (*E*)-1-(aryl)-3-(1*H*-indol-3-yl)prop-2-en-1-ones.



Scheme 2: Synthesis of 3-(aryl)-5-(1*H*-indol-3-yl)-2-pyrazolines and Pyrazoles.

from this, two broad singlets present at δ 7.63, 7.84, 7.96 and 10.46, 10.84, 11.21 ppm were assigned to NH of pyrazole and indole moieties. The signals due to NH disappeared when D_2O was added.

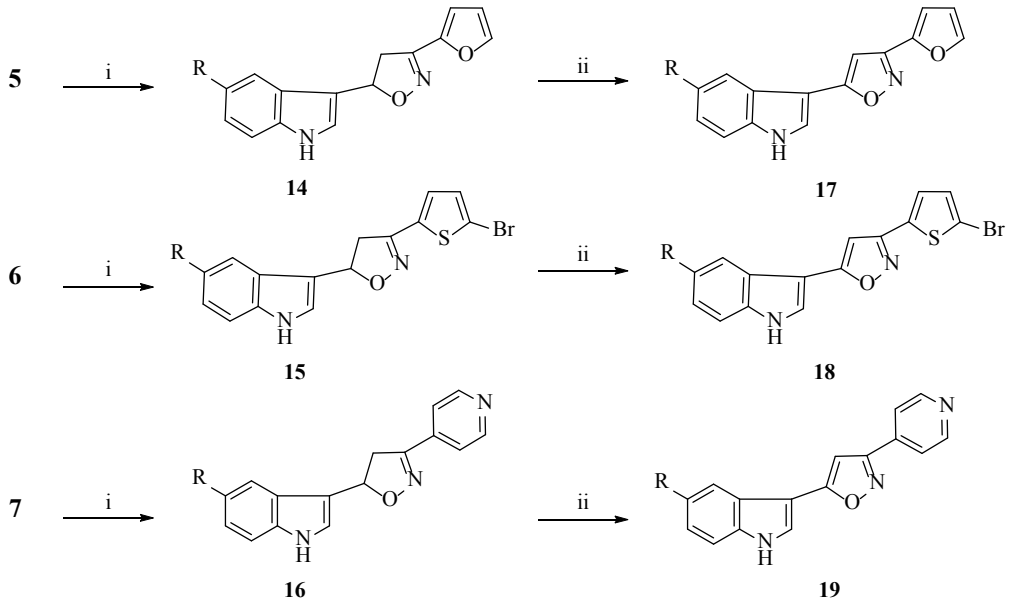
Similarly, the cyclocondensation of **5**, **6** and **7** with hydroxylamine hydrochloride in ethanol under ultrasonication yielded 3-(furan-2-yl)-5-(1H-indol-3-yl)-2-isoxazoline (**14**), 3-(5-bromothiophen-2-yl)-5-(1H-indol-3-yl)-2-isoxazoline (**15**) and 3-(pyridin-2-yl)-5-(1H-indol-3-yl)-2-isoxazoline (**16**), respectively. The ^{1}H NMR spectra of compounds **14a**, **15a** and **16a** displayed an AMX splitting pattern due to isoxazoline ring protons. The three double doublets present at δ 4.98, 4.52, 3.26 (**14a**); at 5.12, 4.57, 3.41 (**15a**) and 5.20, 4.62, 3.52 (**16a**) ppm were assigned to H_A, H_M and H_X, respectively. Besides, the broad singlet observed at δ 10.48 (**14a**), 10.71 (**15a**) and 11.96 ppm (**16a**) was attributed to NH of indole which disappeared on deuteration. Furthermore, the aromatized products 3-(furan-2-yl)-5-(1H-indol-3-yl)isoxazole **(17)**, 3-(5-bromothiophen-2-yl)-5-(1H-indol-3-yl)isoxazole **(18)** and 3-(pyridin-2-yl)-5-(1H-indol-3-yl)isoxazole **(19)** were obtained by the dehydrogenation of compounds **14**, **15** and **16** with chloranil in xylene under ultrasonication (Scheme 3). The ^{1}H NMR spectra of compounds **17a**, **18a** and **19a** presented a singlet due to $C_{4'}$-H at much downfield region and merged with aromatic protons. Moreover, a broad singlet observed at δ 10.72, 10.89 and 11.34 ppm was assigned to NH of indole ring which disappeared when D_2O was added. In fact, the target compounds were obtained in shorter reaction times with high yield under ultrasonication when compared with conventional method. The structures of all the synthesized compounds were further ascertained by IR, ^{13}C NMR, HRMS and microanalyses.

Biological evaluation

Antimicrobial activity: The compounds **8**-**19** were evaluated for antimicrobial activity at two concentrations (50 and 100 µg/well) by agar well diffusion and broth dilution methods. The results regarding the antibacterial activity presented in Table 1 and Figure 1 indicated that Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) were more susceptible against the tested compounds than Gram-negative bacteria (*Pseudomonas aureginosa* and *Klebsiella pneumoniae*). The compounds **8b**, **9a**, **10a** and **14b** displayed low activity whereas the compounds **8a** and **14a** showed no activity. Amongst all the compounds **12b** (39 µg/well) and **18b** (41 µg/well) displayed higher antibacterial activity than the standard Chloramphenicol (38 µg/well) particularly against B. subtilis. However, the remaining compounds showed moderate to good activity. The compounds having pyrazole (**11**-**13**) and isoxazole units (**17**-**19**) displayed higher antibacterial activity than pyrazoline (**8**-**10**) and isoxazoline rings (**14**-**16**). The presence of electron withdrawing groups on the aromatic ring enhanced the activity.

All the tested compounds **8**-**19** inhibited the spore germination against the fungi *Aspergillus niger* and *Penicillium chrysogenum* except **8a** and **14a** (Table 2 and Figure 2). In general, all the compounds showed comparatively higher antifungal activity against *A. niger* than *P. chrysogenum*. Amongst all the tested compounds **12b** (37 µg/well) and **18b** (39 µg/well) displayed higher antifungal activity than the standard drug (36 µg/well). It was observed that thiophene linked compounds (**9**, **12**, **15** and **18**) inhibited the spore germination against the tested fungi when compared with the other compounds. The MIC, MBC and MFC values of the tested compounds are shown in Table 3. The compounds **12b** and **18b** exhibited low MIC values. The MBC value of compounds **12b** and **18b** is 2 × MIC in case of *B. subtilis* and MFC value of **12b** and **18b** is 2 × MIC in case of *A. niger*.

The structure-activity relationship of the tested compounds revealed that thiophene linked compounds showed greater activity than furan and pyridine moieties. Further, it was observed that aromatized heterocycles **11**-**13** and **17**-**19** showed greater antimicrobial



Scheme 3: Synthesis of 3-(aryl)-5-(1*H*-indol-3-yl)-2-isoxazolines and Isoxazoles.

Compound	Zone of inhibition (mm)							
	Gram-positive bacteria				Gram-negative bacteria			
	S. aureus		*B. subtilis*		*P. aeruginosa*		*K. pneumoniae*	
	50 µg/well	100 µg/well	50 µg/well	100 µg/well	50 µg/well	100 µg/well	50 µg/well	100 µg/well
8a	-	-	-	-	-	-	-	-
8b	-	7 ± 1	-	8 ± 3	-	-	-	7 ± 1
9a	11 ± 2	13 ± 1	12 ± 3	14 ± 3	8 ± 2	10 ± 1	11 ± 2	14 ± 1
9b	17 ± 1	19 ± 3	20 ± 1	22 ± 2	16 ± 3	18 ± 1	20 ± 3	21 ± 1
10a	10 ± 1	12 ± 3	11 ± 2	14 ± 1	8 ± 1	11 ± 3	7 ± 2	9 ± 1
10b	15 ± 2	17 ± 1	16 ± 3	19 ± 2	12 ± 3	15 ± 1	13 ± 1	16 ± 2
11a	13 ± 1	15 ± 2	22 ± 1	25 ± 3	9 ± 2	11 ± 1	16 ± 3	18 ± 2
11b	23 ± 3	25 ± 3	27 ± 2	30 ± 2	16 ± 1	19 ± 3	25 ± 2	27 ± 1
12a	19 ± 2	21 ± 1	25 ± 2	28 ± 3	15 ± 3	17 ± 1	23 ± 2	25 ± 3
12b	25 ± 2	28 ± 2	35 ± 1	39 ± 1	21 ± 1	24 ± 3	29 ± 2	32 ± 1
13a	17 ± 3	19 ± 1	25 ± 1	28 ± 1	14 ± 3	16 ± 1	21 ± 2	24 ± 3
13b	24 ± 1	27 ± 3	29 ± 3	32 ± 1	19 ± 1	21 ± 3	25 ± 2	28 ± 1
14a	-	-	-	-	-	-	-	-
14b	-	8 ± 2	-	10 ± 1	-	-	-	9 ± 3
15a	15 ± 1	17 ± 2	19 ± 3	21 ± 1	14 ± 3	17 ± 2	18 ± 1	20 ± 3
15b	22 ± 3	25 ± 1	27 ± 2	28 ± 1	20 ± 1	23 ± 3	24 ± 1	26 ± 2
16a	13 ± 1	15 ± 2	17 ± 2	19 ± 1	13 ± 2	15 ± 2	16 ± 1	19 ± 3
16b	19 ± 3	21 ± 3	25 ± 3	27 ± 3	17 ± 1	19 ± 3	22 ± 3	25 ± 2
17a	16 ± 3	18 ± 1	23 ± 1	27 ± 3	11 ± 2	13 ± 1	18 ± 3	20 ± 2
17b	27 ± 2	29 ± 1	29 ± 3	32 ± 1	19 ± 3	22 ± 2	29 ± 1	33 ± 3
18a	22 ± 1	25 ± 1	28 ± 3	30 ± 2	17 ± 2	19 ± 1	24 ± 3	27 ± 2
18b	28 ± 3	31 ± 3	37 ± 1	41 ± 1	23 ± 1	26 ± 2	32 ± 3	35 ± 1
19a	18 ± 3	20 ± 1	26 ± 2	29 ± 3	12 ± 2	15 ± 3	26 ± 1	29 ± 2
19b	25 ± 2	31 ± 1	32 ± 1	34 ± 2	20 ± 3	23 ± 2	27 ± 1	30 ± 3
Chloramphenicol	33 ± 1	35 ± 3	34 ± 2	38 ± 3	27 ± 1	30 ± 3	40 ± 2	42 ± 1
Control (DMSO)	-	-	-	-	-	-	-	-

- No activity; ± Standard deviation

Table 1: The *in vitro* antibacterial activity of compounds 8-19.

Compound	Zone of inhibition (mm)			
	A. niger		*P. chrysogenum*	
	50 µg/well	100 µg/well	50 µg/well	100 µg/well
8a	-	-	-	-
8b	9 ± 3	11 ± 3	-	8 ± 2
9a	13 ± 2	15 ± 1	8 ± 1	10 ± 3
9b	20 ± 3	22 ± 2	17 ± 1	19 ± 2
10a	12 ± 3	14 ± 2	7 ± 1	9 ± 2
10b	16 ± 1	19 ± 3	14 ± 2	16 ± 2
11a	14 ± 2	17 ± 3	10 ± 1	12 ± 3
11b	25 ± 1	28 ± 1	18 ± 2	21 ± 3
12a	24 ± 1	26 ± 2	15 ± 3	17 ± 2
12b	34 ± 3	37 ± 2	26 ± 1	29 ± 3
13a	19 ± 1	21 ± 2	12 ± 1	14 ± 3
13b	28 ± 3	31 ± 1	19 ± 2	23 ± 1
14a	-	-	-	-
14b	11 ± 1	13 ± 2	8 ± 2	10 ± 3
15a	18 ± 2	21 ± 1	13 ± 1	15 ± 3
15b	28 ± 1	30 ± 2	22 ± 3	24 ± 3
16a	14 ± 2	18 ± 3	10 ± 3	13 ± 2
16b	23 ± 1	26 ± 1	20 ± 2	22 ± 3
17a	15 ± 1	18 ± 1	11 ± 3	14 ± 1
17b	27 ± 3	30 ± 2	23 ± 1	26 ± 1
18a	29 ± 3	32 ± 2	16 ± 1	19 ± 2
18b	35 ± 2	39 ± 3	28 ± 1	31 ± 1
19a	23 ± 2	25 ± 3	13 ± 2	15 ± 1
19b	32 ± 1	34 ± 2	25 ± 3	27 ± 2
Ketoconazole	33 ± 2	36 ± 3	35 ± 1	38 ± 3
Control (DMSO)	-	-	-	-

- No activity; ± Standard deviation

Table 2 The *in vitro* antifungal activity of compounds **8-19**.

Compound	Minimum inhibitory concentration MIC (MBC/MFC) µg/well					
	S. aureus	*B. subtilis*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*P. chrysogenum*
12b	50(200)	6.25(12.5)	50(>200)	100(>200)	12.5(25)	25(100)
18b	25(100)	6.25(12.5)	50(200)	100(>200)	12.5(25)	25(100)
Chloramphenicol	12.5	6.25	6.25	6.25	-	-
Ketoconazole	-	-	-	-	6.25	12.5

- No activity; ± Standard deviation

Table 3: MIC, MBC and MFC of compounds **12b** and **18b**.

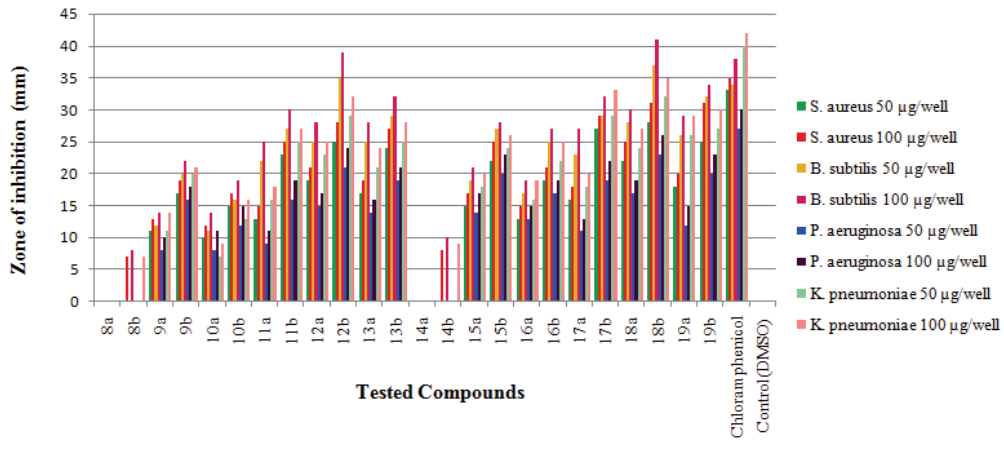


Figure 1: The in vitro antibacterial activity of compounds 8-19.

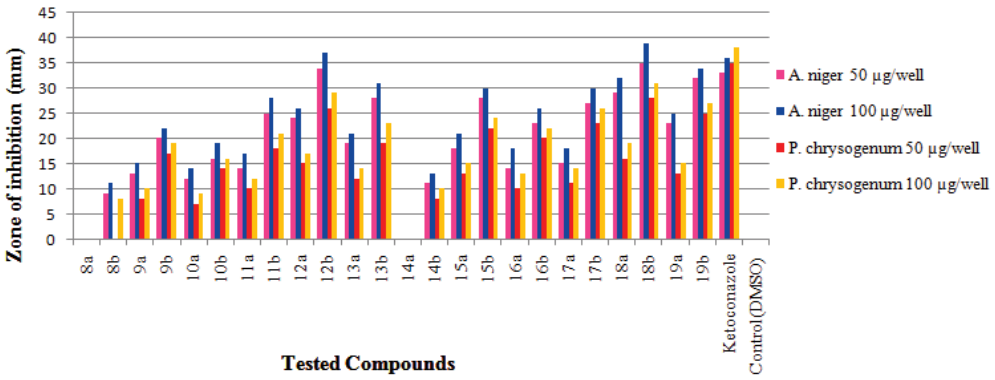


Figure 2: The *in vitro* antifungal activity of compounds **8**-**19**.

activity than non-aromatized compounds **8**-**10** and **14**-**16**. Moreover aromatized heterocyclic compounds having isoxazole unit displayed slightly higher activity than pyrazole unit. This is may be due to the presence of electron withdrawing oxygen atom. It was also noticed that compounds having electron withdrawing fluoro substituent on aromatic ring enhanced the activity when compared with the unsubstituted ones. Besides, the compounds having more number of electron withdrawing groups showed increased antimicrobial activity. Amongst all the compounds **12b** and **18b** were found to be potential antimicrobial agents particularly against *Bacillus subtilis* and *Aspergillus niger*. This result indicates that compounds **12b** and **18b** can be used as lead compounds in the future studies.

Conclusion

A new class of 3,5-disubstituted pyrazoles and isoxazoles were prepared from the Michael acceptors 1-furanyl / thiophenyl / pyridinyl-3-indole-prop-2-en-1-ones under ultrasonication and evaluated for antimicrobial activity. In fact, the target compounds were obtained in shorter reaction times with high yield under ultrasonication when compared with conventional method. Amongst all the tested compounds 12b and 18b displayed promising antimicrobial activity particularly against *Bacillus subtilis* and *Aspergillus niger*. The presence of electron withdrawing fluoro substituent on the aromatic ring enhanced the activity than the unsubstituted ones. Furthermore, compounds with more number of electron withdrawing groups showed higher antimicrobial activity. This result indicates that compounds 12b and 18b can be used as lead compounds in the future studies.

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