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Synthesis and Antimicrobial Activity of Pyrazolyl Triazoles

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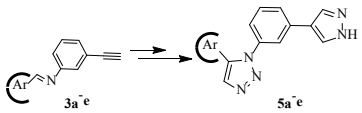
Abstract

Pyrazolyl triazoles (5) were prepared by 1,3-dipolar cycloaddition of diazomethane to Schiff base - 3-ethynyl-*N*-(heteroarylmethylene)aniline (3), followed by oxidation with I_2 in DMSO and studied their antimicrobial activity. The pyridine and dichloropyridine substituted compounds 3d, 5c and 5d are potential antibacterial agents against *Bacillus subtilis* and 3d is a potential antifungal agent against *Penicillium chrysogenum*.



- The compounds **5** were prepared by 1,3-dipolar cycloaddition of diazomethane to **3** followed by oxidation with I_2 in DMSO.
- Pyridine and dichloropyridine substituents enhanced the antimicrobial activity.
  3d, **5c**, **5d**; MBC = 6.25 µg/mL against *Bacillus subtilis*
  5d; MBC = 6.25 µg/mL against *Pseudomonas aeruginosa*
  3d; MFC = 12.5 µg/mL against *Penicillium chrysogenum*

Keywords: Schiff base; Pyrazole; Triazole; Antimicrobial activity

Introduction

The Schiff bases are the core motifs in medicinal and pharmaceutical fields and exhibit antimicrobial [1], antioxidant [2], anticancer [3] and pesticidal [4] properties. They have also find applications as synthetic intermediates to synthesize a variety of heterocycles [5]. Further the azole derivatives are prominent players in pharmaceutical research. Some pyrazole containing drugs viz., Ramifenazone, Antipyrine, Phenylbutazone, Celebrex, Pyrazofurin, Fipronil, Rimonabant, Novalgine are used as drugs (Figure 1). Moreover, 1,2,3-triazole containing entities such as Tazobactam and Cefatrizine are clinically used for the treatment of bacterial infections (Figure 1). Thus molecules with azole motif in different pharmacological agents have made it an indispensible anchor for the articulation of new drugs. The pyrazoles were prepared by the reaction of hydrazines with 1,3-dicarbonyl compounds [6,7] and ynones [8]. Moreover, 1,3-dipolar cycloaddition of nitrile imines and diazoalkanes to alkynes produced pyrazoles [9]. On the other hand, 1,3-dipolar cycloaddition of azides with alkynes under thermal conditions, in the presence of copper or ruthenium catalysts resulted in 1,2,3-triazoles [10-12]. Transition-metal-free synthesis of 1,2,3-triazoles was reported by the cyclocondensation of organic azides with terminal alkynes in the presence of a catalytic amount of tetramethylammonium hydroxide [13].

Metal-free triazole synthesis was also reported by the modification of Sakai reaction of an amine and α,α-dichlorotosylhydrazones [14]. On the basis of these observations and our continued interest to develop novel heterocycles [15,16] it is proposed to construct new molecules having pyrazole and 1,2,3-triazole motifs at 1,3-positions of the phenyl moiety and to study their antimicrobial activity.

Experimental Protocols

Melting points were recorded on a Mel-Temp apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC (silica gel H, BDH, ethyl acetate/hexane, 1:3). The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer

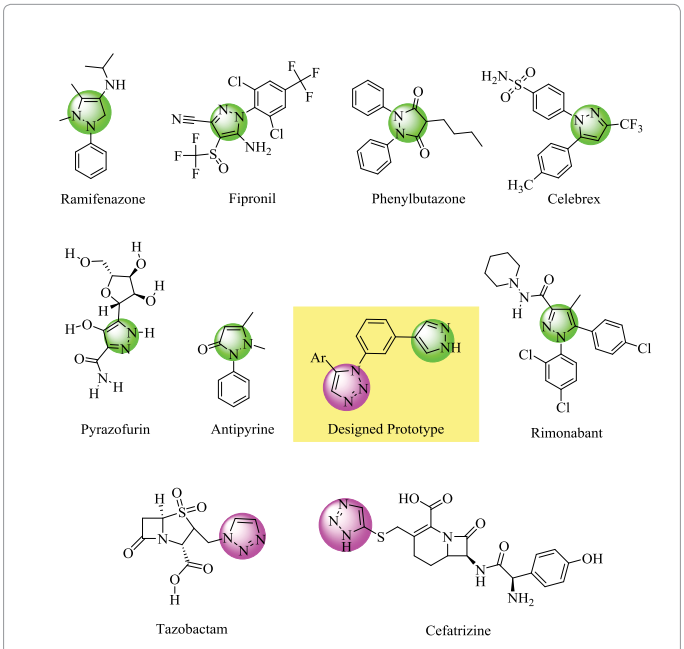


Figure 1: Drugs containing pyrazole and triazole moieties.

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as KBr pellets and the wave numbers were mentioned in cm^{-1}. The ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker-400 spectrometer operating at 400 and 100 MHz, respectively. The chemical shifts are reported in δ (ppm) using TMS as an internal standard. The high-resolution mass spectra were recorded on micromass Q-TOF micromass spectrometer using electrospray ionization. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The progress of the reaction was monitored by TLC using silica gel plates and components were visualized under UV light (254 and 365 nm). 3-Aminophenylacetylene (**1**) was purchased from Sigma-Aldrich, India.

General procedure for the synthesis of 3-ethynyl-N-(heteroarylmethylene)aniline (3a-e)

An equimolar (1 mmol) quantity of 3-aminophenylacetylene and heteroaldehyde in absolute ethanol (20 mL) was refluxed on an oil bath for 4-6 hrs in the presence of a catalytic amount of glacial acetic acid. After completion of reaction (monitored by TLC), the solution was cooled, diluted with water (20 mL) and extracted with ethyl acetate (3 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the resultant solid was purified by column chromatography (silica gel, 60-120 mesh) using hexane-ethyl acetate (4:1) as eluent.

3-Ethynyl-N-(1H-pyrrol-2-ylmethylene)aniline (3a): Yield 82%; m.p. 125-127°C; IR (KBr, cm^{-1}): 1597 (C=N); ^1H NMR (400 MHz, DMSO-d_6): 4.18 (s, 1H, ≡C-H), 6.24 (t, 1H, C_4-H, J=6.4 Hz), 6.48 (d, 1H, C_3-H, J=6.4 Hz), 6.96 (d, 1H, C_5-H, J=6.4 Hz), 7.05-7.35 (m, 4H, Ar-H), 8.71 (bs, 1H, NH-pyrrole), 8.81 (s, 1H, N=CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 81.9 (-C≡CH), 82.8 (-C≡CH), 111.8, 119.2, 122.2, 124.6, 124.9, 127.8, 129.8, 130.6, 131.2, 148.6 (C-2, C-3, C-4, C-5, aromatic carbons), 155.7 (N=CH) ppm; HRMS (m/z): 217.2257 $[M+Na]^+$; Anal. Calcd. for $C_{13}H_{10}N_2$: C, 80.39; H, 5.19; N, 14.42%; Found: C, 80.28; H, 5.23; N, 14.51%.

3-Ethynyl-N-(furan-3-ylmethylene)aniline (3b): Yield 83%; m.p. 130-132°C; IR (KBr, cm^{-1}): 1599 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 3.99 (s, 1H, ≡CH), 6.72 (d, 1H, C_4-H, J=5.5 Hz), 6.91-7.57 (m, 4H, Ar-H), 7.69 (d, 1H, C_5-H, J=5.5 Hz), 7.92 (s, IH, C_2-H), 9.13 (s, IH, N=CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ : 80.9 (-C≡CH), 82.1 (-C≡CH), 113.8, 114.2, 121.2, 124.1, 126.6, 129.2, 130.9, 140.2, 139.8, 143.6 (C-2, C-3, C-4, C-5 aromatic carbons), 159.2 (N=CH) ppm; HRMS (m/z): 218.2099 $[M+Na]^+$; Anal. Calcd. for $C_{13}H_9NO$: C, 79.98; H, 4.65; N, 7.17%; Found: C, 79.89; H, 4.62; N, 7.27%.

3-Ethynyl-N-(pyridin-3-ylmethylene)aniline (3c): Yield 80%; m.p. 128-130°C; IR (KBr, cm^{-1}): 1595 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 4.09 (s, 1H, ≡C-H), 7.02-7.66 (m, 4H, Ar-H), 7.79 (t, 1H, C_5-H, J=4.2 Hz), 8.42 (d, 1H, C_4-H, J=4.2 Hz), 8.76 (d, 1H, C_6-H, J=4.2 Hz), 8.98 (s, 1H, C_2-H), 9.16 (s, 1H, N=CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 81.4 (-C≡CH), 82.5 (-C≡CH), 122.8, 123.9, 124.7, 125.2, 127.6, 129.5, 130.1, 131.8, 133.7, 147.2, 149.1, 151.9 (C-2, C-3, C-4, C-5, C-6, aromatic carbons), 154.8 (N=CH) ppm; HRMS (m/z): 229.2370 $[M+Na]^+$; Anal. Calcd. For $C_{14}H_{10}N_2$: C, 81.53; H, 4.89; N, 13.58%; Found: C, 81.64; H, 4.94; N, 13.65%.

3-Ethynyl-N-(3,5-dichloropyridin-4-ylmethylene)aniline (3d): Yield 78%; m.p. 133-135°C; IR (KBr, cm^{-1}): 1564 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 4.19 (s, 1H, ≡C-H), 7.01-7.81 (m, 4H, Ar-H), 9.07 (s, 2H, C_2-H, C_6-H), 9.53 (s, 1H, N=CH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 82.2 (-C≡CH), 83.1 (-C≡CH), 123.4, 124.2, 125.3, 128.9, 129.5, 130.1, 131.3, 137.1, 138.2, 147.8, 148.6, (C-2, C-3, C-4, C-5, C-6, aromatic carbons), 155.6 (N=CH) ppm; HRMS (m/z): 298.1209 $[M+Na]^+$; Anal. Calcd. for $C_{14}H_8Cl_2N_2$: C, 61.12; H, 2.93; N, 25.77%; Found: C, 61.19; H, 2.96; N, 25.64%.

3-Ethynyl-N-(1H-indol-3-ylmethylene)aniline (3e): Yield 79%; m.p. 135-137°C; IR (KBr, cm^{-1}): 1585 (C=N); ^1H NMR (400 MHz, DMSO-d_6): δ 4.03 (s, 1H, ≡C-H), 7.12-8.01 (m, 9H, Ar-H), 8.92 (s, 1H, N=CH), 11.39 (bs, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 81.9 (-C≡CH), 82.8 (-C≡CH), 103.2, 111.3, 118.2, 120.9, 121.6, 122.1, 124.2, 126.7, 127.2, 129.7, 130.9, 131.1, 137.3, 143.7 (aromatic carbons), 160.2 (N=CH) ppm; HRMS (m/z): 267.2858 $[M+Na]^+$; Anal. Calcd. For $C_{17}H_{12}N_2$: C, 83.58; H, 4.95; N, 11.47%; Found: C, 83.49; H, 4.99; N, 11.39%.

General procedure for the synthesis of 1-(3-(1H-Pyrazol-4-yl) phenyl)-5-(heteroaryl)-4,5-dihydro-1H-1,2,3-triazole (4a-e)

An ice cold ethereal solution of diazomethane (15 mL, 0.4 mmol) and triethylamine (0.1 mL) was added to Schiff base (**3**) (0.1 mmol) in dichloromethane (7 mL). The reaction mixture was kept at -15C for 44-48 hrs. The solvent was removed on a rotary evaporator and the resultant solid was recrystallized from ethanol.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5-(1H-pyrrol-2-yl)-4,5-dihydro-1H-1,2,3-triazole (4a): Yield 72%; m.p. 163-165°C; IR (KBr, cm^{-1}): 1595 (C=N), 1622 (C=C), 3296 (NH); ^1H NMR (400 MHz, DMSO-d_6): δ 2.14 (dd,1H, $C_{5''}$-H, H_X, J_{AX}=7.1 Hz, $J_{MX=}$11.3 Hz), 2.75 (dd, 1H, $C_{5'}$,-H, H_M, J_{AM}=13.3 Hz, J_{MX}=11.3 Hz), 3.95 (dd,1H, $C_{4''}$-H, H_A, J_{AM}=13.3 Hz, J_{AX}=7.1 Hz), 5.73 (d, 1H, C_3-H, J=6.8 Hz), 6.00 (t, 1H, C_4-H, J=6.8 Hz), 6.70 (d, 1H, C_5-H, J=6.8 Hz), 7.16-7.41 (m, 4H, Ar), 8.25 (s, 1H, $C_{3'}$-H), 8.53 (s, 1H, $C_{5'}$-H), 9.01 (bs, 1H, NH-pyrrole), 10.75 (bs, 1H, NH- pyrazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 58.9 (C-5''), 74.8 (C-4''), 108.7, 110.0, 111.5, 115.9, 118.7, 119.9, 120.3, 122.7, 124.3, 125.3, 128.0, 129.9, 130.1 (C-2, C-3, C-4, C-5, C-3', C-4', C-5', aromatic carbons) ppm; HRMS (m/z): 301.3077 $[M+Na]^+$; Anal. Calcd. For $C_{15}H_{14}N_6$: C, 64.73; H, 5.07; N, 30.20%; Found: C, 64.80; H, 5.01; N, 30.26%.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5-(furan-3-yl)-4,5-dihydro-1H-1,2,3-triazole (4b): Yield 70%; m.p. 170-172°C; IR (KBr, cm^{-1}): 1568 (C=N), 1630 (C=C), 3140 (NH); ^1H NMR (400 MHz, DMSO-d_6): 2.30 (dd, 1H, $C_{5''}$, H_X, J_{AX}=7.0 Hz, J_{MX}=11.1 Hz), 2.81 (dd, 1H, $C_{5''}$, H_M, J_{AM}=13.1 Hz, $J_{MX=}$11.1 Hz), 3.83 (dd, 1H, $C_{4''}$, H_A, J_{AM}=3.1 Hz, J_{AX}=7.0 Hz), 6.28 (d, 1H, C_4-H, J=4.7 Hz), 7.15 (d, 1H, C_5-H, J=4.7 Hz), 7.25 (s, 1H, C_2-H), 7.35-7.54 (m, 4H, Ar), 8.32 (s, 1H, $C_{3'}$-H), 8.59 (s, 1H, $C_{5'}$-H), 10.89 (bs, 1H, NH-pyrazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 58.6 (C-5''), 74.2 (C-4''), 105.6, 108.9, 110.5, 112.7, 116.3, 117.1, 120.1, 123.2, 125.3, 130.2, 135.2, 137.5, 141.1 (C-2, C-3, C-4, C-5, C-3', C-4', C-5', aromatic carbons) ppm; HRMS (m/z): 302.1033 $[M+Na]^+$; Anal. Calcd. for $C_{15}H_{13}N_5O$: C, 64.51; H, 4.69; N, 25.07%; Found: C, 64.58; H, 4.62; N, 25.16%.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5-(pyridin-3-yl)-4,5-dihydro-1H-1,2,3-triazole (4c): Yield 68%; m.p. 168-170°C; IR (KBr, cm^{-1}): 1585 (C=N), 1642 (C=C), 3138 (NH); ^1H NMR (400 MHz, DMSO-d_6): δ 1.90 (dd, 1H, $C_{5''}$, H_X, J_{AX}=6.9 Hz, J_{MX}=10.9 Hz), 2.56 (dd, 1H, $C_{5''}$, H_M, J_{AM}=12.9 Hz, J_{MX}=10.9 Hz), 3.60 (dd, 1H, $C_{4''}$, H_A, J_{AM}=12.9 Hz, J_{AX}=6.9 Hz), 7.10-7.42 (m, 4H, Ar), 7.56 (t, 1H, C_5-H, J=4.5 Hz), 7.86 (s, 1H, C_4-H, J=4.5 Hz), 8.14 (s, 1H, $C_{3'}$-H), 8.43 (d, 1H, C_6-H, J=4.5 Hz), 8.52 (s, 1H, $C_{5'}$-H), 8.62 (s, 1H, C_2-H), 10.70 (bs, IH, 2H, pyrazole) ppm; ^{13}C

NMR (100 MHz, DMSO-d_6): δ 57.9 (C-5''), 73.0 (C-4''), 116.2, 117.2, 118.3, 120.2, 121.3, 122.2, 123.4, 124.9, 125.4, 129.3, 133.0, 133.5, 146.6, 148.2 (C-2, C-3, C-4, C-5, C-6, C-3', C-4', C-5', aromatic carbons) ppm; HRMS (m/z): 313.3189 $[M+Na]^+$; Anal. Calcd. for $C_{16}H_{14}N_6$: C, 66.19; H, 4.86; N, 28.95%; Found: C, 66.27; H, 4.81; N, 29.04%.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5-(3,5-dichloropyridine-3-yl)-4,5-dihydro-1H-1,2,3-triazole (4d): Yield 66%; m.p. 174-176°C; IR (KBr, cm^{-1}): 1604 (C=N), 1635 (C=C), 3199 (NH); ^{1}H NMR (400 MHz, DMSO-d_6): 2.11 (dd, 1H, $C_{5''}$, H_X, J_{AX}=7.2 Hz, J_{MX}=11.2 Hz), 2.80 (dd, 1H, $C_{5''}$, H_M, J_{AM}=13.0 Hz, J_{MX}=11.2 Hz), 4.01 (dd,1H, $C_{4''}$, H_A, J_{AM}=13.0 Hz, J_{AX}=7.0 Hz), 7.30-7.53 (m, 4H, Ar), 8,43 (s, 1H, $C_{3'}$-H), 8.92 (s, 2H, C_2-H, C_6-H), 8.95 (s, 1H, $C_{5'}$-H), 11.10 (bs, 1H, NH-pyrazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 57.9 (C-5''), 73.1 (C-4''), 117.2, 118.5, 119.5, 121.1, 122.5, 124.3, 125.6, 129.7, 131.9, 133.4, 142.9, 144.7, 146.4 (C-2, C-3, C-4, C-5, C-6, C-3', C-4', C-5', aromatic carbons) ppm; HRMS (m/z): 382.2027 $[M+Na]^+$; Anal. Calcd. For $C_{16}H_{12}Cl_2N_6$: C, 53.56; H, 3.37; N, 23.40%; Found: C, 53.50; H, 3.33; N, 23.32%.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5-(1H-indole-3-yl)-4,5-dihydro-1H-1,2,3-triazole (4e): Yield 66%; m.p. 174-176°C; IR (KBr, cm^{-1}): 1583 (C=N), 1620 (C=C), 3209 (NH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.01 (dd, 1H, $C_{5''}$, H_X, J_{AX}=7.1 Hz, J_{MX}=11.1 Hz), 2.53 (dd, 1H, $C_{5''}$, H_M, J_{AM}=13.1Hz, J_{MX}=11.1 Hz), 3.79 (dd, 1H, $C_{4''}$, H_A, J_{AM}=13.1 Hz, J_{AX}=7.2 Hz), 7.09-8.03 (m, 9H, Ar), 8.38 (s, 1H, $C_{3'}$-H), 8.85 (s, 1H, $C_{5'}$-H), 10.15 (bs, 1H, NH, indole), 10.85 (bs, 1H, NH-pyrazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 59.0 (C-5''), 73.5 (C-4''), 110.3, 111.9, 118.2, 119.9, 120.9,122.5, 125.1, 125.8, 126.1, 127.2, 128.1, 128.9, 129.3, 130.4, 131.2, 132.7, 135.5 (C-3', C-4', C-5', aromatic carbons) ppm; HRMS (m/z): 351.3680 $[M+Na]^+$; Anal. Calcd. for $C_{19}H_{16}N_6$: C, 69.50; H, 4.91; N, 25.59%; Found: C, 69.59; H, 4.96; N, 25.66%.

General procedure for the Synthesis of 1-(3-(1H-pyrazol-4-yl)phenyl)-5-(heteroaryl)-1H-1,2,3-triazole (5a-e)

The compound **4** (0.3 mmol) and iodine (2 mg, 2.2 mol%) were dissolved in dimethyl sulfoxide (8 mL) and refluxed for 3-4 hrs. The solution was poured onto crushed ice and the resultant solid was filtered, dried and purified by column chromatography (silica gel, 60-120 mesh) using hexane-ethyl acetate (3:1) as eluent.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5-(1H-pyrrol-2-yl)-1H-1,2,3-triazole (5a): Yield 66%; m.p. 174-176C; IR (KBr, cm^{-1}): 1592 (C=N), 1631 (C=C), 3312 (NH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.03 (d, IH, C_3-H, J=7.2 Hz), 6.20 (t, 1H, C_4-H, J=7.2 Hz), 6.98 (d, 1H, C_5-H, J=7.2 Hz), 7.39-7.73 (m, 4H, Ar), 8.03 (s, 1H, $C_{5''}$-H), 8.30 (s, 1H, $C_{3'}$-H), 8.73 (s, 1H, $C_{5'}$-H), 9.12 (bs, 1H, NH-pyrrole), 10.81 (bs, 1H, NH-pyrazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 106.8 (C-3), 111.5 (C-4), 116.1, 120.1, 125.2, 126.5, 127.1, 128.1, 128.9, 129.6, 130.2, 130.9, 132.8, 133.5, 134.0 (C-2, C-5, C-3', C-4', C-5', C-4'', C-5'', aromatic carbons) ppm; HRMS (m/z): 299.2919 $[M+Na]^+$; Anal. Calcd. for $C_{15}H_{12}N_6$: C, 65.21; H, 4.38; N, 30.42%. Found: C, 65.14; H, 4.34; N, 30.34%.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5-(furan-3-yl)-1H-1,2,3-triazole (5b): Yield 64%; m.p. 178-180C; IR (KBr, cm^{-1}): 1594 (C=N), 1638 (C=C), 3186 (NH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.31 (d, 1H, C_4-H, J=5.9 Hz), 7.25 (d, 1H, C_5-H, J=5.9 Hz), 7.41 (s, 1H, C_2-H), 7.51-7.89 (m, 4H, Ar), 8.01 (s, 1H, $C_{5''}$-H), 8.24 (s, 1H, $C_{3'}$-H), 8.71 (s, 1H, $C_{5'}$-H), 10.95 (bs, 1H, NH-pyrazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 107.8, 108.9, 119.8, 122.7, 125.2, 126.7, 127.9, 128.7, 129.5, 130.8, 131.5, 132.7, 133.8, 138.5, 144.1 (C-2, C-3, C-4, C-5, C-3', C-4' C-5', C-4'', C-5'', aromatic carbons) ppm; HRMS (m/z): 300.0845 $[M+Na]^+$; Anal. Calcd. for $C_{15}H_{11}N_5O$: C, 64.97; H, 4.00; N, 25.26%; Found: C, 65.05; H, 4.05; N, 25.18%.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5-(pyridin-3-yl)-1H-1,2,3-triazole (5c): Yield 61%; m.p. 175-177°C; IR (KBr, cm^{-1}): 1590 (C=N), 1654 (C=C), 3299 (NH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.43-7.75 (m, 4H, Ar), 7.87 (t, 1H, C_5-H, J=4.8 Hz), 7.91 (s, 1H, $C_{5''}$-H), 8.42 (d, 1H, C_4-H, J=4.8 Hz), 8.49 (s, 1H, $C_{3'}$-H), 8.70 (d, 1H, C_6-H, J=4.8 Hz), 8.83 (s, 1H, $C_{5'}$ -H), 9.24 (s, IH, C_2-H), 10.74 (bs, 1H, NH-pyrazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 119.7, 122.7, 124.3, 126.1, 126.9, 127.8, 128.1, 128.9, 129.1, 130.6, 131.7, 132.8, 133.7, 134.5, 147.9, 149.1 (C-2, C-3, C-4, C-5, C-6, C-3', C-4', C-5', C-4'', C-5'', aromatic carbons) ppm; HRMS (m/z): 311.2954 $[M+Na]^+$; Anal. Calcd. for $C_{16}H_{12}N_6$: C, 66.66; H, 4.20; N, 29.15%; Found: C, 66.78; H, 4.16; N, 29.26%.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5(3,5-dichloropyridin-4-yl)-1H-1,2,3-triazole (5d): Yield 63%; m.p. 181-183°C; IR (KBr, cm^{-1}): 1568 (C=N), 1645 (C=C), 3256 (NH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.62-7.88 (m, 4H, Ar-H), 8.01 (s, 1H, $C_{5''}$-H), 8.53(s, 1H, $C_{3'}$-H), 8.91 (s, 1H, $C_{5'}$-H), 8.96 (s, 2H, C_2-H, C_6-H), 11.18 (bs, 1H, NH-pyrazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 112.4, 117.2, 120.2, 126.5, 127.1, 128.3, 129.0, 129.9, 130.8, 131.6, 132.8, 133.7, 135.2, 143.3, 147.1 (C-2, C-3, C-4, C-5, C-6, C-3', C-4', C-5', C-4'', C-5'', aromatic carbons) ppm; HRMS (m/z): 380.1857 $[M+Na]^+$; Anal. Calcd. for $C_{16}H_{10}Cl_2N_6$: C, 53.80; H, 2.82; N, 23.53%; Found: C, 53.89; H, 2.85; N, 23.66%.

1-(3-(1H-Pyrazol-4-yl)phenyl)-5-(1H-indole-3-yl)-1H-1,2,3-triazole (5e): Yield 62%; m.p. 194-196°C; IR (KBr, cm^{-1}): 1581 (C=N), 1630 (C=C), 3226 (NH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.22-8.21 (m, 9H, Ar-H), 8.01 (s, 1H, C_5-H), 8.62 (s, 1H, $C_{3'}$-H), 9.01 (s, 1H, $C_{5'}$-H), 10.39 (bs, 1H, NH-indole), 11.01 (bs, 1H, NH-pyrazole) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 111.2, 112.3, 117.3, 119.8, 120.5, 121.2, 123.4, 125.7, 126.4, 127.1, 127.8, 128.5, 129.1, 129.8, 130.1, 132.5, 133.7, 134.2, 136.7 (C-3', C-4', C-5', C-4'', C-5'', aromatic carbons) ppm; HRMS (m/z): 349.3435 $[M+Na]^+$; Anal. Calcd. for $C_{19}H_{14}N_6$: C, 69.92; H, 4.32; N, 25.75%; Found: C, 70.01; H, 4.37; N, 25.64%.

In vitro antimicrobial assay

The *in vitro* antimicrobial studies were determined by agar well diffusion method against test organisms [17-20]. Nutrient broth (NB) plates were swabbed with 24 hrs old broth culture (100 µl) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each petri plate. Different concentrations of DMSO dissolved compounds (50, 75 and 100 µg/well) were added into the wells using micropipette. The standard antibiotics- Chloramphenicol for antibacterial activity and Ketoconazole for antifungal activity (as positive control) were tested against the pathogens simultaneously. 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 hrs for bacteria and at 28°C for 48 hrs for fungi. After an appropriate incubation, the diameter of zone of inhibition of each well was measured. The experiment was repeated twice and the average values were calculated for eventual antibacterial activity.

Broth dilution test was used to determine MIC of the samples [21,22]. Freshly prepared NB was used as diluents. The 24 hrs old culture of the bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and the fungi *Aspergillus niger* and *Penicillium chrysogenum* were diluted 100-folds in NB (100 µl bacterial cultures in 10 ml NB). Increasing concentrations of the 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 the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37°C for 24 hrs for bacteria and at 28°C for 48 hrs

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) [23] and minimum fungicidal concentration (MFC) [24] 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 NB (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 hrs, respectively. After incubation, the lowest concentration that kills the tested organisms was noted as MBC (for bacteria) or MFC (for fungi) at which no visible growth was observed.

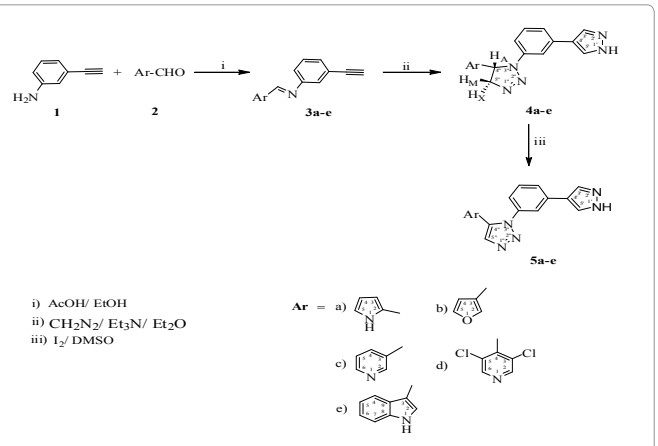
Biological activity

The compounds **3-5** were evaluated for antibacterial and antifungal activities at three different concentrations 50, 75 and 100 µg/well. Bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and fungi *Aspergillus niger*, *Penicillium chrysogenum* were obtained from Department of Microbiology, SV University, Tirupati.

Results and Discussion

Chemistry

The synthetic pathway adopted to achieve 3-ethynyl-N-(heteroarylmethylene)aniline (**3**), 1-(3-(1H-pyrazol-4-yl)phenyl)-5-(heteroaryl)-4,5-dihydro-1H-1,2,3-triazole (**4**) and 1-(3-(1H-pyrazol-4-yl)phenyl)-5-(heteroaryl)-1H-1,2,3-triazole (**5**) is shown in Scheme 1. The Schiff base (**3**) was prepared by the reaction of 3-aminophenylacetylene (Sigma-Aldrich) with heteroaldehydes in the presence of glacial acetic acid in ethanol. The ^{1}H NMR spectrum of **3a** displayed two singlets at δ 4.18, 8.81 due to -C≡CH and -CH=N, two doublets at 6.49, 6.96 due to C_3-H and C_5-H, a triplet at 6.24 due to C_4-H and a broad singlet at 8.71 ppm due to NH in addition to the signals of aromatic protons. The 1,3-dipolar cycloaddition of dipolar reagents across the double bond is one of the facile methods for the synthesis of five membered heterocycles. Although there are wide reports of the addition of diazomethane to acetylenes to get pyrazoles, the reaction of imines with diazomethane to develop 1,2,3-triazoles is sparsely reported [17]. We have synthesized a vulnerable synthetic intermediate **3** having



Scheme 1: Synthesis of 3-ethynyl-*N*-(heteroarylmethylene)aniline/1-(3-(1*H*-pyrazol-4-yl)phenyl)-5-(heteroaryl)-4,5-dihydro-1*H*-1,2,3-triazole/1-(3-(1*H*-pyrazol-4-yl)phenyl)-5-(heteroaryl)-1*H*-1,2,3-triazole.

both acetylene and imine functionalities which paves the way for the development of pyrazole and 1,2,3-triazole units in one pot under more facile and mild reaction conditions. Thus the treatment of compound **3** with diazomethane in the presence of triethylamine in ether at 0-15°C resulted in 1-(3-(1H-pyrazol-4-yl)phenyl)-5-(heteroaryl)-4,5-dihydro-1H-1,2,3-triazole (**4**). The ^{1}H NMR spectrum of **4a** showed two singlets at δ 8.21, 8.53 due to $C_{3'}$-H and $C_{5'}$-H of pyrazole. The methylene and methine protons of 1,2,3-triazoline displayed AMX splitting pattern. The double doublets observed at 2.14, 2.75 and 3.95 were assigned to H_X, H_M and H_A, respectively. The coupling constants $J_{AM=}$13.3, $J_{AX=}$7.1, $J_{MX=}$11.3 Hz indicated that H_A, H_M are cis, H_A, H_X are trans and H_M, H_X are geminal. The pyrrole ring protons C_3-H, C_4-H and C_5-H exhibited a doublet at 5.72, a triplet at 5.99 and a doublet at 6.70, respectively. In addition two broad singlets were observed at 9.01 and 10.75 ppm due to NH of pyrrole and pyrazole which disappeared on deuteration. Aromatization of 1,2,3-triazoline with I_2 in DMSO resulted in 1-(3-(1H-pyrazol-4-yl)phenyl)-5-(heteroaryl)-1H-1,2,3-triazole (**5**). The absence of AMX splitting pattern and the presence of a singlet at δ 8.03 due to $C_{5''}$-H of triazole indicated that aromatization occurred. In addition to this signals corresponding to pyrrole ring protons appeared at downfield region, C_3-H at 6.03, C_4-H at 6.20 and C_5-H at 6.98 ppm which confirmed the extended conjugation. All the new compounds are further characterized by IR, ^{13}C NMR, mass and elemental analysis.

Biological evaluation

***In vitro* antimicrobial activity:** The results of antibacterial activity presented in Table 1 and Figure 2 revealed that except **3b**, **4a** and **4b**, the remaining compounds are susceptible towards the tested bacteria. The compounds displayed higher activity on *B. subtilis* amongst Gram-positive bacteria and on *P. aeruginosa* amongst Gram-negative bacteria. The pyrazolyl triazoles (**5**) exhibited higher activity than pyrazolyl triazolines (**4**) and phenylacetylene Schiff bases (**3**). However the latter compounds displayed more activity than **4**. Amongst the compounds **5a-5e** those having pyridine **5c** and **5d** exhibited higher activity. In fact **5d** showed slightly more activity than **5c** which may be due to the presence of electron withdrawing chloro substituent. On the other hand, **5e** with indole exhibited higher activity than those having pyrrole **5a** and furan **5b**. Amongst latter compounds **5a** showed greater activity than **5b**. Similar effect of substituents was observed in compounds **3a-d** and **4a-d** also. The antibacterial activity exhibited by **5c** and **5d** was greater than the standard drug Chloramphenicol on *B. subtilis* at

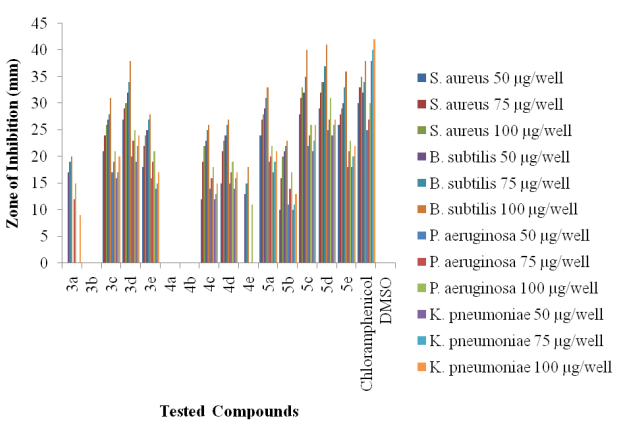


Figure 2: The *in vitro* antibacterial activity of compounds 3a-5e.

Compound	Diameter of zone of inhibition (mm)											
	Gram- positive bacteria						Gram-negative bacteria					
	S. aureus			*B. subtilis*			*P. aeruginosa*			*K. pneumoniae*		
	50 (µg/well)	75 (µg/well)	100 (µg/well)	50 (µg/well)	75 (µg/well)	100 (µg/well)	50 (µg/well)	75 (µg/well)	100 (µg/well)	50 (µg/well)	75 (µg/well)	100 (µg/well)
3a	-	-	-	17 ± 3.2	19 ± 1.1	20 ± 1.1	-	12 ± 1.5	15 ± 1.8	-	-	9 ± 1.4
3b	-	-	-	-	-	-	-	-	-	-	-	-
3c	21 ± 1.2	24 ± 2.2	26 ± 1.8	27 ± 3.1	28 ± 1.9	31 ± 1.9	17 ± 2.5	19 ± 1.8	21 ± 1.1	16± 1.8	17 ± 2.3	20 ± 1.5
3d	27 ± 1.8	29 ± 3.3	30 ± 3.2	32 ± 2.2	34 ± 1.8	38 ± 2.5	20 ± 2.2	23 ± 2.2	25 ± 3.2	19 ± 2.6	22 ± 3.2	24 ± 2.2
3e	18 ± 1.8	22 ± 1.1	24 ± 2.3	25 ± 2.2	27 ± 2.1	28 ± 2.3	16 ± 1.7	18 ± 1.2	20 ± 1.9	14 ± 0.8	15 ± 1.1	17 ± 1.1
4a	-	-	-	-	-	-	-	-	-	-	-	-
4b	-	-	-	-	-	-	-	-	-	-	-	-
4c	12 ± 1.3	19 ± 2.1	22 ± 1.1	23 ± 2.3	25 ± 1.1	26 ± 1.8	14 ± 1.3	16 ± 2.2	18 ± 1.8	12 ± 2.2	13 ± 1.8	15 ± 1.8
4d	15 ± 2.2	21 ± 1.8	23 ± 2.1	24 ± 1.1	26 ± 1.2	27 ± 1.4	15 ± 1.5	17 ± 1.7	19 ± 2.1	14 ± 1.1	16 ± 1.2	17 ± 1.3
4e	-	-	-	13 ± 1.4	15 ± 2.7	18 ± 2.1	-	-	11 ± 1.5	-	-	-
5a	24 ± 1.3	27 ± 2.1	28 ± 3.2	29 ± 2.1	31 ± 2.0	33 ± 2.2	18 ± 1.9	20 ± 2.5	22 ± 1.8	17 ± 0.8	19 ± 1.9	21 ± 2.8
5b	10 ± 1.8	16 ± 1.8	20 ± 2.2	21 ± 1.8	22 ± 2.3	23 ± 0.9	11 ± 1.2	14 ± 1.9	17 ± 1.2	10 ± 1.9	11 ± 2.2	13 ± 1.8
5c	28 ± 1.3	31 ± 2.1	33 ± 3.2	33 ± 2.3	35 ± 3.1	40 ± 1.3	22 ± 2.1	24 ± 1.3	26 ± 1.1	21 ± 2.1	23 ± 2.1	26 ± 3.3
5d	29 ± 2.1	32 ± 1.3	34 ± 2.2	34 ± 1.3	37 ± 2.1	41 ± 2.1	25 ± 0.9	27 ± 1.8	30 ± 1.2	24 ± 2.8	26 ± 2.6	27 ± 1.7
5e	26 ± 2.2	28 ± 2.2	29 ± 3.4	30 ± 2.1	33 ± 2.2	36 ± 3.1	19 ± 1.3	21 ± 3.5	23 ± 1.2	18 ± 3.1	20 ± 2.3	22 ± 2.1
Chloramphenicol	30 ± 3.3	33 ± 1.1	35 ± 2.1	32 ± 3.2	34 ± 2.1	38 ± 1.2	25 ± 3.2	27 ± 2.1	30 ± 2.2	38 ± 1.1	40 ± 2.2	42 ± 3.1
Control (DMSO)	-	-	-	-	-	-	-	-	-	-	-	-
(-) No activity; (±) Standard deviation												

Table 1: The *in vitro* antibacterial activity of compounds 3a- 5e by agar-well diffusion method.

Compound	Diameter of zone of inhibition (mm)					
	Fungi					
	A. niger			*P. chrysogenum*		
	50 (µg/well)	75 (µg/well)	100 (µg/well)	50 (µg/well)	75 (µg/well)	100 (µg/well)
3a	19 ± 3.1	21 ± 1.4	24 ± 2.1	20 ± 1.3	22 ± 0.8	25 ± 2.3
3b	14 ± 3.2	15 ± 1.9	17 ± 3.2	16 ± 1.9	18 ± 3.1	19 ± 2.4
3c	25 ± 2.2	27 ± 1.6	30 ± 1.7	26 ± 2.1	27 ± 2.1	29 ± 1.4
3d	30 ± 1.7	32 ± 2.6	34 ± 1.7	36 ± 1.1	38 ± 1.4	39 ± 2.2
3e	21 ± 1.8	23 ± 2.5	27 ± 0.7	24 ± 1.9	26 ± 1.1	28 ± 1.5
4a	-	9 ± 2.9	10 ± 0.8	-	-	11 ± 1.1
4b	-	-	9 ± 32	-	9 ± 1.2	10 ± 1.2
4c	15 ± 1.5	17 ± 2.1	19 ± 2.1	15 ± 1.8	19 ± 2.2	21 ± 1.8
4d	16 ± 2.6	18 ± 2.3	20 ± 1.7	18 ± 0.8	20 ± 1.5	22 ± 3.2
4e	-	13 ± 1.8	15 ± 2.4	-	-	16 ± 1.2
5a	12 ± 2.8	14 ± 2.8	16 ± 2.3	14 ± 0.9	16 ± 2.4	17 ± 1.8
5b	-	-	11 ± 1.8	-	10 ± 1.8	13 ± 1.1
5c	18 ± 1.9	20 ± 1.3	23 ± 3.2	22 ± 1.5	23 ± 2.1	26 ± 3.2
5d	27 ± 1.4	30 ± 3.4	32 ± 0.9	28 ± 0.9	32 ± 0.9	33 ± 0.8
5e	17 ± 2.2	19 ± 3.2	21 ± 2.7	19 ± 1.9	22 ± 1.8	24 ± 2.8
Ketoconazole	31 ± 2.2	33 ± 3.5	36 ± 2.8	35 ± 1.8	36 ± 2.2	38 ± 3.1
Control (DMSO)	-	-	-	-	-	-
(-) No activity; (±) Standard deviation						

Table 2: The *in vitro* antifungal activity of compounds **3a- 5e** by agar-well diffusion method.

all tested concentrations. However, **3d** displayed equal activity to the standard drug on *B. subtilis* and **5d** on *P. aeruginosa*.

All the compounds inhibited spore germination of the tested fungi. The Schiff bases (**3**) displayed higher activity than pyrazolyl triazolines (**4**) and pyrazolyl triazoles (**5**). Amongst the latter compounds **5** showed greater activity than **4**. Pyridine and dichloropyridine substituted compounds exhibited more activity than pyrrole, furan and indole substituted ones. The compound **3d** displayed higher activity

Compound	Minimum inhibitory concentration MIC (MBC/MFC) µg/well					
	Staphylococcus aureus	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Aspergillus niger*	*Penicillium chrysogenum*
3d	25 (100)	6.25 (12.5)	12.5 (50)	50 (200)	50 (200)	6.25 (12.5)
5c	25 (100)	6.25 (12.5)	12.5 (50)	25 (100)	100 (>200)	12.5 (50)
5d	12.5 (25)	6.25 (12.5)	6.25 (12.5)	12.5 (50)	50 (200)	12.5 (50)
5e	50 (200)	12.5 (50)	25 (100)	25 (100)	100 (>200)	12.5 (50)
Chloramphenicol	12.5	6.25	6.25	6.25	-	-
Ketoconazole	-	-	-	-	6.25	12.5

(-) No activity

Table 3: MIC, MBC and MFC of compounds **3d**, **5c**, **5d** and **5e**.

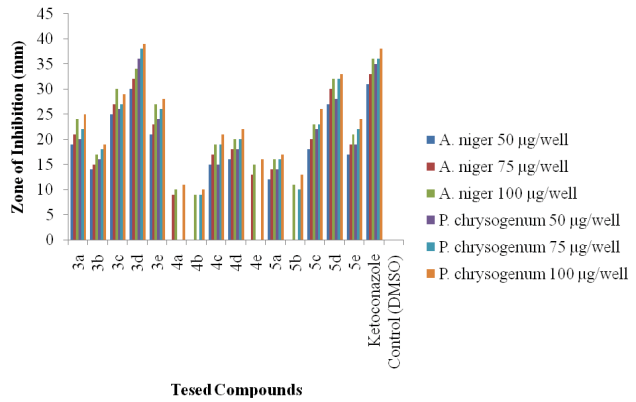


Figure 3: The *in vitro* antifungal activity of compounds **3a-5e.**

on *P. chrysogenum* than the standard drug Ketoconazole at all tested concentrations (Table 2 and Figure 3).

MIC, MBC and MFC of the compounds 3d, 5c, 5d and 5e: The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the compounds tested are listed in Table 3. MIC is the lowest concentration of an antimicrobial that inhibit the visible growth of a microorganism (But it is not sure that the microorganisms are completely killed). The MBC/MFC is the lowest concentration of antibiotic required to kill a particular bacterium/fungus. The MBC/MFC is assayed by performing an additional set of steps once the MIC is determined. The antimicrobials are usually regarded as bactericidal/fungicidal if the MBC/MFC is not greater than four times the MIC [18]. The compounds **3d**, **5c** and **5d** exhibited low MIC and the MBC is 2 × MIC towards *B. subtilis* whereas **5d** displayed MBC is 2 × MIC towards *P. aeruginosa* also. On the other hand, the compound **3d** showed low MIC and MFC is 2 × MIC on *P. chrysogenum*. The structure- activity relationship of the compounds revealed that the aromatized compounds **5** displayed higher activity than non- aromatized heterocylcles **4**. The compounds having pyridine showed more activity and the activity enhanced by the presence of electron withdrawing chloro substituent.

Conclusion

A variety of pyrazolyl triazoles (**5**) were prepared by exploitation of acetylene and azomethine moieties present in 3-ethynyl-N-(heteroarylmethylene)aniline (**3**) by 1,3-dipolar cycloaddition of diazomethane followed by oxidation with I_2 in DMSO. Pyridine substituted compounds showed more activity than those with pyrrole, furan and indole moieties. In fact, pyridine substituted Schiff base and pyrazolyl triazoles **3d**, **5c** and **5d** exhibited potential antibacterial activity against *Bacillus subtilis* and **5d** also on *Pseudomonas aeruginosa*. Further **3d** displayed potential antifungal activity on *Penicillium chrysogenum*.

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References

1. Cleiton MS, Daniel LS, Luzia VM, Rosemeire BA, Maria AR, et al. (2011) Schiff bases: A short review of their antimicrobial activities. J Advan Res 2: 1-8.
2. Valentina P, Ilango K, Deepthi M, Harusha P, Pavani G, et al. (2009) Antioxidant Activity of Some Substituted 1, 2, 4 - Triazo-5-thione Schiff base. J Pharm Sci Res 1: 74-77.
3. Mokhles MA, Ammar AL, Hanan AM, Samia AM, Mamdouh MA, et al. (2016) Synthesis, anticancer activity and molecular docking study of Schiff base complexes containing thiazole moiety. Beni-suefuniversity J Basic and Applied Sci 5: 85-96.
4. Ali MM, Jesmin M, Salam SMA, Khanam JA, Islam MF, et al. (2009) Pesticidal Activities of Some Schiff Bases Derived from Benzoin, Salicyl- aldehyde, Aminophenol and 2,4 Dinitrophenyl Hydrazine. J Sci Res 1: 641-646.
5. Hsiu MK, Wan PK, Yu TH, Gene HL, Chung KL (2016) Mesogenic heterocycles derived from quinoxaline Schiff Bases. Tetrahedron 72: 6321-6333.
6. Ying Li, Chun-E D (2015) Efficient synthesis of fused pyrazoles via simple cyclization of o-alkynylchalcones with hydrazine. Chinese Chem Lett 26: 623-626.
7. Haijun L, Zhiheng H, Xingwei G, Wenjuan L, Xuhui Z, et al. (2009) Iron-Catalyzed Selective Oxidation of N-Methyl Amines: Highly Efficient Synthesis of Methylene-Bridged bis-1,3-Dicarbonyl Compounds. Org Let 11: 4176-4179.
8. Mukund Pramanik MD, Ruchir K, Namrata R (2014) Synthesis of 3-carbonyl pyrazole-5- phosphonates via 1,3-dipolar cycloaddition of BestmanneOhira reagent with ynones Tetrahedron 70: 5214-5220.
9. Science of synthesis: (2002) Houben-Weyl methods of molecular transformations 12: 85.
10. Huisgen R (1963) 1,3-Dipolar Cycloadditions Past and Future. Angew Chem 2: 565-632.
11. Duen-Ren H, Ting-Chun K, Yu-Kai Li, Richmond L, Kuo-Wei H (2010) Electronic effects of ruthenium-catalyzed [3þ2]-cycloaddition of alkynes and azides. Tetrahedron 66: 9415-9420.
12. Hyojin C, Kyongkyu L, Dae Yoon C (2017) Synthesis of N-unsubstituted 1,2,3-triazoles via aerobic oxidative N-dealkylation using copper(II) acetate. Tetrahedron 73: 2878-2885.
13. Kwok SW, Fotsing JR, Rodionov VO, Fokin VV (2010) Transition-Metal-Free Catalytic Synthesis of 1,5-Diaryl-1,2,3-triazoles. Org Lett 12: 4217-4219.
14. Van Berkel SS, Brauch S, Gabriel L, Henze M, Strak S, et al. (2012) Traceless Tosylhydrazone-Based Triazole Formation: A Metal-Free Alternative to Strain-Promoted Azide–Alkyne Cycloaddition. Angew Chem Int Ed 51: 1-5.
15. Ramachandra Reddy P, Padmaja A, Padmavathi V (2014) Synthesis of

Heteroaryl Thiazolidinones and Azetidinones under Conventional and Ultrasonication Methods. J Heterocyclic Chem 52: 1474-1482.

16. Reddy PR, Seenaiah D, Padmaja A, Padmavathi V, Siva Krishna N (2015) Synthesis, antioxidant, and cytotoxic activities of bis(oxazolyl/thiazolyl/imidazolyl) amidomethanesulfonyl Acetamides. Med Chem Res 24: 86-98.
17. Yuliya VR, Ludmyla VB, Petro PO, Anatoly DS (2013) Cycloaddition of N-substituted imines of trifluoropyruvate with diazomethane: Efficient synthesis of 2-(trifluoromethyl) aziridine-2-carboxylates. J Fluorine Chem 148: 14-18.
18. Azoro C (2002) Antibacterial activity of crude extract of Azadirachta indica on Salmonella typhi. World J Biotechnol 3: 347-357.
19. Janovska D, Kubikova K, Kokoska L (2003) Screening for Antimicrobial Activity of Some Medicinal Plants Species of Traditional Chinese Medicine. J Food Sci 21: 107-110.
20. Bishnu J, Sunil L, Anuja S (2009) Antibacterial Property of Different Medicinal Plants: Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana. J Sci Eng Technol 5: 143-150.
21. National Committee for Clinical Laboratory Standards (1993) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 3rd edn. Approved Standard, NCCLS Publication M7-A3, Villanova, PA, USA.
22. National Committee for Clinical Laboratory Standards (1992) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Proposed Standard, NCCLS Document M27-P, Villanova, PA, USA.
23. French GL (2006) Bactericidal agents in the treatment of MRSA infections-the potential role of daptomycin. J Antimicrob Chemother 58: 1107.
24. Mounyr B, Moulay S, Koraichi IS (2016) Methods for in vitro evaluating antimicrobial activity. Journal of Pharmaceutical Analysis 6: 71-79.