SYNTHESIS AND PHARMACOLOGICAL STUDIES OF NEW PYRAZOLE ANALOGUES

K. Nandini[1], Y. B. Basavaraju[1]* and N. K. Hemanth Kumar[2]

[1]*Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysuru-570006, Karnataka, India*

[2]*Department of Studies in Botany, University of Mysore, Manasagangotri, Mysuru-570006, Karnataka, India.*

Abstract: A new class of pyrazole analogues was synthesized by three step reaction in good yields. Initially selected chalcones were treated with hydrazine hydrate in alkali medium through claisen condensation reaction. Further, chloroacetyl pyrazoline derivatives were synthesized by condensation of pyrazoline derivatives with chloroacetyl chloride followed by coupling of different secondary amines afforded target compounds. The structures of newly synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and MASS as data. The synthesized compounds were screened for their biological activities.

***Keywords*:** Pyrazole, chalcone, antibacterial activity and antifungal activity.

INTRODUCTION

Pyrazole derivatives are an important class of heterocyclic compounds that are known to exhibit important biological properties. They have displayed wide range of biological activities as antibacterial, anticancer, antimicrobial and fungi static[1-6]. Promoted with the above mentioned studies and our research interest in the synthesis and biological evaluation of novel pyrazole analogues[7].

In the present work three step synthetic strategies were employed for the preparation of pyrazole analogues. In the first step, Pyrazole derivatives are obtained by cyclisation of chalcones in presence of hydrazine hydrate in absolute ethanol by refluxing for 10-12hrs[8]. Further treatment with chloroacetyl chloride obtains a set of cloroacetyl pyrazole derivatives[9]. In the final step, compound was coupled with different secondary amines in presence of diethyl ether accomplished the desired series of pyrazole analogues[10]. The structures of compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, and MASS spectroscopic techniques.

EXPERIMENTAL

Materials and methods

All the reagents and solvents were purchased from Merck, Alfa aiser and sigma Aldrich chemical AR grade and used as provided. Thin layer chromatography (TLC) analysis was performed with alumina sheets precoated with silica gel and SiO_2, 60-120 mesh (Merck) was used for column chromatography. ^{1}H NMR and ^{13}C NMR spectra were obtained by bruker spectrometer in the (DMSO-d_6) solvent with TMS an internal reference. The mass spectra were recorded on LC-MS-Agilent 1100 series mode. Elemental analysis was performed on Leco CHNS-932 analyzer. Melting points were obtained on a reichert thermo pan melting point apparatus, equipped with a microscope and are uncorrected.

Synthesis

General procedure for the preparation of Pyrazoline derivatives (2a, 2b)

A mixture of chalcone **1(a,b)** (5milimoles) and hydrazine hydrate (5milimoles) were dissolved in absolute alcohol (30mL) and refluxed for 10-12hours. The reaction mixture was poured into crushed ice and stirred well; the solid thus obtained was filtered off and washed with water. Progress of the reaction was monitored by TLC using hexane: ethyl acetate (8:2 ratio) mixture as mobile phase. After the completion of the reaction, the reaction mixture was washed with 10% HCl solution followed by water; the organics were dried over anhydrous sodium sulphate. The yellow solid was obtained by removing solvent using a rotary evaporator at room temperature affords pyrazole derivatives **(2a, 2b)**.

5-methyl-3-phenyl-4,5-dihydro-1H-pyrazole ***(2a)***

The white solid, m.p.: 184-190 ^{0}C, Yield-86%. IR (KBr) γ_{max} (cm^{-1}): 3036 aryl (C-H) and 1618 (C=N); 1331(C-N), 1264(CH_2Cl); ^{1}H NMR (DMSO-d_6 400MHz) δppm: 7.57-7.69 (m, 5H, Ar-CH), 7.20 (s, 1H, N-H of pyrazoline), 2.95-3.23 (dd, 2H, CH_2 of pyrazoline, J= 4.5, 5.9 Hz), 2.7 (t, 1H, CH of pyrazoline, J= 4.0 Hz), 1.4 (s, 3H,CH_3); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm: 145.4, 142.1, 128.3, 125.9, 49.5, 40.2, 20.0; MS (ESI) m/z: 160.10 (M^+). Anal. Calcd. for $C_{10}H_{12}N_2$: C, 74.97; H, 7.55; N, 17.48; found: C, 74.98; H, 7.58; N, 17.54%.

3,5-diphenyl-4,5-dihydro-1H-pyrazole ***(2b)***

Pale yellow solid, m.p.: 172-174 ^{0}C, Yield-87%. IR (KBr) γ_{max} (cm^{-1}): 3321 (N-H) and 1619 (C=N); ^{1}H NMR (DMSO-d_6 400MHz) δppm: 7.32-7.70 (m, 10H, Ar CH), 7.19 (s, 1H, N-H of pyrazoline), 3.62-3.89 (dd, 2H, CH_2 of pyrazoline, J= 6.0, 6.6 Hz), 4.82(t, 1H, CH of pyrazoline, J= 8.0 Hz); ^{13}C NMR (DMSO-d_6 100MHz) δppm: 145.4, 142.1, 128.3, 125.9, 49.5, 40.2, 20.0; MS (ESI) m/z: 222.12 (M^+). Anal. Calcd. for $C_{15}H_{14}N_2$: C, 81.05; H, 6.35; N, 12.60; found: C, 81.12; H, 6.29; N, 12.74 %.

***General procedure for the preparation of chloroacetyl Pyrazoline derivatives* (3a, 3b)**

Chloroacetyl pyrazoline derivatives **3(a, b)** were prepared by dissolving pyrazoline derivatives **2(a, b)** (0.01mole) in a beaker containing 30ml of dry benzene placed on a mechanical stirrer. In another beaker containing 30ml of dry benzene with chloroacetyl chloride (0.01mole) was added drop wise to the beaker containing pyrazoline derivative. The stirring continued vigorously until the reaction mixture is so thick (3-4hrs). Then the reaction mixture is removed and crushed ice was added, after few hours the product was filtered off and washed with water. Finally the product obtained was recrystallized from ethanol.

2-chloro-1-(3-methyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(3a)***
The white solid, m.p.: 172-174 ºC, Yield-87%. IR (KBr) γ_{max} (cm^{-1}): 3034 (Aryl C-H), 1682 (C=O), 1332 (C-N) and 1619 (C=N), 1265 (CH_2Cl); ^1H NMR (DMSO-d_6 400MHz) δ ppm: 7.39-7.52(m, 5H, Ar-H), 5.1(t, 1H, CH of pyrazoline, J= 8.0 Hz) 3.62 (s, 2H, CH_2), 2.95-3.23 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 5.5 Hz), 2.31 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:167.2, 146.7. 140.5, 128.1, 128.4, 64.8, 43.2, 39.7, 20.1; MS (ESI) m/z: 236.04 (M^+). Anal. Calcd. for $C_{12}H_{13}ClN_2O$: C, 60.89; H, 5.54; found: C, 60.72; H, 5.95%.

2-chloro-1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***(3b)***
The brown solid, m.p.: 172-174 ºC, Yield-79%. IR (KBr) γ_{max} (cm^{-1}): 3036 (Aryl C-H), 1681 (C=O), 1331 (C-N) and 1619 (C=N), 1264 (CH_2Cl); ^1H NMR (DMSO-d_6 400MHz) δppm: 7.22-7.76(m, 10H, Ar-H), 4.7(t, 1H, CH of pyrazoline, J= 8.0 Hz), 3.93 (s, 2H, CH_2), 3.65-3.69 (dd, 2H, CH_2 of pyrazoline, J= 5.0, 6.5 Hz); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:165.2, 151.8. 141.6, 136.3, 131.1, 128.4, 126.5, 65.5, 43.1, 40.2; MS (ESI) m/z: 298.04 (M^+). Anal. Calcd. for $C_{17}H_{15}ClN_2O$: C, 68.34; H, 5.06; found: C, 68.32; H, 5.09%.

General procedure for the preparation of Pyrazole analogues (4a-e) and (5a-e)
The Pyrazole analogues **(4a-e)** and **(5a-e)** were obtained by the reaction of **(3a, 3b)** with different heterocyclic compounds **(scheme-1**) in ethyl acetate and in presence of triethylamine as base. The reaction mixture was refluxed for about 4hrs, after the completion of the reaction, reaction mass was cooled to room temperature and distilled off. The resultant residue is treated with water (30ml) with stirring. The separated solid was filtered, washed with water and dried**.**

1-(3-methyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-(piperazin-1-yl)ethanone ***(4a)***
The yellow solid, m.p.: 192-194 ºC, Yield-81%. IR (KBr) γ_{max} (cm^{-1}): 3096 (Aryl C-H), 3328 (N-H), 1550 (C=C), 1690 (C=O), 1128 (C-N) and 1620 (C=N)); ^1H NMR (DMSO-d_6 400MHz) δppm: 7.20-7.42 (m, 5H, Ar-H), 4.8 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.32 (s, 2H, CH_2), 2.90-3.23 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 4.5 Hz), 2.61 (t, 4H, J= 4.0 Hz, CH_2 of piperazine), 2.36 (t, 4H, J= 4.0 Hz, CH_2 of piperazine), 2.08 (s,3H, CH_3), 1.82 (s, 1H, N-H of piperazine); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:166.2, 146.8. 140.6, 128.3, 126.1, 65.8, 60.0, 57.6, 46.4, 39.6, 20.1; MS (ESI) m/z: 286.18 (M^+). Anal. Calcd. for $C_{16}H_{22}N_4O$: C, 67.11; H, 7.74; found: C, 67.23; H, 7.72%.

1-(3-methyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-morpholinoethanone ***(4b)***
The white solid, m.p.: 184-189 ºC, Yield-84%. IR (KBr) γ_{max} (cm^{-1}): 3097 (Aryl C-H), 1549 (C=C), 1692 (C=O), 1130 (C-N) and 1622 (C=N); ^1H NMR (DMSO-d_6 400MHz) δppm: 7.32-7.45 (m, 5H, Ar-H), 4.85 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.68 (t, 4H, methylene group neighboring to oxygen atom in morpholine), 3.25 (s, 2H, CH_2), 2.91-3.14 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 4.5 Hz), 2.46 (t, 4H, methylene group in morpholine), 2.2(s, 3H, CH_3); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:169.2, 147.8. 140.6, 142.2, 127.3, 126.1, 66.2, 59.6, 55.6, 39.7, 20.2; MS (ESI) m/z: 287.16 (M^+). Anal. Calcd. for $C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; found: C, 66.83; H, 7.39%.

1-(3-methyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-(piperidin-1-yl)ethanone ***(4c)***
The white solid, m.p.: 196-199 ºC, Yield-80%. IR (KBr) γ_{max} (cm^{-1}): 3099 (Aryl C-H), 1128 (C-N) and 1620 (C=N), 1548 (C=C), 1691 (C=O); ^1H NMR (DMSO-d_6 400MHz) δppm: 7.27-7.49 (m, 5H, Ar-H), 4.86 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.27 (s, 2H, CH_2), 2.92-3.28 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 4.5 Hz), 2.41 (t, 4H, J= 3.0 Hz, methylene group in pyridine); 2.14 (s, 3H, CH_3), 1.59 (t, 2H, J= 3.0 Hz, methylene group in piperidine), 1.55 (t, 4H, J= 3.0 Hz, methylene group in piperidine); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:169.1, 148.0. 141.6, 128.3, 126.1, 66.0, 59.1, 57.6, 39.9, 25.2, 24.3, 20.1; MS (ESI) m/z: 285.18 (M^+). Anal. Calcd. for $C_{17}H_{23}N_3O$: C, 71.55; H, 8.12; found: C, 71.52; H, 8.14%.

1-(3-methyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-thiomorpholinoethanone ***(4d)***
The yellow solid, m.p.: 187-190 ºC, Yield-84%. IR (KBr) γ_{max} (cm^{-1}): 3094 (Aryl C-H), 1127 (C-N) and 1622 (C=N), 1549 (C=C), 1695 (C=O), 589 (C-S); ^1H NMR (DMSO-d_6 400MHz) δppm: 7.35-7.49 (m, 5H, Ar-H), 4.93 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.27 (s, 2H, CH_2), 2.97-3.12 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 4.5 Hz), 2.74 (t, 4H, methylene group of thiomorpholine), 2.56 (t, 4H, methylene group neighboring to sulfur atom in thiomorpholine), 2.21 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:168.8, 147.3. 141.5, 128.2, 126.5, 66.3, 60.4, 59.2, 40.1, 28.2, 20.2; MS (ESI) m/z: 303.14 (M^+). Anal. Calcd. for $C_{16}H_{21}N_3OS$: C, 63.33; H, 6.98; found: C, 63.35; H, 6.81%.

1-(3-methyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-(pyrrolidin-1-yl)ethanone ***(4e)***
The white solid, m.p.: 189-194 ºC, Yield-82%. IR (KBr) γ_{max} (cm^{-1}): 3090 (Aryl C-H), 1126(C-N) and 1624 (C=N), 1545(C=C), 1693 (C=O); ^1H NMR (DMSO-d_6 400MHz) δppm: 7.28-7.45 (m, 5H, Ar-H), 4.85 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.29 (s, 2H, CH_2), 2.95-3.21 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 4.5 Hz), 2.55 (t, 4H, methylene group nighboring to N-atom of pyrrolidine), 2.13 (s, 3H, CH_3), 1.72 (t, 4H, methylene group of pyrrolidine); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:169.1, 147.5. 141.6, 128.6, 126.3, 66.1, 60.8, 59.1, 40.2, 23.2, 20.3; MS (ESI) m/z: 271.17 (M^+). Anal. Calcd. for $C_{16}H_{21}N_3O$: C, 70.82; H, 7.80; found: C, 70.75; H, 7.84%.

1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-(piperazin-1-yl)ethanone ***(5a)***
The brown solid, m.p.: 196-200 ºC, Yield-79%. IR (KBr) γ_{max} (cm^{-1}): 3092 (Aryl C-H), 3330(N-H), 1125(C-N) and 1626 (C=N), 1547(C=C), 1691 (C=O); ^1H NMR (DMSO-d_6 400MHz) δppm: 7.32-7.75 (m, 10H, Ar-H), 4.92 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.71 4.18 (dd, 2H, CH_2 of pyrazoline, J= 5.0, 5.5 Hz), 3.27 (s, 2H, CH_2), 2.67 (t, 4H, J= 3 Hz, methylene group in piperazine), 2.39 (t, 4H, J= 3 Hz, methylene group in piperazine), 1.95 (t, 1H, N-H of piperazine); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:169.1, 151.5, 141.6, 136.3, 131.6, 128.2, 126.6, 65.9, 59.7, 57.2, 45.4, 39.1; MS (ESI) m/z: 348.20 (M^+). Anal. Calcd. for $C_{21}H_{24}N_4O$: C, 72.39; H, 6.94; found: C, 72.41; H, 6.92%.

1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-morpholinoethanone ***(5b)***
The white solid, m.p.: 191-194 °C, Yield-80%. IR (KBr) γ_{max} (cm^{-1}): 3091 (Aryl C-H), 3332 (N-H), 1124 (C-N) and 1627 (C=N), 1548 (C=C), 1690 (C=O); ^{1}H NMR (DMSO-d_6 400MHz) δppm: 7.34-7.65(m, 10H, Ar-H), 4.95 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.77-3.96 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 4.5 Hz), 3.68 (t, 4H, J= 4.0 Hz methylene group neighboring to oxygen atom in morpholine), 3.31 (s, 2H, CH_2), 2.59 (t, 4H, J= 3.0 Hz methylene group in morpholine); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:169.2, 151.4, 141.5, 136.4, 131.5, 128.2, 126.6, 66.7, 64.8, 59.2, 56.4, 39.0; MS (ESI) m/z: 349.18 (M^+). Anal. Calcd. for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; found: C, 72.11; H, 6.65%.

1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-(piperidin-1-yl)ethanone ***(5c)***
The brown solid, m.p.: 193-197 °C, Yield-83%. IR (KBr) γ_{max} (cm^{-1}): 3093 (Aryl C-H), 3331(N-H), 1126(C-N) and 1623 (C=N), 1550(C=C), 1691(C=O); ^{1}H NMR (DMSO-d_6 400MHz) δppm: 7.31-7.75 (m, 10H, Ar-H), 4.92 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.6-3.9 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 4.5 Hz), 3.29 (s, 2H, CH_2), 2.43 (t, 4H, methylene group in pyridine), 1.61 (t, 2H, J= 3.0 Hz, methylene group in piperidine), 1.55 (t, 4H, J= 3.0 Hz, methylene group in piperidine); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:169.0, 151.3, 141.5, 136.3, 131.4, 129.0, 127.2, 65.7, 59.2, 57.4, 39.2, 25.5, 24.5; MS (ESI) m/z: 347.20 (M^+). Anal. Calcd. for $C_{22}H_{25}N_3O$: C, 76.05; H, 7.25; found: C, 76.11; H, 7.24%.

1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-thiomorpholinoethanone ***(5d)***
The brown solid, m.p.: 193-197 °C, Yield-83%. IR (KBr) γ_{max} (cm^{-1}): 3093 (Aryl C-H), 3331 (N-H), 1126 (C-N) and 1623 (C=N), 1550 (C=C), 1691 (C=O); ^{1}H NMR (DMSO-d_6 400MHz) δppm: 7.33-7.65(m, 10H, Ar-H), 4.94 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.65-3.90 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 5.5 Hz), 3.23 (s, 2H, CH_2), 2.75 (t, 4H, methylene group of thiomorpholine), 2.59 (t, 4H, methylene group neighboring to sulfur atom in thiomorpholine); ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:169.2, 151.5, 141.4, 136.2, 131.3, 128.5, 126.2, 65.5, 60.5, 59.2, 39.1, 28.1; MS (ESI) m/z: 347.20 (M^+). Anal. Calcd. for $C_{21}H_{23}N_3OS$; C, 69.01; H, 6.34; found: C, 69.10; H, 6.32%.

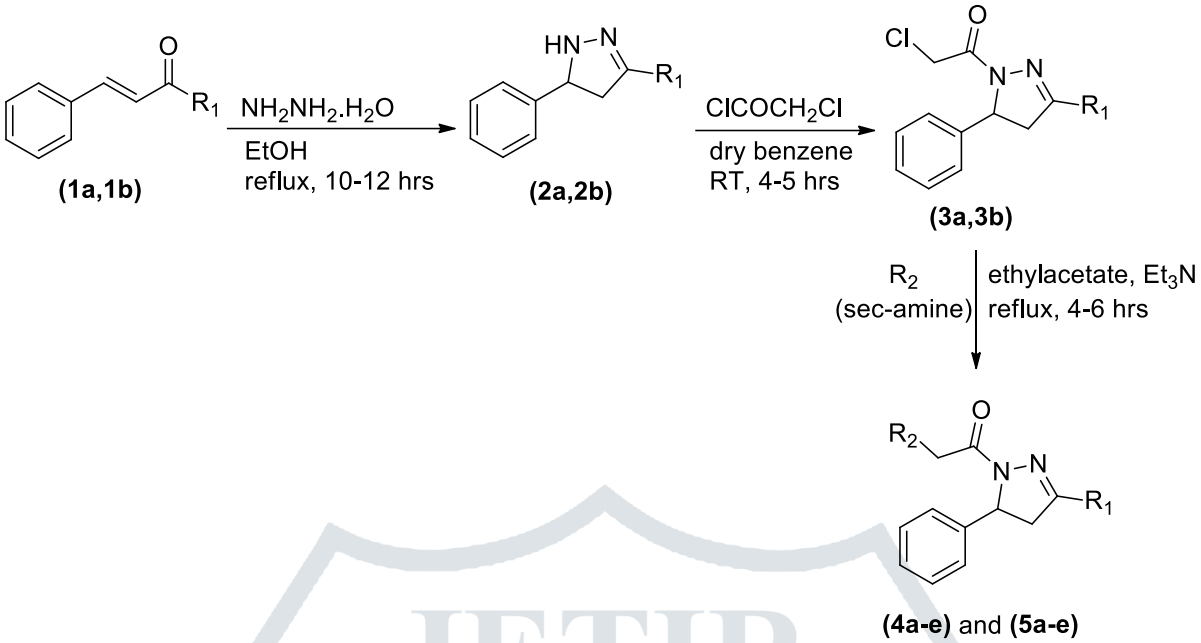
1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-(pyrrolidin-1-yl)ethanone ***(5e)***
The brown solid, m.p.: 185-190 °C, Yield-82%. IR (KBr) γ_{max} (cm^{-1}): 3091 (Aryl C-H), 3335(N-H), 1127 (C-N) and 1625 (C=N), 1551 (C=C), 1690 (C=O); ^{1}H NMR (DMSO-d_6 400MHz) δppm: 7.16-7.73(m, 10H, Ar-H), 4.91 (t, 1H, CH of pyrazoline, J= 5.0 Hz), 3.70-3.86 (dd, 2H, CH_2 of pyrazoline, J= 4.0, 4.8 Hz), 3.39 (s, 2H, CH_2), 2.56 (t, 4H, J= 3.0 Hz methylene group nighboring to N-atom of pyrrolidine),1.71 (t, 4H, J= 3.0 Hz methylene group of pyrrolidine),; ^{13}C NMR (DMSO-d_6 100MHz) δ ppm:169.0, 151.3, 141.2, 136.1, 131.1, 128.2, 126.4, 65.7, 60.4, 59.1, 39.5, 23.1; MS (ESI) m/z: 333.18 (M^+). Anal. Calcd. for $C_{21}H_{23}N_3O$; C, 75.65; H, 6.95; found: C, 75.71; H, 6.91%.

Antimicrobial studies
Antibacterial activity
The antimicrobial assay was performed by agar disc diffusion method[12-14].For antibacterial activity, the molten Mueller Hinton Agar (HiMedia) was inoculated with the 100 µl of the inoculum (1 x 108 Cfu) and poured into the sterile Petri plates (Himedia). For agar disc diffusion method, the disc (0.7 cm) (Hi-Media) was saturated by using 10.0 mg/ml of the test compound in 100ml of dimethylformamide (DMF), allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C for 24 hrs. Antibacterial activity of all the novel pyrazole analogues (4a-e) and (5a-e) was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi antibiotic zone scale). In this study medium with solvent dimethylformamide (DMF) was used as a negative control whereas Chloromphenicol (standard antibacterial drug) was positive control. The experiments were performed in triplicates and standard deviation was calculated.

Antifungal activity
Disc diffusion method was performed to evaluate antifungal activity of novel pyrazole analogues **(4a-e)** and **(5a-e)** by dissolving in dimethylformamide (DMF)[15]. In this method, antifungal activities of chemical compounds were evaluated by using one week old culture of the mold as inoculums. The molten Mueller Hinton Agar (HiMedia) was inoculated with the 100 µl of the inoculum (1 x 108 Cfu) and poured into the sterile Petri plates (Himedia) and the disc (0.7cm) (Hi-Media) was saturated with 100 µl of 10.0 mg/ml of the test compound in the dimethylformamide (DMF), allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 25^{0}C for 7 days. Antifungal activity of all the novel pyrazole analogues **(4a-e)** and **(5a-e)** was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi antibiotic zone scale). Dimethylformamide (DMF) was used as a negative control where as Nystatin (standard antifungal drug) was positive control. The experiments were performed in triplicates and standard deviation was calculated.
The antibacterial activity of newly synthesized compounds **(4a-e)** and **(5a-e)** were determined by disc diffusion method on nutrient agar media. In this work using as a control against *E. faecalis*, *K. pneumonia*, *E. aerugenes*, *P. aerugenosa* and *A. faecalis* were used to investigate the activity. The test compounds were dissolved in Dimethylformamide (DMF) at concentration of 10 mg/ml using Chloromphenicol as standard drug. The antifungal activity of the compounds were carried out against *F. verticillioides* and *A. niger* at concentration of 10 mg/ml using Nystatin as standard drug. Dimethylformamide (DMF) was used as negative control. The antimicrobial activity data is reported in **table 1** and **2**. In the antimicrobial activity, the synthesized compounds showed good and moderate activity against all the bacterial and fungal strains.

RESULTS AND DISCUSSION
Chemistry
Generally, chalcones are considered to be useful intermediates in several cyclization reactions to produce different types of heterocyclic compounds of diverse biological importance, according to the reactants used and the reaction conditions[11]. 4-phenyl-3-buten-2-one **1a** and benzaldehyde chalcones **1b** are separately treated with hydrazine hydrate in ethanol furnished 5-methyl-3-phenyl-4, 5-dihydro-1H-pyrazole **2a** and 3,5-diphenyl-4,5-dihydro-1H-pyrazole **2b**. In the next step pyrazole derivatives treated with chloroacetyl chloride in the presence of dry benzene as solvent afforded 2-chloro-1-(5-methyl-3-phenyl-4,5-dihydro-1H-

pyrazol-1-yl)ethanone **3a** and 2-chloro-1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone **3b**. Finally the conjugation of chloroacetyl pyrazole derivatives with different secondary amines in presence of triethylamine as base and ethyl acetate as solvent accomplished the desired pyrazole analogues **(4a-e)** and **(5a-e).**



Scheme-1: The reaction pathway for the synthesis of pyrazole analogues **4(a-e)** and **5(a-e)**

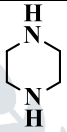
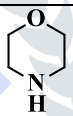
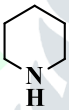
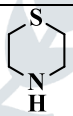
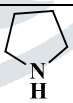
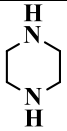
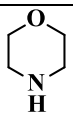
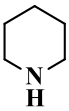
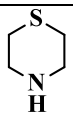
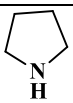
Compound No.	Entry R_1	Entry R_2
4a	CH_3	
4b	CH_3	
4c	CH_3	
4d	CH_3	
4e	CH_3	
5a	C_6H_5	
5b	C_6H_5	
5c	C_6H_5	
5d	C_6H_5	
5e	C_6H_5	

Table-1: Antibacterial activity of the compounds (4a-e) and (5a-e). Inhibitory zone (diameter) mm of the synthesized compounds against tested bacterial strains by disc diffusion method

Comp. No.	*E. faecalis*	*K.pneumonia*	*E.aerogenes*	*P.aeruginosa*	*A. faecalis*
Conc. in mg/mL	10.0	10.0	10.0	10.0	10.0
Control	00	00	00	00	00
Chloramphenicol	18.3 ± 0.07	18.7 ± 0.06	19.4 ± 0.06	18.8 ± 0.06	18.6 ± 0.02
4a	15.8 ± 0.04	14.5 ± 0.02	13.1 ± 0.02	12.7 ± 0.06	13.4 ± 0.04
4b	17.1 ± 0.02	15.2 ± 0.02	13.6 ± 0.03	14.8 ± 0.04	13.2 ± 0.04
4c	12.4 ± 0.01	10.3 ± 0.04	11.5 ± 0.02	10.1 ± 0.04	09.9 ± 0.06
4d	10.3 ± 0.04	09.3 ± 0.02	09.5 ± 0.01	08.8 ± 0.01	09.1 ± 0.01
4e	12.8 ± 0.01	11.6 ± 0.02	12.2 ± 0.01	12.3 ± 0.04	13.1 ± 0.01
5a	15.6 ± 0.04	14.2 ± 0.02	12.5 ± 0.04	12.8 ± 0.02	13.2 ± 0.04
5b	17.4 ± 0.02	15.7 ± 0.02	13.5 ± 0.03	13.3 ± 0.04	14.0 ± 0.01
5c	11.4 ± 0.01	10.1 ± 0.06	11.0 ± 0.01	10.6 ± 0.02	09.8 ± 0.04
5d	09.8 ± 0.02	08.9 ± 0.04	09.3 ± 0.06	08.1 ± 0.02	09.4 ± 0.04
5e	13.3 ± 0.01	12.6 ± 0.02	11.5 ± 0.04	12.1 ± 0.01	13.4 ± 0.01

Values are means of triplicates. Standard 10 mg/disc.

Table-2: Antifungal activity of the compounds (4a-e) and (5a-e). Inhibitory zone (diameter) mm of the synthesized compounds against tested fungal strains by disc diffusion method.

Comp. No.	*F.verticillioides*	*A. niger*
Conc. in mg/mL	10.0	10.0
Control	00	00
Nystatin	22.0 ± 0.06	18.0 ± 0.04
4a	15.7 ± 0.04	14.4 ± 0.02
4b	17.4 ± 0.01	15.8 ± 0.04
4c	12.1 ± 0.01	10.6 ± 0.04
4d	10.3 ± 0.02	9.5 ± 0.04
4e	14.1 ± 0.01	12.5 ± 0.02
5a	14.8 ± 0.02	14.3 ± 0.04
5b	16.7 ± 0.04	15.9 ± 0.02
5c	11.6 ± 0.02	10.1 ± 0.04
5d	09.9 ± 0.04	09.3 ± 0.02
5e	13.5 ± 0.01	12.3 ± 0.02

Values are Mean of triplicates. Standard 10 mg/disc

CONCLUSION

In conclusion, we have synthesized a series of pyrazole analoues **(4a-e)** and **(5a-e)** in good yields and evaluated their antimicrobial activity. Among the synthesized analogues, Compounds **4a, 4e, 5a, 5e** exhibited considerable activity against all antibacterial and antifungal strains Compounds **4b, 5b** showed good activity and remaining compounds **4c, 4d 5c, 5d** exhibited least activity against all the bacterial and fungal strains at concentration of 10.0 mg/mL compared to standard drug Chloramphenicol and Nystatin respectively.

Acknowledgments

University Grants Commission, New Delhi is gratefully acknowledged for proving grant under RGNF for SC. IOE, University of Mysore is acknowledged for providing spectral facilities. University of Mysore, Mysuru is acknowledged.

REFERENCES

[1] Jin, W., Yu, H. and Yu, Z. 2011. Regioselective synthesis of multisubstituted pyrazoles *via* cyclocondensation of β-thioalkyl-a,b-unsaturated ketones with hydrazines. Tetrahedron Letters, 52:5884-5887.

[2] Chandrakantha, B., Arun, M.I., Prakash, S.,Shrikrishna, I., Shridhar, M. and Hoong, K.F. 2012. Synthesis, characterization and antimicrobial activity of novel ethyl 1-(*N*-substituted)-5-phenyl-1*H*-pyrazole-4-carboxylate derivatives. Medicinal Chemistry Research, 21:2702-2708.

[3] Abdel-Aziz, H.A., EI-Zahabi, H.S.A. and Dawood, K.M. 2010. Microwave-assisted synthesis and *in-vitro* anticancer activity of 1,3,4-triaryl-5-*N*-arylpyrazole-carboxamides. European Journal of Medicinal Chemistry, 45:2427-2432.

[4] Abdel-Aziz, H.A., Mekaway, A.A.I. and Dawood, K.M. 2009. Convenient synthesis and antimicrobial evaluation of some novel 2-substituted-3-methylbenzofuran derivatives. European Journal of Medicinal Chemistry, 44:3637-3644.

[5] Sridar, R., Perumal, P.T., Etti, S. and Shanmugam, G. 2004. Design, synthesis and anti-microbial activity of 1*H*-pyrazole carboxylates. Bioorganic Medicinal Chemistry Letters, 14:6035-6040.

[6] Wagna, W., Wardakhan, Nadia, A. and Louca, J. 2007. Chilean Chemical Society, 52:1145-1149.

[7] Mohamad, Y. and Payal, J. 2011. Synthetic and biological studies of pyrazolines and related heterocyclic compounds. Arabian Journal of Chemistry, 7:553-596.

[8] Kavitha, N.V., Kalpana Divekar, Brahmani Priyadarshini, Gajanan, S. and Manjunath, M. 2011. Synthesis and antimicrobial activities of some new pyrazole derivatives. Dar Pharma Chemic, 3:55-62.

[9] Sanmati K. Jain and P. Mishra. 2004. Preparation and evaluation of some 1,3,4-thiadiazoles as diuretic agents. Indian Journal of Chemistry, 43:84-88.

[10] Rangaswamy, J., Vijay Kumar, H., Harini, S.T. and Nagaraja Naik. 2017. Functionalized 3-(benzofuran-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole scaffolds: A new class of antimicrobials and antioxidants. Arabian Journal of Chemistry, 10:S2685–S2696.

[11] Yadav, P.P., Gupta, P., Chaturvedi, A.K., Shukla, P.K. and Maurya, R. 2005. Synthesis of 4-hydroxy-1-methylindole and benzo[*b*]thiophen-4-ol based unnatural flavonoids as new class of antimicrobial agents. Bioorganic Medicinal Chemistry, 13:1497-1505.

[12] Umesha, B., Basavaraju, Y.B., Mahendra, C., Shivakumara, S.B., Poornachandra Raoc, K. and Krishna, M.H. 2014. Synthesis and biological activity of novel nitrogen containing analogues of podophyllotoxin. Indo American Journal of Pharmaceutical Research, 4:905-914.

[13] Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45:493-496

[14] Srisurang, T., Churairatana, N. and Samaniya, S. 2002. Modified antimicrobial disc susceptibility testing for nutritionally-variant streptococci. Southeast Asian Journal of Tropical Medicine and Public Health, 33:151-154.

[15] Umesha, B. and Basavaraju, Y.B. 2014. Synthesis and Pharmacological Studies of New Pyrazole Analogues of Podophyllotoxin. Russian Journal of Bioorganic Chemistry, 40:467-476.