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Synthesis of novel pyrazolic analogues of chalcones as potential antibacterial and antifungal agents

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A B S T R A C T

The present includes the synthesis of pyrazoline derivatives using chalcones and phenyl hydrazine in the presence of ethanol. It also reveals that 2-pyrazoline complexes are physiologically active and may be used in a variety of therapeutic functions. FT-IR, ^{1}H-NMR, ^{13}C-NMR, LC-MS, and elemental analyses were used to characterise newly synthesised phenyl-pyrazoline derivatives. The antimicrobial activity of the synthesised compounds was assessed using the agar well diffusion assay and the Minimum Inhibition Concentration (MIC). Compounds **4b**, **4f**, and **4h** have exhibited remarkable antibiotic action against bacterial strains such as *Staphylococcus aureus, Bacillus subtilis, Salmonella. Typhi, and Shigella sp*. On the other hand, compound **4h** had significant antifungal activity against *Candida albicans* and *Aspergillus fusarium* and was a highly promising agent when compared to standard nystatin.

1. Introduction

Antimicrobial resistance (AMR) is a major threat to human health worldwide.[1] The development of antibacterial treatments that involve oxidative stress as part of their mechanism of action has received attention in recent years.[2] Antimicrobial activity and drug-molecular target interaction are essential in the treatment of pathogenic microbial illnesses (e.g., malaria, cough, TB, plague).[3] Drug interactions can dramatically reduce microorganism resistance[4] antibiotic resistance has become common. The development of innovative, highly active medicines with adequate active sites for strong interaction with the target is required for the treatment of resistant microbial infections. Molecular hybridization, which combines two or more active pharmacophoric components to form a new hybrid molecule with increased potency[5,6] is a potential technique for the design and development of novel biological molecules. The existence of conjugated phenyl and the -C=N-NC- moity in chalcone derived pyrazoline contribute to electron delocalization and resonance production, both of which are significant for the molecule's stability and biological activity. It has been demonstrated that the presence of electron donating and electron withdrawing groups as pyrazoline substituents has unique antibacterial effect.[7]

Chalcone is a well-known natural substance[8] derivative made up of α,β-unsaturated ketones and phenyl group. These compounds have a -C=O-CH=CH- keto ethylenic moiety in their structure. Chalcones are used to synthesise heterocyclic compounds and undergo several chemical processes. By reacting aromatic aldehydes with aryl ketones in the presence of condensing agents[9-15] a diverse spectrum of chalcone derivatives can be synthesised.[16] In their aromatic rings, they have a delocalized -electron configuration.[17] It is a critical mechanism for the synthesis of new heteroaromatic compounds,[18] includes epoxide,[19] pyrimidine,[20] pyrazoline[21] and pyrazole.[22] Substitutes on both aromatic rings influenced solubility factor and binding interactions significantly.[23] The α, β-unsaturated ketone has been identified as the responsible molecule for

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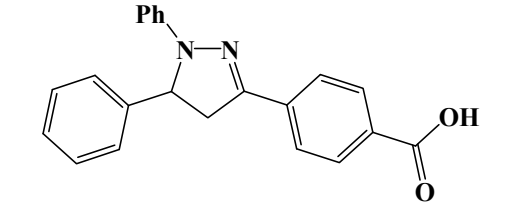
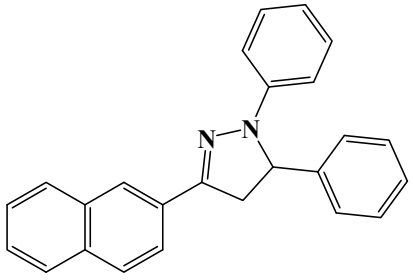
antibacterial and antifungal properties in chalcone and because it is readily convinced to stabilise both rings, these active chalcone derivatives show outstanding activity in drug discovery.[24]

For many decades, pyrazoline and thiazole scaffolds have been extensively studied and discovered to have a diverse range of chemical reactivity and pharmacological activities.[25] These five-membered heterocyclic compounds have been found to have antibacterial activity as well as the ability to delocalize free radicals and produce stable DPPH fragments.[26] Pyrazole compounds have also been shown to exhibit anti-microbial,[27] anti-viral,[28] anti-diabetic,[29] anti-convulsant,[30] anti-oxidant,[31] anti-HIV,[32] anti-inflammatory,[33] Alzheimer's disease,[34] and anti-cancer properties.[35] Previous studies have shown that 3,5-diaryl-4,5-dihydro-1H-pyrazole derivatives, which are representatives of the 2-pyrazoline class, have been increasingly studied using a variety of structural manipulations and that the steric and electronic properties of the various substituents at different positions, in addition to the chirality aspects of these molecules, have a significant effect on activity.[36] The N-N bond link of the pyrazoline ring is thought to be important in their biological activity.[37,38] Because of their low cost, nontoxicity, reducing waste and recycling characteristics,[39,40] they have been used as a heterogeneous catalyst for a variety of organic processes.[41] Here, we synthesized certain pyrazoline compounds with improved bioactive potential that have multiple heterocyclic moieties which are naturally bioactive. Utilizing conventional method; the entire library of compounds was synthesized. Additionally, to increase the biological potential, A literature survey reveals that many of the pyrazolines were prepared and proven that these scaffolds have tremendous biological potential **Fig. 1**.

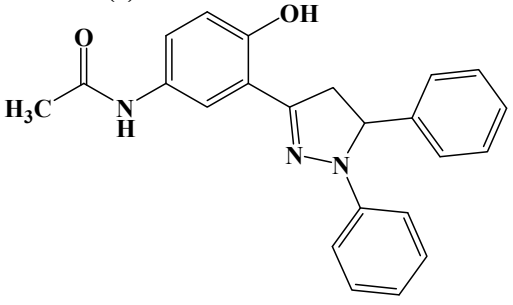


4-(1,5-Diphenyl-4,5-dihydro-1*H*-pyrazol-3-yl) -benzoic acid



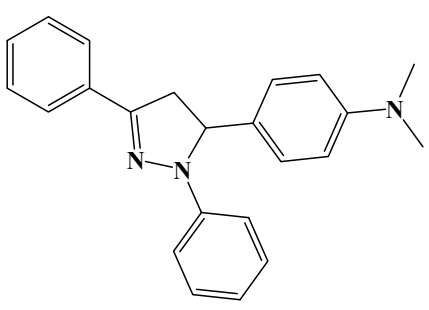
3-Naphthalen-2-yl-1,5-diphenyl-4,5-dihydro-1*H*-pyrazole

(1)



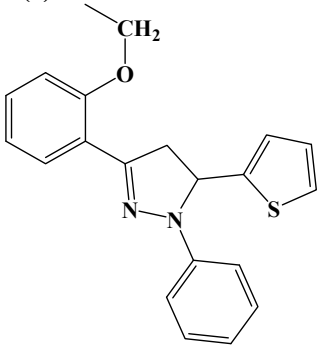
N-[3-(1,5-Diphenyl-4,5-dihydro-1*H*-pyrazol -3-yl)-4-hydroxy-phenyl]-acetamide

(2)



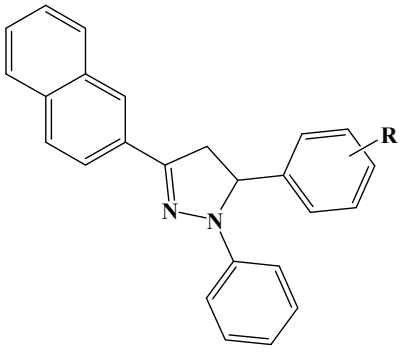
[4-(2,5-Diphenyl-3,4-dihydro-2*H*-pyrazol-3-yl) -phenyl]-dimethyl-amine

(3)



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

(4)



2-naphthyl pyrazoline derivative

(5) **(6)**

Fig 1. Phenyl pyrazoline structures with diverse biological functions (1-6)

2. Results and discussion

2.1. Chemistry

We aimed to obtain a novel 3-(2,5-Diphenyl-4,4-dihydro-2*H*-pyrazol-3-yl)-1*H*-quinoline-2-ones by ring closure of 1-methyl-3((*E*)-(3-oxo-3-phenylprop-1-enyl)quinoline-2(1*H*)-one based on the assessed utility of pyrazolines.[42,43] The synthesis of chalcones **(3a-i)** and their derivatives **(4a-i)** was carried out as shown in Scheme. Compounds **(3a-i)** were obtained with a 40-80% yield by the equimolar condensation of acetophenones and aldehyde in basic medium. The compound **(3a)** was characterized by IR study of the chalcones revealed a distinct band in the region 3146.39 cm^{-1} and 2709.66 cm^{-1} which corresponds to aromatic and aliphatic C-H stretching frequencies. The presence of -C=O was validated by an absorption band at 1635.87 cm^{-1} generated by a α,β-unsaturated carbonyl system. The usual C=C absorption band emerges at 1560.40 cm^{-1}. The 400 MHz ^{1}H-NMR spectra of all the compounds revealed a singlet at δ 3.79 ppm integrating for three protons of the group linked to the quinoline ring N-CH_3. The characteristic α,β-unsaturated protons of chalcones appeared as two doublets at δ 7.96 ppm and 8.46 ppm. Multiplets of ten aromatic protons were observed between 7.29 and 8.10 ppm. ^{13}C-NMR spectra of all the compounds showed signals at δ 29.87 ppm for quinoline ring N-CH_3. At 184.27 ppm the C=O signal was detected and other aromatic carbons appeared in the region at δ 76.84-141.82 ppm. Mass spectra all the compounds displayed a molecular ion peak that corresponded to their molecular formula.

The essential substituted pyrazoline scaffold **(4a-i)** was formed by treating chalcones **(3a-i)** with phenyl hydrazine in the presence of alkaline medium. The **Scheme 1** depicts the synthesis pathway for pyrazoline derivatives. IR, Mass, ^{1}H-NMR, and ^{13}C-NMR spectrum investigations verified the production of phenyl pyrazoline **(4a)**. The FT-IR spectra of pyrazoline revealed an absorption peak of aromatic and aliphatic C-H stretching at 3008.8 cm^{-1} and 2757.5 cm^{-1} respectively. The distinctive peak for the C=O linked to the pyrazoline ring was seen at 1640.4 cm^{-1}. The peaks at 1621.7 cm^{-1} 1460.1 $cm^{-1}$and 1042.16 $cm^{-1}$are attributable to C=N, C=C and C-N respectively. The ^{1}H-NMR spectra of the compounds revealed two sharp singlets at 2.71 ppm, 1.23 ppm integrating for three protons of pyrazoline -CH, -CH_2 with coupling constant and 3.71 ppm for quinoline ring N-CH_3. The pyrazoline -CH_2 protons with coupling constant at 7.7 Hz to a triplet. The pyrazoline -CH proton with coupling constant at 7.6 Hz to a doublet. Similarly, a multiplet of fifteen protons was seen at 8.66-7.47 ppm indicating presence of aromatic protons. The ^{13}C-NMR spectra it was confirmed that the most characteristic signal appearance peak at δ 22.24 was attributed to (N-CH_3) and other aromatic carbons appeared in the region at δ 114.08-169.33 ppm. Further, the structure was confirmed by its mass spectrum. It gave a molecular ion peak at m/z 379.45. Similarly, the series of pyrazolines **(4b-i)** showed a ^{1}H NMR, ^{13}C NMR, IR the disappearance of the alkenyl (CH=CH) and carbonyl (C=O) group was found to be absent and the appearance of pyrazoline ring supports the formation of expected compounds.

All the newly synthesized compounds **(4a-i)** were evaluated for their antimicrobial activity by agar well diffusion assay against two Gram positive (*S. aureus*, *B. subtilis*), two Gram negative bacteria (*S. Typhi* and *Shigella*) and (*C. albican* and *Fusarium*) fungal stains. Many of the compounds show comparable activity and some of them exhibit potent activity compared to standard (Gentamicin and Fliconazole). Comparing the series of all the synthesized compounds, the compound number **4b**, **4f**, and **4h** showed the significant inhibition against bacterial strains with zones of inhibition values between 10 and 12 mm, as compared to standard 14 mm and 10 mm, the results were summarized in **Table 1**. From the above results, we planned to carry out MIC of **4b**, **4f**, and **4h**in different concentrations ranging from 25-100 µg/mL, the results were summarized in **Table 1 and 2**.

2.2. Spectral detail

2.2.1. 3-(2,5-Diphenyl-4,4-dihydro-2H-pyrazol-3-yl)-1H-quinoline-2-one (4a)

Yellow solid, M.P. 191ºC, IR (cm^{-1}): 3008.8 (Ar-C-H), 2757.5 (Aliphatic C-H), 1640.4 (C=O), 1621.7 (C=N), 1460.1 (C=C), 1042.16 (C-N); ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.66 (s, 2H), 8.30 (d, *J* = 15.7 Hz, 2H), 8.03 (d, *J* = 8.0 Hz, 2H), 7.86 (d, *J* = 15.7 Hz, 2H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.35 (t, *J* = 7.5 Hz, 1H), 3.71 (s, 3H), 2.71 (p, *J* = 7.7 Hz, 1H), 1.23 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (101 MHz, Chloroform-d) δ 169.33, 161.12, 155.63, 139.42, 139.0, 133.68, 131.45, 130.52, 129.21, 128.84, 128.31, 128.20, 126.30, 126.77, 122.47, 120.15, 114.08, 57.02, 40.59, 29.68, 22.24; LC-MS (ESI Positive): *m/z* = $(M_+H)_+$): 379.45; Anal. Calcd for $C_{25}H_{21}N_3$: C, 79.13; H, 5.58; N, 11.07. Found: C, 79.18; H, 5.65; N, 11.12.

2.2.2. 1-Methyl-3-[5-(3-nitro-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-1H-quinolin-2-one (4b)

Brown solid, M.P. 224ºC, IR (cm^{-1}): 3047.1 (Ar-C-H), 2931.6 (Aliphatic C-H), 1643.8 (C=O), 1591.6 (C=N), 1460.0 (C=C), 1382.13 (C-NO_2), 1035.8 (C-N); ^{1}H NMR (400 MHz, DMSO-D_6) 8.22 – 8.00 (m, 2H), 7.78 – 7.62 (m, 2H), 7.66 – 7.56 (m, 1H), 7.56 (d, *J* = 1.7 Hz, 1H), 7.58 – 7.41 (m, 3H), 7.45 – 7.22 (m, 2H), 7.18 (td, *J* = 9.0, 7.2 Hz, 3H), 7.02 (d, *J* = 8.1 Hz, 2H), 6.75 (t, *J* = 7.3 Hz, 1H), 5.58 (dd, *J* = 12.6, 6.2 Hz, 1H), 3.96 (dd, *J* = 17.7, 12.7 Hz, 1H), 3.68 (s, 3H), 3.21 (dd, *J* = 17.7, 6.2 Hz, 2H); ^{13}C NMR (101 MHz, Chloroform-d) δ 169.60, 160.93, 153.30, 148.58, 139.47, 137.6, 134.90, 133.34, 132.27, 130.77, 129.89, 129.84, 129.25, 128.33, 126.68, 126.34, 124.67, 122.56, 121.55, 120.00, 114.13, 58.01, 39.99, 29.65, 22.25; LC-MS (ESI Positive): *m/z* = $(M_+H)_+$): 424.45; Anal. Calcd for $C_{25}H_{20}N_4$: C, 70.74; H, 4.75; N, 13.20. Found: C70.78; H, 4.80; N, 13.26.

2.2.3. 1-Methyl-3-[5-(4-nitro-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-1H-quinolin-2-one (4c)

Brown solid, M.P. 229ºC, IR (cm^{-1}): 3017.8 (Ar-C-H), 2919.8 (Aliphatic C-H), 1687.1 (C=O), 1660.7 (C=N), 1417.6 (C=C), 1392.6 (C-NO_2), 1100.8 (C-N); ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.67 (s, 1H), 8.59 – 8.50 (m, 0H), 8.42 – 8.34 (m, 2H), 8.31 (s, 1H), 8.27 (d, *J* = 1.4 Hz, 2H), 8.25 (d, *J* = 2.0 Hz, 1H), 7.88 (d, *J* = 15.7 Hz, 1H), 7.79 – 7.65 (m, 2H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.33 (t, *J* = 7.4 Hz, 1H), 3.96 (dd, *J* = 17.7, 12.7 Hz, 1H), 3.68 (s, 3H), 3.21 (dd, *J* = 17.7, 6.2 Hz, 2H); ^{13}C NMR (101 MHz, Chloroform-D) δ 169.68, 160.92, 153.26, 148.55, 139.49, 137.6, 137.59, 134.92, 130.82, 129.84, 129.26, 128.36, 127.44, 126.68, 126.35, 124.09, 122.59, 120.00, 114.17, 77.48, 77.16, 76.84, 58.18, 39.91, 29.67, 22.23; LC-MS (ESI Positive): *m/z* = $(M_+H)_+$): 424.45; Anal. Calcd for $C_{25}H_{20}N_4$: C, 70.74; H, 4.75; N, 13.20. Found: C, 70.80; H, 4.79; N, 13.27.

2.2.4. 3-[5-(4-Amino-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-1-methyl-1H-quinolin-2-one (4d)

Dark brown solid, M.P. 231ºC, IR (cm^{-1}): 3436.3 (C-NH)3080.8 (Ar-C-H), 2922.2 (Aliphatic C-H), 1813.8 (C=O), 1636.6 (C=N), 1447.0 (C=C), 1093.2 (C-N); δ 8.56 (s, 1H), 8.20 (d, *J* = 15.6 Hz, 1H), 7.83 (d, *J* = 8.6 Hz, 2H), 7.78 – 7.69 (m, 3H), 7.65 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 2H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 6.60 (d, *J* = 8.6 Hz, 2H), 6.15 (s, 2H), 3.96 (dd, *J* = 17.7, 12.7 Hz, 1H), 3.67 (s, 3H), 3.21 (dd, *J* = 17.7, 6.2 Hz, 2H);); ^{13}C NMR (101 MHz, Chloroform-d) δ 169.30, 155.22, 140.08, 139.10, 133.82, 130.57, 129.19, 128.32, 128.00, 127.65, 126.30, 122.52, 120.12, 119.46, 114.11, 57.02, 40.54, 29.68, 24.85, 22.21; LC-MS (ESI Positive): *m/z* = $(M_+H)_+$): 394.47; Anal. Calcd for $C_{25}H_{22}N_4$: C, 76.12; H, 5.62; N, 14.20. Found: C, 76.16; H, 5.67; N, 14.24.

2.2.5. 3-[5-(4-Methoxy-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-1-methyl-1H-quinolin-2-one (4e)

Yellow solid, M.P. 250ºC, IR (cm^{-1}): 3040.9 (Ar-C-H), 2889.9 (Aliphatic C-H), 2833.0 (0-CH_3), 1856.4 (C=O), 1639.1 (C=N), 1455.3 (C=C), 1109.2 (C-N); ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.49 (s, 1H), 8.16 (dd, *J* = 25.2, 7.6 Hz, 2H), 7.74 – 7.64 (m, 2H), 7.61 – 7.53 (m, 3H), 7.22 (q, *J* = 9.0 Hz, 3H), 7.07 (d, *J* = 7.6 Hz, 2H), 6.80 (t, *J* = 7.3 Hz, 1H), 5.63 (dd, *J* = 12.7, 6.3 Hz, 1H), 4.00 (dd, *J* = 17.7, 12.6 Hz, 2H), 3.72 (s, 3H), 3.25 (dd, *J* = 17.7, 6.2 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-d) δ 169.12, 161.47, 161.17, 155.41, 139.39, 137.62, 133.52, 133.37, 132.26, 130.71, 130.47, 129.72, 129.54, 129.20, 128.37, 124.09, 122.44, 120.17, 114.22, 114.06, 56.84, 55.52, 40.70, 29.67, 22.22; LC-MS (ESI Positive): *m/z* = $(M_+H)_+$): 409.48; Anal. Calcd for $C_{26}H_{23}N_3$: C, 76.26; H, 5.66; N, 12.17. Found: C, 76.31; H, 5.70; N, 12.21.

2.2.6. 1-Methyl-3-(2-phenyl-5-p-tolyl-3,4-dihydro-2H-pyrazol-3-yl)-1H-quinolin-2-one (4f)

Yellow solid, M.P. 239ºC, IR (cm^{-1}): 3196.0 (Ar-C-H), 2999.5 (Aliphatic C-H), 1760.8 (C=O), 1658.6 (C=N), 1495.8 (C=C), 1033.2 (C-N); ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.49 (s, 1H), 8.15 (dd, *J* = 25.2, 7.6 Hz, 2H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 6.3 Hz, 1H), 7.59 (d, *J* = 6.9 Hz, 1H), 7.55 (d, *J* = 7.5 Hz, 2H), 7.22 (q, *J* = 9.0 Hz, 3H), 7.06 (d, *J* = 7.6 Hz, 2H), 6.79 (t, *J* = 7.3 Hz, 1H), 5.62 (dd, *J* = 12.7, 6.3 Hz, 1H), 3.99 (dd, *J* = 17.7, 12.6 Hz, 2H), 3.72 (s, 3H), 3.25 (dd, *J* = 17.7, 6.2 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-D) δ 169.24, 161.14, 155.74, 140.86, 139.38, 138.57, 133.52, 130.71, 130.48, 129.54, 129.19, 128.65, 126.71, 127.68, 126.70, 122.44, 120.16, 114.06, 56.84, 40.68, 29.67, 22.22, 21.64; LC-MS (ESI Positive): *m/z* = $(M_+H)_+$): 393.48; Anal. Calcd for $C_{26}H_{23}N_3$: C, 79.36; H, 5.89; N, 10.68. Found: C, 79.41; H, 5.92; N, 10.72.

2.2.7. 3-[5-(4-Chloro-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-1-methyl-1H-quinolin-2-one (4g)

Yellow solid, M.P. 196ºC, IR (cm^{-1}): 3047.1 (Ar-C-H), 2931.6 (Aliphatic C-H), 1649.3 (C=O), 1619.5 (C=N), 1593.4 (C=C), 1360.5 (C-N); ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.60 (s, 1H), 8.24 (d, *J* = 15.6 Hz, 2H), 7.87 (d, *J* = 8.7 Hz, 2H), 7.77 (d, *J* = 15.6 Hz, 2H), 7.72 – 7.65 (m, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.34 (t, *J* = 7.5 Hz, 1H), 6.64 (d, *J* = 8.9 Hz, 1H), 6.19 (s, 2H), 3.71 (s, 3H); ^{13}C NMR (101 MHz, Chloroform-D) δ 169.37, 161.05, 154.51, 139.43, 136.42, 134.05, 130.61, 130.34, 129.99, 129.22, 129.09, 129.01, 127.99, 126.83, 126.74, 122.50, 120.08, 114.10, 57.35, 40.35, 29.66, 22.21; LC-MS (ESI Positive): *m/z* = $(M_+H)_+$): 413.90; Anal. Calcd for $C_{25}H_{20}N_3$: C, 72.55; H, 4.87; N, 10.15. Found: C, 72.60; H, 4.90; N, 10.20.

2.2.8. 3-[5-(2-Hydroxy-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-1-methyl-1H-quinolin-2-one (4h)

Yellow solid, M.P. 204ºC, IR (cm^{-1}): 3162.0 (OH), 3033.3 (Ar-C-H), 2909.3 (Aliphatic C-H), 1643.7 (C=O), 1585.1 (C=N), 1381.8 (C=C), 1030.13 (C-N);^{1}H NMR (400 MHz, DMSO-D_6) δ 8.63 (s, 1H), 8.26 (d, *J* = 15.7 Hz, 1H), 8.03 – 7.95 (m, 2H), 7.82 (d, *J* = 15.7 Hz, 1H), 7.76 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.67 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1H), 7.55 (d, *J* = 8.5 Hz, 1H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.35 – 7.27 (m, 1H), 3.68 (s, 3H), 2.68 (q, *J* = 7.6 Hz, 2H), 1.19 (t, *J* = 7.6 Hz, 3H).^{13}C NMR (101 MHz, Chloroform-D) δ 168.36, 160.98, 158.13, 157.84, 139.48, 134.39, 132.44, 130.77, 129.92, 129.52, 129.28, 128.75, 128.40, 126.21, 122.60, 120.00, 119.90, 117.12, 115.27, 114.14, 55.87, 40.85, 29.69, 22.37; LC-MS (ESI Positive): *m/z* = $(M_+H)_+$): 395.45; Anal. Calcd for $C_{25}H_{21}N_3$: C, 75.93; H, 5.35; N, 10.63. Found: C, 75.97; H, 5.40; N, 10.69.

2.2.9. 3-[5-(4-Ethyl-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-1-methyl-1H-quinolin-2-one (4i)

Yellow solid, M.P. 220°C, IR (cm^{-1}): 3059.9 (Ar-C-H), 2918.4 (Aliphatic C-H), 1639.7 (C=O), 1584.0 (C=N), 1415.6 (C=C), 1010.1 (C-N); ^{1}H NMR (400 MHz, DMSO-D_6) δ 8.63 (s, 1H), 8.26 (d, J = 15.7 Hz, 1H), 8.03 – 7.95 (m, 2H), 7.82 (d, J = 15.7 Hz, 1H), 7.76 (dd, J = 7.8, 1.6 Hz, 1H), 7.67 (ddd, J = 8.6, 7.1, 1.6 Hz, 1H), 7.55 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 8.1 Hz, 2H), 7.35 – 7.27 (m, 1H), 3.68 (s, 3H), 2.68 (q, J = 7.6 Hz, 2H), 1.19 (t, J = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-D) δ 169.25, 161.15, 155.75, 147.20, 139.39, 133.51, 130.72, 130.48, 129.81, 129.62, 129.19, 128.89, 128.50, 128.38, 126.83, 126.73, 122.45, 120.16, 114.06, 56.84, 40.69, 29.68, 28.97, 22.22, 15.56; LC-MS (ESI Positive): m/z = $(M_+H)_+$): 407.51; Anal. Calcd for $C_{27}H_{25}N_3$: C, 79.58; H, 6.18; N, 10.31. Found: C, 79.62; H, 6.23; N, 10.37.

2.3. Antimicrobial activity

All the synthesized compounds were evaluated for their antimicrobial activity by agar well diffusion assay against two Gram-positive bacteria (*S. aureus*, *B. subtilis*), and two Gram-negative bacteria (*S. Typhi* and *P. aeruginosa*) using gentamicin as the standard; whereas the antifungal activity by agar well diffusion assay against two strains (*C. albicans and Fusarium)* were evaluated using fluconazole as the standard. The results suggested that many compounds showed comparable activities with some of them exhibiting potent antimicrobial activity in comparison to the standards.

From the results obtained by zone of inhibition studies, it was seen that among the series of all the synthesized compounds, compounds **4b**, **4f** and **4h** showed the excellent inhibition against the bacterial and fungal strains with zone of inhibition values ranging between 10 and 14 mm, in comparison with the standard. Taking a cue from these results, a Minimum inhibitory concentration (MIC) experiment was carried out for compounds **4b**, **4f** and **4h** in different concentrations ranging from 25 mg/mL to 100 mg/mL. Analysis of the values from the MIC experiment revealed that the aforementioned compounds exhibited potent antibacterial activities between 25 and 100 µg/mL. A systematic analysis of the data from the overall results showed that the synthesized compounds are capable of exhibiting enhanced antibacterial activity, against the Gram positive and the Gram-negative bacteria. The results showing the measurement of zones of inhibition created by these compounds in millimeters, and the values representing the MIC values of *in vitro* antimicrobial screening are summarized in **Table 1** &**Table 2** respectively.

Table 1. Antimicrobial activity (ZIC) of title compounds **(4a-i)** and reference drugs

Compounds	Zone of inhibition (in mm)					
	Antibacterial				Antifungal	
	S. aureus	*B. subtilis*	*S. Typhi*	*P. aeruginosa*	*C. albicans*	*Fusarium*
4a	8	9	8	9	9	8
4b	11	11	11	12	9	9
4c	10	10	11	10	8	7
4d	7	8	9	8	8	8
4e	6	6	6	7	7	7
4f	13	12	13	12	8	8
4g	9	9	8	9	6	5
4h	10	10	10	11	8	6
4i	9	8	8	9	8	8
Gentamicin	12	15	8	9	-	-
Fluconazole	-	-	-	-	10	14

Table 2. Minimum inhibitory concentration of the compound **4b**, **4f** and **4h**.

Culture	Minimum inhibitory concentration (MIC)				
	Compound	(25 µg/mL)	(50 µg/mL)	(75 µg/mL)	(100 µg/mL)
S. aureus	4b	24.17	32.96	56.04	79.12
	4f	27.47	43.95	67.03	86.81
	4h	20.87	30.76	37.36	43.35
B. subtilis	4b	20.98	51.85	64.19	85.18
	4f	19.75	49.38	70.37	87.65
	4h	8.64	46.91	67.90	58.02
S. Typhi	4b	17.85	35.71	52.38	76.92
	4f	9.52	33.33	51.19	78.02
	4h	11.90	39.28	48.80	63.73
P. aeruginosa	4b	12.5	27.5	55.0	83.75
	4f	11.25	22.5	60.0	80.0
	4h	5.0	21.25	41.25	55.0

3. Conclusion

Here, we have synthesized a novel chalcone by condensation of N-methyl quinoline 3-carbaldehyde with substituted ketone (acetophenone) and piperidine in alkaline medium, followed by chalcone Claisen condensation **(3a-i)**. Based on our earlier research, the ring closure of chalcones with phenyl hydrazine allows the novel pyrazoline family to have a potential substituted ring. As a result, the majority of pyrazoline ligands have favoured the poly dentate system of N-N donor atoms. The synthesis and antimicrobial screening of novel pyrazole chalcones **(4a-i)** were done as part of our present research work focusing on the discovery of new bioactive heterocyclic compounds. The antimicrobial evaluation data indicated that among the nine compounds examined, derivatives **4b, 4f** and **4h** demonstrated strong activity patterns against antibacterial and antifungal strains with notable values. This will be validated in the future plan.

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Declaration of Competing Interest

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4. Materials and Methods

4.1. General

All the reagents and chemicals were purchased from commercial sources obtained to be and utilised without purification. Thin-layer chromatography (TLC) was employed to monitor the completions of reactions at regular intervals utilising aluminium sheets (E. Merck) precoated with GF254 silica gel with 0.2 mm layer thickness, n-hexane: ethyl acetate (7:3) as a solvent, and detected in ultraviolet light. Melting points and other physical constants were calculated using an open capillary tube and are uncorrected. Shimadzu IR Affinity 1S (ATR) FT-IR spectrophotometer was used to validate the IR spectra of the synthesised compounds, and absorption frequencies are provided in cm^{-1}. The ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded using a Bruker Advance II 400 spectrophotometer. Chemical shifts (δ) are determined in ppm downfield using TMS as an internal standard and DMSO-$d_6$as a solvent at a sophisticated analytical equipment facility at Karnataka University's SAIF department in Dharwad, India.

4.2. General procedure

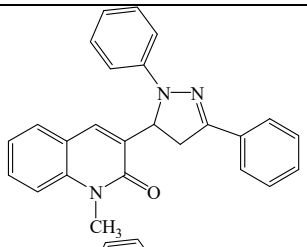
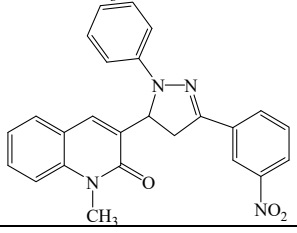
4.2.1. Synthesis of the compounds (3a-i)

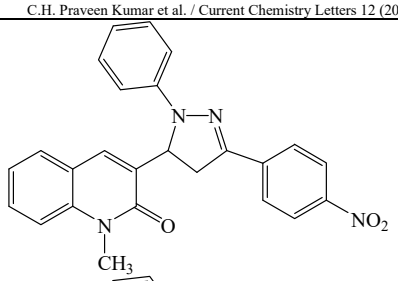
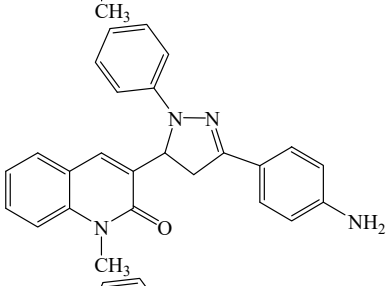
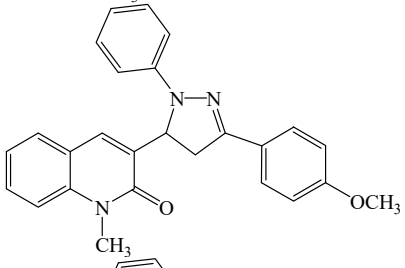
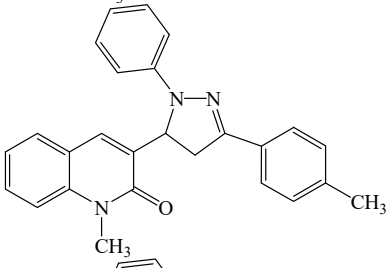
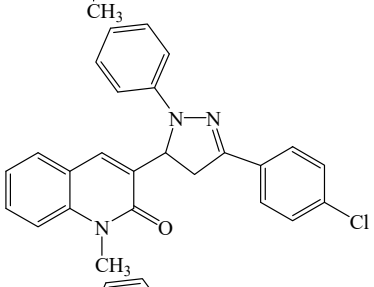
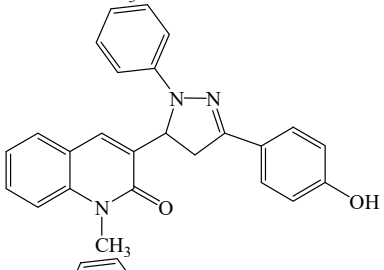
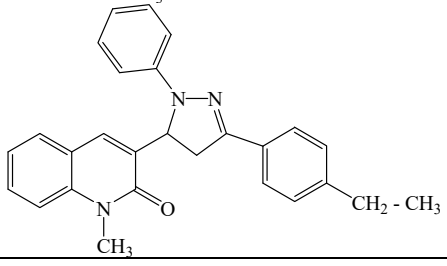
1,2-dihydro-1-methyl-2-oxoquinoline-3-carbldehyde **(1)** (0.01 mol) and 2 mL piperidine solution were added to a solution of substituted ketones **(2a)** (0.01 mol) in 10 mL absolute ethanol the mixture was refluxed for 5 hours. The progress of the reaction was monitored by TLC using n-hexane and ethyl acetate as eluent. The solution was then left at room temperature overnight. The solid crystals were separated by filtering, washed with absolute ethanol, dried and purified by recrystallization with pure ethanol were illustrated in **Scheme 1**. Compounds **(4b-i)** have also been synthesised in a similar manner and.

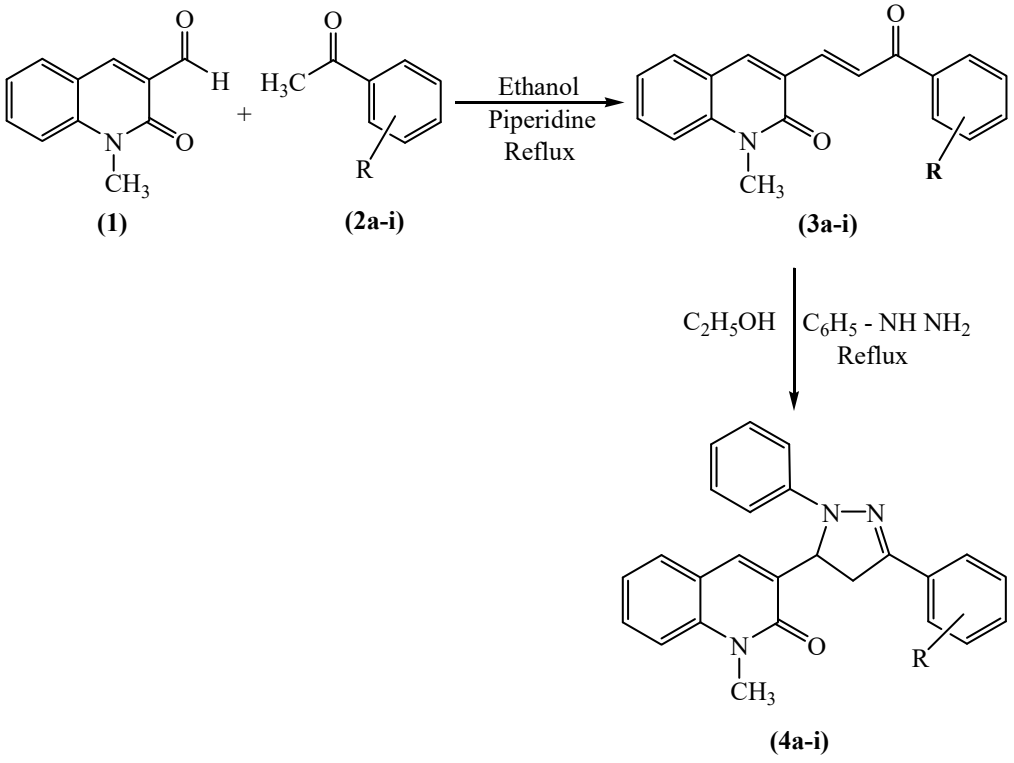
4.2.2. Synthesis of the compounds (4a-i)

To synthesise the 3-(2,5-Diphenyl-4,4-dihydro-2*H*-pyrazol-3-yl)-1*H*-quinoline-2-ones **(4a-i)**, In 25 mL of absolute ethanol, a combination of 1 mmol of compound **(3a-i)** and 2 mmol of phenyl hydrazine was dissolved and refluxed for 8 hours. TLC was used to monitor the development of the reaction, with n-hexane and ethyl acetate as eluents. The mixture was then placed in a beaker with 50 mL of ice-cold water. The obtained precipitate is filtered, washed with cold water and dried. Recrystallization of the product in absolute ethanol to get pure pyrazolines was illustrated in **Scheme 1**. A similar method was used to synthesise compounds **(4b-i)** and physical data of the final compounds are Illustrated in **Table 3**.

Table 3. Physical data of the final compounds

Compound No.	Structure	% Yield
4a		76.3
4b		80.6

4c		78.4
4d		83.0
4e		92.4
4f		94.3
4g		68.9
4h		76.5
4i		83.2



(1) **(2a-i)** **(3a-i)** **(4a-i)**

R= -H, -3-NO_2, -4NO_2, -4-NH_2, -4-OCH_3, -4-CH_3, -4-Cl, -2-OH, -4-CH_2-CH_3

Scheme 1. Synthesis of 3-(2,5-Diphenyl-4,4-dihydro-2*H*-pyrazol-3-yl)-1*H*-quinoline-2-ones from chalcone derivatives

5. Biological evaluation

5.1. Collection of microorganisms

Test bacterial cultures were procured from Microbial Type Culture Collection (MTCC) of Institute of Microbial Technology, Chandigarh. Cultures of Gram-positive bacteria *S. aureus* (MTCC 7443), *B. subtilis* (MTCC 121) and Gram-negative *S. Tyaphi* (MTCC 7410) and *P. aeruginosa* (MTCC 1036) were grown on nutrient agar media used for antibacterial activity assay. *C. albicans* (MTCC 3017), *Fusarium* (MTCC 1372) were grown on nutrient agar media used for antifungal activity assay.

5.2. Antibacterial assay

Antibacterial activity of chemical compounds was tested for dual-culture agar diffusion assay (Nostro *et al*., 2000) with some modifications. Petri dishes were prepared by pouring 20 ml of sterilized Nutrient agar media under aseptic conditions and allowed to solidify. After solidification of the media, 100 µL of standardized test microbial inoculums of Gram-positive bacteria and Gram-negative bacteria were spread uniformly using a glass loop. Concentration 1 mg/mL was tested, diluting the sample with DMSO was added to plates for diffusion of antibacterial compounds, thereafter plates were incubated at 37 ºC for 24 hours. The diameter of the inhibition zone around the well is measured in millimetres (mm) and the average of three repeated agar discs were taken to assess the strength of antibacterial activity. Gentamicin was considered standard.

5.3. Antifungal assay

Antifungal activity of different compounds was tested by agar well diffusion assay (Zhang *et al*., 2009) with some modifications. Petridishes were prepared by pouring 20 mL of sterilized PDA media under aseptic conditions and allowed to solidify. After solidification of the media, 100 µL of standardized test *Candida albicans and Fusarium* was spread uniformly using sterile L-shaped loop. a 6 mm diameter agar is drawn from plate to form a well using sterile cork borer. Antifungal Fluconazole was used as positive control, DMSO as negative control. After keeping at 4 ºC for 4 hours for the diffusion of antibacterial metabolites, thereafter plates were incubated at 28 ºC for 72 hours. The diameter of the inhibition zone around the well is measured in millimetre (mm) and the average of three repeated agar discs were taken to assess the strength of antibacterial activity.

5.4. MIC (Minimum inhibitory concentration)

Test microbial inoculum for MIC antibacterial assay were prepared according to Clinical Laboratory Standards Institute (CLSI, 2005). For the growth method, a loop is used to touch the top of three to five colonies of the same morphological type from an agar plate culture. This is suspended in 10 mL of a sterile Mueller Hinton broth (MHB) aseptically and incubated at 37 °C. The turbidity of the actively growing cells was adjusted to the 0.5 McFarland standard (at 625 nm, 0.08-0.01 absorbance in UV-VIS Spectrophotometer) using sterile broth to produce an standardized microbial of approximately 1-2×10^8 CFU/mL. 25-100 mg/mL of 100 µL is added to each plate for studying minimum inhibitory concentration at 625 mm.

This work clarifies that chemical compounds have a lot of applications as reported before in different scientific papers.

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