SYNTHESIS AND ANTI-MICROBIAL ACTIVITIES OF 1,3,5-TRISUBSTITUTED-PYRAZOLE DERIVATIVES CONTAINING A PYRIDYL MOIETY

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ABSTRACT

Claisen condensation of ethyl isonicotinate with different acetophenones gave the corresponding pyridyl- β -diketones, while the treatment with hydrazine hydrate yielded 3,5-disubstituted-1*H*-pyrazoles, which then converted 1,3,5-trisubstituted-pyrazole derivatives containing a pyridyl moiety by N-acylation with acyl chloride. The structures of all newly synthesized compounds are established by FTIR, ^1H NMR, mass spectroscopy and elemental analysis, and in the case of compound **2e**, analyzed by single-crystal X-ray diffraction further. The anti-microbial activities of the title compounds have been tested by disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae* and *Rhizoctnia solani*. The results showed that compounds **3c** and **4c** exhibited good inhibitory activity against all the tested organisms.

Key words: 1,3,5-Trisubstituted-pyrazole, β -Diketone, Synthesis, Anti-microbial activity

1. INTRODUCTION

Pyrazole derivatives are an interesting class of organic compounds, which are found to be associated with diverse pharmacological properties such as anti-microbial [1,2], anti-inflammatory [3], antihypertensive [4], antidepressant [5], antiviral [6] and anticancer [7] activities. The synthesis and applications of these compounds have received considerable attention in recent years [8-10]. In addition, the pyridyl ring is also a prominent heterocyclic scaffold in lot of bioactive molecules. Many pyridine-based compounds have been reported to exhibit versatile bioactivities, such as insecticidal, fungicidal, anticancer and antibacterial activities [11-13]. Keeping this in view, we choose the pyridyl ring as one of the aryl substituents about the central pyrazole ring to synthesize a series of new 1,3,5-trisubstituted-pyrazole derivatives containing a pyridyl moiety. Herein we synthesized some novel 1-acyl-3-(4-pyridyl)-5-aryl-pyrazole derivatives and presented the initial results of anti-microbial activities against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae* and *Rhizoctnia solani* of the title compounds.

2. RESULTS AND DISCUSSION

2.1 Chemistry

The synthesis of 1,3,5-trisubstituted-pyrazole derivatives is conducted as outlined in Scheme 1. The pyridyl- β -diketones **1a-e** were prepared via Claisen condensation of the required methyl ketones with ethyl isonicotinate in benzene using sodium amide as the condensing agent. The structures of **1a-e** were established under the basis of their elemental analysis and spectral data. Their ^1H NMR spectra exhibited the single peaks at $\delta = 4.60$ -4.68 attributed to the keto-CH₂ protons, the single peaks at $\delta = 6.83$ -6.93 assigned to the vinylic protons in enol and the single peaks at $\delta = 16.52$ -16.68 due to the enolic OH protons. The IR spectra data also demonstrated the presence of the C=O and enolic C=C stretching vibrations at 1600-1591 cm⁻¹ and 1545-1511 cm⁻¹ in the pyridyl- β -diketones, respectively.

Treatment of the pyridyl- β -diketones **1a-e** with hydrazine monohydrate in refluxing ethanol for 3h gave the 1*H*-pyrazoles **2a-e**. The IR spectra for compounds **2a-e** displayed the typical bands of the N-H stretching vibrations at 3135-3115 cm⁻¹ and their ^1H NMR spectra exhibited the single peaks at $\delta = 6.85$ -7.00 due to the 4-position protons of 1*H*-pyrazoles, but the N-H resonance of the pyrazole ring were not observed in CDCl₃. This is in agreement with the result in the literature [14]. In the case of **2e**, the structure was additionally solved by single-crystal X-ray diffraction. There are two molecules (**A** and **B**) in the asymmetric unit in the crystal structure. The labeled displacement ellipsoid plots of these two molecules are shown in Fig. 1. The comparison between molecule (**A**) and molecule (**B**) shows no significant differences in bond lengths and bond angles. However, their torsion angles and dihedral angles between the

phenyl ring and pyrazole ring exhibit some differences in the molecules. The torsion angles C6-C1-C7-C8, C2-C1-C7-C8, C6-C1-C7-C12, C2-C1-C7-C12, C11-C10-C15-C13 and C9-C10-C15-C13 are 153.16 (0.19)°, -25.36 (0.29)°, -26.02 (0.28)°, 155.47 (0.21)°, -27.05 (0.31)° and 150.25 (0.21)° in molecule (**A**), respectively, while C21-C26-C27-C28, C25-C26-C27-C28, C21-C26-C27-C32, C25-C26-C27-C32, C31-C30-C33-C34 and C29-C30-C33-C34 are 147.95 (0.21)°, -31.02 (0.29)°, -31.47 (0.29)°, 149.56 (0.20)°, -20.95 (0.31)° and 158.28 (0.19)° in molecule (**B**), respectively. The dihedral angles for the two benzene rings and the benzene ring with the pyrazole ring are 25.86° and 25.97° in molecule (**A**), respectively, but in molecule (**B**), the dihedral angles are 31.30° and 21.65°, respectively.

Scheme 1. The synthetic routes for compounds **3a-e** and **4a-e**.

The title compounds **3a-e** and **4a-e** were obtained by N-acylation of the 1*H*-pyrazoles **2a-e** with acyl chloride in the presence of triethylamine. Their structures were confirmed by elemental analysis, FTIR, ^1H NMR and mass spectroscopy. Their IR spectra showed the absorption bands at 1740-1710 cm⁻¹ attributed to the C=O stretching vibration of the N-acylation products. Their ^1H NMR spectra revealed the single peaks at $\delta = 6.73$ -7.19 corresponding to the vinylic protons of the pyrazole ring. The molecular ion peaks (M⁺) for these compounds were observed in accordance with the Nitrogen Rule. The mass spectra of the title compounds were compared to confirm elemental compositions.

2.2 Anti-microbial activity

The screening results of *in vitro* anti-microbial activities of compounds **3a-e** and **4a-e** are summarized in Table 1. All test data were the average values from triplicate runs. It is obvious from the data that these compounds possess

inhibitory activities to a certain degree against the tested microorganisms. It was observed that compounds **3c** and **4c** exhibited much higher activities against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae* and *R. solani* among all synthesized compounds, but compounds **3d** and **4d** revealed much lower activities. By the preliminary structure-activity relationship analysis, it was concluded that compounds **3a-e** had slightly better anti-microbial activities than compounds **4a-e** attributed to the strong electron-withdrawing property of the N1 acetyl group in the pyrazole nucleus [15]. Especially, the pyrazole derivatives with 4-fluorophenyl moiety at 5-position of the pyrazole ring showed potent anti-microbial activities against the tested microorganisms, being significantly higher than the rest (R = phenyl, 4-methylphenyl, 4-methoxyphenyl and 4-phenylphenyl). As a result, it indicated that the electronic nature of the substituent groups at para positions in the phenyl ring played a significant role in anti-microbial activities.

Fig. 1 View of compound **2e** with the atom-labeling scheme and 50% probability displacement ellipsoids.

Table 1 Anti-microbial data of 1,3,5-trisubstituted-pyrazole derivatives.

Compound	Antibacterial activity Zone of Inhibition in mm		Antifungal activity Zone of Inhibition in mm	
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. oryzae</i>	<i>R. solani</i>
3a	14	15	16	13
3b	11	12	12	10
3c	22	21	23	22
3d	9	10	12	11
3e	16	16	15	16
4a	13	15	14	12
4b	11	9	10	12
4c	20	21	22	20
4d	10	9	10	8
4e	17	15	16	15
Norflaxacin	31	30	—	—
Triadimefon	—	—	28	30

3. CONCLUSIONS

In conclusion, a series of new 1,3,5-trisubstituted-pyrazole derivatives containing a pyridyl moiety were synthesized by Claisen condensation, cyclization and N-acylation and their structures were characterized by FTIR, ¹H NMR, MS spectroscopy and elemental analysis. The anti-microbial activities of these compounds were evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Pyricularia oryzae* and *Rhizoctnia solani*. The results showed that these compounds showed certain degree of anti-bacterial and anti-fungal activities and compounds **3c** and **4c** were much higher active against the organisms employed among all synthesized compounds. Thus, this study provides a lead for synthesis and evaluation of more pyrazole derivatives for anti-microbial

activity as the same could lead to the discovery of some potential agents.

4. EXPERIMENTAL

Melting points were determined using X-4 digital melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet FTIR 5700 spectrophotometer with KBr pellets. The ¹H NMR spectra were measured on an advance III™ 300 MHz NB Digital NMR spectrometer in CDCl₃ solution with TMS as internal standard. Electrospray ionization mass spectra (ESI-MS) were performed with a Finnigan LCQ Advantage Max spectrometer. The elemental analysis (C, H, N) was carried out on a Perkin-Elmer 2400 elemental analyzer. Reagents were of analytical grade and were used without further purification.

4.1 Typical procedure for the synthesis of the pyridyl-β-diketones

To a suspension of sodium amide (1.4 g, 36 mmol) and ethyl isonicotinate (6.65 g, 44 mmol) in benzene (50 mL), A solution of the aryl methyl ketones (22 mmol) in benzene was added dropwise under stirring at 50 °C. The reaction mixture was refluxed for about 7 h until the dark yellow product precipitated. The precipitate was filtered off and washed with 5 % acetic acid until pH = 6. The crude products were recrystallized from ethanol (95 %) to give the pyridyl-β-diketones (**1a-e**).

4.1.1 1-Phenyl-3-(4-pyridyl)-1,3-propandione (**1a**)

Colorless needles, yield 70.5 %, mp 81-82 °C; ¹H NMR (CDCl₃, 300 MHz) δ: 4.65 (s, 0.08H, keto CH₃), 6.89 (s, 1H, enol CH), 7.50-7.63 (m, Ar-H, 3H), 7.79 (d, 2H, Py-H, *J* = 5.7 Hz), 8.01 (d, 2H, Ar-H, *J* = 7.8 Hz), 8.80 (d, 2H Py-H, *J* = 5.7 Hz), 16.55 (br s, 1H, enol OH) ppm; IR (KBr) n: 3419 (w), 3089 (m), 3040 (m), 2920 (m), 1591 (s), 1542 (s), 1480 (m), 1401 (m), 1290 (m), 1228 (m), 1064 (m), 764 (s), 692 (s) cm⁻¹; ESI-MS *m/z*: 226.13 [M+1]⁺. Anal. calcd. for C₁₄H₁₁NO₂: C 74.65, H 4.92, N 6.22; found C 74.89, H 4.88, N 6.25.

4.1.2 1-(4-Methylphenyl)-3-(4-pyridyl)-1,3-propandione (**1b**)

Yellow crystals, yield 68.2 %, mp 102-103 °C; ¹H NMR (CDCl₃, 300 MHz) δ: 2.44 (s, 3H, CH₃), 4.62 (s, 0.02H keto CH₃), 6.86 (s, 1H, enol CH), 7.31 (d, 2H, Ar-H, *J* = 8.1 Hz), 7.78 (d, 2H, Py-H, *J* = 5.7 Hz), 7.91 (d, 2H, Ar-H, *J* = 8.1 Hz), 8.79 (d, 2H, Py-H, *J* = 5.7 Hz), 16.61 (br s, 1H, enol OH) ppm; IR (KBr) n: 3434 (w), 3108 (m), 3029 (m), 2919 (m), 1599 (s), 1534 (s), 1486 (s), 1296 (m), 1231 (m), 1056 (m), 982 (m), 839 (m), 792 (s) cm⁻¹; ESI-MS *m/z*: 240.05 [M+1]⁺. Anal. calcd. for C₁₅H₁₃NO₂: C 75.30, H 5.48, N 5.85; found C 75.66, H 5.43, N 5.90.

4.1.3 1-(4-Fluorophenyl)-3-(4-pyridyl)-1,3-propandione (**1c**)

Yellow powder, yield 72.6 %, mp 113-114 °C; ¹H NMR (CDCl₃, 300 MHz) δ: 4.62 (s, 0.03H keto CH₃), 6.84 (s, 1H, enol CH), 7.20 (t, 2H, Ar-H, *J* = 8.4 Hz), 7.78 (d, 2H, Py-H, *J* = 5.7 Hz), 8.01-8.06 (m, 2H, Ar-H), 8.80 (d, 2H, Py-H, *J* = 6.0 Hz), 16.52 (br s, 1H, enol OH) ppm; IR (KBr) n: 3438 (w), 3075 (m), 3021 (m), 1600 (s), 1545 (m), 1507 (m), 1299 (m), 1227 (s), 1161 (m), 844 (m), 787 (s) cm⁻¹; ESI-MS *m/z*: 244.07 [M+1]⁺. Anal. calcd. for C₁₄H₁₀FO₂: C 69.13, H 4.14, N 5.76; found C 69.27, H 4.11, N 5.79.

4.1.4 1-(4-Methoxyphenyl)-3-(4-pyridyl)-1,3-propandione (**1d**)

Bright yellow powder, yield 68.4 %, mp 121-122 °C; ¹H NMR (CDCl₃, 300 MHz) δ: 3.90 (s, 3H, OCH₃), 4.60 (s, 0.03H keto CH₃), 6.83 (s, 1H, enol CH), 7.00 (d, 2H, Ar-H, *J* = 8.7 Hz), 7.78 (d, 2H, Py-H, *J* = 6.0 Hz), 8.00 (d, 2H, Ar-H, *J* = 9.0 Hz), 8.79 (d, 2H, Py-H, *J* = 6.0 Hz), 16.68 (br s, 1H, enol OH) ppm; IR (KBr) n: 3443 (w), 3031 (m), 2960 (m), 2900 (m), 2840 (w), 1598 (s), 1511 (m), 1311 (m), 1260 (s), 1177 (s), 1018 (m), 843 (w), 786 (s) cm⁻¹; ESI-MS *m/z*: 256.21 [M+1]⁺. Anal. calcd. for C₁₅H₁₃NO₃: C 70.58, H 5.13, N 5.49; found C 70.81, H 5.09, N 5.56.

4.1.5 1-(4-Phenylphenyl)-3-(4-pyridyl)-1,3-propandione (**1e**)

Yellow powder, yield 70.1 %, mp 134-135 °C; ¹H NMR (CDCl₃, 300 MHz) δ: 4.68 (s, 0.03H keto CH₃), 6.93 (s, 1H, enol CH), 7.40-7.52 (m, 3H, Ar-H), 7.66 (d, 2H, Ar-H, *J* = 7.8 Hz), 7.74 (d, 2H, Ar-H, *J* = 8.1 Hz), 7.82 (d, 2H, Py-H, *J* = 5.1 Hz), 8.09 (d, 2H, Ar-H, *J* = 8.4 Hz), 8.81 (d, 2H, Py-H, *J* = 5.4 Hz), 16.61 (br s, 1H, enol OH) ppm; IR (KBr) n: 3445 (w), 3041 (m), 1592 (s), 1517 (s), 1485 (s), 1409 (m), 1303 (s), 1217 (s), 1058 (m), 993 (m), 847 (s), 768 (s) cm⁻¹; ESI-MS *m/z*: 302.28 [M+1]⁺. Anal. calcd. for C₂₀H₁₅NO₂: C 79.72, H 5.02, N 4.65; found C 79.94, H 4.98, N 4.68.

4.2 Typical procedure for the synthesis of the 1H-pyrazoles

Hydrazine hydrate (6.0 mmol) was added dropwise to a solution of the required pyridyl-β-diketone (5.0 mmol) in 30 mL absolute ethanol under stirring and the mixture was refluxed at 78 °C for 3h. Then the mixture was cooled to room temperature and the solvent was removed by evaporation under reduced pressure to form a precipitate. The precipitate was recrystallized from ethanol (95 %) to obtain the 1H-pyrazoles (**2a-e**).

4.2.1 3-(4-Pyridyl)-5-phenyl-1H-pyrazole (**2a**)

Colorless powder, yield 88.9 %, mp 202-204 °C; IR (KBr): n 3115 (b, s), 3010 (m), 1602 (s), 1487 (m), 1467 (m), 1427 (m), 1391 (m), 1183 (m), 1057

(μ), 1000 (m), 957 (m), 826 (m), 759 (s), 708 (s) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 6.96 (s, 1H, C=CH), 7.39-7.50 (m, 3H, Ar-H), 7.67 (d, 2H, Ar-H, $J = 7.5$ Hz), 7.72 (d, 2H, Py-H, $J = 4.8$ Hz), 8.68 (d, 2H, Py-H, $J = 4.5$ Hz) ppm; ESI-MS: m/z 221.16 M^+ ; Anal. calcd. for $\text{C}_{14}\text{H}_{11}\text{N}_3$: C 76.00; H 5.01; N 18.99; found C 76.24; H 4.97; N 19.03.

4.2.2 3-(4-Pyridyl)-5-(4-methylphenyl)-1H-pyrazole (2b)

Colorless powder, yield 91.3 %, mp 210-211 $^{\circ}\text{C}$; IR (KBr): n 3116 (m), 3071 (m), 3006 (m), 2920 (w), 2855 (w), 1604 (s), 1510 (m), 1461 (m), 1185 (m), 1063 (m), 965 (m), 865 (w), 822 (s) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 2.38 (s, 3H, CH_3), 6.89 (s, 1H, C=CH), 7.21 (d, 2H, Ar-H, $J = 7.8$ Hz), 7.53 (d, 2H, Ar-H, $J = 7.8$ Hz), 7.66 (d, 2H, Py-H, $J = 4.8$ Hz), 8.63 (d, 2H, Py-H, $J = 5.1$ Hz) ppm; ESI-MS: m/z 235.07 M^+ ; Anal. calcd. for $\text{C}_{15}\text{H}_{13}\text{N}_3$: C 76.57; H 5.57; N 17.86; found C 76.80; H 5.52; N 17.89.

4.2.3 3-(4-Pyridyl)-5-(4-fluorophenyl)-1H-pyrazole (2c)

Colorless powder, yield 90.5 %, mp 189-191 $^{\circ}\text{C}$; IR (KBr): n 3135 (b, s), 3048 (m), 1607 (s), 1507 (s), 1458 (m), 1232 (s), 1168 (m), 1099 (m), 1064 (m), 973 (m), 836 (s), 800 (s), 698 (m) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 6.90 (s, 1H, C=CH), 7.13 (t, 2H, Ar-H, $J = 8.1$ Hz), 7.64 (d, 2H, Ar-H, $J = 8.7$ Hz), 7.67 (d, 2H, Py-H, $J = 5.1$ Hz), 8.66 (d, 2H, Py-H, $J = 4.8$ Hz) ppm; ESI-MS: m/z 239.14 M^+ ; Anal. calcd. for $\text{C}_{14}\text{H}_9\text{FN}_3$: C 70.28; H 4.21; N 17.56; found C 70.50; H 4.18; N 17.60.

4.2.4 3-(4-Pyridyl)-5-(4-methoxyphenyl)-1H-pyrazole (2d)

Colorless needles, yield 90.5 %, mp 196-197 $^{\circ}\text{C}$; IR (KBr): n 3117 (m), 3072 (m), 3026 (m), 2915 (w), 2845 (w), 1607 (s), 1509 (s), 1458 (m), 1290 (m), 1252 (s), 1180 (s), 1029 (m), 964 (m), 828 (s), 793 (m), 697 (m) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 3.85 (s, 3H, OCH_3), 6.85 (s, 1H, C=CH), 6.96 (d, 2H, Ar-H, $J = 7.8$ Hz), 7.58 (d, 2H, Ar-H, $J = 8.1$ Hz), 7.69 (d, 2H, Py-H, $J = 3.3$ Hz), 8.65 (d, 2H, Py-H, $J = 2.1$ Hz) ppm; ESI-MS: m/z 251.10 M^+ ; Anal. calcd. for $\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}$: C 71.70; H 5.21; N 16.72; found C 71.92; H 5.17; N 16.74.

4.2.5 3-(4-Pyridyl)-5-(4-phenylphenyl)-1H-pyrazole (2e)

Colorless powder, yield 92.8 %, mp 267-269 $^{\circ}\text{C}$; IR (KBr): n 3133 (m), 3031 (m), 1603 (s), 1480 (m), 1424 (m), 1351 (m), 1226 (m), 1181 (m), 1058 (m), 1000 (m), 955 (m), 837 (s), 762 (s), 693 (m) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 7.00 (s, 1H, C=CH), 7.40-7.42 (m, 3H, Ar-H), 7.47 (d, 2H, Ar-H, $J = 7.2$ Hz), 7.50 (d, 2H, Ar-H, $J = 6.9$ Hz), 7.65 (d, 2H, Ar-H, $J = 7.8$ Hz), 7.72 (d, 2H, Py-H, $J = 6.3$ Hz), 8.69 (d, 2H, Py-H, $J = 5.1$ Hz) ppm; ESI-MS: m/z 297.11 M^+ ; Anal. calcd. for $\text{C}_{20}\text{H}_{15}\text{N}_3$: C 80.78; H 5.08; N 14.13; found C 80.99; H 5.04; N 14.18.

4.3 Typical procedure for the synthesis of the 1-acetyl-pyrazoles

Acetyl chloride (4.0 mmol) was added dropwise to a mixture of the corresponding 1H-pyrazoles (2.0 mmol) and triethylamine (4.0 mmol) in 20 mL THF. The mixture was refluxed for 5h until the precipitate was formed. The precipitate was filtered and the filtrate was concentrated to obtain a solid product. The solid was recrystallized from ethanol-cyclohexane (1:1) to give the 1-acetyl pyrazoles (3a-e).

4.3.1 1-Acetyl-3-(4-pyridyl)-5-phenylpyrazole (3a)

Yellow powder, yield 87.2 %, mp 129-131 $^{\circ}\text{C}$; IR (KBr): n 3089 (m), 3005 (m), 2936 (w), 2855 (w), 1740 (s), 1620 (m), 1557 (m), 1491 (m), 1434 (m), 1368 (m), 1299 (s), 1219 (m), 1105 (m), 1026 (m), 945 (s), 831 (m), 761 (s), 690 (s) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 2.83 (s, 3H, CH_3), 6.85 (s, 1H, C=CH), 7.42-7.52 (m, 5H, Ar-H), 8.02 (d, 2H, Py-H, $J = 6.0$ Hz), 8.76 (d, 2H, Py-H, $J = 6.0$ Hz) ppm; ESI-MS: m/z 263.75 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{16}\text{H}_{13}\text{N}_3\text{O}$: C 72.99; H 4.98; N 15.96; found C 73.26; H 4.94; N 15.99.

4.3.2 1-Acetyl-3-(4-pyridyl)-5-(4-methylphenyl) pyrazole (3b)

Yellow powder, yield 86.6 %, mp 138-139 $^{\circ}\text{C}$; IR (KBr): n 3085 (m), 3024 (m), 2922 (w), 2860 (w), 1731 (s), 1673 (m), 1614 (m), 1561 (w), 1494 (w), 1421 (m), 1370 (m), 1331 (s), 1230 (m), 946 (m), 816 (s) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 2.42 (s, 3H, CH_3), 2.81 (s, 3H, CH_3), 6.78 (s, 1H, C=CH), 7.29 (d, 2H, Ar-H, $J = 8.7$ Hz), 7.39 (d, 2H, Py-H, $J = 6.0$ Hz), 7.77-7.80 (m, 2H, Ar-H), 8.68 (d, 2H, Py-H, $J = 6.0$ Hz) ppm; ESI-MS: m/z 277.84 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}$: C 73.63; H 5.45; N 15.15; found C 73.85; H 5.41; N 15.18.

4.3.3 1-Acetyl-3-(4-pyridyl)-5-(4-fluorophenyl) pyrazole (3c)

Bright yellow powder, yield 84.8 %, mp 142-143 $^{\circ}\text{C}$; IR (KBr): n 3100 (m), 3010 (m), 2960 (w), 2870 (w), 1739 (s), 1608 (m), 1506 (m), 1425 (m), 1371 (m), 1321 (s), 1225 (s), 1160 (m), 1107 (m), 947 (m), 817 (s) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 2.81 (s, 3H, CH_3), 6.79 (s, 1H, C=CH), 7.09-7.19 (m, 2H, Ar-H), 7.41-7.45 (m, 2H, Ar-H), 7.87 (d, 2H, Py-H, $J = 5.1$ Hz), 8.69 (d, 2H, Py-H, $J = 5.1$ Hz) ppm; ESI-MS: m/z 281.88 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{16}\text{H}_9\text{FN}_3\text{O}$: C 68.32; H 4.30; N 14.94; found C 68.56; H 4.27; N 14.98.

4.3.4 1-Acetyl-3-(4-pyridyl)-5-(4-methoxyphenyl) pyrazole (3d)

Yellow powder, yield 83.6 %, mp 158-159 $^{\circ}\text{C}$; IR (KBr): n 3106 (m), 3000

(w), 2985 (w), 2840 (w), 1735 (s), 1670 (m), 1610 (m), 1505 (m), 1427 (m), 1367 (m), 1300 (s), 1250 (s), 1180 (m), 1109 (m), 1029 (s), 945 (s), 810 (s) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 2.81 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 6.73 (s, 1H, C=CH), 6.94-7.01 (m, 2H, Ar-H), 7.39 (d, 2H, Ar-H, $J = 7.8$ Hz), 7.76-7.84 (m, 2H, Py-H), 8.68-8.71 (m, 2H, Py-H) ppm; ESI-MS: m/z 293.85 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}_2$: C 69.61; H 5.15; N 14.33; found C 69.89; H 5.11; N 14.37.

4.3.5 1-Acetyl-3-(4-pyridyl)-5-(4-phenylphenyl) pyrazole (3e)

Yellow crystals, yield 85.9 %, mp 166-168 $^{\circ}\text{C}$; IR (KBr): n 3090 (w), 3035 (w), 2920 (w), 2860 (w), 1740 (s), 1609 (m), 1559 (m), 1485 (m), 1421 (m), 1368 (m), 1295 (s), 1216 (m), 1108 (m), 1017 (m), 946 (m), 832 (s) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 2.84 (s, 3H, CH_3), 6.83 (s, 1H, C=CH), 7.38-7.56 (m, 5H, Ar-H), 7.63-7.72 (m, 4H, Ar-H), 7.82-7.98 (m, 2H, Py-H), 8.68-8.74 (m, 2H, Py-H) ppm; ESI-MS: m/z 339.89 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}$: C 77.86; H 5.05; N 12.38; found C 78.11; H 5.01; N 12.42.

4.4 Typical procedure for the synthesis of the 1-benzoyl-pyrazoles

Same procedure as for the 1-acetyl-pyrazoles, but the reaction mixture with the required 1H-pyrazoles (2.0 mmol), triethylamine (4.0 mmol) and benzoyl chloride (4.0 mmol). The 1-benzoyl pyrazoles (4a-e) were obtained.

4.4.1 1-Benzoyl-3-(4-pyridyl)-5-phenylpyrazole (4a)

Yellow powder, yield 87.6 %, mp 111-113 $^{\circ}\text{C}$; IR (KBr): n 3066 (m), 1710 (s), 1633 (s), 1521 (m), 1488 (m), 1446 (m), 1405 (m), 1295 (s), 1241 (m), 1193 (m), 1076 (m), 903 (s), 818 (s), 766 (s) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 7.13 (s, 1H, C=CH), 7.47-7.59 (m, 3H, Ar-H), 7.70-7.75 (m, 2H, Ar-H), 8.02-8.05 (m, 3H, Ar-H), 8.18 (d, 2H, Ar-H, $J = 7.2$ Hz), 8.35 (d, 2H, Py-H, $J = 6.6$ Hz), 8.85 (d, 2H, Py-H, $J = 5.7$ Hz) ppm; ESI-MS: m/z 326.03 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{21}\text{H}_{15}\text{N}_3\text{O}$: C 77.52; H 4.65; N 12.91; found C 77.80; H 4.61; N 12.95.

4.4.2 1-Benzoyl-3-(4-pyridyl)-5-(4-methylphenyl) pyrazole (4b)

Yellow powder, yield 82.6 %, mp 130-131 $^{\circ}\text{C}$; IR (KBr): n 3079 (m), 2940 (w), 2885 (w), 1713 (s), 1633 (s), 1497 (m), 1435 (m), 1317 (s), 1185 (m), 900 (s), 820 (s), 719 (m) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 2.41 (s, 3H, CH_3), 7.11 (s, 1H, C=CH), 7.37 (d, 2H, Ar-H, $J = 8.1$ Hz), 7.53-7.60 (m, 2H, Ar-H), 7.70-7.77 (m, 3H, Ar-H), 8.04 (d, 2H, Ar-H, $J = 7.2$ Hz), 8.34 (d, 2H, Py-H, $J = 4.5$ Hz), 8.84 (d, 2H, Py-H, $J = 5.4$ Hz) ppm; ESI-MS: m/z 340.25 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}$: C 77.86; H 5.05; N 12.38; found C 78.13; H 5.01; N 12.41.

4.4.3 1-Benzoyl-3-(4-pyridyl)-5-(4-fluorophenyl) pyrazole (4c)

Yellow powder, yield 85.2 %, mp 128-139 $^{\circ}\text{C}$; IR (KBr): n 3058 (m), 1721 (s), 1634 (s), 1601 (m), 1491 (s), 1434 (m), 1318 (s), 1232 (m), 1166 (m), 1099 (m), 906 (m), 818 (s), 719 (m) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 7.09 (s, 1H, C=CH), 7.17 (t, 2H, Ar-H, $J = 8.4$ Hz), 7.45-7.50 (m, 2H, Ar-H), 7.54 (t, 2H, Ar-H, $J = 7.8$ Hz), 7.71-7.76 (m, 1H, Ar-H), 8.02-8.10 (m, 2H, Ar-H), 8.31 (d, 2H, Py-H, $J = 6.0$ Hz), 8.80 (d, 2H, Py-H, $J = 6.0$ Hz) ppm; ESI-MS: m/z 344.00 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{21}\text{H}_{14}\text{FN}_3\text{O}$: C 73.46; H 4.11; N 12.24; found C 73.69; H 4.08; N 12.28.

4.4.4 1-Benzoyl-3-(4-pyridyl)-5-(4-methoxyphenyl) pyrazole (4d)

Yellow powder, yield 80.0 %, mp 96-97 $^{\circ}\text{C}$; IR (KBr): n 3086 (m), 2994 (m), 2830 (m), 1717 (s), 1633 (s), 1497 (s), 1422 (m), 1322 (s), 1293 (s), 1255 (s), 1184 (s), 1107 (m), 1031 (m), 953 (m), 902 (s), 814 (s), 715 (m) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 3.85 (s, 3H, OCH_3), 6.94 (d, 2H, Ar-H, $J = 6.9$ Hz), 7.11 (s, 1H, C=CH), 7.41 (d, 2H, Ar-H, $J = 8.7$ Hz), 7.55 (t, 2H, Ar-H, $J = 7.5$ Hz), 7.68-7.74 (m, 1H, Ar-H), 8.02 (d, 2H, Ar-H, $J = 7.2$ Hz), 8.34 (d, 2H, Py-H, $J = 6.6$ Hz), 8.90 (d, 2H, Py-H, $J = 6.3$ Hz) ppm; ESI-MS: m/z 356.05 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{22}\text{H}_{17}\text{N}_3\text{O}_2$: C 74.35; H 4.82; N 11.82; found C 74.62; H 4.78; N 11.86.

4.4.5 1-Benzoyl-3-(4-pyridyl)-5-(4-phenylphenyl) pyrazole (4e)

Colorless powder, yield 84.3 %, mp 136-138 $^{\circ}\text{C}$; IR (KBr): n 3062 (m), 3012 (m), 1711 (s), 1632 (s), 1603 (m), 1516 (m), 1488 (m), 1447 (m), 1409 (m), 1295 (s), 1200 (s), 949 (m), 902 (s), 830 (s), 767 (m), 716 (s) cm^{-1} ; ^1H NMR (300 MHz, CDCl_3): δ 7.19 (s, 1H, C=CH), 7.36-7.49 (m, 3H, Ar-H), 7.54-7.76 (m, 9H), 8.07 (d, 2H, Ar-H, $J = 6.9$ Hz), 8.35 (d, 2H, Py-H, $J = 6.3$ Hz), 8.87 (d, 2H, Py-H, $J = 6.3$ Hz) ppm; ESI-MS: m/z 402.39 $[\text{M}+1]^+$; Anal. calcd. for $\text{C}_{27}\text{H}_{19}\text{N}_3\text{O}$: C 80.78; H 4.77; N 10.47; found C 80.94; H 4.73; N 10.50.

4.5 Procedure for anti-microbial activity

The synthesized compounds (3a-e and 4a-e) were screened for their *in vitro* antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and antifungal activity against *Pyricularia oryzae* and *Rhizoctonia solani*. The anti-microbial activity was performed by disc diffusion method [16, 17] at a concentration of 50 mg L^{-1} . Muller-Hinton agar (Hi-Media) was employed as culture medium and DMSO was used as solvent control for anti-microbial activity. Norfloxacin and Triadimefon were used as standard for antibacterial

and antifungal activities, respectively. The inhibition zones were measured in mm at the end of an incubation period of 24 h at 37 °C for bacteria and 72 h at 24 °C for fungi.

SUPPLEMENTARY MATERIAL

Crystallographic data have been deposited with the Cambridge Crystallographic Data Center. Supplementary publication No. CCDC 965134. Copies of data may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336033; email: deposit@ccdc.cam.ac.uk).

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