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SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL AND PHYTOTOXIC SCREENING OF 1-AROYL-3,5-DIARYLPYRAZOLINE DERIVATIVES

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Abstract: Pyrazolines are biologically and pharmaceutically very active scaffolds. Derivatives of (3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)(phenyl)methanone were synthesized by the cyclization of chalcones (**1a-c**) with substituted benzyl hydrazides (**2a-e**) using a few drops of piperidine as catalyst. Structures of all the synthesized compounds were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectrometric analysis. All the pyrazolines were subjected to antimicrobial and phytotoxic assays. Compound **3a** and **3c** showed maximum antimicrobial activities while all the synthesized compounds were active acc. to their phytotoxic assays.

Keywords: antimicrobial, chalcones, phytotoxic, pyrazolines

With an increase in the resistance of the microorganisms to the existing drugs, a need for the development of new synthetic routes for the synthesis of biologically and industrially significant compounds is increasing day by day (1). In this regard, organic synthesis is playing a significant role in the development of new molecules with broad spectrum of activities (2). Due to significant contribution of heterocyclic compounds for the treatment of many infectious diseases they have attained a special attention of many scientists. With the increase in world population, a need for the improvement in food growth is also increasing. Heterocycles are also playing an important role in the field of insecticides and pesticides synthesis (3-5). Among them pyrazolines derivatives are pharmacologically active compounds with a broad spectrum of activities including antimicrobial (6), antioxidant, anticancerous (7), anti hypertensive, antifungal, and malarial (8) insecticidal, (9) herbicidal and anti-inflammatory activities (7, 10-12).

With all this significant importance, we have synthesized some 1-aroyl-3,5-diarylpyrazolines under standard reaction conditions (13).

RESULTS AND DISCUSSION

Derivatives of (3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)(phenyl)methanone were synthesized by the reaction of substituted hydrazides (**2a**-**e**) with substituted chalcones (**1a**-**c**) in the presence of piperidine as a catalyst in dry ethanol (13, 14). All the synthesized compounds were subjected to determination of antimicrobial and antifungal activities using agar well diffusion method (with concentration of 5 mg/mL) and disc diffusion method (with the same concentration), respectively. Phytotoxic activities were carried out on the surface of the broad leaves of Physalis plant. In the FTIR spectral data, stretching for pyrazoline ring was observed at 2800-2934 cm^{-1} (15). ^{1}H NMR spectra structures were confirmed due to the presence of a stereogenic center at 5 position of the pyrazoline ring, three characteristic peaks were observed for 4Ha, 4Hb and 5H protons. A doublet of doublets was observed at δ 5.2-5.4 ppm (J = 10.1, 5.5 Hz) for 5H proton and also at δ 4.1-4.25 (11.9, 9.7 Hz) ppm and δ 3.9-4.0 (10.2, 4.1 Hz) ppm for 4Ha and 4Hb protons of the pyrazoline ring, respectively (16, 17) . In ^{13}C NMR spectra, signal for C-5 carbon at δ 59-62 ppm and that for C-4 carbon at δ 43 ppm were observed (8, 18, 19). In mass fragmentation pattern of pyrazolines, the molecular ion peak and base peak derived from benzoyl group were observed. In the antibacterial bioassay, maximum inhibition was observed by compounds **3a** and **3c** with two methoxy electron donating groups at 3 and 5 positions of ring 'A' and a para chloro group on ring 'C' of the pyrazoline. In

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case of antifungal activities, the maximum percentage inhibition was observed for the same compounds, while all other pyrazolines showed moderate inhibition against yeast cells. From above observations it is found that molecules with methoxy and chloro groups shows more inhibition as compared to the others (20). In the case of phytotoxic activities, pale yellowing of the leaves were observed, which is due to binding of the compound with the chemoreceptor of the Physalis plants leaves (21). During phytotoxic assay of pyrazolines, almost all the compounds could actively bind with the chemoreceptor and have the potential to inhibit the growth of plant by ceasing photosynthesis (Table 1)

EXPERIMENTAL

Melting points were recorded using a digital Gallenkamp (SANYO) model MPD BM 3.5 apparatus and are uncorrected. ^{1}H NMR spectra were determined for $CDCl_3$ solutions at 300 MHz and ^{13}C NMR spectra were recorded for the same solutions at 75 MHz using a Bruker AM-300 spectrophotometer. FTIR spectra were recorded using an FTS 3000 MX spectrophotometer; mass spectra (EI, 70 eV) were recorded on a GC-MS instrument, Agilent Technology USA. Rf values were deretmined for mobile phase: petroleum ether : ethyl acetate (3 : 2, v/v). All compounds were purified by thin layer chromatography using silica gel from Merck (Germany).

General procedure for the synthesis of 1-aroyl-3,5-disubstituted pyrazolines (3a-h)

Ethanolic solution of appropriate chalcone (**1a-c**, 0.5 mol) and hydrazide (**2a-e**, 0.5 mol) were refluxed for two hours in the presence of few drops of piperidine as catalyst. Completion of reaction was confirmed by TLC. Upon completion, the resulting mixture was concentrated and purified by using precoated TLC chromatography (13).

(±)-(5-(4-Chlorophenyl)-3-(2-hydroxy-5-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(3,5-dimethoxyphenyl)methanone (3a)

Yield: 92%; R_f 0.9; m.p. 117-118^{o}C. IR (KBr, cm^{-1}): 3315, 2932, 2812, 1712, 1621, 1596, 1437, 1243; ^{1}H NMR ($CDCl_3$, δ, ppm): 6.9-7.7 (m, 10H arom.), 5.2 (dd, 1H, *J* = 9.0, 3.9 Hz, H5), 4.4 (dd, 1H, *J* = 5.8, 3.1 Hz, H4a), 4.2 (dd, 1H, *J* = 6.1, 3.3 Hz, H4b), 3.9 (s , 6H), 1.6 (s, 3H); ^{13}C NMR ($CDCl_3$, δ, ppm): 168 (CO), 162 (C-3), 61 (C-5), 43 (C-4). EIMS (m/z, %): 450 [M^{+}] (12), 452 (4), 285 (10), 165 (100), 133 (42).

(±)-(5-(4-Chlorophenyl)-3-(2-hydroxy-5-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(3,4,5-trimethoxyphenyl)methanone (3b)

Table 1. Antimicrobial and phytotoxic activities of compounds **3a-h**.

Compound	Antibacterial		Antifungal *S. cerevisiae*		Phytotoxic activity
	E. coli	*B. subtilis*	(ZI)	(PI)	
3a	7	16	0.4	66.66	Pale yellow
3b	8	11	0.5	58.33	Pale yellow
3c	6	16	0.4	66.6	Pale yellow
3d	9	14	0.8	33.3	Pale yellow
3e	7	12	0.9	25.0	Pale yellow
3f	12	14	0.5	58.33	Pale yellow
3g	5	13	0.5	58.3	Pale yellow
3h	9	15	0.4	66.6	Pale yellow
Negative control (acetone)	-	-	-	-	-
Kanamycin	-	20	-	-	-
Ampicillin	15	-	-	-	-
Fluconazone	-	-	12	100	-

Concentration used: 5 mg/mL; -: no activity, ZI (zone of inhibition, radius, mm), PI: percent inhibition
PI = 100 - fungal growth in sample (cm) / fungal growth in control (cm) × 100.

Yield: 85%; R_f 0.9; m.p. 101-102°C. IR (KBr, cm^{-1}): 3311, 2965, 2913, 1713, 1622, 1583, 1431, 1257. ^1H NMR ($CDCl_3$, δ, ppm): 6.9-7.7 (m, 12H arom.), 5.2 (dd, 1H, *J* = 9.6, 3.9 Hz, H5), 4.3 (dd, 1H, *J* = 5.7, 3.3 Hz, H4a,), 4.2 (dd, 1H, *J* = 6.1, 3.3 Hz, H4b), 3.9 (s , 9H), 1.6 (s, 3H); ^{13}C NMR ($CDCl_3$, δ, ppm): 167 (CO), 163 (C-3), 59 (C-5), 43 (C-4). EIMS (m/z, %): 460 (21), 462 (5) [M^+], 265 (34), 195 (100), 133 (56).

(±)-(5-(4-Chlorophenyl)-3-(2-hydroxy-5-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(3,5-dimethoxy-4-methylphenyl)methanone (3c)

Yield: 78%; R_f 0.9; m.p. 141-142°C. IR (KBr, cm^{-1}): 3017, 2965, 2911, 1707, 1621, 1597, 1433, 1257 cm^{-1}; ^1H NMR ($CDCl_3$, δ, ppm): 6.9-7.7 (m, 12H arom.), 5.4 (dd, 1H, *J* = 9.6, 3.3, H5,), 4.2 (dd, 1H, *J* = 5.7, 3.3, H4a), 4.1 (dd, 1H, *J* = 5.7, 3.3, H4b), 3.9 (s , 6H), 2.348 (s, 3H); ^{13}C NMR ($CDCl_3$, δ, ppm): 170 (CO), 161 (C-3), 58 (C-5), 43 (C-4). EIMS (m/z, %): 462 (37), 464 (53) [M^+], 285 (21), 179 (100), 133 (49).

(±)-(2E,4E)-1-(5-(4-chlorophenyl)-3-(2-hydroxy-5-methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-5-phenylpenta-2,4-dien-1-one (3d)

Yield: 85%; R_f 0.9; semisolid. IR (KBr, cm^{-1}): 3012, 2966, 2917, 1722, 1665, 1571, 1433; ^1H NMR ($CDCl_3$, δ, ppm): 6.6-7.30 (m, 12H arom.), 7.4 (d, 1H, *J* = 6.3 Hz, $H_ß$), 7.1 (d, 1H, *J* = 3.4 Hz, $H_α$), 6.9 (d, 1H, *J* = 4.2 Hz, $H_γ$), 6.6 (d, 1H, *J* = 3.6 Hz, $H_δ$), 5.4 (dd, 1H, *J* = 9.6, 3.3 Hz, H5), 4.1 (dd, 1H *J* =5.7, 3.3 Hz, H4a), 3.9 (dd, 1H, *J* = 5.7, 3.3 Hz, H4b), 2.5 (s, 3H, CH3); ^{13}C NMR ($CDCl_3$, δ, ppm): 166 (CO), 157 (C-3), 61 (C-5), 43 (C-4). EIMS (m/z, %): 442 (21), 444 (11) [M^+], 133 (47), 90(100).

(±)-(3-(2-Hydroxy-5-methylphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(3,4,5-trimethoxyphenyl)methanone (3e)

Yield: 80%; R_f 0.8; m.p. 170-171°C. IR (KBr, cm^{-1}): 3121, 2964, 2911, 1706, 1675, 1595, 1431; ^1H NMR ($CDCl_3$, δ, ppm): 6.9-7.7 (m, 9H arom.), 5.1 (dd, 1H, *J* = 9.0, 3.3 Hz, H5), 4.24 (dd, 1H, *J* = 5.7, 3.6 Hz, H4a), 4.1 (dd, 1H, *J* = 5.7, 3.6 Hz, H4b), 3.9 (s, 12H, OCH3), 1.6 (s, 3H); ^{13}C NMR ($CDCl_3$, δ, ppm): 167 (CO), 162 (C-3), 60 (C-5), 43 (C-4); EIMS (m/z, %): 540 (11), 542 (32) [M^{+o}], 345 (12), 196 (10), 195 (100).

(±) (3,5-Dimethoxy-4-methylphenyl)(3-(2-hydroxy-5-methylphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)methanone (3f)

Yield: 83%; R_f 0.6; oil. IR (KBr, cm^{-1}): 3021, 2963, 2943, 1700, 1686, 1595, 1435; ^1H NMR ($CDCl_3$, δ, ppm): 6.9-7.7 (m, 9H arom.), 5.1 (dd, 1H, *J* = 9.0, 3.3 Hz, H5), 4.24 (dd, 1H, *J* = 5.7, 3.6 Hz, H4a), 4.1 (dd, 1H, *J* = 5.7, 3.6 Hz, H4b), 3.9 (s, 9H, OCH_3), 2.52 (s, 3H, CH3), 1.6 (s, 3H); ^{13}C NMR ($CDCl_3$, δ, ppm): 168 (CO), 161 (C-3), 59 (C-5), 43 (C-4). EIMS (m/z, %): 524 (11), 526 (25) [M^+], 345 (21), 196 (27), 179 (100).

(±)-(3,5-Dimethoxyphenyl)[3-(2-hydroxy-5-methylphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]methanone (3g)

Yield: 78%; R_f 0.8; m.p. 160-161°C. IR (KBr, cm^{-1}): 3021, 2963, 2919, 1742, 1656, 1595,1441; ^1H NMR ($CDCl_3$, δ, ppm): 6.9-7.7 (m, 10H arom.), 5.1 (dd, 1H, *J* = 9.0, 3.3 Hz, H5), 4.2 (dd, 1H, *J* = 5.1, 3.6 Hz, H4a), 4.0 (dd, 1H, *J* = 5.1, 3.6 Hz, H4a), 3.9 (s, 9H, OCH_3), 1.6 (s, 3H); ^{13}C NMR ($CDCl_3$, δ, ppm): 168 (CO), 162 (C-3), 61 (C-5), 43 (C-4). EIMS (m/z, %): 510, 512 [M^+] (32), 345 (11), 196 (29), 165 (100).

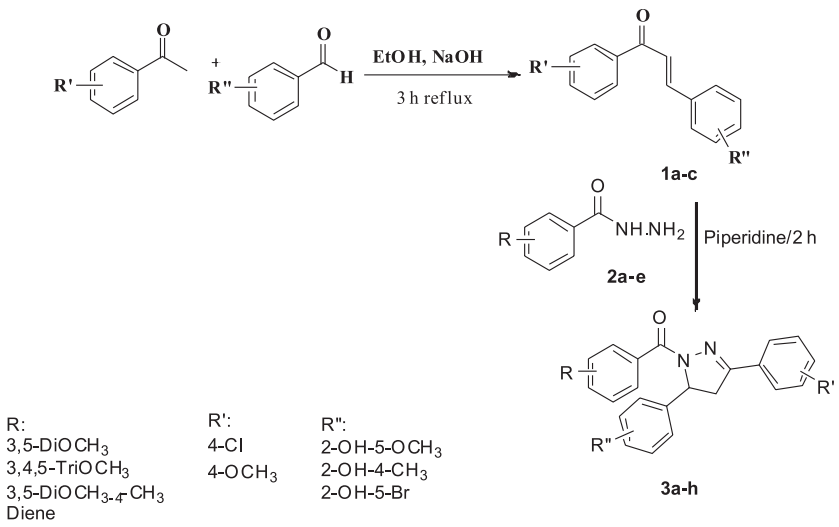
(±) (2E,4E)-1-[3-(5-bromo-2-hydroxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl]-5-phenylpenta-2,4-dien-1-one (3h)

Yield: 82%; R_f 0.9; oil. IR (KBr, cm^{-1}): 3023, 2961, 2920, 1698, 1656, 1595,1441; ^1H NMR ($CDCl_3$, δ, ppm): 6.6-7.30 (m, 12H arom.), 7.4 (d, 1H, *J* = 6.3 Hz, $H_ß$), 7.1 (d, 1H, *J* = 3.4 Hz, $H_α$,), 6.9 (d, 1H, *J* = 4.2 Hz, $H_γ$), 6.6 (d, 1H, *J* = 3.6 Hz, $H_δ$), 5.4 (dd, *J* = 9.6, 3.3 Hz, H5), 4.1 (dd, *J* = 5.7, 3.3 Hz, H4a), 3.9 (dd, *J* = 5.7, 3.3 Hz, H4b); ^{13}C NMR ($CDCl_3$, δ, ppm): 168 (CO), 162 (C-3), 61 (C-5), 43 (C-4); EIMS (m/z, %): 502, 504 [M^{+o}] (19), 198 (23), 90 (100).

Biological assay

Antibacterial activities

Antibacterial *in vitro* bioassays were conducted by using agar well diffusion method (22) against Gram-positive (*Bacillus subtilus*) and Gram-negative (*Escherichia coli*) strains of bacteria, ampicillin (against Gram-negative) and kanamycin (against Gram-positive) were used as standards. For analysis, concentrations of 3 mg/mL and 5 mg/mL of compounds dissolved in 1 mL of acetone were used. Experiments were carried out on Mueller-Hinton plates. Laboratory strain bacteria (*Bacillus subtilis*, *Escherichia coli*) were grown to log phase in LB (1% yeast extract, 1% peptone and 1% dextrose) at 37°C for overnight with constant shaking. Cultures were spread onto the plates, then wells were made by using cork borer (4 mm). Wells were loaded with: 3 mg/mL and 5 mg/mL solution of compounds in acetone, negative control acetone and positive



Scheme 1. Synthesis of 1-aroyl-3,5-diarylpyrazoline derivatives

control ampicillin and kanamycin with the help of 5 µL micropipette. Plates were incubated for 24 h at 37°C. Tests were repeated three times. Most significant results were obtained using a concentration of 5 mg/mL. After 24 h incubation time, the zones of inhibition were evaluated in mm scale and compared with standard drugs (22).

Antifungal activities

Antifungal activities were carried out against yeast (*Saccharomyces cerevisiae*). Fluconazole was used as standard drug. Concentrations of 3 mg/mL and 5 mg/mL of compounds were used for the antifungal analysis. For antifungal activities, plates were loaded with sample solutions, standard drug and negative control acetone with the help of 5 µL micropipette and incubated for 24 h at 28°C. Tests were repeated three times. Concentration of 5 mg/mL showed most significant results. After 24 h incubation time, the zones of inhibition were examined and percentage of fungal inhibition was calculated and compared with reference to the standard drugs (23) according to equation:.

% of fungal inhibition = 100 - fungal growth in test sample (cm) / fungal growth in control (cm) ×100.

Phytotoxic activities

All the synthesized compounds were tested for their phytotoxic activities as an effect of chemicals on the growth inhibition of plant. Leaves of Physalis plant (the fruit of this plant is called Cape gooseberry used as a decorative of cakes) were used for the above test. Solutions of compounds (3 and 5 mg/mL) were applied on the broad leaves of the Physalis plants. Plants were left for 24 h, then phytotoxic affects of the compounds were observed by blackening or pale yellowing of the leave (21).

CONCLUSION

Successful synthesis of the derivatives of (3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)(phenyl)-methanone were carried out. Structures of all the synthesized compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometric analysis. All the compounds were subjected to their antibacterial, antifungal and phytotoxic assays. Among them, compounds **3a** and **3c** showed maximum inhibition against the bacterial and fungal strains while all compound were active for their phytotoxic activities.

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