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SYNTHESIS OF NOVEL PYRAZOLINES BEARING TRICHLOROPHENYL MOIETY AS ANTIMICROBIAL AGENTS

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ABSTRACT

In view of developing novel and effective antimicrobial agents, novel series of pyrazolines (4a-g and 5a-g) bearing 2,3,4-trichlorophenyl moiety were synthesized by treating 1-(2,3,4-trichlorophenyl)-3-(4-aryl)-2-propene-1-ones (3) with hydrazine hydrate and formic acid/ acetic acid (Schemes 1 and 2). The newly synthesized compounds were characterized by elemental analysis and spectroscopic studies and screened for their *in vitro* antibacterial and antifungal activities. The screening studies revealed that the novel pyrazoline derivatives (4a-g and 5a-g) exhibited moderate to good activity.

KEY WORDS*:* **Formylpyrazoline, Acetylpyrazoline, Antimicrobial Activity, Spectroscopic Data.**

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INTRODUCTION

Pyrazolines are five-member prominent heterocyclic compounds containing two nitrogen atoms at 1 and 2 positions. Pyrazoline derivatives are the electron rich nitrogen heterocycles which play an important role in the diverse biological activities. Considerable attention has been focused on the pyrazolines and substituted pyrazolines due to their interesting biological activities like antimicrobial[1,2], antifungal[3], anti-inflammatory[4,5], antimycobacterial[6], anti-depressant[7-11], ulcerogenic, anaesthetic and analgesic properties[12-14], dual lipoxygenase/cycloxygenase inhibitor[15]. Literature reveals the prominence of pyrazolines. Based on the literature review attention is given to the synthesis and structural aspects of pyrazolines on the basis of their elemental analysis, IR, ^{1}H-NMR spectral studies and thereby the compounds were screened for their antimicrobial activity.

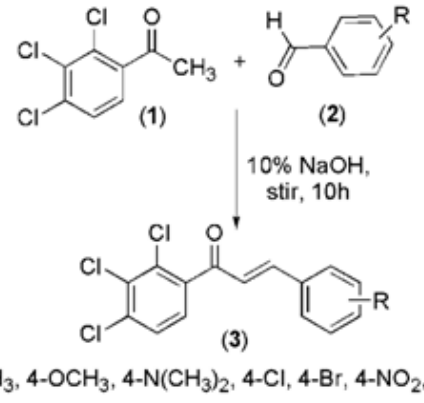
MATERIALS AND METHODS

The newly synthesized products were confirmed by their spectral analysis. The chemicals used for the synthesis of novel pyrazolines were of standard quality. 2,3,4-trichloroacetophenone, different benzaldehydes were procured from Sigma-Aldrich, Bengaluru, India, Potassium hydroxide, Hydrazine hydrate, formic acid and acetic acid from Spectrochem, Mumbai-India. Melting points were determined in an open capillary tube and are uncorrected. FT-IR spectra were recorded in KBr on a SHIMADZU-FTIR Infrared Spectrometer, ^{1}H-NMR were recorded on Brucker Avil HD-300 MHz-FT-NMR at 400 MHz in CDCl3, Elemental analysis was carried out on a Euro-E-300, Mass spectra (FAB mass) was recorded on a JEOL SX 102/DA-6000 Mass Spectrometer using argon/xenon (6 kv, 10 mA). Completion of the reaction was monitored by thin layer chromatography (TLC) using Merck silica gel 60 F_{254} coated alumina plates. The synthetic pathway is presented in Schemes 1 and 2 and in spectroscopic data. The data of the novel pyrazolines are presented in Tables 1 and 2 and the biological activity data are tabulated in Tables 3 and 4

REACTION SCHEMES

Scheme 1

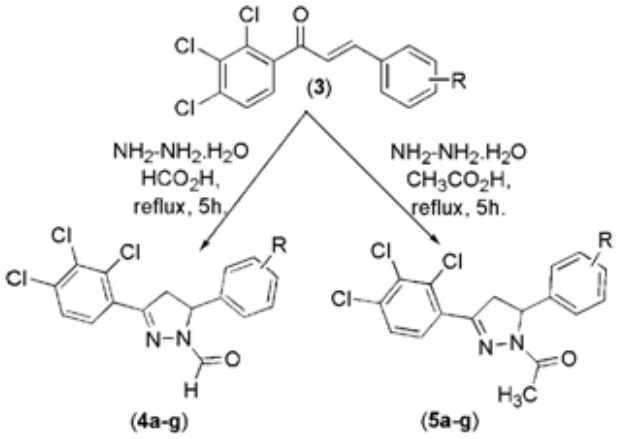
Synthesis of 1-(2, 3, 4-trichlorophenyl)-3-(4-aryl)-2-propene-1-ones (3a-g)



R=4-CH_3, 4-OCH_3, 4-$N(CH_3)_2$, 4-Cl, 4-Br, 4-NO_2, 4-OH.

(Scheme 1)

Scheme 2
Synthesis of N-formylpyrazolines and N-acetylpyrazolines (4a-g and 5a-g)



R= 4-CH_3, 4-OCH_3, 4-$N(CH_3)_2$, 4-Cl, 4-Br, 4-NO_2, 4-OH.

(Scheme 2)

General Procedure for the Synthesis of (2E)-3-phenyl-1-(2,3,4-trichlorophenyl)prop-2-en-1-one (3a-g).

2,3,4-Trichloroacetophenone (0.01 mol) was dissolved in ethanol or in a mixture of Dimethylformamide and ethanol. Then, Sodiumhydroxide (5mL, 10%) solution and different aromatic aldehyde (0.01 mol) were added to the resulting solution with continuous stirring. The reaction mixture was stirred for 6 hours and was allowed to stand overnight. The solid separated out was filtered off, dried and recrystallised from a mixture of Dimethylformamide and ethanol (Scheme 1). The characterization data of the title compounds are given in Table 1.

General Procedure for the Synthesis of N-formylpyrazolines and N-acetylpyrazolines (4a-g and 5a-g).

Hydrazine hydrate (90%, 5mL) was added drop-wise to a mixture of propenone (3) (0.01 mol) and formic acid (25mL) or acetic acid (25mL). The reaction mixture was refluxed for 5 hours, cooled and poured on to crushed ice. The resulting pyrazolines were collected by filtration and recrystallized from a mixture of dimethyl formamide and ethanol (Scheme 2). The characterization data of N-formyl/N-acetylpyrazolines are given in Table 2.

RESULTS AND DISCUSSION

(2*E*)-3-(aryl)-1-(2,3,4-trichlorophenyl)prop-2-en-1-one (**3a-g**) were synthesized through Claisen-Schmidt condensation, which is an important step in formation of C-C bond for the synthesis of propenones (Chalcones). It is normally carried out by the use of strong bases such as NaOH or KOH in polar solvents (MeOH or DMF). The synthesized chalcone derivatives were treated with hydrazine hydrate and formic/acetic acid to obtain N-formyl/N-acetyl pyrazolines (4a-g and 5a-g). The structure of the synthesized compounds was confirmed by IR, ^{1}H-NMR, Mass spectra and Elemental analysis.

SPECTROSCOPIC DATA

4a: FT-IR (KBr, cm^{-1}): 3039 (Ar-H), 2894 (C-H), 1657(N-CHO), 1604, 1594 and 1554 (C=N and C=C), 834, 751 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.10 (s, 3H, CH_3), 2.20 (s, 3H, -N-CO-CH_3) 3.22 and 3.16 (dd, 2H, *J*=2.4 Hz), 3.83 and 3.91(dd, 2H, *J*=11.2 Hz), 5.51 and 5.53 (dd, 1H, *J*=4.4 Hz, methine proton of pyrazoline), 7.80 (d, 2H, *J* = 8.8 Hz 2,3,4-trichlorophenyl) 7.35 (d, 2H, *J*=8.8 Hz, 2,3,4-trichlorophenyl), 7.17 (d, 2H, *J*=8.2 Hz, 4-methylphenyl), 7.03 (d, 2H, J=8.2 Hz 4-methylphenyl). FAB MS (*m/z*, %): 368 (M^+, 65), 369 (M^++1, 100), 370 (M^++2, 78), 372 (M^++4, 54), 374 (M^++6, 36).

4b: IR (KBr, cm^{-1}): 3010 (Ar-H), 2924 (C-H), 1655 (N-CHO), 1594, 1571 and 1514 (C=N and C=C), 814, 722 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 3.90 (s, 3H, OCH_3), 3.34 and 3.41(dd, 2H, *J*=4.7 Hz), 3.95 and 4.01 (dd, 2H, *J*=11.7 Hz), 5.53 and 5.49 (dd, 1H, *J* = 4.6 Hz, methine proton of pyrazoline), 6.88 and 7.20 (2d, 4H, *J*=8.6 Hz, 4-methoxyphenyl), 7.45 and 7.62 (2d, 2H, *J*=8.5 Hz, 2,3,4-trichlorophenyl), 8.91 (s, 1H, N-CHO). FAB MS (*m/z*, %): 384 (M^+, 46), 385 (M^++1, 100), 386 (M^++2, 61), 388 (M^++4, 48), 390 (M^++6, 18).

4c: IR (KBr, cm^{-1}): 3067 (Ar-H), 2974 (C-H), 1651 (N-CHO), 1594, 1548 and 1517 (C=N and C=C), 823, 738 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.93 (s, 6H,-N-N-dimethyl), 3.42 and 3.66 (dd, 2H, *J*=4.6 Hz), 3.89 and 3.95 (dd, 2H, *J*=11.7 Hz), 5.46 and 5.49 (dd, 1H, *J* = 4.5 Hz, methine proton of pyrazoline), 6.68 and 7.14 (2d, 2H, *J*=8.6 Hz, N,N-dimethylamino phenyl), 7.61 and 7.45 (2d, 2H, *J*=8.6 Hz, 2,3,4-trichlorophenyl), 8.92 (s, 1H, N-CHO). FAB MS (*m/z*, %): 397 (M^+, 74), 398 (M^++1, 100), 399 (M^++2, 61), 401 (M^++4, 55), 403 (M^++6, 22).

4d: IR (KBr, cm^{-1}): 3033 (Ar-H), 2867(C-H), 1658 (N-CHO), 1601, 1493 and 1413 (C=N and C=C), 817, 898 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 3.33 and 3.39 (dd, 2H, *J*=4.9 Hz), 3.92 and 3.96 (dd, 2H, *J*=4.1 Hz), 5.50 and 5.54(dd, 1H, *J*=4.8 Hz, methine proton of pyrazoline), 7.20 and 7.41 (2d, 4H, *J*=8.1 Hz, 4-chlorophenyl), 7.46 and 7.63 (2d, 2H, *J*=8.3 Hz, 2,3,4-trichlorophenyl), 9.12 (s, 1H, N-CHO). FAB MS (*m/z*,

%): 388 (M^+, 32), 389 (M^++1, 100), 390 (M^++2, 63), 392 (M^++4, 44), 394 (M^++6, 22), 249 (24).

4e: IR (KBr, cm^{-1}): 3056 (Ar-H), 2965 (C-H), 1656 (N-CHO), 1591, 1558 and 1532 (C=N and C=C), 814, 729 (C-Cl), 683 (C-Br). ^{1}H-NMR ($CDCl_3$, δ ppm): 3.25 and 3.31 (dd, 2H, *J*=4.6 Hz), 3.89 and 3.95 (dd, 2H, *J*=11.9 Hz), 5.52 and 5.56 (dd, 1H, *J*=4.6 Hz, methane proton of pyrazoline), 7.11 and 7.43 (2d, 2H, *J*=8.3 Hz, 2,3,4-trichlorophenyl), 7.59 and 7.46 (2d, 2H, *J*=8.5 Hz, 4-bromophenyl), 9.02 (s, 1H, N-CHO). FAB MS (*m/z*, %): 432 (M^+, 69), 433 (M^++1, 100), 434 (M^++4, 57).

4f: IR (KBr, cm^{-1}) 3018 (Ar-H), 2925 (C-H), 1656 (N-CHO), 1595, 1538 and 1512 (C=N and C=C), 1548 and 1353 (NO_2, asymmetric and symmetric stretch), 806, 757 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 3.43 and 3.37 (dd, 2H, *J*=5.2 Hz), 3.95 and 4.02 (dd, 2H, *J*=12.16 Hz), 5.62 and 5.66 (dd, 1H, *J*=5.0 Hz, methine proton of pyrazoline), 7.41 and 7.48 (d, 2H, *J*=8.5 Hz, 2,3,4-trichlorophenyl), 7.64 and 8.23 (d, 2H, *J*= 8.5Hz,4-nitrophenyl), 9.05(s, 1H,N-CHO). FAB MS (*m/z*, %): 397 (M^+, 55), 398 (M^++1, 100), 401(M^++4, 47).

4g: IR (KBr, cm^{-1}): 3478 (OH, broad peak), 3065 (Ar-H), 2935 (saturated C-H), 1655 (N-CHO), 1598, 1496 and 1460 (C=N and C=C), 857, 791(C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 3.36 and 3.64 (dd, 2H, *J*=4.8 Hz), 3.88 and 3.94 (dd, 2H, *J*=4.8 Hz), 5.31 (s, 1H, OH), 5.43 and 5.47 (dd, 1H, *J*=4.6 Hz, methine proton of pyrazoline), 7.43 and 7.22 (2d, 4H, *J*=8.3 Hz, 4-OH-phenyl), 9.44 and 8.76 (2d, 4H, *J*=10.0 Hz, 2,3,4-trichlorophenyl), FAB MS (*m/z*, %): FAB MS (*m/z*, %): 369 (M^+, 72), 370 (M^++1, 100), 371 (M^++2, 61), 373 (M^++4, 54), 375 (M^++6, 43).

5a: IR (KBr, cm^{-1}): 3047 (Ar-H), 2968, 2823 (saturated C-H), 1673 (N-CO-CH_3), 1579, 1558 and 1466 (C=N and C=C), 812, 843 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.10 (s, 3H, CH_3), 2.38 (s, 3H, CO-CH_3), 3.25 and 3.17 (dd, 2H, *J*=2.4 Hz), 3.80 and 3.92 (dd, 2H, *J*=11.9 Hz), 5.51 and 5.57 (dd, 1H, *J*=4.4 Hz, methine proton of pyrazoline), 7.19 and 7.05 (2d, 4H, *J*=8.4 Hz, 4-methylphenyl), 7.39 and 7.83 (2d, 4H, *J*=8.8 Hz, 2,3,4-trichlorophenyl). FAB MS (*m/z*, %): 382 (M^+, 81), 383 (M^++1, 100), 384 (M^++2, 66), 386 (M^++4, 53), 388 (M^++6.

5b: IR (KBr, cm^{-1}): 3018 (Ar-H), 2927, 2853 (saturated C-H), 1670 (N-CO-CH_3), 1579, 1542 and 1486 (C=N and C=C), 835, 873 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.36 (s, 3H, CO-CH_3), 3.89 (s, 3H, OCH_3), 3.32 and 3.43 (dd, 2H, *J*=4.6 Hz), 3.92 and 4.06 (dd, 2H, *J*=4.8 Hz), 5.46 and 5.58 (dd, 1H, *J*=4.6 Hz, methine proton of pyrazoline), 6.84 and 7.19 (2d, 4H, *J*=8.3Hz, 4-methoxyphenyl), 7.41 and 7.60 (2d, 2H, *J*=8.5Hz, 2,3,4-trichlorophenyl). FAB MS (*m/z*, %): 398 (M^+, 63), 399 (M^++1, 100), 400 (M^++2, 56), 402 (M^++4, 45), 404 (M^++6, 33), 288(26), 253(21).

5c: IR (KBr, cm^{-1}): 3067 (Ar-H), 2930, 2867 (saturated C-H), 1653 (N-CO-CH_3), 1547, 1532 and 1521 (C=N and C=C), 827, 748 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.37 (s, 3H, CO-CH_3), 2.94 (s, 6H,-N-N-dimethyl), 3.43 and 3.67 (dd, 2H, *J*=4.6 Hz), 3.88 and 3.93 (dd, 2H, *J*=11.7 Hz), 5.45 and 5.48 (dd, 1H, *J*=4.5 Hz, methine proton of pyrazoline), 6.68 and 7.14 (2d, 2H, *J*=8.6 Hz, N,N-dimethylamino phenyl), 7.60 and 7.44 (2d, 2H, *J*=8.6 Hz, 2,3,4-trichlorophenyl). FAB MS (*m/z*, %): 411 (M^+, 74), 412 (M^++1, 100), 413 (M^++2, 61), 414 (M^++4, 55), 416 (M^++6, 22).

5d: IR (KBr, cm^{-1}): 3078 (Ar-H), 2925 (saturated C-H), 1670 (N-CO-CH_3), 1573, 1516 and 1491 (C=N and C=C), 822, 8871 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.47 (s, 3H, CO-CH_3), 3.25 and 3.31 (dd, 2H, *J*=2.3 Hz), 3.89 and 3.95 (dd, 2H, *J*=11.9 Hz), 5.54 and 5.58 (dd, 1H, *J*=4.6 Hz, methine proton of pyrazoline), 7.17 and 7.30 (2d, 4H, *J*=8.4 Hz, 4-chlorophenyl), 7.44 and 7.59 (2d, 2H, *J*=8.5 Hz, 2,3,4-trichlorophenyl). FAB MS (*m/z*, %): 402 (M^+, 67), 403 (M^++1, 100), 404 (M^++2, 32), 406 (M^++4, 41), 408 (M^++6, 16), 281(26), 247(18).

5e: IR (KBr, cm^{-1}): 3075 (Ar-H), 2927 (saturated C-H), 1670 (N-CO-CH_3), 1573, 1488 and 1461 (C=N and C=C), 871, 819 (C-Cl), 629 (C-Br). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.38 (s, 3H, CO-CH_3), 3.21 and 3.29 (dd, 2H, *J*=4.6 Hz), 3.86 and 3.92 (dd, 2H, *J*=11.9 Hz), 5.49 and 5.53 (dd, 1H, *J*=4.6 Hz, methine proton of pyrazoline), 7.09 and 7.39 (2d, 2H, *J*=8.3 Hz, 4-bromophenyl), 7.42 and 7.53 (2d, 2H, *J*=8.5 Hz, 2,3,4-trichlorophenyl). FAB MS (*m/z*, %): 446 (M^+, 50), 447 (M^++1, 100), 448 (M^++2, 61), 450 (M^++4, 37).

5f: IR (KBr, cm^{-1}): 3063 (Ar-H), 2936 (saturated C-H), 1672 (N-CO-CH_3), 1576, 1483 and 1467 (C=N and C=C), 1535 and 1348 (NO_2, asymmetric and symmetric stretch), 851, 822 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.39 (s, 3H, CO-CH_3), 3.41 and 3.34 (dd, 2H, *J*=5.2 Hz), 3.92 and 4.05 (dd, 2H, *J*=12.0 Hz), 5.64 and 5.69 (dd, 1H, *J*=5.0 Hz, methine proton of pyrazoline), 7.43 and 7.51 (2d, 4H, *J*=8.5 Hz, 2,3,4-trichlorophenyl), 7.68 and 8.32 (2d, 4H, *J*=8.6 Hz, 4-nitrophenyl); FAB MS (*m/z*, %): FAB MS (*m/z*, %): 411 (M^+, 69), 412 (M^++1, 100), 413 (M^++2, 53), 415 (M^++4, 44), 417 (M^++6, 35).

5g: IR (KBr, cm^{-1}): 3469 (OH, broad peak), 3041 (Ar-H), 2965 (saturated C-H), 1670 (N-CO-CH_3), 1571, 1485 and 1460 (C=N and C=C), 866, 838 (C-Cl). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.35 (s, 3H, CO-CH_3), 3.32 and 3.54 (dd, 2H, *J*=4.6Hz), 3.78 and 3.86 (dd, 2H, *J*=4.6 Hz), 5.36 (s, 1H, OH), 5.48 and 5.53 (dd, 1H, *J*= 5.0 Hz, methine proton of pyrazoline), 7.37 and 7.19 (2d, 4H, *J*=8.3 Hz, 4-OH-phenyl), 7.53 and 7.68 (2d, 4H, *J*=8.6 Hz, 2,3,4-trichlorophenyl). FAB MS (*m/z*, %): 382 (M^+, 72), 383 (M^++1, 100), 384 (M^++2, 61), 386 (M^++4, 54), 388 (M^++6, 43).

Table 1
Characterization data of Propen-1-ones

Compound	R	MF	M.W.	m. p.(°C)
3a	4-CH_3	$C_{16}H_{11}OCl_3$	325.35	243
3b	4-OCH_3	$C_{16}H_{11}O_2Cl_3$	341.29	142
3c	-$N(CH_3)_2$	$C_{17}H_{14}ONCl_3$	354.35	100
3d	4-Cl	$C_{15}H_8OCl_4$	345.81	165
3e	4-Br	$C_{15}H_8OCl_3Br$	390.26	173
3f	4-NO_2	$C15H8NO_3Cl_3$	356.35	189
3g	4-OH	$C_{15}H_9O_2Cl_3$	327.35	178

Table 2
Characterization Data of N-Formylpyrazolines and N-Acetylpyrazolines

Samples	R	m. p. (°C) (Yield %)	MF (MW)
4a	4-CH_3	238 (79)	$C_{17}H_{13}N_2OCl_3$ (367.35)
4b	4-OCH_3	152 (78)	$C_{17}H_{13}N_2O_2Cl_3$ (383.65)
4c	$N(CH_3)_2$	156 (75)	$C_{18}H_{16}N_3OCl_3$ (396.70)
4d	4-Cl	139 (69)	$C_{16}H_{10}N_2OCl_4$ (388.08)
4e	4-Br	163 (72)	$C_{16}H_{10}N_2OBrCl_3$ (432.53)
4f	4-NO_2	141 (81)	$C_{16}H_{10}N_3O_3Cl_3$ (398.63)
4g	4-OH	186 (78)	$C_{16}H_{11}N_2O_2Cl_3$ (369.63)
5a	4-CH_3	110 (72)	$C_{18}H_{15}N_2OCl_3$ (381.68)
5b	4-OCH_3	102 (68)	$C_{18}H_{15}N_2O_2Cl_3$ (397.68)
5c	$N(CH_3)_2$	119 (65)	$C_{19}H_{18}N_3OCl_3$ (410.72)
5d	4-Cl	93 (63)	$C_{17}H_{12}N_2OCl_4$ (402.10)
5e	4-Br	86 (60)	$C_{17}H_{12}N_2OBrCl_3$ (446.55)
5f	4-NO_2	154 (81)	$C_{17}H_{12}N_3O_3Cl_3$ (412.66)
5g	4-OH	190 (63)	$C_{17}H_{13}N_2O_2Cl_3$ (383.65)

ANTIBACTERIAL ACTIVITY

The newly synthesized compounds (4a-g) and (5a-g) were screened for their antibacterial activities against *E. coli* (ATCC-25922), *S. aureus* (ATCC-25923) *P. aeruginosa (ATCC-27853)* and *K. pneumoniae (recultured)* bacterial strains by the Disc Diffusion method.[16, 17] Discs measuring 6.25 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140 °C for an hour. The test compounds were prepared with different concentrations using *N, N*-dimethyl formamide (DMF). Exactly 1mL containing 100 times the amount of chemical in each disc was added to each bottle, containing 100 discs. The discs of each concentration were placed in nutrient agar medium inoculated with fresh bacterial strains separately. The plates were incubated at 37 °C for 24h. The renowned drug Ciprofloxacin was the Reference Standard. Solvent and growth controls were kept separately and the diameter of zone of inhibition was measured. The results were tabulated in Table 3 and Fig. 1.

ANTIFUNGAL ACTIVITY

All the newly synthesized compounds (4a-g) and (5a-g) were screened for their antifungal activity against *C. albicans* (NICM No.3100*), A. fumigatus* (NICM No.902), *A. Flavus* (NICM No.524) *and P. marneffei* (recultured) in DMSO by serial dilution method.[18, 19] Sabourauds agar (prepared by dissolving peptone (1g), D-glucose (4g) and agar (2g) in distilled water (100mL) and adjusting the pH to 5.7) was used as medium for fungal growth. Normal saline was used to make spore suspension of fungal strains (i.e., a loopful of particular fungal stain was transferred to 3 mL of saline in order to obtain a suspension of corresponding species). Prepared Sabourauds agar media (20mL) was poured in to each Petri dish. Excess of media was decanted and the plates were dried by placing in an incubator for 1h. Wells were made on these seeded agar plates used an agar punch and labeled. A 10µg/mL solution of the test compound in dimethylsulfoxide (DMSO) was then added into each of these labeled wells. A control was also prepared in the same way using DMSO. The petri dishes were then incubated at 37 °C for 3 to 4 days. The Diameter of the inhibition zone (ZOI Test)

and the Minimum Inhibitory Concentrations (MIC Test) were determined in comparison with the Reference Standard Ciclopiroxolamine. The results are tabulated in Table 4 and Fig. 2.

Table 3
Antibacterial Activity of N-Formylpyrazolines and N-Acetylpyrazolines

Samples (50 µg/disc) /bacterial species	Diametre of Inhibition Zone (mm±SD)[A]			
	Bacterial strains			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Escherichia coli*
4a	**19±0.41**	**17±0.27**	**25±0.67**	**19±0.58**
4b	13±0.49	11±0.42	10±0.29	15±0.31
4c	18±0.35	14±0.40	12±0.22	14±0.27
4d	12±0.27	10±0.32	15±0.37	13±0.30
4e	**19±0.27**	**16±0.38**	**23±0.29**	**19±0.42**
4f	10±0.29	13±0.31	12±0.23	11±0.54
4g	**18±0.51**	**17±0.42**	**25±0.38**	**18±0.31**
5a	11±0.48	15±0.36	13±0.32	11±0.33
5b	14±0.28	17±0.29	19±0.25	12±0.49
5c	**17±0.29**	**18±0.35**	**22±0.24**	**20±0.33**
5d	14±0.26	11±0.31	13±0.31	17±0.33
5e	13±0.41	10±0.27	11±0.28	19±0.41
5f	**17±0.34**	**18±0.32**	**25±0.21**	**18±0.26**
5g	19±0.24	17±0.27	23±0.31	20±0.29
Ciprofloxacin	**19±0.57**	**18±0.53**	**25±0.39**	**20±0.42**

Note: [A]Mean values of 3 trials. Ref. Std: Ciprofloxacin (10 µg/disc).

Table 4
Antifungal Activity of N-Formylpyrazolines and N-Acetylpyrazolines

Samples (50 µg/disc) /fungal species	Diameter of Inhibition Zone (mm±SD)[A]			
	Antifungal strains			
	A. fumigatus	*A. flavus*	*C. albicans*	*P. marneffei*
4a	**22±0.31**	**17±0.21**	**19±0.58**	**20±0.42**
4b	13±0.38	11±0.31	20±0.28	15±0.33
4c	**20±0.37**	**18±0.41**	**19±0.24**	**19±0.26**
4d	**22±0.25**	**17±0.33**	**18±0.35**	**20±0.32**
4e	11±0.27	17±0.32	11±0.26	15±0.40
4f	14±0.28	173±0.33	20±0.25	13±0.51
4g	**22±0.46**	**16±0.44**	**19±0.32**	**18±0.35**
5a	**21±0.43**	**18±0.29**	**18±0.34**	**20±0.36**
5b	15±0.26	13±0.31	17±0.29	15±0.45
5c	11±0.27	12±0.28	13±0.27	17±0.32
5d	17±0.29	18±0.34	12±0.32	12±0.31
5e	**223±0.38**	**17±0.29**	**20±0.26**	**19±0.38**
5f	16±0.32	13±0.31	17±0.25	11±0.22
5g	**21±0.21**	**18±0.23**	**18±0.28**	**19±0.25**
Ciclopiroxolamine	**22±0.48**	**18±0.41**	**20±0.36**	**20±0.45**

Note: [A]Mean values of 3 trials. Ref. Std: Ciclopiroxolamine (10 µg/disc).

Figure 1
Antibacterial Activity of Novel Pyrazolines (4a-g and 5a-g)

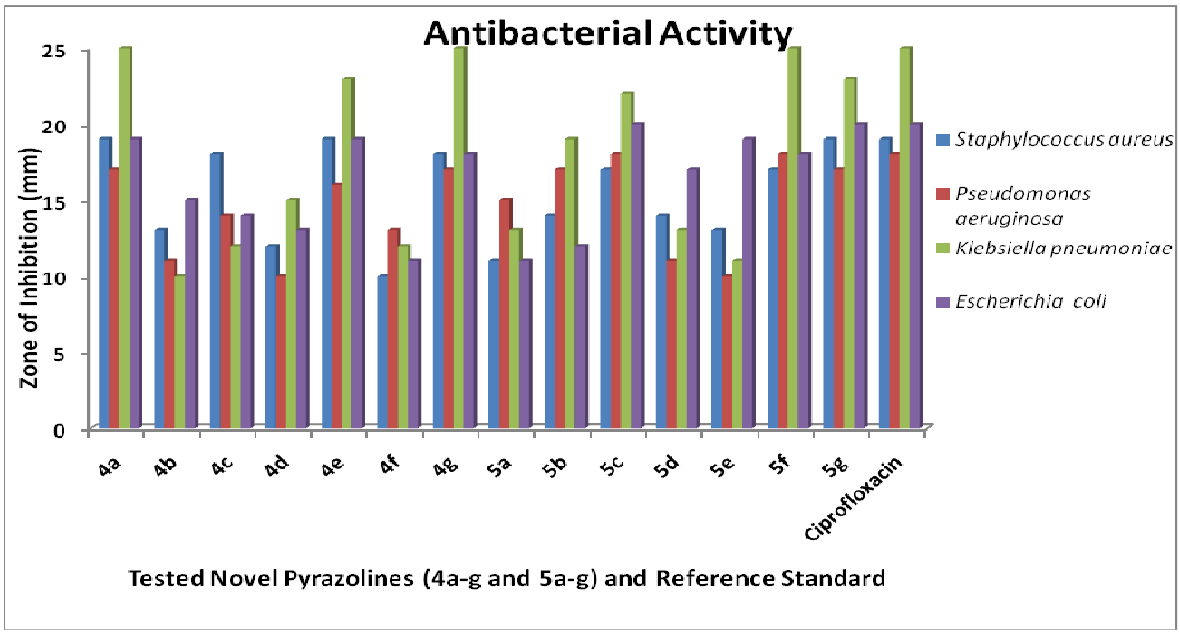
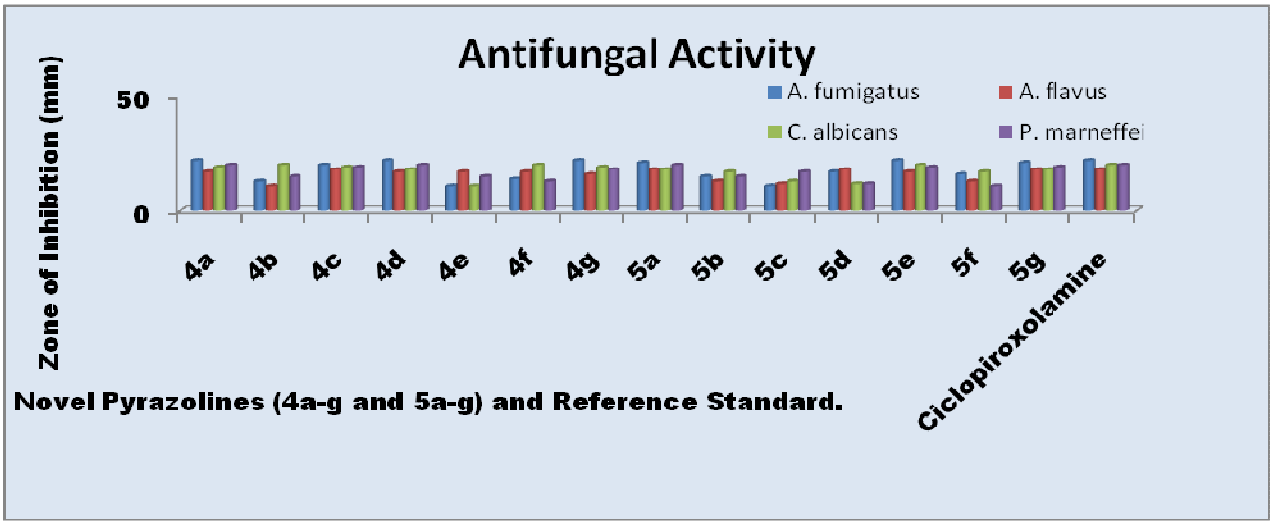


Figure 2
Antifungal Activity of Novel Pyrazolines (4a-g and 5a-g)



CONCLUSION

The investigation of the antibacterial and antifungal screening studies revealed that all tested compounds (4a-g) and (5a-g) showed moderate to good inhibition in respective solvents used for testing. The compounds 4a, 4e, 4g, 5c, 5f and 5g showed comparatively good activity against all the bacterial strains. The good activity can be attributed to the presence of pharmacologically active groups 4-methyl, 4-bromo, 4-hydroxy and 4-nitro which are directly attached to the phenyl ring of the pyrazoline ring system. The 4a, 4c, 4d, 4g, 5a, 5e and 5g showed comparatively good activity against all the tested fungal strains. The groups 4-methyl, 4- N,N-dimethyl, 4-bromo, 4-chloro and 4-hydroxy which are directly attached to the phenyl ring of the N- formyl pyrazoline and N-acetyl pyrazoline ring were responsible for the good antifungal activity.

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