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Novel Heterocyclic Chalcone Compounds Containing Acylated Pyrazole: Synthesis, Characterization, and Antibacterial Activity

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KEYWORDS

Chalcone-pyrazoline derivatives, Antifungal, Antibacterial, Acylated Pyrazoline

ABSTRACT:

In this study, a novel series of 1-[3-(4-fluoro-3-methylphenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethan-1-one derivatives (**3a-i**) were synthesized, and their chemical structures were studied by ^{1}H NMR, IR, and mass spectroscopy. TLC was used to examine the products that were isolated to determine their level of purity. The results of this study show that these derivatives have interesting properties. The discs diffusion method was used to test the in vitro antimicrobial activity of the synthesized compounds against Escherichia coli (MCC 2412), *Staphylococcus aureus* (MCC 2408), *Bacillus subtilis* (MCC 2010), *Pseudomonas aeruginosa* (MCC 2080), *Saccharomyces cerevisiae* (MCC 1033), and *Candida albicans* (MCC 1439).

Introduction:

Fungal infections are a significant health issue affecting individuals globally [1]. The advent of antibiotics for combating fungus and other microorganisms has led to pharmaceutical-resistant infections. The use of pharmacological agents, including Fluconazole, Azathioprine, Methotrexate, Voriconazole, Cyclosporine, Itraconazole, Posaconazole, Micafungin, Flucytosine, Caspofungin, Anidulafungin, and other others, is efficacious in combating fungal infections. However, it has been observed that the administration of these pharmaceuticals in conjunction with other medications has resulted in the occurrence of adverse effects [2, 3]. Because of their wide variety of pharmacological properties, including antibacterial, anticancer, antifungal, anti-inflammatory, antioxidative, and others [4, 5], pyrazole derivatives are among the chemicals that can act against fungus. In the crystalline structure of fluconazole [6-8], the presence of an F-N interaction between fluoro-1 and the two nitrogen (N1 and N-2) atoms indicates the presence of two combined N, F-pharmacophore sites, and removing or modifying this topology could hinder or enhance its various pharmacological activities.

Several studies [9–12] observed that various organic compounds had enhanced antifungal efficacy against numerous microbial strains. Encouraged by this, a series of heterocyclic bis-armed pyrazole derivatives were produced by focusing on the terminal aryl electron rich with different phenyl ring substituents. Due to pyrazolines taking on a wide variety of forms, they have proven to be quite beneficial in the field of drug research [9-13]. This research reports the synthesis and antibacterial activity of a novel family of 1-[3-(4-fluoro-3-methylphenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl]ethan-1-one and its derivatives. This work continues our search for novel biologically active novel heterocyclic chalcone compounds containing acylated pyrazole.

Table 1: Commercially marketed pyrazoline medications that are now used in clinical practice.

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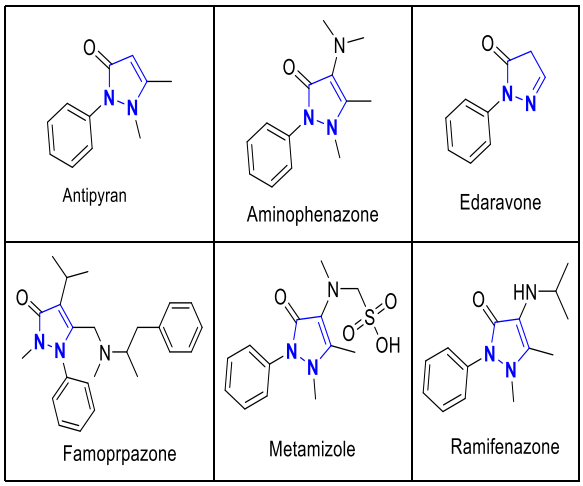
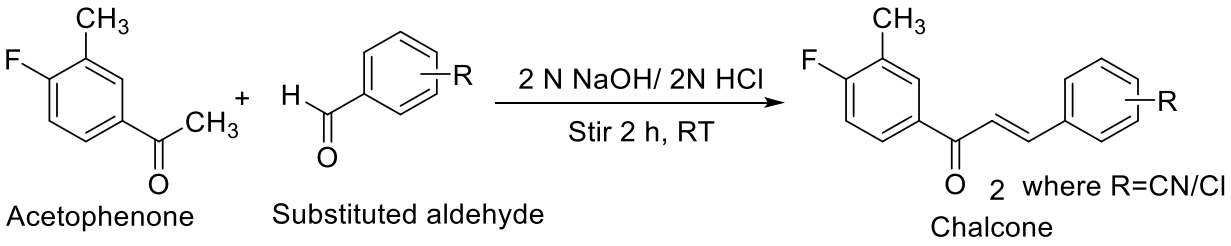


Experimental

Materials and methods

The Barnstead Electrothermal 9100 melting point was used to determine the melting points (uncorrected). The deuterated chloroform ($CDCl_3$) served as the solvent for the ^1H NMR spectra that were recorded on a Bruker Avance spectrometer at 400 MHz. The values for the chemical shift were presented in δ (ppm) scales. Infrared (IR) spectra were collected for analysis using a spectrometer manufactured by Brucker. The HRMS was captured using a Bruker IMPACT HD instrument. To monitor and detect the chemicals, thin-layer chromatography (TLC) alumina sheets precoated with silica gel (0.2 mm thickness) were utilized. The spots on the sheets were seen under a UV light at 254 nm. There was no need for additional purification because all of the chemicals and reagents used were analytical grade.

General procedure for synthesis of chalcones (2a–i):
In ethanol (25 mL), a mixture of 4-fluoro-3-methylacetophenone (**1**) (10 mmol) and cyano- and chloro-substituted benzaldehydes (**a-i**) (10 mmol) was mixed. The combination received was treated with 15 mL of an aqueous solution of sodium hydroxide at a concentration of 2N dropwise at 0±5 °C. After completion of the addition remove the ice bath and raise the reaction mass temperature to RT, the reaction mixture was agitated for 0.5 to 2h at room temperature. 2N HCl was used to achieve the desired pH level for the reaction mass. After the solid result was filtered, washed with water, and dried to yield the solid, the corresponding chalcones (**2a–i**) were prepared through the process of recrystallization using ethanol. TLC was used to check whether or not the reaction was successful [14].



Scheme 1: General procedure for the synthesis of chalcones **(2a–i)**

General procedure for the synthesis of pyrazolines derivatives (3a–i):
A solution containing a combination of chalcones (**2a–h**) (10 mmol) with the required amount of hydrazine hydrate (20 mmol) in glacial acetic acid was subjected to reflux for an extended period. The reaction mixture underwent a cooling process and was subsequently transferred into crushed ice. The resulting residue was then separated using filtration, followed by a washing and drying procedure, resulting in the formation of compounds (**3a–h**). The advancement of the reaction was monitored using thin-layer chromatography (TLC) [15].

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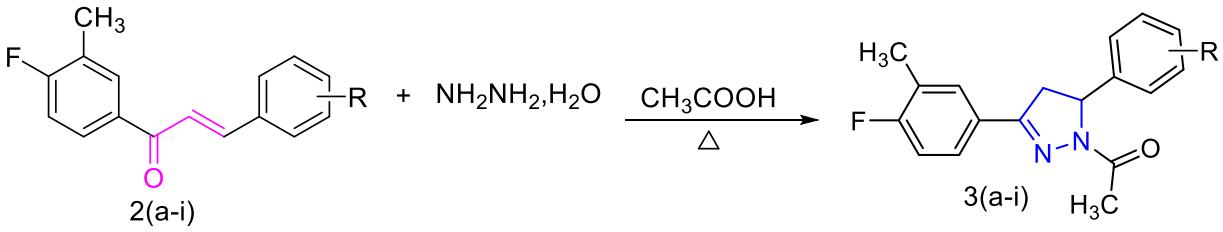

Scheme 2: General procedure for the synthesis of acylated pyrazolines derivatives **(3a–i)**

2-[1-acetyl-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]benzonitrile (3a):
White solid, yield: (214 mg, 66.67 %), m. p: 188±1 °C; IR (cm^{-1}): 3063(CO–CH_3), 2924 (-CH_3), 2229 (CN), 1719 (C=O), 1630 (C=N), 1502/1400 (C=C aromatic ring), 1362 (C-F), 1012 (N-N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.331 (*s*, 3H, Co-CH_3), 2.414 (*s*, 3H, -CH_3), 3.112-3.156 (*dd*, 1H, pyrazole-H, *J* = 11.44, 7.95 Hz), 3.774-3.848 (*dd*, 1H, pyrazole-H, *J* = 11.44, 4.26 Hz), 5.622-5.621 (*dd*, 1H, pyrazole-H, *J* = 8.06, 4.27 Hz), 7.073-7.117 (*dd*, 1H, Ar-H, *J* = 8.40, 0.54 Hz), 7.473-7.638 (*m*, 7H, Ar-H). HRMS: MS (ESI, m/z) $[M+H]^+$: calcd.: 321.35; Observed: 321.5542. Anal. calcd for $C_{19}H_{16}FN_3O$: C, 71.01; H, 13.02; F, 5.91; N, 13.08; O, 4.98.

3-[1-acetyl-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]benzonitrile (3b):
White solid, yield: (249 mg, 71.55 %), m. p: 183±1 °C; IR (cm^{-1}): 3065(CO–CH_3), 2926 (-CH_3), 2229 (CN), 1725 (C=O), 1605 (C=N), 1501/1411 (C=C aromatic ring), 1363 (C-F), 1037 (N-N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.331 (*s*, 3H, Co-CH_3), 2.414 (*s*, 3H, -CH_3), 3.112-3.156 (*dd*, 1H, pyrazole-H, *J* = 11.44, 7.95 Hz), 3.774-3.848 (*dd*, 1H, pyrazole-H, *J* = 11.44, 4.26 Hz), 5.622-5.621 (*dd*, 1H, pyrazole-H, *J* = 8.06, 4.27 Hz), 7.073-7.117 (*dd*, 1H, Ar-H, *J* = 8.40, 0.54 Hz), 7.473-7.638 (*m*, 7H, Ar-H). HRMS: MS (ESI, m/z) $[M+H]^+$: calcd.: 321.35; Observed: 322.9871. Anal. calcd for $C_{19}H_{16}FN_3O$: C, 71.01; H, 13.02; F, 5.91; N, 13.08; O, 4.98.

4-[1-acetyl-3-(4-fluoro-3-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl]benzonitrile (3c):
White solid, yield: (256 mg, 73.56 %), m. p: 178±1 °C; IR (cm^{-1}): 3038(CO–CH_3), 2920 (-CH_3), 2227 (CN), 1652 (C=O), 1605 (C=N), 1502/1409 (C=C aromatic ring), 1331 (C-F), 1016 (N-N); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.356 (*s*, 3H, CO-CH_3), 2.458 (*s*, 3H, -CH_3), 3.102-3.158 (*dd*, 1H, pyrazole-H, *J* = 8.05, 7.77 Hz), 3.775-3.849 (*dd*, 1H, pyrazole-H, *J* = 7.76, 4.25 Hz), 5.616-5.658 (*dd*, 1H, pyrazole-H, *J* = 8.06, 4.27 Hz), 7.069-7.114 (*dd*, 1H, Ar-H, *J* = 8.42, 0.54 Hz), 7.299-7.381 (*ddd*, 2H, Ar-H, *J* = 8.61, 1.65, 0.40 Hz), 7.550-7.615 (*dd*, 1H, Ar-H, *J* = 8.47, 0.55 Hz), 7.646-7.666 (*ddd*, 3H, Ar-H, *J* = 5.59, 1.95, 0.45 Hz). HRMS: MS (ESI, m/z) $[M+H]^+$: calcd.: 321.35; Observed: 322.9874. Anal. calcd for $C_{19}H_{16}FN_3O$: C, 71.01; H, 5.02; F, 5.91; N, 13.08; O, 4.98.

1-[5-(2, 3-dichlorophenyl)-3-(4-fluoro-3-methylphenyl)-4, 5-dihydro-1H-pyrazol-1-yl]ethan-1-one (3d):
White solid, yield: (256 mg, 70.01 %), m. p: 199±1 °C; IR (cm^{-1}): 3067(CO–CH_3), 2925 (-CH_3), 1739 (C=O), 1605 (C=N), 1502/1402 (C=C aromatic ring), 1323 (C-F), 1010 (N-N), 818 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.343 (*s*, 3H, CO-CH_3), 2.520 (*s*, 3H, -CH_3), 3.056 (*dd*, 1H, pyrazole-H, *J* = 13.66, 8.01 Hz), 3.841-3.915 (*dd*, 1H, pyrazole-H, *J* = 13.69, 4.25 Hz), 5.930-5.970 (*dd*, 1H, pyrazole-H, *J* = 8.06, 4.26 Hz), 6.989-7.008 (*dd*, 1H, Ar-H, *J* = 8.42, 0.55 Hz), 7.171-7.211 (*dd*, 1H, Ar-H, *J* = 1.87, 0.51 Hz), 7.300 (*dd*, 1H, Ar-H, *J* = 8.00, 1.10 Hz), 7.403-7.422 (*dd*, 1H, Ar-H, *J* = 8.33, 1.86 Hz), 7.548-7.616 (*dd*, 1H, Ar-H, *J* = 7.77, 1.11 Hz). HRMS: MS (ESI, m/z) $[M+H]^+$: calcd.: 365.23; Observed: 363.1474. Anal. calcd for $C_{18}H_{15}Cl_2FN_3O$: C, 59.19; H, 4.14; Cl, 19.41; F, 5.20; N, 7.67; O, 4.38.

1-[5-(2, 4-dichlorophenyl)-3-(4-fluoro-3-methylphenyl)-4, 5-dihydro-1H-pyrazol-1-yl]ethan-1-one (3e):
White solid, yield: (272 mg, 74.52 %), m. p: 188±1 °C; IR (cm^{-1}): 3067(CO–CH_3), 2925 (-CH_3), 1739 (C=O), 1605 (C=N), 1502/1402 (C=C aromatic ring), 1323 (C-F), 1010 (N-N), 818 (C-Cl); ^{1}H NMR (400 MHz,

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$CDCl_3$): δ 2.300 (*s*, 3H, CO-CH_3), 2.449 (*s*, 3H, -CH_3), 3.050 (*dd*, 1H, pyrazole-H, *J* = 13.66, 8.00 Hz), 3.810 (*q*, 1H, pyrazole-H, *J* = 13.71, 4.26 Hz), 5.858-5.899 (*t*, 1H, pyrazole-H, *J* = 8.03, 4.44 Hz), 7.015-7.051 (*dd*, 1H, Ar-H, *J* = 8.38, 0.49 Hz), 7.073-7.095 (*dd*, 1H, Ar-H, *J* = 8.29, 1.59 Hz), 7.212-7.232 (*dd*, 1H, Ar-H, *J* = 1.87, 0.52 Hz), 7.450 (*dd*, 1H, Ar-H, *J* = 1.63, 0.51 Hz), 7.545-7.559 (*dd*, 1H, Ar-H, *J* = 8.33, 1.88 Hz), 7.601-7.618 (*dd*, 1H, Ar-H, *J* = 8.28, 0.53 Hz). HRMS: MS (ESI, m/z) $[M+H]^+$: calcd.: 365.23; Observed: 364.2769. Anal. calcd for $C_{18}H_{15}Cl_2FN_3O$: C, 59.19; H, 4.14; Cl, 19.41; F, 5.20; N, 7.67; O, 4.38.

1-[5-(2, 5-dichlorophenyl)-3-(4-fluoro-3-methylphenyl)-4, 5-dihydro-1H-pyrazol-1-yl]ethan-1-one (3f):

White solid, yield: (263 mg, 73.42 %), m. p: 183±1°C; IR (cm^{-1}): 3088(CO–CH_3), 2925 (-CH_3), 1740 (C=O), 1586 (C=N), 1502/1438 (C=C aromatic ring), 1362 (C-F), 1008 (N-N), 817 (C-Cl); ^1H NMR (400 MHz, $CDCl_3$): δ 2.345 (*s*, 3H, CO-CH_3), 2.523 (*s*, 3H, -CH_3), 2935 (*dd*, 1H, pyrazole-H, *J* = 13.66, 8.03 Hz), 3.820-3.894 (*q*, 1H, pyrazole-H, *J* = 13.60, 4.22 Hz), 5.866-5.907 (*dd*, 1H, pyrazole-H, *J* = 8.00, 4.21 Hz), 7.039-7.199 (*dd*, 2H, Ar-H, *J* = 8.44, 0.53 Hz), 7.220-7.262 (*dd*, 1H, Ar-H, *J* = 1.87, 0.55 Hz), 7.295-7.373 (*dd*, 1H, Ar-H, *J* = 8.10, 0.53 Hz), 7.547-7.624 (*dd*, 2H, Ar-H, *J* = 8.39, 1.88 Hz). HRMS: MS (ESI, m/z) $[M+H]^+$: calcd.: 365.23; Observed: 365.2291. Anal. calcd for $C_{18}H_{15}Cl_2FN_3O$: C, 59.19; H, 4.14; Cl, 19.41; F, 5.20; N, 7.67; O, 4.38.

1-[5-(2, 6-dichlorophenyl)-3-(4-fluoro-3-methylphenyl)-4, 5-dihydro-1H-pyrazol-1-yl]ethan-1-one (3g):

White solid, yield: (298 mg, 81.64 %), m. p: 194±1 °C; IR (cm^{-1}): 3052(CO–CH_3), 2929 (-CH_3), 1741 (C=O), 1581 (C=N), 1501/1432 (C=C aromatic ring), 1320 (C-F), 1017 (N-N), 821 (C-Cl); ^1H NMR (400 MHz, $CDCl_3$): δ 2.360 (*s*, 3H, CO-CH_3), 2.392 (*s*, 3H, -CH_3), 3.275-3.40 (*q* 1H, pyrazole-H, *J* = 8.44, 8.00 Hz), 3.665-3.742 (*q*, 1H, pyrazole-H, *J* = 8.26, 4.26 Hz), 6.225-6.278 (*q*, 1H, pyrazole-H, *J* = 8.00, 4.26 Hz), 7.069-7.091 (*q*, 1H, Ar-H, *J* = 8.42, 0.55 Hz), 7.113-7.192 (*dd*, 1H, Ar-H, *J* = 1.86, 0.53 Hz), 7.277-7.295 (*dd*, 1H, Ar-H, *J* = 8.06, 1.20 Hz), 7.542-7.556 (*dd*, 1H, Ar-H, *J* = 8.00, 0.49 Hz), 7.563-7.570 (*dd*, 1H, Ar-H, *J* = 8.11, 0.89 Hz), 7.575-7.661 (*dd*, 1H, Ar-H, *J* = 1.20, 0.51 Hz). HRMS: MS (ESI, m/z) $[M+H]^+$: calcd.: 365.23; Observed: 365.0028. Anal. calcd for $C_{18}H_{15}Cl_2FN_3O$: C, 59.19; H, 4.14; Cl, 19.41; F, 5.20; N, 7.67; O, 4.38.

1-[5-(3, 4-dichlorophenyl)-3-(4-fluoro-3-methylphenyl)-4, 5-dihydro-1H-pyrazol-1-yl]ethan-1-one (3h):

White solid, yield: (255 mg, 69.86 %), m. p: 182±1 °C; IR (cm^{-1}): 3066(CO–CH_3), 2925 (-CH_3), 1587 (C=O), 1587 (C=N), 1502/1402 (C=C aromatic ring), 1321 (C-F), 1013 (N-N), 819 (C-Cl); ^1H NMR (400 MHz, $CDCl_3$): δ 2.359 (*s*, 3H, CO-CH_3), 2.469 (*s*, 3H, -CH_3), 3.091-3.148 (*dd* 1H, pyrazole-H, *J* = 8.33, 7.99 Hz), 3.730-3.804 (*dd*, 1H, pyrazole-H, *J* = 8.48, 4.27 Hz), 5.518-5.480 (*dd*, 1H, pyrazole-H, *J* = 8.06, 4.20 Hz), 7.072-7.125 (*dd*, 3H, Ar-H, *J* = 8.00, 3.33 Hz), 7.283-7.372 (*dd*, 1H, Ar-H, *J* = 1.96, 0.54 Hz), 7.615 (*dd*, 1H, Ar-H, *J* = 8.39, 1.88 Hz), 7.634 (*dd*, 1H, Ar-H, *J* = 1.22, 0.49 Hz). HRMS: MS (ESI, m/z) $[M+H]^+$: calcd.: 365.23; Observed: 368.1157. Anal. calcd for $C_{18}H_{15}Cl_2FN_3O$: C, 59.19; H, 4.14; Cl, 19.41; F, 5.20; N, 7.67; O, 4.38.

1-[5-(2, 3, 5-trichlorophenyl)-3-(4-fluoro-3-methylphenyl)-4, 5-dihydro-1H-pyrazol-1-yl]ethan-1-one (3i):

White solid, yield: (302 mg, 75.39 %), m. p: 196±1°C; IR (cm^{-1}): 3055(CO–CH_3), 2927 (-CH_3), 1695 (C=O), 1585 (C=N), 1515/1444 (C=C aromatic ring), 1329 (C-F), 1019 (N-N), 817 (C-Cl); ^1H NMR (400 MHz, $CDCl_3$): δ 2.361 (*s*, 3H, CO-CH_3), 2.395 (*s*, 3H, -CH_3), 3.276-3.456 (*dd* 1H, pyrazole-H, *J* = 8.45, 8.01 Hz), 3.666-3.745 (*dd*, 1H, pyrazole-H, *J* = 8.20, 4.25 Hz), 6.222-6.277 (*dd*, 1H, pyrazole-H, *J* = 8.00, 4.26 Hz), 7.069-7.091 (*dd*, 1H, Ar-H, *J* = 8.40, 0.53 Hz), 7.115-7.190 (*dd*, 1H, Ar-H, *J* = 1.86, 0.53 Hz), 7.277-7.295 (*dd*, 1H, Ar-H, *J* = 8.06, 1.20 Hz), 7.542-7.556 (*dd*, 1H, Ar-H, *J* = 8.00, 0.49 Hz), 7.563-7.570 (*dd*, 1H, Ar-H, *J* = 8.11, 0.89 Hz). HRMS: MS (ESI, m/z) $[M+H]^+$: calcd.: 399.67; Observed: 402.6042. Anal. calcd for $C_{18}H_{14}Cl_3FN_3O$: C, 54.09; H, 3.53; Cl, 26.61; F, 4.75; N, 7.01; O, 4.75.

Antimicrobial screening:

Antimicrobial activity of the synthesized compounds was evaluated in vitro using four bacterial strains (*Escherichia coli* (MCC 2412), Bacillus subtilis (MCC 2010), *Staphylococcus aureus* (MCC 2408),

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Pseudomonas aeruginosa (MCC 2080)), two fungal strains (*Saccharomyces cerevisiae*, MCC 1033, and *Candida albicans*, MCC 1439). ZOI (zone of inhibition) and MIC (minimum inhibitory concentration) values were used to describe the antibacterial efficacy of the test substances. DMF served as a negative control, while *streptomycin* and *fluconazole* served as the study's reference drugs. The following approach was used to conduct the tests in triplicate.

Autoclaved Petri dishes were filled with sterilized bacterial (nutrient agar) and fungal (sabouraud dextrose agar) growth medium. In addition, 100 µl inocula of each test organism were swabbed onto the agar plates in a sterile environment. Adsorption was followed by creating wells of 6 mm diameter using a sterile metallic borer and filling them with solutions of the working substances (128 µg/20 µL). After 48 hours of incubation at 28 °C, the ZOI was determined. After incubation at 28 °C for 48 hours, the MIC values for each chemical were determined using the broth double-dilution method with a 100 µl inoculum of each fungal culture [16, 17].

Results and discussion:

The condensation of 4-fluoro-3-methylacetophenone (**1**) with cyano- and chloro-substituted benzaldehydes (**a-i**) carried place in the presence of 2N sodium hydroxide and ethanol throughout the synthesis of chalcones (**2a–1**) according to **Scheme 1**. Pyrazoline derivatives (**3a–h**) were synthesized through a cyclization reaction that involved chalcones (**3a–h**) and hydrazine hydrate (**Scheme 2**) in the presence of glacial acetic acid.

FT-IR, ^{1}H NMR, and mass spectrometry were used to characterize the novel heterocyclic chalcone compounds containing acylated pyrazoles (**3a–h**). The presence of –C=N and carbonyl C=O groups was determined from the IR spectrum of compounds (**3a–h**), which displayed distinctive bands at around 1566–1630 cm^{-1} and 1652–1741 cm^{-1}, respectively [18, 19]. The IR of the compounds (**3a–h**) displayed distinctive bands with wavelengths ranging from 2924–2929 cm^{-1}. These bands were developed by the C–H sp^3 stretching in the –$COCH_3$ group [20]. A band at 1320-1363 cm^{-1} in the IR spectra of acylated pyrazoles (**3a-i**) is indicative of C-F stretching of the aromatic ring. Bands at 1008-1037 cm^{-1} was observed in the acylated pyrazoles (**3a–i**), indicating the presence of a (N-N) group. Absorption bands at 1501-1502 and 1400–1411 cm^{-1} were likewise present in compounds **3a–i**, indicating the presence of the C=C aromatic ring. Identifiable bands at 2227-2229 cm^{-1}, attributed to the stretching vibration of the -CN group, were also observed in the IR spectrum of the compounds (**3a-c**) [21]. At a range of 817–821 cm^{-1}, the infrared spectrum of the pyrazoles (**3d–i**) displayed a band that was characteristic of the stretching of the C-Cl group [22].

The ^{1}H NMR spectra of the compounds (**3a-i**) confirmed their structures. Compounds **3a–i** had a singlet of acyl group $COCH_3$ protons at d 2.300–2.360 ppm in their ^{1}H NMR patterns. The singlet readings that added up for three protons in the high field region (δ2.414–2.523 ppm) were found to be -CH_3 protons [23]. Compounds **3a-i** exhibited a pair of doublet-of-doublet resonances at δ2.935-3.158 ppm (J = 7.95-8.05 Hz, J = 7.77-18.15 Hz) and δ3.774-3.915 ppm (J = 4.25-11.60 Hz, J = 7.76-18.15 Hz) for the CH_2 protons of the pyrazoline ring [23]. The Hx-7 also showed up as a doublet-of-doublet at δ5.622-6.278 (J = 0.54–4.44 Hz, J = 8.06–8.40 Hz). In the range from 7.015-7.666ppm [22-25], multiplets of five or six protons in the aromatic area were seen.

The mass spectrum confirmed the presence of M^+ in the region, with a m/z measurement of 320.9945-398.2661, providing more evidence for the structure's accuracy.

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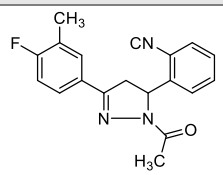
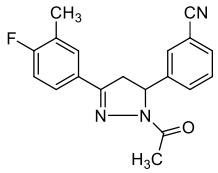
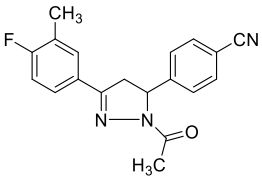
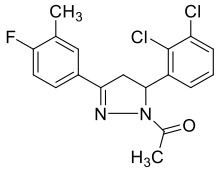
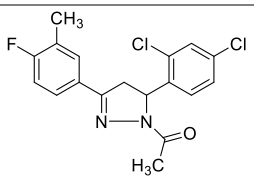
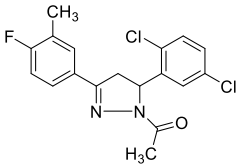
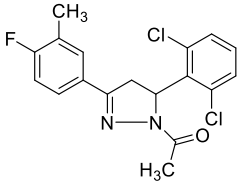
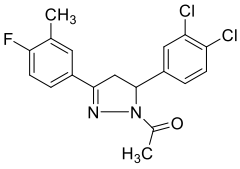
Table 2: Structures, melting points, and molecular weight of compounds **3a-i**

Comp Code	MW	Formula	MP	Structure
3a	321.39	$C_{19}H_{16}FN_3O$	188±1 °C	
3b	321.39	$C_{19}H_{16}FN_3O$	183±1°C	
3c	321.39	$C_{19}H_{16}FN_3O$	178±1 °C	
3d	365.23	$C_{18}H_{15}Cl_2FN_3O$	199±1°C	
3e	365.23	$C_{18}H_{15}Cl_2FN_3O$	188±1°C	
3f	365.23	$C_{18}H_{15}Cl_2FN_3O$	183±1°C	
3g	365.23	$C_{18}H_{15}Cl_2FN_3O$	194±1°C	
3h	365.23	$C_{18}H_{15}Cl_2FN_3O$	182±1°C	

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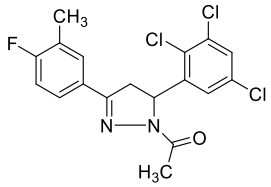
3i	399.67	$C_{18}H_{14}Cl_3FN_3O$	196±1 °C	

Antibacterial activities:

The antimicrobial activities of newly synthesized compounds were assessed in vitro against gram-positive bacteria, specifically *Bacillus subtilis* and *Staphylococcus aureus*, as well as gram-negative bacteria, including *Escherichia coli*, and *Pseudomonas aeruginosa*. The disc diffusion method (zone inhibition) was employed for this determination. As presented in **Table 3**, some of the newly synthesized compounds showed minimal inhibitory concentrations (MIC, µg/mL) and inhibition zone (mm) against all the screened gram-positive bacteria and gram-negative bacteria. It can be seen in **Figure 1** that some of the compounds **3a, 3b, 3c, 3e, 3g,** and **3h** showed higher activity than *Streptomycin* against *S. aureus* (MIC, 25-80 µg/mL). Compounds **3a, 3b, 3d,** and **3g** displayed higher antimicrobial activities than reference drugs against the *P. aeruginosa* bacteria. Also, the reference drug showed more inhibitory activities than all of the newly synthesized compounds against *B. subtilis* bacteria. On the other hand, compounds **3a, 3c**, and **3g** exhibited more inhibitory activities than *Streptomycin* against *E. coli*, gram-negative bacteria.

Table 3: Antibacterial studies of **3a-3i** compounds

Compound	**Antibacterial Activity (zone of inhibition)**			
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
3a	14	7	12	13
3b	11	9	11	12
3c	10	8	12	8
3d	9	7	10	16
3e	14	7	0	9
3f	8	7	0	0
3g	12	9	12	23
3h	11	8	7	0
3i	8	10	0	8
Streptomycin	8	10	11	11

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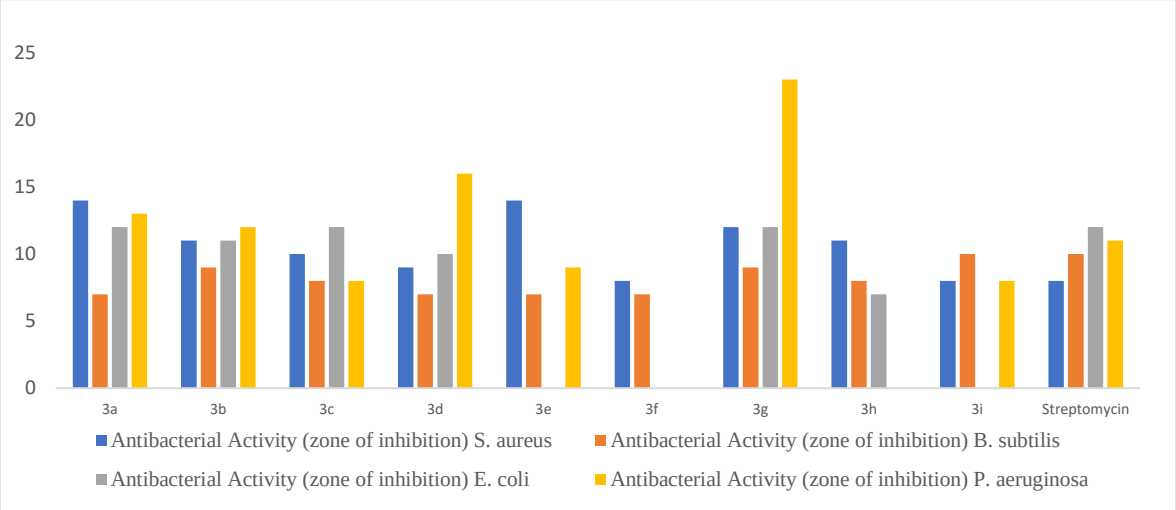

Figure 1: Antibacterial studies of **3a-i** compounds

Antifungal activity:

Antifungal activity against *C. albican*, and *S. cerevisiae* was determined at 50 mg/L and *fluconazole* as positive control. The results are listed in **Table 4**. The activity of compounds **3c**, and **3d** against *C. albican* is higher than that of control. Among them, compounds **3c**, **3d**, **3e**, and **3g** exhibited good activity against *S. cerevisiae*.

Table 4: Antifungal activities of compounds **3a-i**

Compound	Antibacterial Activity (zone of inhibition/mm)	
	C. albican	***S. cerevisiae***
3a	8	8
3b	9	6
3c	13	12
3d	12	11
3e	0	11
3f	8	6
3g	7	20
3h	0	6
3i	0	7
Fluconazole	11	10

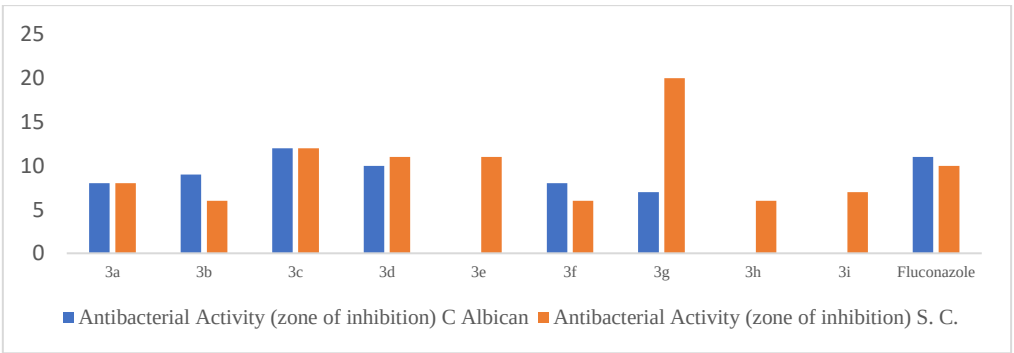

Figure 2: Antifungal activity of compounds **3a-i**

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Conclusion:

The main objective of this research is to expand the existing knowledge base with novel, useful compounds containing the pyrazoline structure. To assess their in vitro biological activity, we have developed and synthesized 9 novel pyrazoline-derived compounds. The structures of these compounds were analyzed using FT-IR, ^{1}H NMR spectrum spectroscopy, and HRMS. The next step was to test the efficacy of the pyrazolines (**3a-i**) against a variety of bacteria, including Gram-positive (*Bacillus subtilis* MCC 2010 and *Staphylococcus aureus* MCC 2408) and Gram-negative (*E. coli* MCC 2412 and *Pseudomonas aeruginosa* MCC 2080) strains. The chemicals proved to be efficient against a wide variety of bacteria, and their antibacterial activity was even higher than that of *streptomycin*.

References:

1. Goussault, H., Salvator, H., Catherinot, E., Chabi, M. L., Tcherakian, C., Chabrol, A., ... & Couderc, L. J. (2019). Primary immunodeficiency-related bronchiectasis in adults: comparison with bronchiectasis of other etiologies in a French reference center. *Respiratory Research*, *20*(1), 1-10.
2. Rossi S (ed.), Adelaide: Australian Medicines Handbook, (2006). ISBN 0-9757919-2-3
3. Charlier, C., Hart, E., Lefort, A., Ribaud, P., Dromer, F., Denning, D. W., & Lortholary, O. (2006). Fluconazole for the management of invasive candidiasis: where do we stand after 15 years? *Journal of Antimicrobial Chemotherapy*, *57*(3), 384-410.
4. Singh, V. K., Chaurasia, H., Mishra, R., Srivastava, R., Naaz, F., Kumar, P., & Singh, R. K. (2022). Docking, ADMET prediction, DFT analysis, synthesis, cytotoxicity, antibacterial screening, and QSAR analysis of diarylpyrimidine derivatives. *Journal of Molecular Structure*, *1247*, 131400.
5. Karrouchi, K., Radi, S., Ramli, Y., Taoufik, J., Mabkhot, Y. N., Al-Aizari, F. A., & Ansar, M. H. (2018). Synthesis and pharmacological activities of pyrazole derivatives: A review. *Molecules*, *23*(1), 134.
6. Tantardini, C., Arkhipov, S. G., Cherkashina, K. A., Kil'Met'Ev, A. S., & Boldyreva, E. V. (2016). Crystal structure of a 2: 1 co-crystal of meloxicam with acetylendicarboxylic acid. *Acta Crystallographica Section E: Crystallographic Communications*, *72*(12), 1856-1859.
7. Coro-Bermello, J., López-Rodríguez, E. R., Alfonso-Ramos, J. E., Alonso, D., Ojeda-Carralero, G. M., Prado, G. A., & Moreno-Castillo, E. (2021). Identification of novel thiadiazole derivatives as potentially selective inhibitors towards trypanothione reductase from Trypanosoma cruzi by molecular docking using the numerical index poses ratio Pr and the binding mode analysis. *SN Applied Sciences*, *3*, 1-20.
8. Owoyemi, B. C. D., da Silva, C. C., Diniz, L. F., Souza, M. S., Ellena, J., & Carneiro, R. L. (2019). Fluconazolium oxalate: Synthesis and structural characterization of a highly soluble crystalline form. *CrystEngComm*, *21*(7), 1114-1121.
9. Cetin, A., & Bildirici, I. (2018). A study on the synthesis and antimicrobial activity of 4-acyl-pyrazoles. *Journal of Saudi Chemical Society*, *22*(3), 279-296.
10. Tripathi A. C.; Upadhyay S.; Paliwal S.; Saraf S. K. (2018) N1-benzenesulfonyl-2-pyrazoline hybrids in neurological disorders: Syntheses, biological screening and computational studies. EXCLI J. 17, 126–148.
11. Marinescu, M. (2021). Synthesis of antimicrobial benzimidazole–pyrazole compounds and their biological activities. *Antibiotics*, *10*(8), 1002.
12. Anush, S. M., Vishalakshi, B., Kalluraya, B., & Manju, N. (2018). Synthesis of pyrazole-based Schiff bases of Chitosan: Evaluation of antimicrobial activity. *International journal of biological macromolecules*, *119*, 446-452.
13. Manna K.; Banik U.; Ghosh P.; Das M. (2014) A review on synthesis and pharmacological diversity of isoxazoles and pyrazolines. *Pharmaceut. Sci. 1, 37–49.*
14 Mohammed Rayees Ahmad, V. Girija Sastry, Nasreen Bano, Syed Anwar, (2016), Synthesis of novel chalcone derivatives by conventional and microwave irradiation methods and their pharmacological activities *Arabian Journal of Chemistry 9 (1), S931-S935*
15 Xie, D., Yang, J., Niu, X., Wang, Z., & Wu, Z. (2022). Synthesis and bioactivity evaluation of 5-trifluoromethyl-1 H-pyrazole-4-carboxamide

derivatives as potential anticancer and antifungal agents. *Journal of Heterocyclic Chemistry*, *59*(10), 1759-1767.

16 Kumar, A., Rajeswara Rao, M., Lee, W. Z., & Ravikanth, M. (2017). Hybrid macrocycles of sub-porphyrins and porphyrins. *Organic letters*, *19*(21), 5924-5927.

17 Pfaller, M. A., Espinel-Ingroff, A., Boyken, L., Hollis, R. J., Kroeger, J., Messer, S. A., ... & Diekema, D. J. (2011). Comparison of the broth microdilution (BMD) method of the European Committee on Antimicrobial Susceptibility Testing with the 24-hour CLSI BMD method for testing susceptibility of Candida species to fluconazole, posaconazole, and voriconazole by use of epidemiological cutoff values. *Journal of Clinical Microbiology*, *49*(3), 845-850.

18 Singh, V. K., Mishra, R., Kumari, P., Som, A., Yadav, A. K., Ram, N. K., ... & Singh, R. K. (2022). In silico design, synthesis, and anti-HIV activity of quinoline derivatives as non-nucleoside reverse transcriptase inhibitors (NNRTIs). *Computational Biology and Chemistry*, *98*, 107675.

19 Smith, B. C. (2017). The carbonyl group, part I: introduction. *Spectroscopy*, *32*(9), 31-36.

20 Ignatieva, L. N., Beloliptsev, A. Y., Kozlova, S. G., & Buznik, V. M. (2004). Quantum-chemical study of C n F2 n+ 2 conformers. Structure and IR spectra. *Journal of Structural Chemistry*, *45*, 599-609.

21 Sajan, D., Joe, I. H., & Jayakumar, V. S. (2006). NIR-FT Raman, FT-IR and surface-enhanced Raman scattering spectra of organic nonlinear optic material: p-hydroxy acetophenone. *Journal of Raman Spectroscopy: An International Journal for Original Work in all aspects of Raman Spectroscopy, Including Higher Order Processes, and also Brillouin and Rayleigh Scattering*, *37*(4), 508-519.

22 Choi, S., Park, J., Kwak, K., & Cho, M. (2021). Substituent Effects on the Vibrational Properties of the CN Stretch Mode of Aromatic Nitriles: IR Probes Useful for Time-resolved IR Spectroscopy. *Chemistry–An Asian Journal*, *16*(18), 2626-2632.

23 Dozova, N., Krim, L., Alikhani, M. E., & Lacome, N. (2005). Vibrational spectra and structure of CH3Cl: H2O, CH3Cl: HDO, and CH3Cl: D2O complexes. IR matrix isolation and ab initio calculations. *The Journal of Physical Chemistry A*, *109*(45), 10273-10279.

24 Nyquist, R. A. (2001). *Interpreting infrared, Raman, and nuclear magnetic resonance spectra* (Vol. 2). Academic Press.

25 Mullen, C. A., Strahan, G. D., & Boateng, A. A. (2009). Characterization of various fast-pyrolysis bio-oils by NMR spectroscopy. *Energy & Fuels*, *23*(5), 2707-2718.

26 D.T. Mahajan, V.H. Masand, K.N. Patil, T. Ben Hadda, R.D. Jawarkar, S.D. Thakur, V. Rastija, Bioorg. *Med. Chem. Lett*. 22, 4827–4835 (2012)

27 Macêdo, W. J. C., Braga, F. S., Santos, C. F., Costa, J. D. S., de Melo, G. S., de Mello, M. N., ... & dos Santos, C. B. R. (2015). Antimalarial artemisinins derivatives study: Molecular modeling and multivariate analysis (PCA, HCA, KNN, SIMCA, and SDA). *Journal of Computational and Theoretical Nanoscience*, *12*(10), 3443-3458.

28 Santos, C. B. R., Vieira, J. B., Lobato, C. C., Hage-Melim, L. I., Souto, R. N., Lima, C. S., ... & Carvalho, J. C. T. (2013). A SAR and QSAR study of new artemisinin compounds with antimalarial activity. *Molecules*, *19*(1), 367-399.

29 Ibrahim, Z. Y. U., Uzairu, A., Shallangwa, G. A., & Abechi, S. E. (2021). Application of QSAR method in the design of enhanced antimalarial derivatives of Azetidine-2-carbonitriles, their molecular docking, drug-likeness, and SwissADME properties. *Iranian Journal of Pharmaceutical Research: IJPR*, *20*(3), 254.