ORIGINAL ARTICLE

Synthesis, characterization, and antibacterial evaluation of some new 1,3,5-trisubstituted pyrazole derivatives

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Abstract

Objective: The objective of the paper was to design, synthesis, and characterization of new 1,3,5-trisubstituted-2-pyrazolines derivative and evaluate for antibacterial activity. **Materials and Methods:** The 1,3,5-tri-substituted-2-pyrazolines derivatives have been synthesized by the reaction of chalcone derivatives with succinic hydrazide in the environment of pyridine. A total of 20 compounds have been synthesized and characterized by the infrared, 1H-nuclear magnetic resonance, and mass spectral analysis. Antibacterial activity of the compounds carried out on five Gram-positive bacterial strains, that is, *Staphylococcus aureus*, *Staphylococcus faecalis*, *Bacillus subtilis*, *Proteus vulgaris, and Bacillus pumilus* and two Gram-negative bacterial strains, that is, *Escherichia coli* and *Klebsiella pneumoniae* in two different concentrations, that is, 50 and 100 µg/ml by agar diffusion method using cup-plate method. Norfloxacin and ciprofloxacin were used as standard antibacterial drug. **Results and Discussion:** The data of antibacterial activity against the Gram-positive bacterial strains (*S. aureus*, *S. faecalis, B. subtilis*, *P. vulgaris, and B. pumilus*) suggested the order of activity of compounds: BR-3 >BR-2>BR-1>CL-4>BR-4>CL-3> CL-2>CL-5>CL-6>ME-3>ME-2>ME-4>ME-5>ME-6>ME-7>CL-7>CL-8>CL-1>ME-8>ME-1. The compounds series BR-1 to BR-4 has shown the highest activity. Compound ME-8, CL-8, CL-7, CL-1, ME-5, ME-6, and ME-1 have showed mild activity, compounds CL-2, CL-5, ME-4, CL-6, ME-3, ME-2, and ME-7 showed moderate activity, and compounds BR-3, BR-2, BR-1, CL-4, BR-4, and CL-3 have showed good activity against Gram-negative bacteria. The result data of antibacterial activity suggested that Cl, Br, F, and nitro substitution at the third and fifth position may enhance the antibacterial activity of the compounds but the methyl and methoxy substitution may result in reduction of the activity.

Key words: Agar Diffusion, Antibacterial, Ciprofloxacin, Cup-plate method, Norfloxacin, Pyrazole

INTRODUCTION

As humankind developed throughout the years, the study of infectious diseases and transmission alleviated the mortality rate of many diseases.[1-4] Today, we are much more capable of treating and surviving infection diseases. However, bacterial pathogenesis and virulence factors have allowed for bacterial infections to persist and become problematic.[5-7] Bacterial infections are of particular interest because without all the complexity that multicellular species have, bacteria have found a unique way to survive.[8] Bacteria have the innate ability to spontaneously mutate their DNA while replicating in response to deleterious circumstances and therefore pass this survival instinct to their progeny to ensure survival.[9]

Since their discovery, antimicrobial drugs have played a major role in human health and greatly benefited human existence. However, antimicrobial agents are also among the most frequently misused drugs by the physicians. Widespread and injudicious use of antibiotics has resulted in the emergence of drug resistance and multidrug resistance among pathogens, which in many parts of the world, especially in developing countries, has reached unacceptable levels.[10-12] The limited life span of presently used antimicrobial drugs; alteration in genetic and metabolic level, a faster rate of evolution with varying global temperature; well-documented side effects on prolonged use of antimicrobial and high cost of clinical investigation;

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and drug development are the major concerns among the scientists and the clinicians.

The major obstacle of antibiotic drug resistance demands a renewed effort to develop novel and effective classes of antibiotics with a novel or altered mode of actions. Several recent studies demonstrated drug resistance for their recommended antibiotic therapy with increasing prevalence in parasitic protozoa including *Trypanosoma*, *Plasmodium*, *Toxoplasma gondii*, *Leishmania*, and *Entamoeba*.[13-16] During the early stages of medicinal chemistry development, scientists were primarily concerned with the isolation of medicinal agents found in plants. Today, scientists in this field are also equally concerned with the creation of new synthetic drug compounds.[17]

The drugs which are used for bacterial infection treatment are ineffective by the times, the main reason behind is resistance by bacterial strains. Hence, there are augment needs of new drugs or new chemical modified moieties that have to be effective against the bacterial infection. For searching the moieties that have to be effective against the bacterial infection, we have find the diazoles that are reported to be used as antibacterial agents.[18-21]

Pyrazole is unique in their chemical behavior not only among heterocyclic compounds in general but also among related diazoles. Pyrazole derivatives have been known for more than 80 years, the investigation of their chemistry commended rather slowly. Earlier studies were mainly devoted to the development of synthetic methods.[22] Recently, the attention was focused on the investigation of chemical properties and in particular on the peculiarities of the behavior of pyrazole derivatives and the elucidation of their physicochemical characteristics. Pyrazole derivatives have a long history of application in agrochemicals as herbicides and insecticides also used in pharmaceutical industry as antipyretic and anti-inflammatory.[23]

Nowadays, vast number of compounds with pyrazole nucleus have been reported to show a broad spectrum of biological activity including antimicrobial, antifungal, antioxidant, anti-amoebic, analgesic, antitubercular, neuroprotective, anticancer, antiproliferative, antiviral, anticonvulsant, muscle relaxant, and anti-inflammatory activities.[24] Due to its wide range of biological activity, pyrazoles ring constitutes a relevant synthetic route in pharmaceutical industry. Chalcone is 1,3-diphenyl-2-propene-1-one, in which two aromatic rings are linked by a three carbon α, β-unsaturated carbonyl system. The reaction of 1,3,5-pyrazoline with chalcone derivatives to developed the new derivatives of pyrazoline and evaluated for antibacterial activity against Gram-positive and Gram-negative bacteria.

The objective of the paper was to design, synthesis, and characterization of new 1,3,5-trisubstituted-2-pyrazolines derivative and evaluate for antibacterial potential.

MATERIALS AND METHODS

Chemical p-chloroacetophenone, p-bromoacetophenone, and p-methylacetophenone were purchased from HiMedia, New Delhi. Benzaldehyde, 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-nitrobenzaldehyde, 4-methyl benzaldehyde, and 4-methoxybenzaldehyde were purchased from Chemical Drug House, New Delhi, India. Succinic acid was purchased from Sigma-Aldrich, New Delhi. The chemical used for experimental work was synthetic grade. The melting points of the synthesized compounds were determined in open glass capillaries. Infrared (IR) spectra were recorded on Bruker Alpha IR Spectrometer. Elemental analysis was performed and found that values were within 0.4% of theoretical values. 1H-nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 400 spectrophotometer at 400 MHz, 5 mm multinuclear inverse probe head, low- and high-temperature facility. Mass spectra were recorded using Mass Spectrometer Jeol SX-102 (FAB) by ESI.

Chemistry

Present synthesis comprises

Synthesis of 1,3,5-trisubstituted pyrazole derivatives involves the following steps.

Scheme-I: Synthesis of chalcones by Claisen-Schmidt condensation

Scheme-II: Synthesis of succinic hydrazide and 4-aminobutane hydrazide from corresponding ester

Scheme-III: Reaction of succinic hydrazide with chalcone to form 1,3,5-trisubstituted pyrazole derivatives

Scheme I: Synthesis of chalcones by Claisen-Schmidt condensation

Equimolar quantity (0.05 M) of p-chloroacetophenone and p-methylacetophenone was taken and mixed with equimolar quantity of benzaldehyde and substituted benzaldehyde. The mixture was dissolved in ethanol. The mixture was stirrer for 5 min and added 50% aqueous solution of potassium hydroxide that was added slowly with continues stirring at room temperature for 24 h. The completion of the reaction was monitored by the thin-layer chromatography (TLC). Then, the synthesis is completed, the mixture was poured into the crushed ice, solid product was obtained, but if the solid product was not obtained so acidified with dilute hydrochloric acid.[14] The obtained solid was separated by filtration, dried, and purified by column chromatography using solvent system (hexane:ethyl acetate). The reaction was shown in synthesis scheme-I.

Scheme II: Synthesis of succinic hydrazide

Succinic acid (0.05 M) can be easily converted to succinic hydrazide by reaction with hydrazine hydrate (0.05 M)

in alcohol, then, the reaction mixture was cooled to room temperature, succinic hydrazide separates as solid which was recrystallized using ethanol. The IR spectra denote the peak at 3500.66 (-NH str.); 3313.58 (NH_2 str.); 1658.32(C=O); and 1430–3046.55 (CH-CH). The reaction was monitored by the TLC using hexane:ethyl acetate as mobile phase. Obtained compounds were characterized by IR, 1H-NMR and were found consistent with an expected structure [Figure 1].

Scheme III: Synthesis of 1,3,5-trisubstituted pyrazole

The synthesized chalcone derivatives with equimolar quantity (0.005 M) were mixed with succinic hydrazide (0.005M) in absolute alcohol and addition of small amount of pyridine (0.01 M). The reaction mixture was refluxed at 65°C up to 2–6 h. The reaction was monitored by the TLC using ethyl acetate:hexane as mobile phase. The solvent was completely evaporated and then was poured into the ice-cold water with constant stirring that coverts liquid form into solid product that resulted into the corresponding synthesized product.[21] The synthesis was shown in scheme-II [Figure 2]. This solid was filtered under vacuum and dried. The synthesized compound purified by the column chromatography and was obtained as pale yellow solid color powder.

Antibacterial Screening of the Synthesized Compounds

Antibacterial screening of the synthesized compounds was tested against five Gram positive (*Staphylococcus aureus*, *Staphylococcus faecalis*, *Bacillus subtilis*, *Proteus vulgaris,* and *Bacillus pumilus*) and two Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) organisms using the agar diffusion method**.** Norfloxacin and ciprofloxacin were used as standard drug for compare the efficacy of synthesized compounds against Gram-positive and Gram-negative bacteria, respectively.[25]

Nutrient agar broth medium was used for the preparation of inoculum of the bacteria and nutrient agar was used for the screening method. The test organisms were subcultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with the respective bacterial strain. After incubation at 37°C ± 1°C for 18 h, they were stored in a refrigerator. The nutrient agar medium was sterilized by autoclaving at 121°C (15 lb/sq.inch) for 15 min. The Petri plates, tubes, and flasks plugged with cotton were sterilized in hot air oven at 160°C, for an hour. In each of the sterilized petriplates, pour the agar medium which contains the respective strains of bacteria aseptically. The plates were left at room temperature aseptically to allow the solidification.[26] After solidification, the cups of each of 7 mm diameter were made by scooping out medium with a sterilized cork borer from a Petri dish and labeled accordingly.

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 ml Analar grade) to give a concentration of 1000 g/ml. Norfloxacin solution was also prepared to give a concentration of 1000 g/ml in sterilized distilled water. The pH of all the test solutions and control was maintained in between 2 and 3 using conc. HCl. All the compounds were tested at dose levels of 50 g (0.05 ml) and 100 g (0.1 ml) and DMSO used as a control. The solutions of each test compound, control and reference standard (0.05 ml and 0.1 ml), were added separately in the cups and the plates were kept undisturbed for at least 2 h in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37 ± 1°C for 24 h. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.[27,28] The same procedure adopted for the Gram-negative bacteria screening and ciprofloxacin was used as a standard drug.

RESULTS AND DISCUSSION

Scheme-I

The synthesized compounds were characterized by the infrared spectroscopy and proton NMR spectroscopy and were found reliable with probable structure. Obtained compounds were characterized by IR, 1H-NMR and were found consistent with an expected structure. The IR spectra denote the peak at 1650–1658 (C=O); 1500–1580 (C=C Quadrant of Ar), 761 (mono substituted benzene); 1105 (C-F), 825 (C-Cl),

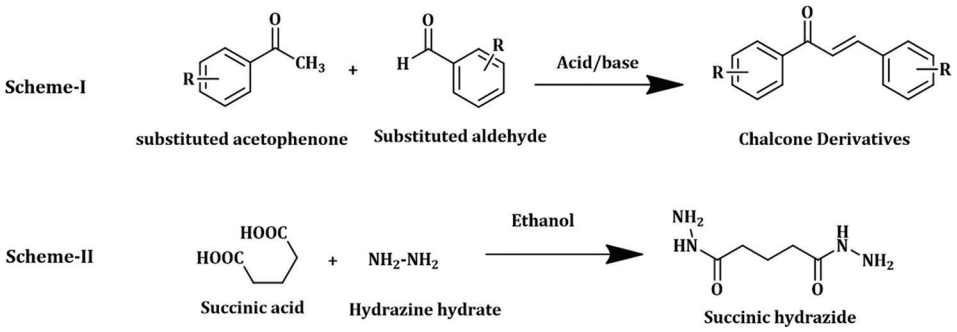

Figure 1: Synthesis scheme-I and scheme-II

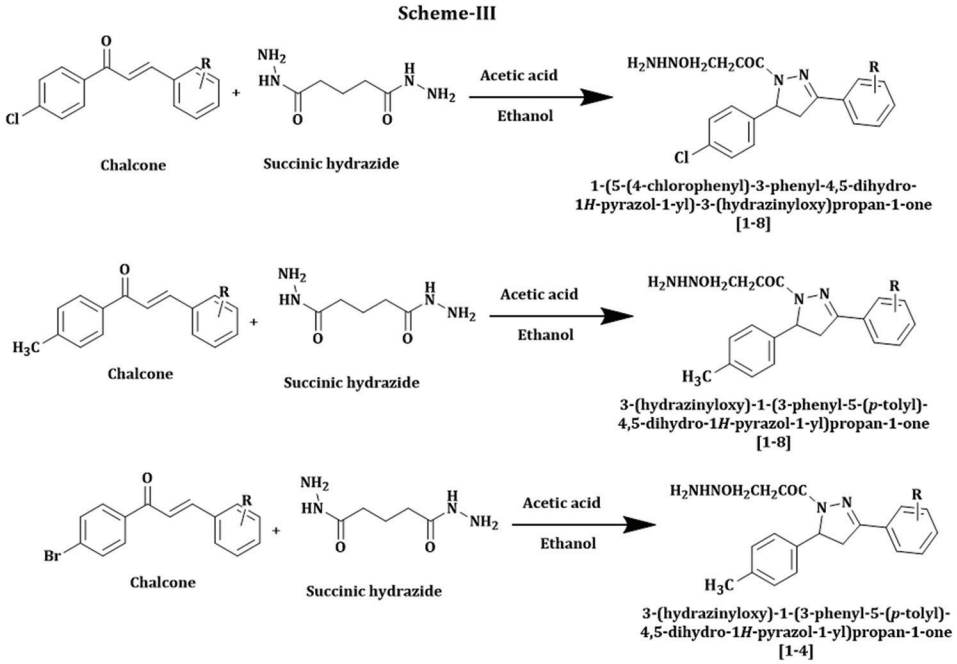

Figure 2: Synthesis scheme-III

1015 (C-Br), and 1160 (OCH_3). These compounds further confirmed by proton NMR revealed the characteristic ethylene protons of the chalcone system in between δ 7.60 (C=O-CH), 6.68–7.90 (Ar-H), and 8.05 (=CH-Ar) confirm the compound. The reaction was monitored by the TLC using hexane:ethyl acetate as mobile phase.

Scheme-III

The synthesized compounds were characterized by the infrared spectroscopy and proton NMR spectroscopy and were found reliable with probable structure. Obtained compounds were characterized by IR, 1-HNMR and were found consistent with an expected structure. The IR spectra demote the peak at 3205.66 (C-H str., aromatic), 1510.25 (C=N), 3042.55 (C-H), 1660.32 (C=O), 1486.20 (C=N), 3502.21 (-NH str.) and 3315.50 (-NH_2 str), 852.22 (C-Cl), 1025.27 (C-Br), 1118.62 (C-F), 1072.46 (C-OCH_3), 1569 (N=O str.), and 1365 (N-O str.). These compounds further confirmed by proton NMR revealed the characteristic protons of the system δ 1.26, 1.28 (4H methylene of pyrazoline), δ 4.81 (4H methylene side chain of pyrazoline), δ 3.60 (1H, dd, pyrazole ring); δ 5.38 (methyl group at phenyl ring), δ 1.50-158 (NH_2), and 8.33 (N-H) confirm the compound. The reaction was monitored by the TLC using hexane:ethyl acetate as mobile phase.

Compound CL-1: 1-(5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy) propan-1-one

Molecular formula: $C_{18}H_{19}ClN_4O_2$; molecular weight: 358.82; TLC (Rf value): 0.38; element (Found/Calc.)%: Nitrogen (15.60/15.61); oxygen (8.90/8.92); IR (cm^{-1}): 3206.66 (C-H str.), 1172.05 -C_6H_5, 1512.25 (C=N str.), 3042.55 (C-H str.), 1665.32 (C=O str.), 1482.20 (C=N str.), 3502.21 (-NH str.), 3312.50 (-NH_2 str.), 852.22 (C-Cl); 1H-NMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.68 (1H, dd, pyrazole ring); δ 1.56 (NH_2), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 7.52–7.67 (m, 2H, Ar–H). FAB mass (m/z): 344.12 (Quasi-molecular ion peak (M+H)+).

Compound CL-2: 1-(5-(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3 (hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}ClFN_4O_2$; molecular weight: 376.81; TLC (Rf value): 0.42; Element (Found/Calc.)%: Nitrogen (14.85/14.87); oxygen (8.48/8.49); IR (cm^{-1}): 3215.66 (C-H str.)

1506.25 (C=N str.), 3032.55 (C-H str.), 1640.32 (C=O str.), 1466.20 (C=N str.), 3509.21 (-NH str.)

3312.50 (-NH_2 str.), 850.22 (C-Cl), 1118.62 (C-F); 1H-NMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.65 (1H, dd, pyrazole ring), δ 1.56 (NH_2), δ 8.30 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 7.36–7.81 (m, 2H, Ar–H). FAB mass (m/z): 376.11 (Quasi-molecular ion peak (M+H)+).

Compound CL-3: 1-(3,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazin-yloxy) propan-1-one

Molecular formula: $C_{18}H_{18}Cl_2N_4O_2$; molecular weight: 393.27; TLC (Rf value): 0.40; Element (Found/Calc.)%:

Nitrogen (14.24/14.25); oxygen (8.12/8.14); IR (cm^{-1}): 3208.66 (C-H str.), 1512.35 (C=N str.), 3052.45 (C-H str.), 1640.32 (C=O str.), 1456.20 (C=N str.), 3515.41 (-NH str.), 3310.20 (-NH_2 str.), 852.22 (C-Cl); 1H-NMR (ppm): δ 1.28 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring), δ 1.56 (NH_2), 8.30 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 7.52–7.98 (m, 2H, Ar–H). FAB mass (m/z): 392.08 (Quasi-molecular ion peak (M+H)+).

Compound CL-4: 1-(3-(4-bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}BrClN_4O_2$; molecular weight: 437.72; TLC (Rf value): 0.45; Element (Found/Calc.)%: Nitrogen (12.78/12.80); oxygen (7.30/7.31); IR (cm^{-1}): 3212.56 (C-H str.),

1514.15 (C=N str.), 3040.45 (C-H str.), 1658.22 (C=O str.), 1479.10 (C=N str.), 3509.16 (-NH str.), 3314.40 (-NH_2 str.), 850.12 (C-Cl), 1020.37 (C-Br); 1H-NMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.78 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 1.56 (NH_2), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 7.58–7.72 (m, 2H, Ar–H). FAB mass (m/z): 438.03 (Quasi-molecular ion peak (M+H)+).

Compound CL-5: 1-(5-(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}ClN_5O_4$; molecular weight: 403.82; TLC (Rf value): 0.36; element (Found/Calc.)%: Nitrogen (17.32/17.34); oxygen (15.80/15.85); IR (cm^{-1}): 3205.66 (C-H str.),

1512.25 (C=N str.), 3040.55 (C-H str.), 1660.32 (C=O str.), 1482.20 (C=N str.), 3509.21 (-NH str.), 3318.50 (-NH2 str.), 850.22 (C-Cl), 1564.62 (N=O str.), 1362.52 (N-O str.); 1H-NMR (ppm): δ 1.24 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.58 (1H, dd, pyrazole ring); δ 1.58 (NH2), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 8.10–8.33 (m, 2H, Ar–H). FAB mass (m/z): 403.10 (Quasi-molecular ion peak (M+H)+).

Compound CL-6: 1-(5-(4-chlorophenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy) propan-1-one

Molecular formula: $C_{19}H_{21}ClN_4O_2$; molecular weight: 372.85; TLC (Rf value): 0.32; element (Found/Calc.)%: Nitrogen (15.02/15.03); oxygen (8.56/8.58); IR (cm^{-1}): 3212.42 (C-H str.)

1512.42 (C=N str.), 3040.52 (C-H str.), 1658.66 (C=O str.), 1474.40 (C=N str.), 3509.25 (-NH str.), 3312.40 (-NH_2 str.), 850.22 (C-Cl); 1H-NMR (ppm): δ 1.28 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.58 (1H, dd, pyrazole ring); δ 2.15 (methyl group at phenyl ring), δ 1.56 (NH_2), 8.30 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 7.28–7.68 (m, 2H, Ar–H). FAB mass (m/z): 372.14 (Quasi-molecular ion peak (M+H)+).

Code No: CL-7: 1-(5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{19}H_{21}ClN_4O_3$; molecular weight: 388.85; TLC (Rf value): 0.30; element (Found/Calc.)%: Nitrogen (14.40/14.41); oxygen (12.32/12.34); IR (cm^{-1}): 3212.66 (C-H str.),

1512.25 (C=N str.), 3040.55 (C-H str.), 1664.32 (C=O str.), 1485.20 (C=N str.), 3509.21 (-NH str.), 3314.50 (-NH_2 str.), 850.22 (C-Cl str.), 1072.46 (-OCH_3); 1H-NMR (ppm): δ 1.28 (4H methylene of pyrazoline), δ 4.83 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 1.56 (NH_2), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 7.30–7.70 (m, 2H, Ar–H), δ 3.81 (-OCH3). FAB mass (m/z): 388.13 (Quasi-molecular ion peak (M+H)+).

Compound CL-8: 1-(5-(4-chlorophenyl)-3-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{20}H_{24}ClN_5O_2$; molecular weight: 401.89; TLC (Rf value) 0.48; element (Found/Calc.)%: Nitrogen (17.42/17.43); oxygen (7.95/7.96); IR (cm^{-1}): 3209.66 (C-H str.)

1512.25 (C=N str.), 3040.55 (C-H str.), 1662.32 (C=O str.), 1481.20 (C=N str.), 3504.21 (-NH str.), 3315.50 (-NH_2 str.), 850.22 (C-Cl); 1HMNR (ppm): δ 1.26 (4H methylene of pyrazoline), δ 4.82 (4H methylene side chain of pyrazoline), δ 3.65 (1H, dd, pyrazole ring); δ 1.54 (NH_2), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 6.65–7.50 (m, 2H, Ar–H), 2.58 ($N(CH_3)_2$). FAB mass (m/z): 401.16 (Quasi-molecular ion peak (M+H)+).

Compound BR-1: 1-(5-(4-bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}BrFN_4O_2$; molecular weight: 421.26; TLC (Rf value): 0.44; element (Found/Calc.)%: Nitrogen (13.28/13.30); oxygen (7.58/7.60); IR (cm^{-1}): 3205.66 (C-H str.), 1510.25 (C=N str.), 3042.55 (C-H str.), 1660.32 (C=O str.), 1486.20 (C=N str.), 3502.21 (-NH str.), 3315.50 (-NH_2 str.), 1025.27 (C-Br), 1118.62 (C-F); 1H-NMR (ppm): δ 1.26 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.68 (1H, dd, pyrazole ring); δ 1.58 (NH_2), 8.32 (N-H), δ 7.18–7.48

(m, 2H, Ar–H), δ 7.52–7.81 (m, 2H, Ar–H). FAB mass (m/z): 420.06 (Quasi-molecular ion peak (M+H)+).

Compound BR-2: 1-(5-(4-bromophenyl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}BrClN_4O_2$; molecular weight: 437.72; TLC (Rf value): 0.54; element (Found/Calc.)%: Nitrogen (12.78/12.80); oxygen (7.28/7.31); IR (cm^{-1}): 3208.26 (C-H str.),

1512.45 (C=N str.), 3040.35 (C-H str.), 1658.22 (C=O str.), 1478.44 (C=N str.), 3509.25 (-NH str.), 3310.35 (-NH_2 str.), 1028.22 (C-Br), 850.25 (C-Cl); 1H-NMR (ppm): δ 1.26 (4H methylene of pyrazoline), δ 4..80 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 1.54 (NH_2), 8.32 (N-H), δ 7.18–7.48 (m, 2H, Ar–H), δ 7.52–7.75 (m, 2H, Ar–H). FAB mass (m/z): 438.03 (Quasi-molecular ion peak (M+H)+).

Compound BR-3: 1-(3,5-bis(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy) propan-1-one

Molecular formula: $C_{18}H_{18}Br_2N_4O_2$; molecular weight: 482.17; TLC (Rf value): 0.55; element (Found/Calc.)%: Nitrogen (11.60/11.62); oxygen (6.62/6.64); IR (cm^{-1}): 3215.45 (C-H str.),

1512.15 (C=N str.), 3040.22 (C-H str.), 1658.42 (C=O str.), 1485.35 (C=N str.), 3509.31 (-NH str.), 3312.27 (-NH_2 str.), 1022.37 (C-Br); 1H-NMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 1.54 (NH_2), 8.32 (N-H), δ 7.18–7.48 (m, 2H, Ar–H), δ 7.58–7.72 (m, 2H, Ar–H). FAB mass (m/z): 481.98 (Quasi-molecular ion peak (M+H)+).

Compound BR-4: 1-(5-(4-bromophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}BrN_5O_4$; molecular weight: 448.27; TLC (Rf value): 0.64; element (Found/Calc.) %: Nitrogen (15.60/15.62); oxygen (14.26/14.28); IR (cm^{-1}): 3208.26 (C-H str.)

1512.35 (C=N str.), 3040.55 (C-H str.), 1658.22 (C=O str.), 1482.18 (C=N str.), 3509.13 (-NH str.), 3312.50 (-NH_2 str.), 1022.27 (C-Br), 1569.25 (N=O str.), 1365.53 (N-O str.); 1H-NMR (ppm): δ 1.26 (4H methylene of pyrazoline), δ 4.82 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 1.54 (NH_2), 8.32 (N-H), δ 7.18–7.48 (m, 2H, Ar–H), δ 8.10–8.30 (m, 3H, Ar–H). FAB mass (m/z): 447.05 (Quasi-molecular ion peak (M+H)+).

Compound ME-1: 3-(hydrazinyloxy)-1-(3-phenyl-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl) propan-1-one

Molecular formula: $C_{19}H_{22}N_4O_2$; molecular weight: 338.40; TLC (Rf value): 0.45; element (Found/Calc.)%: Nitrogen (16.52/16.56); oxygen (9.45/9.46); IR (cm^{-1}): 3205.66 (C-H str.), 1510.25 (C=N str.), 1172.05 C_6H_5, 3042.55 (C-H str.), 1660.32 (C=O str.), 1486.20 (C=N str.), 3502.21 (-NH str.), 3315.50 (-NH_2 str.); 1H-NMR (ppm): δ 1.32 (4H methylene of pyrazoline), δ 4.81 (4H methylene side chain of pyrazoline), δ 3.69 (1H, dd, pyrazole ring); δ 2.15 (methyl group at phenyl ring), δ 1.55 (NH_2), 8.30 (N-H), δ 7.10–7.20 (m, 2H, Ar–H), δ 7.52–7.67 (m, 3H, Ar–H). FAB mass (m/z): 338.17 (Quasi-molecular ion peak (M+H)).

Compound ME-2: 1-(3-(4-fluorophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy) propan-1-one

Molecular formula: $C_{22}H_{20}ClN_3O_4S$; molecular weight 356.39; TLC (Rf value): 0.38; element (Found/Calc.)%: Nitrogen (9.12/9.18); oxygen (13.95/13.98); IR (cm^{-1}): 3202.46 C-H str., 1510.15 (C=N str.), 3038.47 (C-H str.), 1658.34 (C=O str.), 1482.25 (C=N str.), 3515.41 (-NH str.), 3310.20 (-NH_2 str.), 1118.62 (C-F); 1H-NMR (ppm): δ 1.28 (4H methylene of pyrazoline), δ 4.82 (4H methylene side chain of pyrazoline), δ 3.65 (1H, dd, pyrazole ring); δ 5.38 (methyl group at phenyl ring), δ 1.54 (NH_2), 8.32 (N-H), δ 7.10–7.20 (m, 2H, Ar–H), δ 7.36–7.81 (m, 3H, Ar–H). FAB mass (m/z): 356.16 (Quasi-molecular ion peak (M+H)+).

Compound ME-3: 1-(3-(4-chlorophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy) propan-1-one

Molecular formula: $C_{19}H_{21}ClN_4O_2$; molecular weight: 372.85; TLC (Rf value): 0.40; element (Found/Calc.)%: Nitrogen (15.00/15.02); oxygen (8.56/8.58); IR (cm^{-}): 3202.46 (C-H str.), 1520.30 (C=N str.), 3040.55 (C-H str.), 1658.32 (C=O str.), 1482.48 (C=N str.), 3506.16 (-NH str.), 3312.42 (-NH2 str.), 850.22 (C-Cl); 1H-NMR: δ 1.27 (4H methylene of pyrazoline), δ 4.84 (4H methylene side chain of pyrazoline), δ 3.65 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.52 (NH_2), 8.32 (N-H), δ 7.12–7.20 (m, 2H, Ar–H), δ 7.52–7.95 (m, 3H, Ar–H). FAB mass (m/z): 372.14 (Quasi-molecular ion peak (M+H)+).

Compound ME-4: 1-(3-(4-bromophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy) propan-1-one

Molecular formula: $C_{19}H_{21}BrN_4O_2$; molecular weight: 417.30; TLC (Rf value): 0.54; Element (Found/Calc.)%: Nitrogen (13.40/13.43); oxygen (7.65/7.67); IR (cm^{-1}): 3206.32 (C-H str.),

1509.26 (C=N str.), 3040.52 (C-H str.), 1658.30 (C=O str.), 1482.30 (C=N str.), 3509.16 (-NH str.), 3312.40 (-NH_2 str.), 1020.27 (C-Br); 1H-NMR (ppm): δ 1.22 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.58 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.58 (NH_2), 8.29 (N-H), δ 7.15–7.20 (m, 2H, Ar–H), δ 7.58–7.72 (m, 2H, Ar–H). FAB mass (m/z): 416.08 (Quasi-molecular ion peak (M+H)+)

Compound ME-5: 3-(hydrazinyloxy)-1-(3-(4-nitrophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl) propan-1-one

Molecular formula: $C_{19}H_{21}N_5O_4$; molecular weight: 383.40; TLC (Rf value): 0.30; element (Found/Calc.)%: Nitrogen (18.25/18.27); oxygen (16.65/16.69); IR (cm^{-1}): 3202.66 (C-H str.),

1512.20 (C=N str.), 3038.35 (C-H str.), 1658.32 (C=O str.), 1476.20 (C=N str.), 3509.21 (-NH str.), 3312.50 (-NH_2 str.), 1562.25 (N=O str,), 1362.42 (N-O str.); 1H-NMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.52 (NH_2), 8.30 (N-H), δ 7.10–7.20 (m, 2H, Ar–H), δ 8.09–8.33 (m, 2H, Ar–H). FAB mass (m/z): 333.40 (Quasi-molecular ion peak (M+H)+)

Compound ME-6: 1-(3,5-di-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy) propan-1-one

Molecular formula: $C_{20}H_{24}N_4O_2$; molecular weight: 352.43; TLC (Rf value): 0.64; element (Found/Calc.)%: Nitrogen (9.58/9.60); sulfur (7.32/7.33); oxygen (14.60/14.63); IR (cm^{-1}): 3206.66 (C-H str.), 1512.23 (C=N str.), 3040.34 (C-H str.), 1658.32 (C=O str.), 1482.20 (C=N str.),

3506.21 (-NH str.), 3312.50 (-NH_2 str.); 1H-NMR (ppm): δ 1.26 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.65 (1H, dd, pyrazole ring); δ 2.12 (methyl group at phenyl ring), δ 1.53 (NH_2), 8.29 (N-H), δ 7.10–7.20 (m, 2H, Ar–H), δ 7.25–7.71 (m, 2H, Ar–H). FAB mass (m/z): 352.19 (Quasi-molecular ion peak (M+H)+).

Compound ME-7: 3-(hydrazinyloxy)-1-(3-(4-methoxyphenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)propan-1-one

Molecular formula: C20H24N4O3; molecular weight: 368.43; TLC (Rf value): 0.23; element (Found/Calc.)%: Nitrogen (15.19/15.21); oxygen (13.01/13.03); IR (cm^{-1}): 3208.66 (C-H str.),

1514.25 (C=N str.), 3040.55 (C-H str.), 1662.32 (C=O str.), 1485.15 (C=N str.), 3506.18 (-NH str.), 3312.35 (-NH_2 str.), 1074.26 (-OCH_3); 1H-NMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.66 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.56 (NH2), 8.53 (N-H), δ 7.10–7.20 (m, 2H, Ar–H), δ 7.30–7.80 (m, 2H, Ar–H), δ 3.81 (-OCH3). FAB mass (m/z): 368.18 (Quasi-molecular ion peak (M+H)+).

Compound ME-8: 1-(3-(4-(dimethylamino) phenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{21}H_{27}N_5O_2$; molecular weight: 381.47; TLC (Rf value): 0.42; element (Found/Calc.)%: Nitrogen (18.35/18.36); oxygen (8.37/8.39); IR (cm^{-1}): 3204.42 (C-H str.),

1511.38 (C=N str.), 3040.22 (C-H str.), 1658.16 (C=O str.), 1455.18 (C=N str.), 3510.15 (-NH str.), 3312.42 (-NH_2 str.); 1H-NMR (ppm): δ 1.28 (4H methylene of pyrazoline), δ 4.82 (4H methylene side chain of pyrazoline), δ 3.69 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.50-158 (NH_2), 8.33 (N-H), δ 7.15–7.20 (m, 2H, Ar–H), δ 6.68–7.50 (m, 2H, Ar–H), δ 2.58 ($N(CH_3)_2$). FAB mass (m/z): 381.47 (Quasi-molecular ion peak (M+H)+).

Antibacterial Activity

In accordance with the data obtained from antibacterial activity, all the synthesized 1,3,5-trisubstituted pyrazole derivatives (ME1- ME8, CL1-CL8, and BR1-BR4) have showed mild to good activity against tested organisms. Antibacterial activity of the synthesized compounds has been carried out for Gram-positive and Gram-negative bacterial strain separately. The data of antibacterial activity against the Gram-positive bacterial strains (*S. aureus*, *S. faecalis*, *B. subtilis*, *P. vulgaris, and B. pumilus*) suggested the order of activity of compounds: BR-3 >BR-2>BR-1>CL-4>BR-4>CL-3>CL-2>CL-5>CL-6>ME-3>ME-2>ME-4>ME-5>ME-6>ME-7>CL-7>CL-8>CL-1>ME-8>ME-1. Among these 1,3,5-trisubstituted pyrazole derivatives, compound ME-8, ME-1, ME-5, ME-6, ME-7, CL-7, CL-8, and CL-1 shows mild activity and ME-4, CL-5, CL-6, ME-3, and ME-2 showed moderate activity and BR-3, BR-2, BR-1, CL-4, BR-4, CL-3, and CL-2 showed best activity against Gram-positive bacteria. The compounds series BR-1 to BR-4 has shown the highest activity [Table 1].

The data of antibacterial activity against the Gram-negative bacterial strains (*E. coli* and *K. pneumoniae*) suggested the order of activity of compounds: BR-3 >BR-2 > BR-1 > CL-4 > BR-4 > CL-3 > CL-2 > CL-5> ME-4> CL-6 > ME-3 > ME-2 > ME-7 > ME-8 > CL-8 > CL-7>CL-1 > ME-5 >ME-6>ME-1. Compound ME-8, CL-8, CL-7, CL-1, ME-5, ME-6, and ME-1 has showed mild activity, compounds CL-2, CL-5, ME-4, CL-6, ME-3, ME-2, and ME-7 showed moderate activity, and compounds BR-3, BR-2, BR-1, CL-4, BR-4, and CL-3 have showed

good activity against Gram-negative bacteria [Table 2]. Compounds BR-3 (17.02 ± 0.21), BR-2 (16.25 ± 0.24), BR-1 (14.25 ± 0.28), CL-4 (12.02 ± 0.24), BR-4 (11.54 ± 0.25), and CL-3 (10.54 ± 0.26) have shown zone of inhibition in mm in comparison to standard drug (ciprofloxacin, 17.25 + 0.36) which has shown good activity against *E. coli* (Gram-negative bacteria) at 50 µg concentration. Compounds BR-3 (16.02 ± 0.26), BR-2 (15.25 ± 0.22), BR-1 (13.25 ± 0.27), CL-4 (11.02 ± 0.23), BR-4 (10.54 ± 0.23), and CL-3 (09.54 ± 0.27) have shown zone of inhibition in mm in comparison to standard drug (ciprofloxacin, 17.25 + 0.36) which has shown good activity at 50 µg concentration against *K. pneumoniae* (Gram-negative bacteria). The graphical representation of antibacterial activity on Gram-positive bacterial strains was shown in zone of inhibition which is shown in Figures 3-7.

Antibacterial Activity Against Gram-negative Bacteria

Compounds BR-3 (17.02 ± 0.21), BR-2 (16.25 ± 0.24), BR-1 (14.25 ± 0.28), CL-4 (12.02 ± 0.24), BR-4 (11.54 ± 0.25), and CL-3 (10.54 ± 0.26) have shown zone of inhibition in mm in comparison to standard drug (ciprofloxacin, 17.25 + 0.36) which has shown good activity against *E. coli* (Gram-negative bacteria) at 50 µg concentration. Compounds BR-3 (16.02 ± 0.26), BR-2 (15.25 ± 0.22), BR-1 (13.25 ± 0.27), CL-4 (11.02 ± 0.23), BR-4 (10.54 ± 0.23), and CL-3

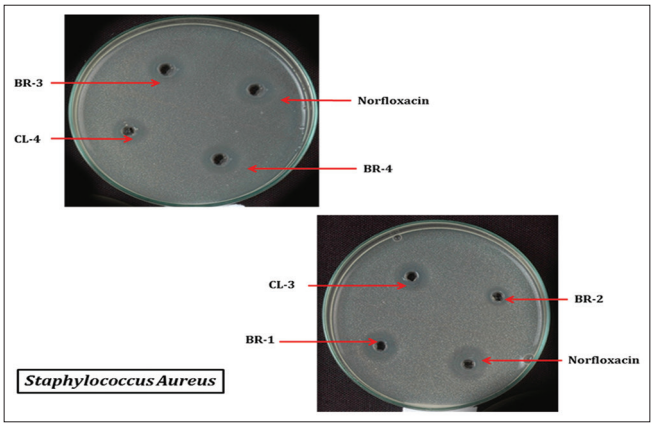

Figure 3: Zone of inhibition of synthesized derivatives against *Staphylococcus aureus*

Table 1: Antibacterial activity of synthesized pyrazole derivatives against Gram-positive bacteria

Compound	Zone of inhibition in mm									
	Staphylococcus aureus		*Staphylococcus faecalis*		*Bacillus subtilis*		*Proteus vulgaris*		*Bacillus pumilus*	
Concentration	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**
ME-1	3.32±0.3	4.22±0.5	6.32±0.6	6.52±0.7	6.22±0.8	6.32±0.4	6.35±0.6	6.25±0.3	6.23±0.3	6.32±0.6
ME-2	8.32±0.5	9.32±0.4	6.32±0.5	7.25±0.5	5.42±0.2	6.25±0.5	6.64±0.5	7.21±0.5	5.16±0.5	6.14±0.4
ME-3	9.32±0.3	10.72±0.6	7.52±0.7	8.64±0.4	6.23±0.5	8.54±0.3	6.35±0.2	7.42±0.6	6.22±0.5	7.13±0.5
ME-4	7.32±0.7	9.42±0.3	8.32±0.1	10.64±0.6	7.32±0.6	9.24±0.7	7.23±0.4	8.36±0.3	6.85±0.4	8.56±0.7
ME-5	6.32±0.2	7.62±0.5	5.62±0.3	6.12±0.3	4.52±0.3	6.56±0.4	6.16±0.5	7.32±0.7	4.46±0.6	6.57±0.5
ME-6	6.32±0.3	6.22±0.2	6.22±0.3	6.32±0.3	6.22±0.3	6.24±0.4	6.12±0.2	6.24±0.3	6.21±0.3	6.17±0.3
ME-7	5.32±0.1	6.72±0.7	6.32±0.5	6.22±0.7	6.42±0.7	6.36±0.2	6.34±0.3	6.23±0.4	6.16±0.3	6.18±0.6
ME-8	4.32±0.6	5.332±0.6	6.42±0.3	6.42±0.2	6.62±0.6	6.25±0.7	6.24±0.4	6.26±0.8	6.19±0.7	6.26±0.7
CL-1	3.52±0.4	4.62±0.2	6.32±0.4	6.32±0.5	6.32±0.7	6.32±0.7	6.32±0.6	6.32±0.8	6.32±0.3	6.32±0.4
CL-2	10.62±0.7	12.62±0.2	10.24±0.5	13.52±0.7	9.62±0.8	11.42±0.4	10.42±0.7	12.72±0.3	9.22±0.5	11.42±0.6
CL-3	11.42±0.6	13.72±0.3	11.52±0.8	15.32±0.5	12.32±0.3	14.62±0.7	12.72±0.2	14.32±0.5	13.32±0.3	15.32±0.2
CL-4	12.72±0.3	14.20±0.8	12.62±0.2	17.72±0.6	14.2±0.5	16.32±0.3	15.42±0.8	17.72±0.7	16.52±0.2	18.72±0.5
CL-5	8.32±0.8	9.32±0.4	9.65±0.3	11.52±0.8	7.62±0.7	9.52±0.9	8.62±0.4	9.62±0.2	7.42±0.6	8.33±0.4
CL-6	7.32±0.8	8.12±0.7	8.65±0.2	10.22±0.4	6.72±0.4	8.22±0.8	7.27±0.3	8.27±0.7	6.23±0.6	7.23±0.3
CL-7	5.22±0.2	6.52±0.3	6.32±0.5	6.32±0.5	6.32±0.2	6.32±0.7	6.32±0.7	6.32±0.8	6.32±0.3	6.32±0.3
CL -8	4.62±0.8	6.72±0.5	6.32±0.7	6.32±0.3	6.32±0.5	6.32±0.4	6.32±0.3	6.32±0.3	6.32±0.8	6.32±0.7
BR-1	12.22±0.6	14.25±0.7	11.42±0.3	13.55±0.4	10.28±0.2	11.44±0.3	11.56±0.5	13.66±0.2	11.23±0.5	10.45±0.7
BR-2	13.45±0.2	17.32±0.2	15.23±0.6	17.35±0.6	13.54±0.7	15.60±0.4	14.20±0.8	17.54±0.7	15.25±0.3	17.52±0.3
BR-3	15.75±0.5	18.65±0.8	16.34±0.7	19.25±0.3	15.20±0.9	17.52±0.5	16.65±0.7	19.05±0.3	17.56±0.8	19.54±0.4
BR-4	11.32±0.3	13.25±0.4	13.38±0.5	15.42±0.2	12.09±0.5	13.47±0.6	13.52±0.3	15.27±0.5	13.25±0.4	14.25±0.2
DMSO (control)	-	-	-	-	-	-				
Norfloxacin	17.22±0.3	19.45±0.5	17.25±0.5	21.52±0.4	16.64±0.3	17.45±0.3	17.64±0.2	20.65±0.4	18.25±0.4	19.33±0.2

Table 2: Antibacterial activity of synthesized pyrazole derivatives against Gram-negative bacteria

Compound	Zone of inhibition in mm			
	Escherichia coli		*Klebsiella pneumoniae*	
	50 µg	100 µg	50 µg	100 µg
ME-1	4.22±0.25	6.25±0.28	3.22±0.23	6.22±0.25
ME-2	7.26±0.25	10.23±0.23	6.26±0.24	8.26±0.25
ME-3	7.56±0.27	10.52±0.25	6.56±0.26	8.56±0.26
ME-4	8.22±0.23	10.20±0.23	7.22±0.24	9.22±0.27
ME-5	4.85±0.28	6.83±0.27	3.85±0.28	6.85±0.25
ME-6	4.65±0.23	5.64±0.25	3.65±0.23	6.65±0.23
ME-7	6.42±0.23	8.44±0.26	5.42±0.22	7.42±0.23
ME-8	6.28±0.23	7.2±0.23	5.28±0.25	7.28±0.22
CL-1	5.12±0.27	7.17±0.27	4.12±0.27	6.12±0.22
CL-2	9.64±0.24	10.62±0.29	8.64±0.23	10.64±0.24
CL-3	10.54±0.26	12.57±0.23	9.54±0.27	11.54±0.28
CL-4	12.02±0.24	14.08±0.22	11.02±0.23	13.02±0.24
CL-5	8.68±0.25	9.62±0.27	7.68±0.28	9.68±0.24
CL-6	8.02±0.22	10.06±0.24	7.02±0.24	9.02±0.25
CL-7	5.85±0.27	7.83±0.26	4.85±0.26	7.85±0.28
CL-8	5.62±0.23	7.65±0.23	4.62±0.23	7.62±0.25
BR-1	14.25±0.28	17.22±0.25	13.25±0.27	12.25±0.28
BR-2	16.25±0.24	19.26±0.28	15.25±0.22	17.25±0.24
BR-3	17.02±0.21	20.09±0.25	16.02±0.26	18.02±0.21
BR-4	11.54±0.25	13.52±0.26	10.54±0.23	12.54±0.23
Ciprofloxacin	17.25±0.36	21.45±0.23	17.64±0.65	20.65±0.26

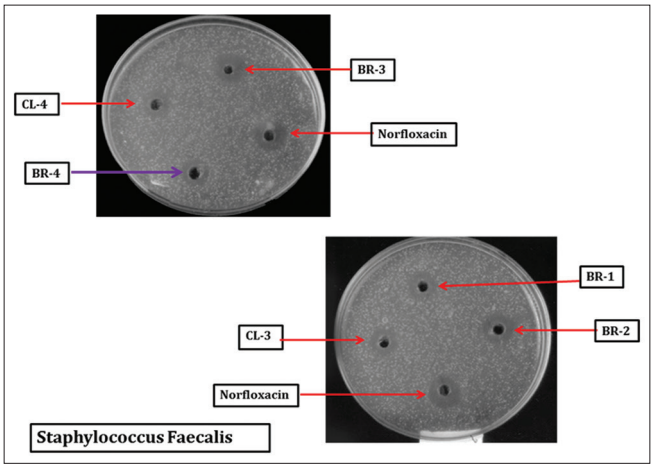

Figure 4: Zone of inhibition of synthesized derivatives against *Staphylococcus faecalis*

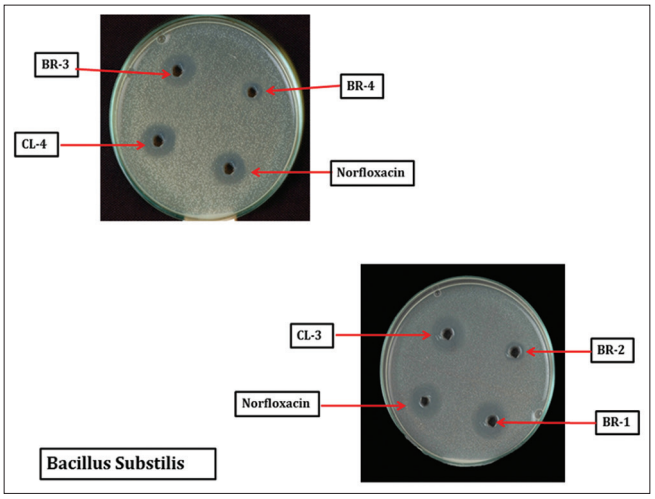

Figure 5: Zone of inhibition of synthesized derivatives against *Bacillus subtilis*

(09.54 ± 0.27) have shown zone of inhibition in mm in comparison to standard drug (ciprofloxacin, 17.25 + 0.36) which has shown good activity at 50 µg concentration against *K. pneumoniae* (Gram-negative bacteria). The graphical representation of zone of inhibition is shown in Figures 8 and 9.

Biological Activity Based on Structure

Antibacterial activity

In accordance with the data obtained from antibacterial activity, all the synthesized 1,3,5-trisubstituted pyrazole

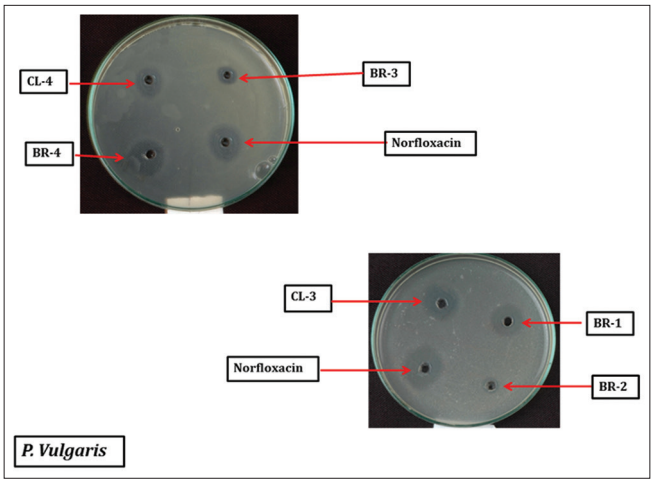

Figure 6: Zone of inhibition of synthesized derivatives against *Proteus vulgaris*

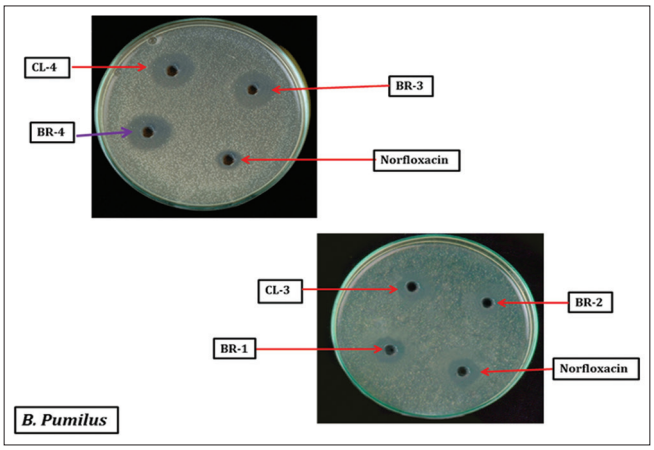

Figure 7: Zone of inhibition of synthesized derivatives against *Bacillus pumilus*

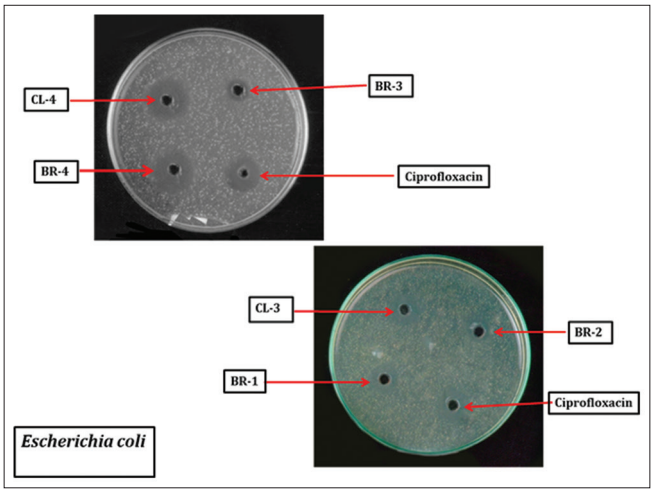

Figure 8: Zone of inhibition of synthesized derivatives against *Escherichia coli*

derivatives (ME1-ME8, CL1-CL8, and BR1-BR4) have shown mild to best activity against tested microbes. Among these 1,3,5-trisubstituted pyrazole derivatives, compounds

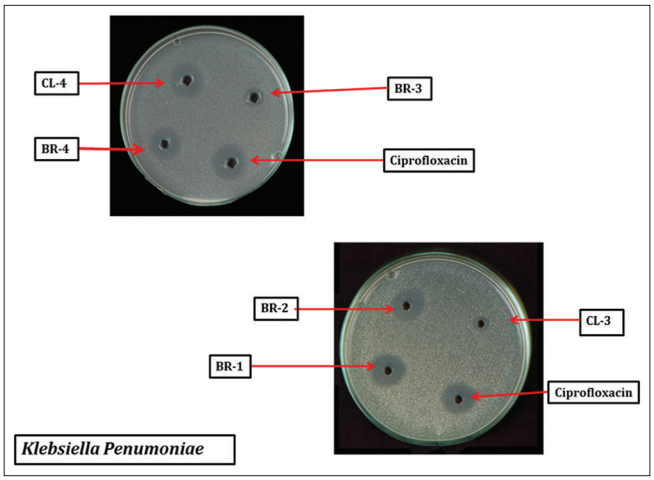

Figure 9: Zone of inhibition of synthesized derivatives against *Klebsiella pneumoniae*

BR-3 (bromophenyl at position 3 and bromophenyl at position 5); BR-2 (bromophenyl at position 3 and chlorophenyl at position 5); BR-1 (bromophenyl at position 3 and fluorophenyl at position 5); CL-4 (bromophenyl at position 3 and chlorophenyl at position 5); BR-4 (bromophenyl at position 3 and nitrophenyl at position 5); and CL-3 (chlorophenyl at position 3 and chlorophenyl at position 5) are essential for the antibacterial activity against Gram-positive and Gram-negative bacteria.

CONCLUSION

All the 2-pyrazolines have been evaluated for their antibacterial activity against *S. aureus*, *S. faecalis*, *B. subtilis*, *P. vulgaris, and B. pumilus* (Gram positive) and *E. coli* and *P. vulgaris* (Gram negative), using agar diffusion method. The results of this evaluation have been compared by taking benzyl penicillin, ciprofloxacin was used as standard. The antibacterial activity data of 2-pyrazolines (BR-3>BR-2>BR-1>CL-4>BR-4>CL-3>CL-2) indicated that the compounds have significant inhibitory activity on all the bacteria at both 50 µg (0.05 ml) and 100 µg (0.1 ml) dose levels when compared with standard. Among all the compounds tested, compounds BR-3, BR-2, BR-1, CL-4, BR-4, and CL-3 possessed maximum activity. These compounds possessed the halogens on the aromatic ring and thus reveal the positive contribution of electron-withdrawing groups to the antibacterial activity.

The presence of electronegative group (Br, Cl, F, and NO_2) either at the third and fifth position of 1,3,5-pyrazoline ring is required for the potent antimicrobial activity. The presence of electronegative group (Br, Cl) at the third and fifth position may necessary for the best activity against bacterial strains, but the addition of F, NO_2 has shown the moderate activity but in case of -CH_3 -OCH_3 substitution may diminish the activity.

The series BR-1 to BR-4 is most active compound of the synthesized compounds. This evident that the presence of bromine in the third and fifth position of pyrazole is essential for the antimicrobial activity and chloro, bromo, fluoro, and nitro group attached at phenyl ring enhance the antimicrobial activity. The result data of antimicrobial activity suggested that Cl, Br, F, and nitro substitution at the third and fifth position may enhance the antimicrobial activity of the compounds, but the methyl and methoxy substitution may resulted in reduction of the activity.

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CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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