Preparation, analysis and antibacterial evaluation of some new 1,3,5-trisubstituted pyrazole derivatives

Abstract

The goal of this study was to develop, synthesise, and characterise a novel 1,3,5-trisubstituted-2-pyrazoline derivative, as well as test its antibacterial activity. The reaction of chalcone derivatives with succinic hydrazide in the presence of pyridine yielded the 1,3,5-tri-substituted-2-pyrazolines derivatives. The IR, 1HNMR, and mass spectral analyses were used to characterise a total of 20 substances. Antibacterial activity of the compounds was tested on five gramme positive bacterial strains (*Staphylococcus aureus, S. Faecalis, Bacillus Substilis, P. Vulgaris, and B. Pumilus)* and two gramme negative bacterial strains (*Staphylococcus aureus, S. Faecalis, Bacillus Substilis, P. Vulgaris, and B. Pumilus)* and two gramme negative i.e. *Escherichia Coli, Klebsiella Penumoniae* in two different concentration i.e. 50 and 100 µg/ml by Agar-diffusion method using Cup-plate method. Standard antimicrobial drugs were Norfloxacin and Ciprofloxacin. The antibacterial activity of substances against gramme positive bacterial strains (Staphylococcus Aureus, Staphylococcus Faecalis, Bacillus Substilis, Pseudomonas Vulgaris, and B. Pumilus) suggested the following order of action: 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>ME-8>ME-1>ME-8>ME-1>ME-8>ME-1>ME-8>ME-1>ME-8>ME-1>ME-8>ME-1>ME-8>ME-1>ME-8>ME-1>ME-8>ME The chemicals of the BR-1 to BR-4 series have the most action. ME-8, CL-8, CL-7, CL-1, ME-5, ME-6, and ME-1 have light activity, whereas CL-2, CL-5, ME-4, CL-6, ME-3, ME-2, and ME-7 have moderate activity. The compounds BR-3, BR-2, BR-1, CL-4, BR-4, and CL-3 have been shown to have excellent action. The result data of antibacterial activity suggested that Cl, Br, F, and Nitro substitution at third and Fifth position may enhance the antbacterial activity of the compounds but the Methyl and methoxy substitution may resulted in reduction of the activity.

Keywords: Antibacterial, Norfloxacin, ciprofloxacin, Agar Diffusion, pyrazole, Cup plate method

INTRODUCTION

The study of infectious illnesses and transmission has reduced the death rate of numerous diseases as mankind has progressed over time. [1-4] Today, we have a far better understanding of how to treat and survive infectious disorders. Bacterial pathogenesis and virulence factors, on the other hand, have allowed bacterial infections to persist and become troublesome. [5-7] Bacterial infections are of particular importance because, in the absence of the complexity that multicellular creatures possess, bacteria have devised a novel means of survival. [8] Bacteria have the intrinsic capacity to spontaneously modify their DNA while reproducing in response to harmful conditions, and hence transmit this survival drive on to their progeny.[9]

Antimicrobial medications have played a significant role in human health and have substantially helped human living since their discovery. Antimicrobial agents, on the other hand, are among the most commonly mishandled medications by doctors. The widespread and indiscriminate use of antibiotics has led in the establishment of drug resistance and multidrug resistance (MDR) among microorganisms, which has reached alarming levels in many areas of the world, particularly in poor nations. [10-12] The short lifespan of currently employed antimicrobial medications; genetic and metabolic changes; a quicker pace of evolution as global temperatures change; Scientists and physicians are concerned about well-documented negative effects from long-term antimicrobial usage, as well as the expensive expense of clinical research and medication development. Antibiotic drug resistance is a critical challenge that necessitates a fresh effort to create new and effective antibiotic classes with unique or changed modes of action. Drug resistance for their indicated antibiotic therapy has been found in parasitic protozoa such as Trypanosoma, Plasmodium, Toxoplasma gondii, Leishmania, and Entamoeba in recent investigations. [13-16] Early on in the development of medicinal chemistry, The isolation of therapeutic compounds found in plants was a major issue for scientists. Today's researchers are equally concerned with the development of novel synthetic medicine molecules. [17] The medications that are used to treat bacterial infections are becoming increasingly ineffective, due to bacterial strain resistance. As a result, there is an increased need for novel medications or chemically modified moieties that are efficient against bacterial infection. We discovered the diazoles, which are claimed to be utilised as antibacterial agents, while searching for moieties that must be efficient against bacterial illness. [18-22] Pyrazole has a chemical behaviour that is unusual not just among heterocyclic compounds, but also among related diazoles. Pyrazole derivatives have been known for over 80 years, but research into their chemistry has been sluggish. Earlier research focused mostly

on the creation of synthetic approaches. [23] The study of chemical properties, particularly the peculiarities of the behaviour of Pyrazole derivatives and the clarification of their physicochemical features, has recently gotten a lot of interest. Pyrazole derivatives have a long history of usage as herbicides and insecticides in agriculture, as well as in the pharmaceutical business as antipyretics and anti-inflammatory agents. [24] A wide range of biological activity has been described for compounds containing pyrazole nucleus, including antibacterial, antifungal, antioxidant, anti-amoebic, analgesic, antitubercular, neuroprotective, anticancer, anti-proliferative, antiviral, anticonvulsant, and muscarinic action. [25] The pyrazoles ring is an important synthesis pathway in the pharmaceutical sector because of its wide variety of biological activities. Chalcone is a 1,3-diphenyl-2-propene-1-one compound with two aromatic rings connected by a three-carbon, -unsaturated carbonyl system. The reaction of 1,3,5-pyrazoline with chalcone derivatives was used to create novel pyrazoline derivatives, which were then tested for antibacterial efficacy against both Gram positive and Gram negative microorganisms. The goal of the study was to develop, synthesise, and characterise novel 1,3,5-trisubstituted-2-pyrazolines derivatives, as well as to assess their antibacterial properties.

MATERIAL AND METHOD

Hi-media, New Delhi, provided the chemicals p-chloroacetophenone, p-bromoacetophenone, and p-methylacetophenone. CDH (Chemical Drug House), New Delhi, India, provided benzaldehyde, 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-nitrobenzaldehyde, 4-methyl benzaldehyde, and 4-methoxy benzaldehyde. Sigma Aldrich, New Delhi, provided the succinic acid. Chemicals of synthetic grade were utilised in the experiments. In open glass capillaries, the melting points of the produced compounds were determined. The Bruker-alpha IR Spectrometer was used to record IR spectra. Elemental analysis was carried out, and the results were determined to be within 0.4 percent of the theoretical values. 1HNMR spectra were recorded on Bruker Avance 400 spectrophotometer at 400 MHz, 5mm multi-nuclear inverse probe head, low and high-temperature facility. Mass Spectra were recorded using Mass Spectrometers 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-chloro acetophenone and p-methyl acetophenone was taken and mixed with equimolar quantity of benzaldehyde and substituted benzaldehyde. The mixture was dissolved in ethanol. The mixture was stirrer for 5 minutes and added 50% aqueous solution of potassium hydroxide was added slowly with continues stirring at room temperature for 24 hrs. The completion of the reaction was monitored by the 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.[26] 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 (0.05M) can be easily converted to succinichydrazide by reaction with hydrazine hydrate (0.05M) in alcohol, then the reaction mixture was cooled to room temperature, succinichydrazide separates as solid which was recrystallized using ethanol. The IR spectra denotes the peak at 3500.66 (-NH str.); 3313.58 (NH_2 str.); 1658.32(C=O); 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, 1HNMR and were found consistent with an expected structure (Image 1).

Scheme III: Synthesis of 1,3,5-tri substituted pyrazole

The synthesized chalcone derivatives with equimolar quantity (0.005 M) was mixed with succinic hydrazide (0.005M) in absolute alcohol and addition of small amount of pyridine (0.01M). The reaction mixture was refluxed at 65°C up to 2-6 hrs. 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 covert liquid form into solid product, that resulted into the corresponding synthesized product.[27] The synthesis was shown in scheme-II (**Image 2)**. This solid was filtered under vacuum and dried. The synthesized compound purified by the column chromatography and were obtained as pale yellow solid colour powder.

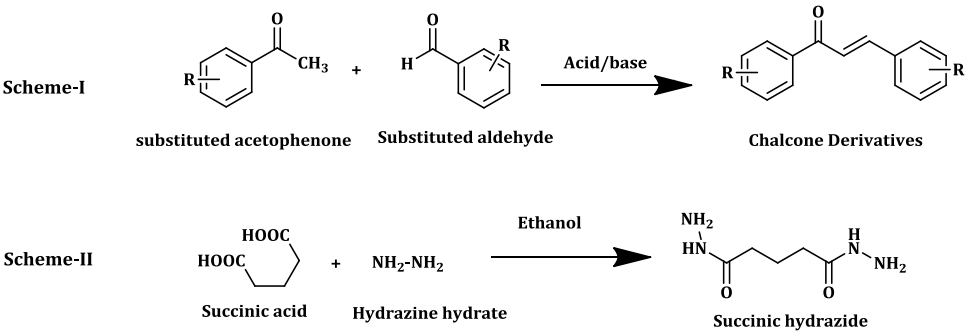

Image 1: Synthesis scheme-I and scheme-II

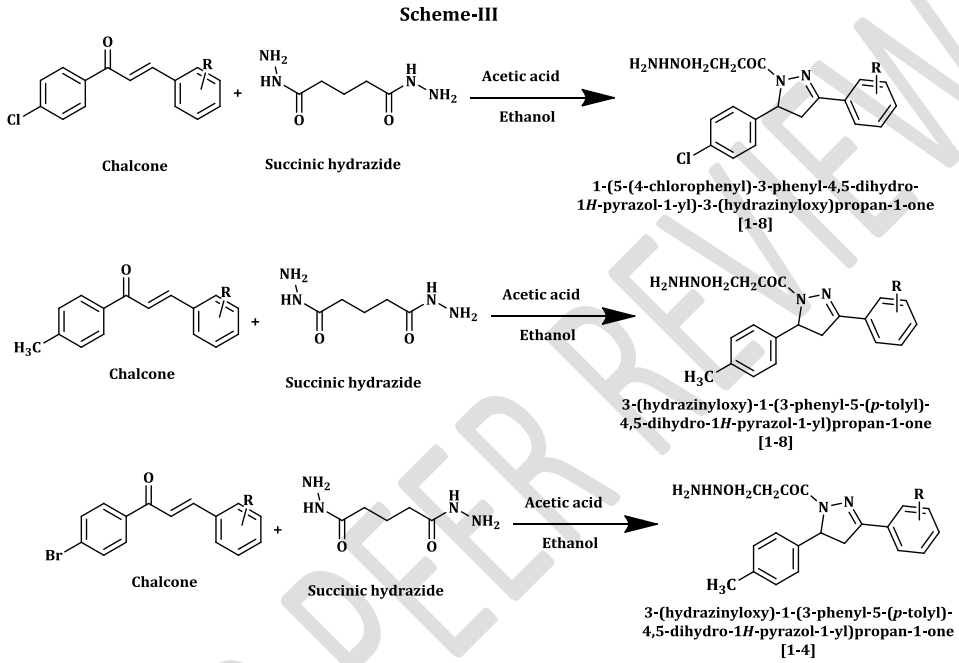

Image 2: Synthesis scheme-III

Antibacterial screening of the synthesized compounds

Anti-bacterial screening of the synthesized compounds were tested against five gram positive (*Staphylococcus Aureus, Staphylococcus Faecalis, Bacillus Substilis, P. Vulgaris and B. Pumilus*) and two gram negative (Escherichia coli and Klebsiella penumoniae) organisms by using the Agar diffusion method**.** Norfloxacin and Ciprofloxacin was used as standard drug for the compare the efficacy of synthesized compounds against gram positive and gram negative bacteria respectively.[28]

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 37ºC ±1ºC for 18 hours, they were stored in a refrigerator. The nutrient agar medium was sterilized by autoclaving at 121ºC (15 lb/sq.inch) for 15 min. The petriplates, tubes and flasks plugged with cotton were sterilized in hot-air

oven at 160ºC, for an hour. Into each sterilized petriplate (20 cm diameter), was poured about 125 ml of molten nutrient agar medium which was already inoculated with the respective strain of bacteria (5 ml of inoculum to 250 ml of nutrient agar medium) aseptically. The plates were left at room temperature aseptically to allow the solidification.[29] After solidification, the cups of each of 7 mm diameter were made by scooping out medium with a sterilized cork borer from a petridish 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 were 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 to 3 by 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 hours 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 hrs. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.[30,31] The same procedure adopted for the gram negative bacteria screening and Ciprofloxacin was used as a standard drug.

RESULT AND DISCUSSION

Scheme-I: Infrared spectroscopy and proton NMR spectroscopy were used to analyse the produced compounds, and they were confirmed to be trustworthy with a likely structure. The obtained compounds were analysed using IR and 1HNMR and found to have a structure that was compatible with what was predicted. The IR spectra show the peak at 1650-1658 (C=O); 1500-1580 (Ar C=C Quadrant), 761 (mono substituted benzene); 1105 (C-F), 825 (C-Cl), 1015 (C-Br), and 1160 (C-Br) (OCH3). These compounds were further validated by proton NMR, which revealed the chalcone system's typical ethylene protons in the range of 7.60 (C=O-CH), 6.68-7.90 (Ar-H), and 8.05 (=CH-Ar). TLC was used to monitor the reaction, which used Hexane: ethyl acetate as the mobile phase.

Scheme-III: The synthesized compounds was characterized by the Infra-red spectroscopy and proton NMR spectroscopy and was found reliable with probable structure. Obtained compounds were characterized by IR, 1HNMR and were found consistent with an expected structure. The IR spectra demotes 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 (-OCH_3), 1569 (N=O str.) and 1365

(N-O str.). These compound 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.

Scheme-I: The synthesized compounds was characterized by the Infra-red spectroscopy and proton NMR spectroscopy and was found reliable with probable structure. Obtained compounds were characterized by IR, 1HNMR and were found consistent with an expected structure. The IR spectra denotes the peak at 1650-1658 (C=O); 1500-1580 (C=C Quadrant of Ar), 761 (mono substituted benzene); 1105 (C-F), 825 (C-Cl), 1015 (C-Br), and 1160 (OCH_3). These compound 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 was characterized by the Infra-red spectroscopy and proton NMR spectroscopy and was found reliable with probable structure. Obtained compounds were characterized by IR, 1HNMR and were found consistent with an expected structure. The IR spectra demotes 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 compound 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); 1HNMR (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 (Quassi-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); 1HNMR (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 (Quassi-molecular ion peak (M+H)+).

Compound CL-3: 1-(3,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)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); 1HNMR (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 (Quassi-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); 1HNMR(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 (Quassi-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.); 1HNMR (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 (Quassi-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); 1HNMR (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 (Quassi-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); 1HNMR (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 (Quassi-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 (Quassi-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); 1HNMR (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 (Quassi-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); 1HNMR (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 (Quassi-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); 1HNMR (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 (Quassi-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.); 1HNMR (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 (Quassi-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.); 1HNMR (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 (Quassi-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); 1HNMR (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 (Quassi-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^{-1}): 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); 1HNMR: 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 (Quassi-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); 1HNMR (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 (Quassi-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.); 1HNMR (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 (Quassi-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); Sulphur (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.); 1HNMR (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 (Quassi-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);1HNMR (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 (Quassi-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.); 1HNMR (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 (Quassi-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, BR1-BR4) have showed mild to good activity against tested organisms. Antibacterial activity of the synthesized compounds has been carried out for Gram +ve and gram -ve bacterial strain separately. The Data of antibacterial activity against the gram positive bacterial strains (*Staphylococcus Aureus, Staphylococcus Faecalis, Bacillus Substilis, 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, 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 (*Escherichia Coli, Klebsiella Penumoniae*) 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 has 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) has shown zone of inhibition in mm in comparison to standard drug (Ciprofloxacin, 17.25+0.36) has shown good activity against *Escherichia coli* (gram negative bacteria) at 50µg 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) has shown zone of inhibition in mm in comparison to standard drug (Ciprofloxacin, 17.25+0.36) has shown good activity at 50µg concentration against *Klebsiella Penumoniae* (gram negative bacteria). The graphical representation of antibacterial activity on gram positive bacterial strains was shown in zone of inhibition was shown in Figure 1 to 5.

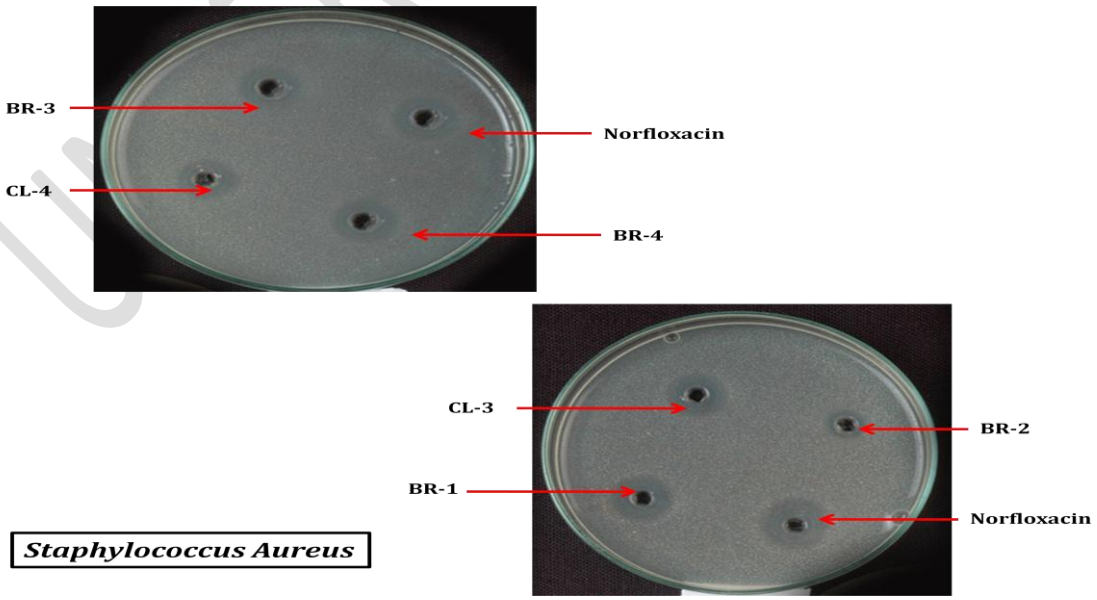

Figure 1: Zone of inhibition of synthesized derivatives against *Staphylococcus aureus*

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

COMPOUND	Zone of inhibition in mm									
	Staphylococcus Aureus		***Staphylococcus Faecalis***		***Bacillus Substilis***		***P. Vulgaris***		***B. 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	08.32±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	05.22±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	04.62±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

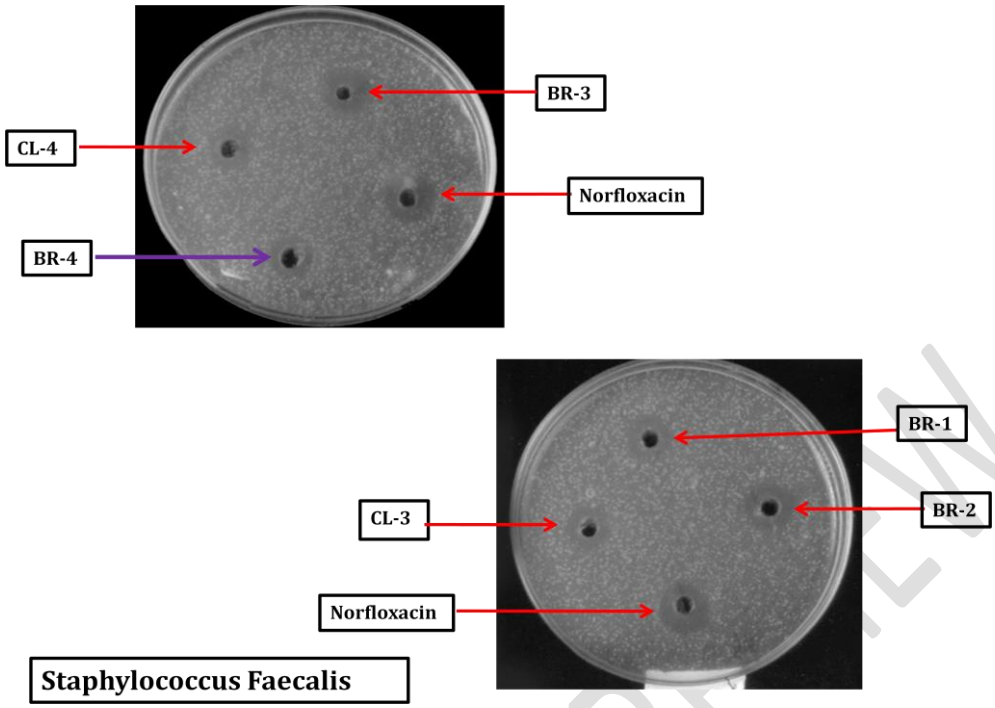

Figure 2: Zone of inhibition of synthesized derivatives against *Staphylococcus Faecalis*

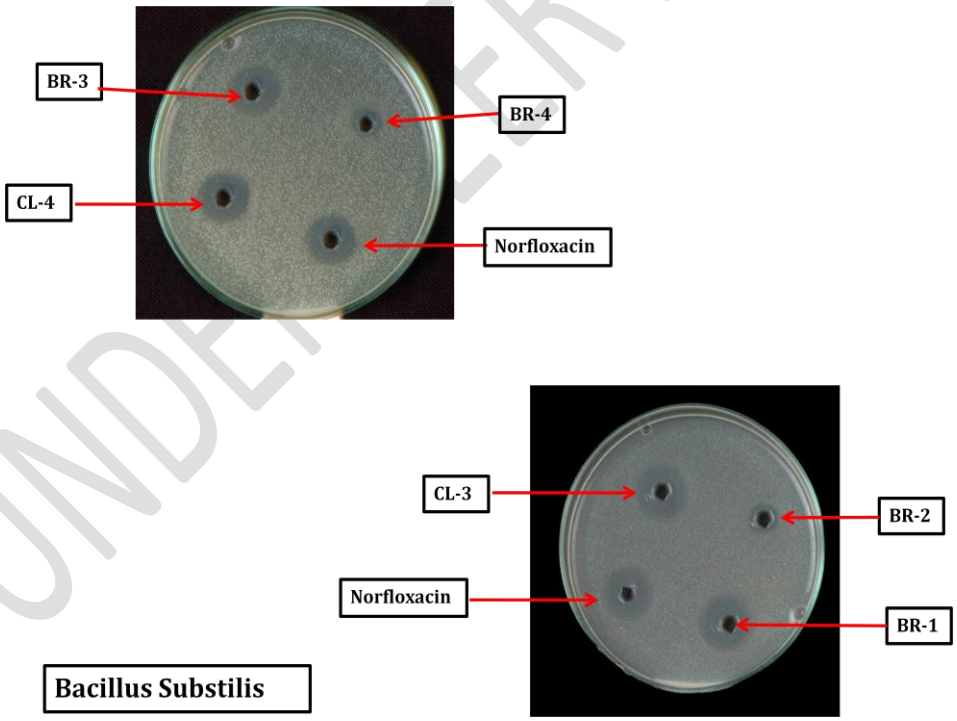

Figure 3: Zone of inhibition of synthesized derivatives against Bacillus Substilis

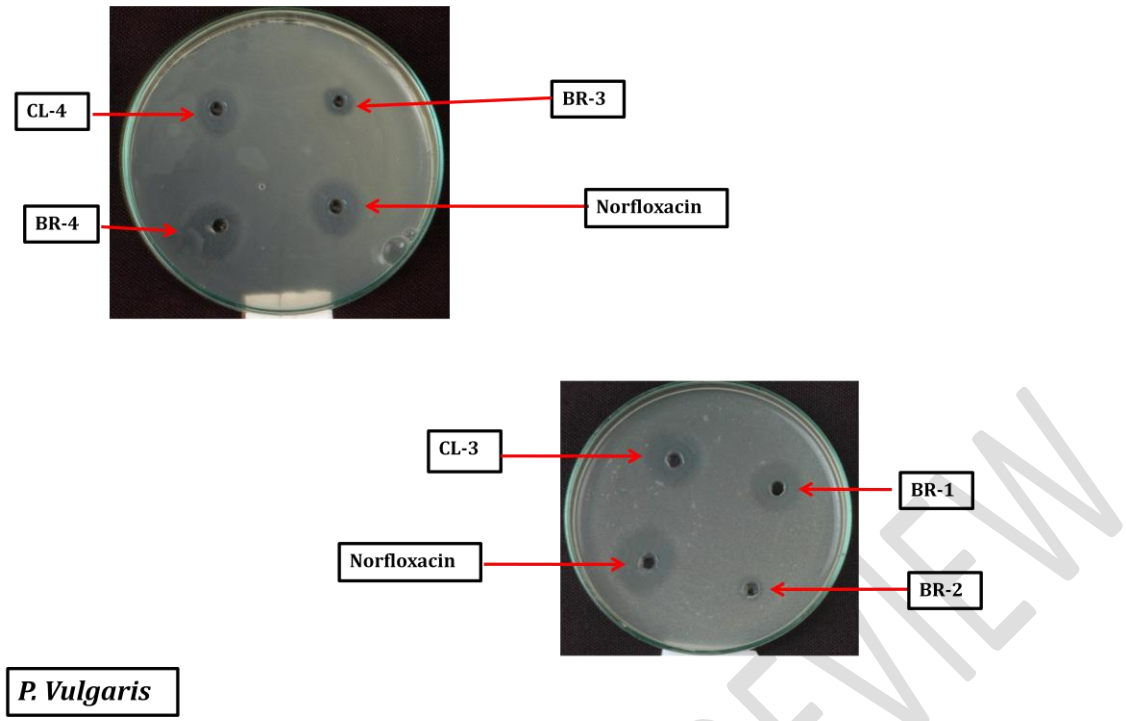

Figure 4: Zone of inhibition of synthesized derivatives against *P. Vulgaris*

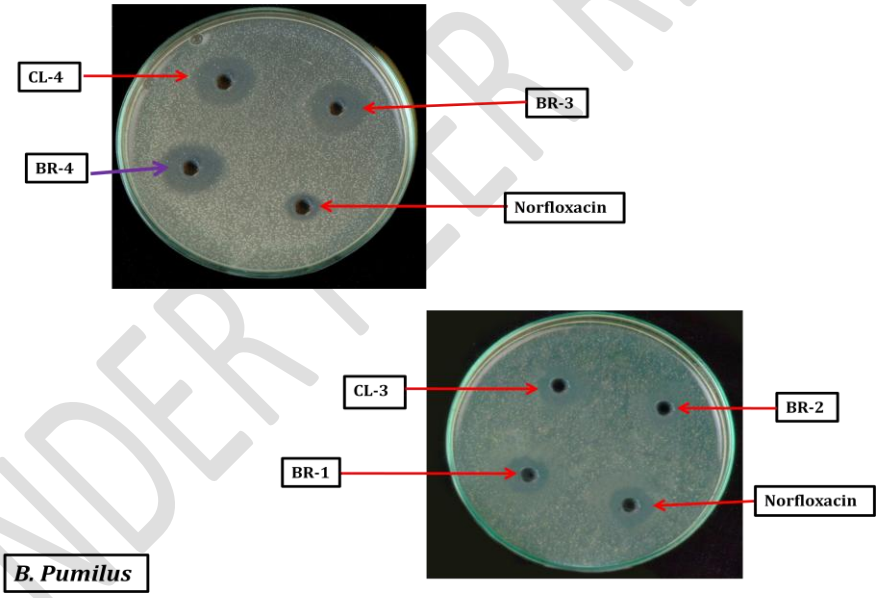

Figure 5: Zone of inhibition of synthesized derivatives against *B. Pumilus.*

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) has shown zone of inhibition in mm in comparison to standard drug (Ciprofloxacin, 17.25+0.36) has shown good activity against *Escherichia coli* (gram negative bacteria) at 50µg 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) has shown zone of inhibition in mm in comparison to

standard drug (Ciprofloxacin, 17.25+0.36) has shown good activity at 50µg concentration against *Klebsiella Penumoniae* (gram negative bacteria). The graphical representation of zone of inhibition was shown in Figure 6 and 7.

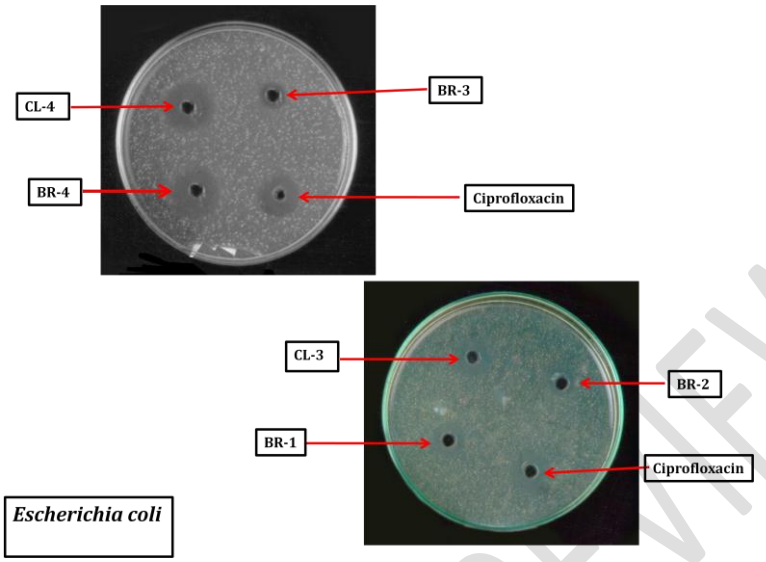

Figure 6: Zone of inhibition of synthesized derivatives against *Escherichia Coli*

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

COMPOUND	Zone of inhibition in mm			
	Escherichia coli		*Klebsiella Penumoniae*	
	50µg	**100µg**	**50µg**	**100µg**
ME-1	04.22±0.25	06.25±0.28	03.22±0.23	06.22±0.25
ME-2	07.26±0.25	10.23±0.23	06.26±0.24	08.26±0.25
ME-3	07.56±0.27	10.52±0.25	06.56±0.26	08.56±0.26
ME-4	08.22±0.23	10.20±0.23	07.22±0.24	09.22±0.27
ME-5	04.85±0.28	06.83±0.27	03.85±0.28	06.85±0.25
ME-6	04.65±0.23	05.64±0.25	03.65±0.23	06.65±0.23
ME-7	06.42±0.23	08.44±0.26	05.42±0.22	07.42±0.23
ME-8	06.28±0.23	07.2±0.23	05.28±0.25	07.28±0.22
CL-1	05.12±0.27	07.17±0.27	04.12±0.27	06.12±0.22
CL-2	09.64±0.24	10.62±0.29	08.64±0.23	10.64±0.24
CL-3	10.54±0.26	12.57±0.23	09.54±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	08.68±0.25	09.62±0.27	07.68±0.28	09.68±0.24
CL-6	08.02±0.22	10.06±0.24	07.02±0.24	09.02±0.25
CL-7	05.85±0.27	07.83±0.26	04.85±0.26	07.85±0.28
CL -8	05.62±0.23	07.65±0.23	04.62±0.23	07.62±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

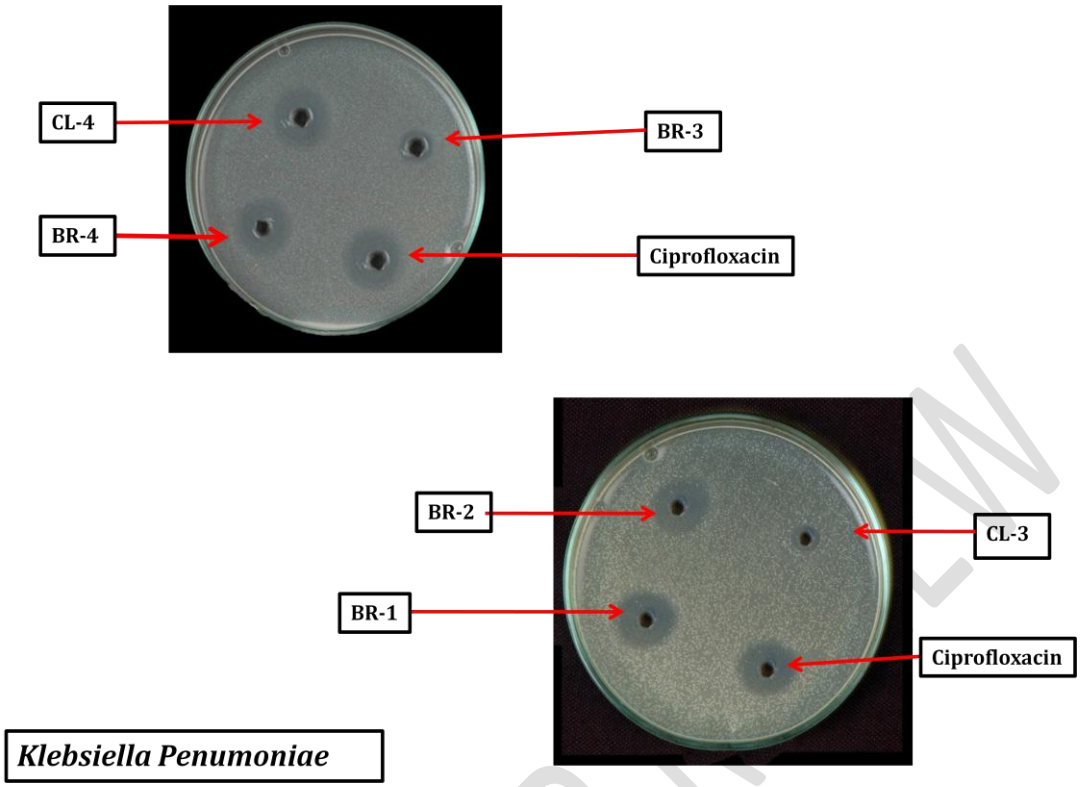

Figure 7: Zone of inhibition of synthesized derivatives against *Klebsiella Penumoniae*

Biological activity based on structure

❖ **Antibacterial activity**

In accordance with the data obtained from antibacterial activity all the synthesized 1,3,5-trisubstituted pyrazole derivatives (ME1- ME8, CL1-CL8, BR1-BR4) have shown mild to best activity against tested microbes. Among these 1,3,5- trisubstituted pyrazole derivatives, compounds BR-3 (Bromo phenyl at position 3 and Bromo phenyl at position 5); BR-2 (Bromo phenyl at position 3 and chloro phenyl at position 5); BR-1 (Bromo phenyl at position 3 and fluoro phenyl at position 5); CL-4 (bromo phenyl at position 3 and chloro phenyl at position 5); BR-4 (Bromo phenyl at position 3 and nitro phenyl at position 5) and CL-3 (chlorophenyl at position 3 and chloro phenyl at position 5) is 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 *Staphylococcus Aureus, Staphylococcus Faecalis, Bacillus Substilis, P. Vulgaris and B. Pumilus* (Gram-positive) and Escherichia coli, Proteus 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

Presence of electronegative group (Br, Cl, F and NO_2) either at third and Fifth position of 1,3,5-pyrazoline ring is required for the potent antimicrobial activity. Presence of electronegative group (Br, Cl) at 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 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Darmon E, Leach DR. Bacterial genome instability. Microbiol Mol Biol Rev 2014;78:1-39.

2. Dobrindt U, Zdziarski J, Salvador E, Hacker J. Bacterial genome plasticity and its impact on adaptation during persistent infection. International Journal of Medical Microbiology 2010;300:363-366.
3. Dobrindt U. Whole genome plasticity in pathogenic bacteria. Current Opinion in Microbiology 2001;4:550-557.
4. Reardon S. WHO warns against 'post-antibiotic' era. Nature 2014;15:135-138.
5. Salton MR, Kim K-S, Baron S. 1996. Medical microbiology. University of Texas Medical Branch at Galveston: Galveston, TX.
6. Abbott A. Scientists bust myth that our bodies have more bacteria than human cells. Nature 2016;19:136-140.
7. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLOS Biology 2016;14:e1002533.
8. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. Current Opinion in Gastroenterology 2015;31:69-75.
9. Finlay BB, McFadden G. Anti-Immunology: Evasion of the Host Immune System by Bacterial and Viral Pathogens. Cell 2006;124:767-782.
10. Schmidt H, Hensel M. Pathogenicity Islands in Bacterial Pathogenesis. Clin Micro Rev 2004;17:14-56.
11. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nat Rev Drug Discov 2007;6:29-40.
12. Reddick LE, Alto Neal M. Bacteria Fighting Back: How Pathogens Target and Subvert the Host Innate Immune System. Mol Cell 2014;54:321-328.
13. Tanitame A, Oyamada Y, Ofuji Kl. Synthesis and antibacterial activity of a novel series of potent DNA gyrase inhibitors: pyrazole derivatives. J Med Chem 2004;47:3693–3696.
14. Yu LG, Ni TF, Gao W, He Y, Wang YY, Cui HW, Yang CG, Qiu WW. The synthesis and antibacterial activity of pyrazole-fused tricyclic diterpene derivatives. Eur J Med Chem 2015; 90:10-20.
15. Chu M-J, Wang W, Ren Z-L, Liu H, Cheng X, Mo K, Wang L, Tang F, Lv X-H. Discovery of novel triazole-containing pyrazole ester derivatives as potential antibacterial agents. Mol 2019;24:1311-1322
16. Ventura TLB, Calixto SD, Abrahim-Vieira BA, Teles de Souza AN, Mello MVP, Rodrigues CR, Mariz Miranda RSD, Muzitano MF. Antimycobacterial and anti-

inflammatory activities of substituted chalcones focusing on an anti-tuberculosis dual treatment approach. Mol 2015;20:8072-8093.

17. Kini S, Bhat AR. Novel 2-pyrazoline derivatives as potential antibacterial and antifungal agents. Ind J Pharm Sci 2008;70(1):105-108.
18. Kumar MP, Ravi TK, Gopalakrishnan S. Antioxidant and antibacterial studies of arylazo pyrazoles and arylhydrazonopyrazolones containing coumarin moiety. Eur J Med Chem 2009;44:4690–94
19. Bawa S, Kumar H. Synthesis of 6-fluoro-2-[4-formyl-3-(substituted phenyl) pyrazol-1-yl]benzothiazoles as potential antibacterial agents. Ind J Het Chem 2005;14:249-50
20. Kumar MP, Ravi TK, Gopalakrishnan S. Antioxidant and antibacterial studies of arylazo pyrazoles and arylhydrazonopyrazolones containing coumarin moiety. Eur J Med Chem 2009;44:4690–94
21. Chetan BP, Mulwar VV. Synthesis and evaluation of certain pyrazolines and related compounds for their anti tubercular, anti bacterial and anti fungal activities. Ind J Chem 2000;44B:232–237.
22. Palkar RB, Master HE. Synthesis of some new 3,5-diarylpyrazoles and their antibacterial activity. Ind J Het Chem 1999;8:315-18.
23. Suma BV, Venkataramana AKR, JudyJays CHS, Madhavan V. Synthesis, characterization, in-vitro antibacterial, anti-inflammatory evaluations of novel 4-quinolone containing pyrazolidinedione derivatives. Int J Chem Tech Res 2010;2(4):2156-62.
24. Hassanzadeh F, Jafari E, Hakimelahi GH, Khajouei MR, Jalali M, Khodarahmi GA. Antibacterial, antifungal and cytotoxic evaluation of some new quinazolinone derivatives. Res Pharm Sci. 2012; 7(2): 87-94.
25. Chougala BM, Samundeeswari S, Holiyachi M, Shastri LA, Dodamani S, Jalalpure S, Dixit SR, Joshi SD, Sunagar VA. Synthesis, characterization and molecular docking studies of substituted 4-coumarinylpyrano[2,3-c]pyrazole derivatives as potent antibacterial and anti-inflammatory agents. Eur J Med Chem 2017;125:101-116.
26. Yu LG, Ni TF, Gao W, He Y, Wang YY, Cui HW, Yang CG, Qiu WW. The synthesis and antibacterial activity of pyrazole-fused tricyclic diterpene derivatives. Eur J Med Chem 2015;90:10–20.
27. Palkar RB, Master HE. Synthesis of some new 3,5-diarylpyrazoles and their antibacterial activity. Ind J Het Chem 1999;8:315-18.

28. Mohammadzadeh T, Sadjjadi S, Habibi P, Sarkari B. Comparison of Agar Dilution, Broth Dilution, Cylinder Plate and Disk Diffusion Methods for Evaluation of Anti-leishmanial Drugs on Leishmania promastigotes. Iran J Parasitol 2012;7(3):43-7.
29. Ben-David A, Davidson CE. Estimation method for serial dilution experiments. J Micro Meth 2014;107:214-221.
30. Chauhan N, Kruppa MD. Standard Growth Media and Common Techniques for Use with Candida albicans. In: Cihlar R.L., Calderone R.A. (eds) Candida albicans. Methods in Molecular Biology. 2009;499:352-358. Humana Press, Totowa, NJ.
31. Bazzaz BSF, Khameneh B, Ostad MRZ, Hosseinzadeh H. In vitro evaluation of antibacterial activity of verbascoside, lemon verbena extract and caffeine in combination with gentamicin against drug-resistant Staphylococcus aureus and Escherichia coli clinical isolates. Avicenna J Phytomed. 2018;8:246-253.