SYNTHESIS, CHARACTERIZATION OF SOME NEW 1,3,5-TRISUBSTITUTED PYRAZOLE DERIVATIVES FOR THEIR ANTIFUNGAL POTENTIAL

Abstract

The aim of the study was to develop, synthesis, and characterise a novel 1,3,5-trisubstituted-2-pyrazolines derivative, as well as to evaluate its antifungal activity. The reaction of chalcone derivatives with succinic hydrazide in the presence of pyridine yielded the 1,3,5-tri-substituted-2-pyrazolines derivatives. Total 20 compounds has been synthesized and characterized by the IR, 1HNMR and mass spectral analysis. Antifungal activity of the compounds carried out onfour fungal strains i.e. *Saccharomyces Cerevisiae, A. Niger, C. Albicans* and *R. Oryzae* in two different concentration i.e. 50 and 100 µg/ml by Agar-diffusion method using Cup-plate method. The usual antifungal medicine was ketoconazole. All of the synthesized 1,3,5-trisubstituted pyrazole compounds (ME1-ME8, CL1-CL8, BR1-BR4) showed medium to best action against examined organisms, according to antifungal activity data. The antifungal activity of compounds against fungal strains (*Saccharomyces cerevisiae, A. Niger, Candida albicans*, and *R. Oryzae*) revealed the following order of action: CL-4 > BR-4 > CL-3 > CL-2 > ME-3> ME-2> CL-5 > CL-6 > ME-4 > ME-5 > ME-6 > ME-7 > CL-7 > CL-8>ME-8 >CL-1 >ME-1 > CL-5 > CL-6 > ME-4 > ME-5 > ME-6 > ME-7 > CL-7 > CL-8>ME-8 >CL-1 >ME-1. Electronegative groups (Br, Cl, F, and NO2) must be present at the third and fifth positions of the 1,3,5-pyrazoline ring for significant antifungal action. The presence of an electronegative group at the third and fifth positions may be required for the best action against bacterial and fungal strains, however the addition of F, NO2 has demonstrated moderate activity, while the substitution of methyl and methoxy groups may reduce the activity. The synthesized compounds in the BR-1 through BR-4 class are the most active.

Keywords: Antifungal, Ketoconazole, Agar Diffusion, pyrazole, Cup plate method

INTRODUCTION

In the contemporary period, sickness from fungal and bacterial origins has increased dramatically. There is an increased need for novel medications or chemically modified moieties that are effective against bacterial and fungal illness. We discovered the diazoles, which are claimed to be employed as antifungal medicines, while searching for moieties that must be efficient against fungal infection. [1] Anti-fungals are used to treat fungal diseases including athlete's foot, ringworm, and candidiasis (thrush), as well as dangerous systemic

infections such cryptococcal meningitis. Antifungal kills the fungal organism without harming the host by taking advantage of distinctions between mammalian and fungal cells. As a result, novel moieties are being sought to aid in the treatment of fungal infections. [2] Pyrazole derivatives have the interest of chemists due to their vast variety of biological characteristics and many practical uses. Pyrazole has been shown to have a wide range of biological effects, including antifungal, anticancer, anti-inflammatory, antifungal, anti-proliferative, anticonvulsant, antioxidant, and antitubercular properties. The pyrazole ring is a useful synthetic target in the pharmaceutical industry because of its wide variety of biological activities. [3] Pyrazole-containing products are commercially available. Celecoxib is an NSAID used to treat rheumatoid arthritis, osteoarthritis, acute pain, and menstruation; Phenzone (phenazon or antipyrine) is an analgesic and antipyretic; Lonazolac is an NSAID; Betazole is an H2 receptor agonist. It is used to assess stomach secretory function in clinical settings. Fipronil is a broad-spectrum pesticide that affects the central nervous system of insects by inhibiting chloride ions from passing through the gamma-Aminobutyric acid (GABA) receptor and glutamate-gated chloride channels, which are both central nervous system components. [4] Chalcone is made through claisen-schmidt condensation of an aromatic aldehyde and a ketone, which is catalyzed by a base or an acid, and then dehydration. Antifungal action is mediated by the presence of a,-unsaturated functional group, which can be changed depending on the kind of substituent on the aromatic rings [14]. As they undergo a range of chemical processes, they also serve as a backbone for the production of numerous heterocyclic compounds. As a result, chalcone plays a crucial role in the production of therapeutic substances. [5] Antifungal agents, antioxidants, anti-inflammatory activity, cytotoxic activity, hypoglycaemic activity, anti-hepatotoxic, antimalarial, antileishmanial, tyrosine inhibitors, and antitumor activities are among the pharmacological activities of many chalcone derivatives of natural or synthetic origin, according to a review of the literature. [6]

The combined synthesis reaction of chalcone with pyrazole moieties may be useful in synthesizing a novel pyrazole derivative, which will aid in the hunt for new antifungal compounds. The aim of the study was to see how effective novel 1,3,5-trisubstituted-2-pyrazolines derivatives in fighting fungus.

MATERIAL AND METHOD

Hi-media, New Delhi, provided the chemicals p-chloroacetophenone, p-bromoacetophenone, and p-methylacetophenone. CDH (Chemical Drug House) in New Delhi, India, provided benzaldehyde, 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-nitrobenzaldehyde, 4-methylbenzaldehyde, and 4-methoxybenzaldehyde. Sigma Aldrich, New Delhi, provided the succinic acid. Chemicals of synthetic grade were utilized 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 ofchalcones by claisen-schmidt condensation

Scheme-II: Synthesis of Succinic hydrazide from correspondingester

Scheme-III: Reaction of Succinic hydrazide with chalcone to form 1,3,5-trisubstituted pyrazolederivatives

Scheme I: Synthesis of chalcones by claisen-schmidt condensation

An equimolar amount of substituted acetophenone (0.05 M) was combined with an equimolar amount of benzaldehyde and substituted benzaldehyde. In ethanol, the mixture was dissolved. The mixture was stirred for 5 minutes before adding a 50 percent aqueous potassium hydroxide solution slowly and stirring for 24 hours at room temperature. The thin layer chromatography (TLC) was used to monitor the reaction's completion. The reaction was then finished and poured over crushed ice, yielding a solid product; however, if the solid product was not produced, it was acidified with dilute hydrochloric acid. [7] 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.

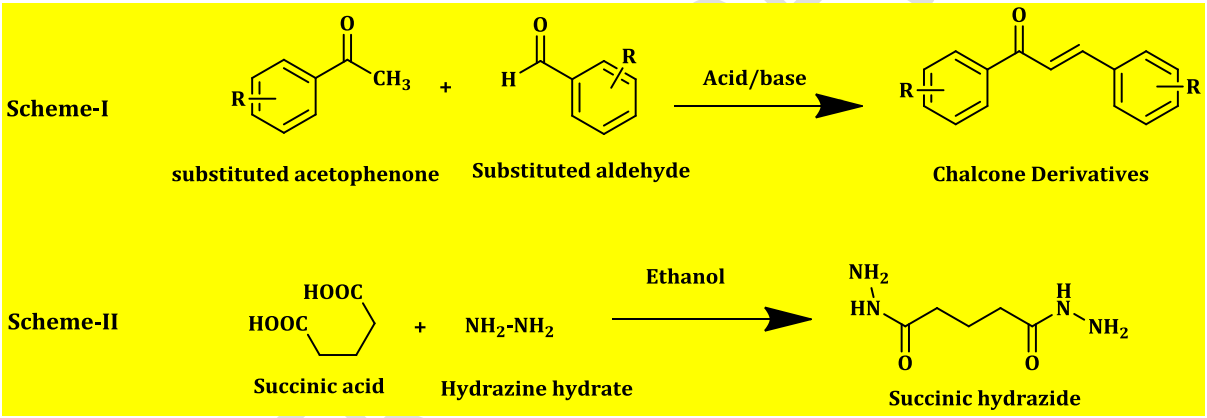
Scheme II: Synthesis of succinic hydrazide

Succinic acid (0.05M) may be readily transformed to succinic hydrazide by reacting it with hydrazine hydrate (0.05M) in alcohol, cooling the reaction mixture to room temperature, and then recrystallizing the succinic hydrazide as a solid. The IR spectra show the peak at 3500.66 (-NH str.); 3313.58 (NH2 str.); 1658.32 (C=O); 1430-3046.55 (-NH str.); 1430-3046.55 (-NH str.); 1430-3046.55 (-NH str.); 1430-3046.55 (-NH str.); 1430-3046.55 (-

(CH-CH). TLC was used to monitor the reaction, which used Hexane: ethyl acetate as the mobile phase. The obtained compounds were analysed using IR and 1HNMR and found to have a structure that was compatible with what was predicted. Scheme-II depicted the synthesis.

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

The synthesized chalcone derivatives were combined in absolute alcohol with equimolar amounts of succinic hydrazide (0.005 M) and a little amount of pyridine (0.01M). The reaction mixture was refluxed for 2-6 hours at 65°C. TLC was used to monitor the reaction, which used ethyl acetate:hexane as the mobile phase. The solvent was entirely evaporated before being placed into ice cold water and constantly stirred to turn the liquid into a solid product, which yielded the synthesized product. [8] This substance was filtered and dried under vacuum. The synthesized compound purified by the column chromatography and were obtained as pale yellow solid colour powder. The synthesis was shown in scheme-III.



Evaluation of antifungal activity

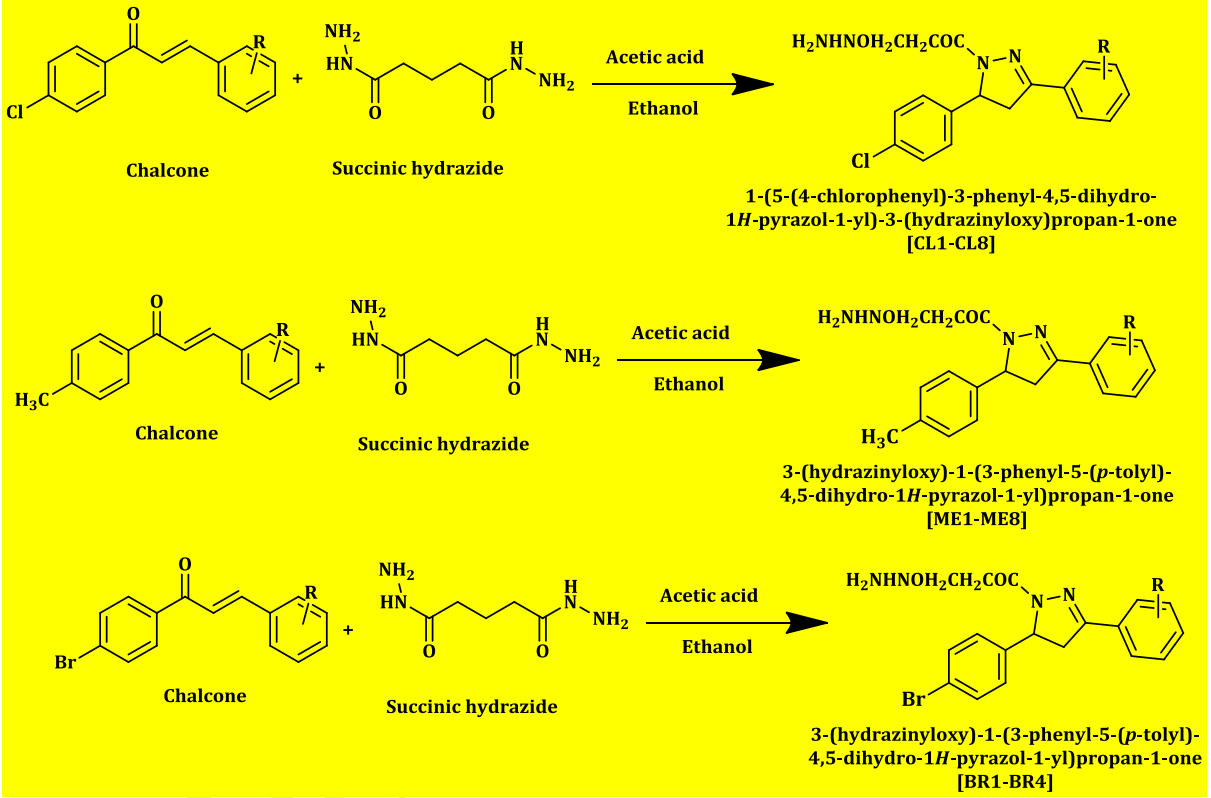
In order to study and evaluate the antifungal activity, reliable and reproducible methods must be used to evaluate them. Agar Diffusion method was used for evaluation of the antifungal activity using Cup-plate method.[9-12]

Antifungal screening of the Synthesized Compounds

The antifungal activity of all 20 produced compounds was tested. Saceharomyces Cerevisiae, Aspergillus Niger, Rhizopus oryzae, and Candida albicans were obtained from IMTECH Chandigarh for the screening. As a standard, ketoconazole was used. Potato-DextroseAgar (PDA) medium was used to sub-culture the test organisms. [13-17] To acquire growth, the tubes containing sterilized media were injected with test fungus and stored at room temperature. They were then refrigerated at 4 degrees Celsius. Cup plate technique was used to test the activity of the derivatives at various concentration levels. The typical medicine was ketoconazole. Each test chemical (5 mg) was diluted in 5 mL Analar grade dimethyl

sulfoxide to provide a 1000 g/mL concentration. Each test compound's solution, control, and reference standards (0.05 ml and 0.1 ml) were put individually in the cups, and the plates were left undisturbed in the refrigerator for at least 2 hours to allow appropriate diffusion of the solution into the PDA medium. After that, the petri dishes were stored at room temperature for 48 hours. After that, using an antibiotic zone reader, the diameter of the zone of inhibition in mm surrounding each of the cups was measured.

Scheme-III



RESULT AND DISCUSSION

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

Molecularformula: $C_{19}H_{22}N_4O_2$; 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

Molecularformula: $C_{22}H_{20}ClN_3O_4S$; 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)+)

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

Molecularformula: $C_{18}H_{18}Br_2N_4O_2$; 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)+)

ANTIFUNGAL ACTIVITY

In accordance with the present data obtained from antifungal 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 *Saccharomyces Cerevisiae, A. Niger C. Albicans, R. Oryzae*.

The Data of antifungal activity against the fungal strains (*Saccharomyces cerevisiae, A. niger, C. albicans, R. oryzae*) suggested the order of activity of compounds: BR-3 >BR-2 > BR-1 > CL-4 > BR-4 > CL-3 > CL-2 > ME-3> ME-2> CL-5 > CL-6 > ME-4 > ME-5 > ME-6 > ME-7 > CL-7 > CL-8>ME-8 >CL-1 >ME-1. Among these 1,3,5-trisubstituted pyrazole derivatives, compound CL-6 > ME-4 > ME-5 > ME-6 > ME-7 > CL-7 > CL-8>ME-8 >CL-1 >ME-1 shows mild activity and compounds CL-2, ME-3, ME-2 and CL-5 has showed moderate activity and BR-3 >BR-2 > BR-1 > CL-4 > BR-4 > CL-3 has shown best activity against all fungi strains (**Table 1**).

[A] Activity against Sacharomyces Cerevisiae*:*

The Compounds BR-3 (14.75±0.53 ;17.65±0.83), BR-2 (12.45±0.28; 16.32±0.26), BR-1 (11.22±0.65; 15.25±0.74), CL-4 (11.72±0.34; 12.20±0.82), BR-4 (10.32±0.32; 12.25±0.45) and CL-3 (10.42±0.6712.72±0.37) has shown zone of inhibition in mm in comparison to standard drug (Clotrimazole, 16±0.34; 21±0.24) has shown good activity against Sacharomyces Cerevisiae (Fungi strains) at 50µg concentration.

The Compounds BR-3 (17.65±0.83), BR-2 (16.32±0.26), BR-1 (15.25±0.74), CL-4 (12.20±0.82), CL-3 (12.72±0.37) and BR-4 (12.25±0.45) has shown zone of inhibition in comparison to standard drug (Clotrimazole, 21±0.24) has shown good activity against Saceharomyces Cerevisiae (Fungi strains) at 100µg concentration.

[B] Activity against *A. Niger*:

Compounds BR-3 (13.34±0.78); BR-2 (12.23±0.63); BR-4 (10.38±0.54); Cl-4 (9.62±0.23) ;CL-3 (8.52±0.85); BR-1 (8.42±0.36) has shown zone of inhibition as compared to standard drug (Clotrimazole, 15±0.45) has shown good activity against *A. Niger* (Fungi strains) at 50µg concentration

Compounds BR-3 (18.25±0.32), BR-2 (16.35±0.65), BR-4 (14.42±0.25); Cl-4 (16.72±0.68), CL-3 (14.32±0.546), BR-1 (12.55±0.42) has shown zone of inhibition comparison to standard drug (Clotrimazole, 20±0.34) has shown good activity against *A. Niger* (Fungi strains) at 100 µg concentration

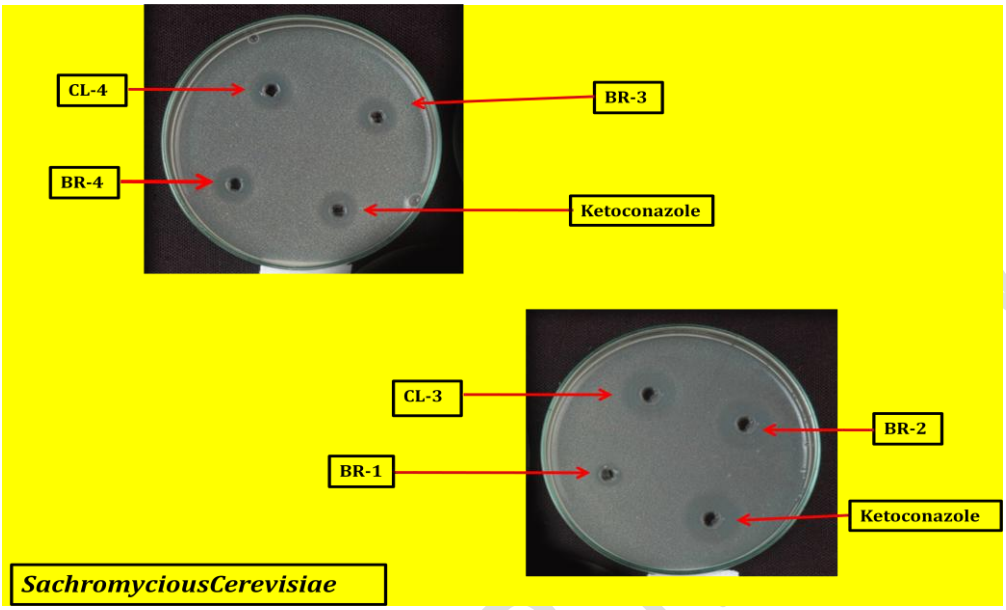


Figure 1: Zone of inhibition of synthesized derivatives against *Staphylococcus Aureus.*

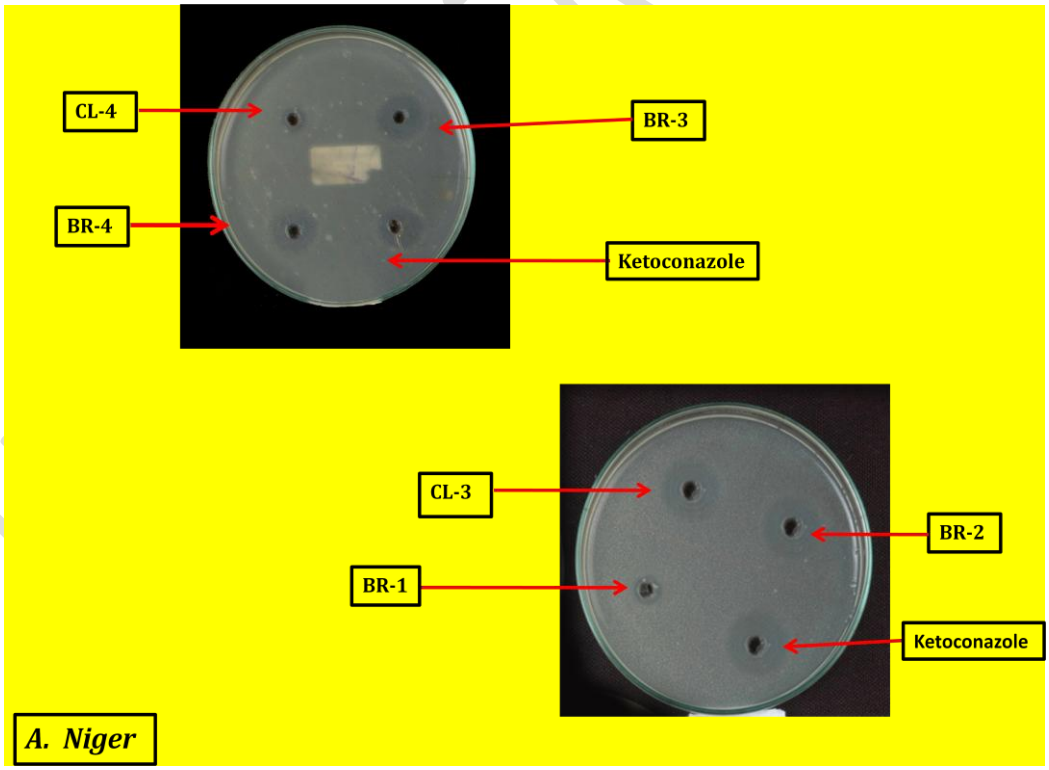


Figure 2: Zone of inhibition of synthesized derivatives against *Staphylococcus aureus*

Table 1: Antifungal activity of synthesized pyrazole derivatives.

COMPOUND	Zone of inhibition in mm							
	Saccharomyces cerevisiae		***A. niger***		***C. albicans***		***R. oryzae***	
Concentration	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**
ME-1	2.32±0.33	3.22±0.52	3.32±0.63	5.52±0.73	5.22±0.83	6.32±0.42	7.35±0.62	9.25±0.33
ME-2	7.32±0.55	7.32±0.45	3.42±0.52	6.25±0.55	4.42±0.24	6.25±0.55	7.64±0.55	9.21±0.55
ME-3	8.32±0.33	8.72±0.64	4.52±0.76	7.64±0.42	5.23±0.52	8.54±0.33	7.35±0.23	9.42±0.63
ME-4	6.32±0.77	8.42±0.36	5.32±0.13	9.64±0.65	6.32±0.66	9.24±0.26	8.23±0.46	10.36±0.37
ME-5	5.32±0.22	6.62±0.53	2.62±0.37	5.12±0.33	4.52±0.33	6.56±0.42	7.16±0.55	9.32±0.74
ME-6	5.32±0.37	5.22±0.27	3.22±0.34	5.32±0.36	4.22±0.37	6.24±0.44	7.12±0.26	8.24±0.32
ME-7	4.32±0.14	5.72±0.72	3.32±0.57	5.22±0.73	4.42±0.72	6.36±0.26	7.34±0.32	8.23±0.46
ME-8	3.32±0.63	4.332±0.67	3.42±0.35	5.42±0.27	4.62±0.65	6.25±0.63	7.24±0.45	8.26±0.83
CL-1	2.52±0.45	3.62±0.25	3.62±0.44	5.32±0.54	4.32±0.73	6.32±0.38	7.32±0.63	8.32±0.87
CL-2	9.62±0.72	11.62±0.23	7.24±0.53	12.52±0.72	7.62±0.54	11.42±0.44	12.42±0.77	14.72±0.38
CL-3	10.42±0.67	12.72±0.37	8.52±0.85	14.32±0.546	10.32±0.36	14.62±0.33	13.72±0.24	16.32±0.53
CL-4	11.72±0.34	12.20±0.82	9.62±0.23	16.72±0.68	12.2±0.52	16.32±0.36	16.42±0.85	19.72±0.75
CL-5	07.32±0.88	8.32±0.44	6.65±0.36	10.52±0.82	5.62±0.26	9.52±0.41	9.62±0.43	11.62±0.22
CL-6	6.32±0.84	7.12±0.75	5.65±0.22	9.22±0.44	4.72±0.49	8.22±0.24	8.27±0.35	10.27±0.75
CL-7	04.22±0.26	5.52±0.32	3.62±0.55	5.32±0.53	4.32±0.23	6.32±0.43	7.32±0.72	8.32±0.87
CL -8	03.62±0.82	5.72±0.56	3.32±0.73	5.44±0.35	4.54±0.45	6.32±0.46	7.32±0.31	8.32±0.33
BR-1	11.22±0.65	15.25±0.74	8.42±0.36	12.55±0.42	8.28±0.22	11.44±0.33	12.56±0.54	15.66±0.25
BR-2	12.45±0.28	16.32±0.26	12.23±0.63	16.35±0.65	11.54±0.54	15.60±0.46	15.20±0.82	19.54±0.72
BR-3	14.75±0.53	17.65±0.83	13.34±0.78	18.25±0.32	13.20±0.35	17.52±0.54	17.65±0.77	21.05±0.31
BR-4	10.32±0.32	12.25±0.45	10.38±0.54	14.42±0.25	10.09±0.52	13.47±0.67	14.52±0.34	17.27±0.54
DMSO (Control)	-	-	-	-	-	-		
Ketoconazole	**15** ±0.32	**20**±0.26	**14**±0.32	**19**±0.23	**13**±0.45	**18**±0.69	**18**±0.30	**22**±0.33

[C] Activity against *C. albicans:*

Compounds BR-3 (13.20±0.35), Cl-4 (12.2±0.52), BR-2 (11.54±0.54), CL-3 (10.32±0.36), BR-4 (10.09±0.52) and BR-1 (8.28±0.22) has shown zone of inhibition comparison to standard drug (Clotrimazole, 14±0.46) has shown good activity against C. Albicans (Fungi strains) at 50µg concentration.

Compounds BR-3 (17.52±0.54), Cl-4 (16.32±0.36), BR-2 (15.60±0.46), CL-3 (14.62±0.33), BR-4 (13.47±0.67) and BR-1 (11.44±0.33) has shown zone of inhibition comparison to standard drug (Clotrimazole, 19±0.65) has shown good activity against C. Albicans (Fungi strains) at 100 µg concentration.

[D] Activity against *R. oryzae:*

Compounds BR-3 (17.65±0.77; Cl-4 (16.42±0.85); BR-2 (15.20±0.82; BR-4 (14.52±0.34); CL-3 (13.72±0.24); BR-1 (12.56±0.54) and CL-2 (14.52±0.34) has shown zone of inhibition comparison to standard drug (Clotrimazole, 18±0.30) has shown good activity against *R. Oryzae* (Fungi strains) at 50µg concentration.

Compounds BR-3 (21.05±0.31); Cl-4 (19.72±0.75); BR-2 (19.54±0.72); BR-4 (17.27±0.54); CL-3 (16.32±0.53); BR-1 (15.66±0.25) and CL-2 (22.±0.23) has shown zone of inhibition comparison to standard drug (Clotrimazole, 22±0.33) has shown good activity against R. Oryzae (Fungi strains) at 100 µg concentration. However, further studies on activity and long term toxicity are to be carried out before any conclusion are drawn, as these categories of drug are known to have potential antifungal activity. Testing on different models can further substantiate the antifungal activity of the synthesized analogues. The graphical representation of zone of inhibition was shown in Figure 1 to 4.

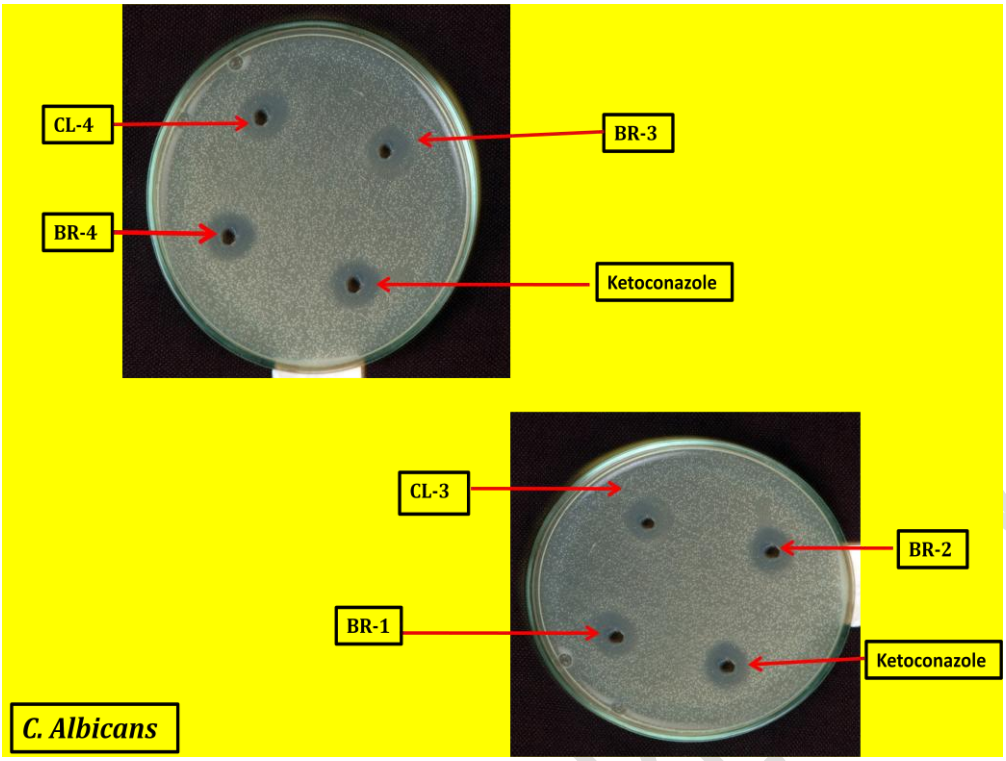


Figure 3: Zone of inhibition of synthesized derivatives against *Staphylococcus aureus*

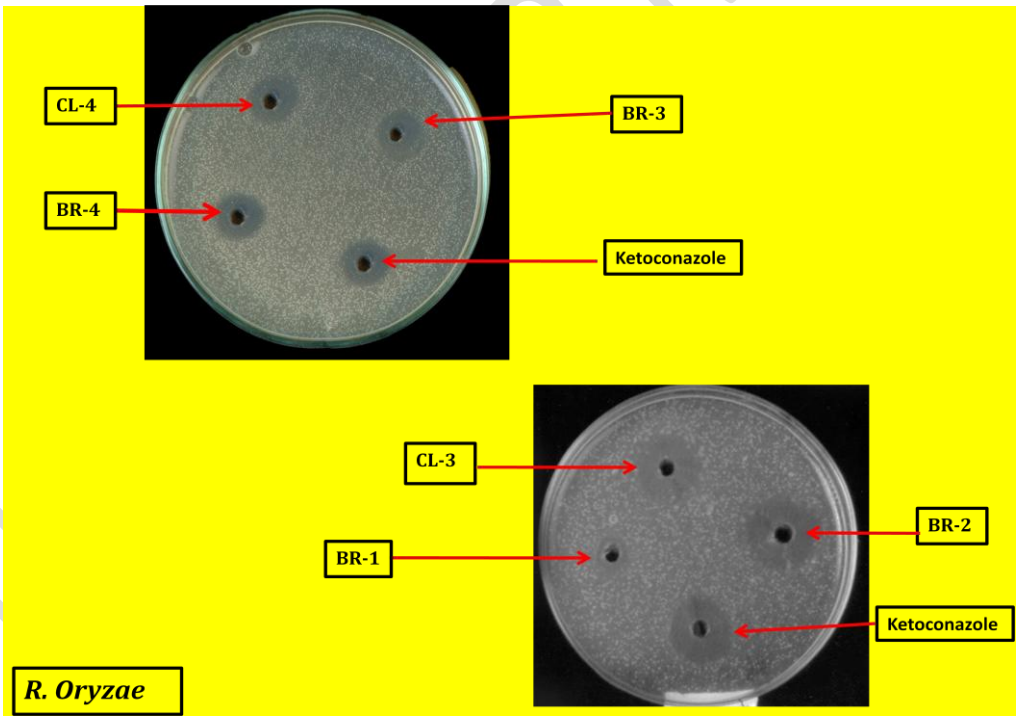


Figure 4: Zone of inhibition of synthesized derivatives against *Staphylococcus Aureus.*

CONCLUSION

Using the Agar diffusion technique, all of the 2-pyrazolines were tested for antifungal activity against *Saccharomyces cerevisiae*, *A. niger*, *C. albicans*, and *R. oryzae*. The outcomes of this study were compared using ketoconazole as a benchmark. When pyrazoline was compared to

standard, the antifungal activity results showed that the compounds exhibit considerable inhibitory effect on all fungal strains at both 50 g (0.05 ml) and 100 g (0.1 ml) dosing levels. Chemicals BR-3, BR-2, BR-1, CL-4, BR-4, and CL-3 had the highest activity of all the compounds examined. These compounds have halogens on the aromatic ring, indicating that electron withdrawing groups to the antifungal activity.

Electronegative groups (Br, Cl, F, and NO_2) must be present at the third and fifth positions of the 1,3,5-pyrazoline ring for significant antifungal action. The presence of an electronegative group (Br, Cl) at the third and fifth positions may be required for the best action against bacterial and fungal strains, however the addition of F, NO_2 has demonstrated moderate activity, while the replacement of -CH3 -OCH3 may reduce the activity. The synthesized compounds in the BR-1 through BR-4 class are the most active. This shows that bromine in the third and fifth positions of pyrazole is required for antifungal action, and that the presence of chloro-, bromo-, fluoro-, and nitrogen groups linked to the phenyl ring enhances antifungal activity. The antifungal activity data revealed that Cl, Br, F, and Nitro substitutions at the third and fifth positions may increase the antifungal activity of the compounds, whilst methyl and methoxy substitutions may decrease it.

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.

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