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Design and synthesis of novel pyrazolines as potent antimicrobial and antioxidant agents

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ABSTRACT

The nitrile imines generated by the oxidative dehydrogenation of aldehyde phenylhydrazones with chloramine-T in ethyl alcohol undergo 1,3-dipolar cycloaddition with benzalacetone in situ to afford 1-(3-aryl-1,4-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanones in good yield. The structure proofs of the synthesized new cycloadducts were provided by IR, ^{1}H NMR, ^{13}C NMR, Mass spectral studies and elemental analysis. The new compounds were evaluated in vitro for their antimicrobial and antioxidant activities.

Key words: Antibacterial, antifungal, antioxidant, chloramine-T, dipolar, nitrile imines.

INTRODUCTION

The five membered heterocycles such as pyrazoles and their analogs are interesting class compounds in pharmaceutical and medicinal chemistry. They were considered as useful synthons in organic synthesis in constructing biologically active molecules. The pyrazoles acts as selective inhibitors of tissue-nonspecific alkaline phosphatase [1]. They were known to exhibit antitubercular [2], antiviral [3], antimicrobial [4], antioxidant [5], antitumor and antiangiogenic properties [6].

A series of structurally related 1*H*-pyrazolyl derivatives were tested for their anti-inflammatory activities, COX-1 and COX-2 inhibitory activities, ulcerogenic effects and acute toxicity [7]. The usual synthesis of pyrazole ring system has been executed in the literature via 1,3-dipolar cycloaddition reactions of alkenes and alkynes with nitrile imines generated *in situ* from aldehyde phenylhydrazones [8]. Chloramine-T was extensively used reagent for *in situ* generation of nitrile imines from aldehyde phenylhydrazones [9].

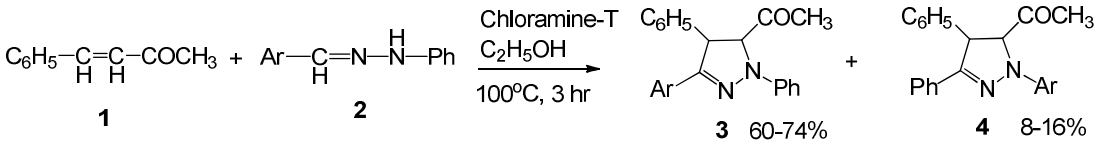
In search of new antimicrobial and antioxidant agents; the present project of synthesizing novel pyrazoles and study their biological properties was undertaken. We herein report the synthesis of series of isomeric pyrazoles (**3, 4**); which involve 1,3-dipoar cycloaddition reaction of benzalacetone (**1**) and nitrile imines generated *in situ* from aldehyde phenylhydrazones (**2**) using chloramine-T (CAT) as catalytic dehydrogenating agent; and the antimicrobial and antioxidant activities of the major products (**3**).

EXPERIMENTAL SECTION

IR spectra were recorded on Shimadzu 8300 spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker supercon 400 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as an internal standard. The Chemical shifts are expressed in δ ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrophotometer

(chemical ionization) and the relative intensities of the important fragments were given in the parenthesis. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyser. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane: ethyl acetate (8:2) as eluent.

In a typical 1,3-dipolar cycloaddition reaction, the nitrile imines generated by the catalytic dehydrogenation of aromatic aldehyde phenylhydrazones (**2**) with chloramine-T in ethyl alcohol were trapped *in situ* with benzalacetone (**1**), the reaction yielded an isomeric mixture of 1-(3-aryl-1,4-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanones (**3**) as major and 1-(1-aryl-3,4-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanones (**4**) as minor products (Scheme-1).



3 or 4 a) Ar = 4-FC_6H_4; b) Ar = 4-ClC_6H_4; c) Ar = 4-$CH_3C_6H_4$; d) Ar = C_6H_5; e) Ar = 4-$OCH_3C_6H_4$; f) Ar = 3,4-$(OCH_3)_2C_6H_3$; g) Ar = Furan-2-yl.

Scheme-1: Reaction pathway for the synthesis of pyrazolines

General procedure for the cycloaddition

A mixture of aldehyde phenylhydrazone (**2**) (4.0 mmol), benzalacetone (**1**) (4.0 mmol) and chloramine-T trihydrate (5.0 mmol) in ethanol was refluxed on water bath for 3 hours. The progress of the reaction was monitored by TLC. After the completion of the reaction, the sodium chloride formed was filtered off, and the solvent was evaporated in vacuo. The residual mass was extracted into ether (25 mL), washed successively with water (2 X 15 mL), 10% sodium hydroxide (2 X 15 mL) and saturated brine solution (1 X 10 mL). The organic layer was dried over anhydrous sodium sulphate and the solvent was evaporated to dryness. The reaction produced light to brown oily mass, which showed two spots corresponding to the cycloadducts (**3** and **4**) in TLC. The products were separated by column chromatography using hexane: ethyl acetate (8:2 v/v) as eluent.

Antibacterial activity

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique [10]. The nutrient broth, which contain logarithmic serially two-fold diluted amount of test compound and controls were inoculated with approximately 5 x 10^5 c.f.u of actively dividing bacteria cells. The bacterial cultures were incubated for 24 hrs at 37^0C and fungi cultures were incubated for 72 hrs at 37^0C, the growth was monitored visually and spectrophotometrically. The lowest concentration required to arrest the growth of bacteria and fungi was regarded as minimum inhibitory concentration (MIC). The test compounds were screened for their antibacterial activity against Gram-negative bacteria species *Escherichia coli*, *Salmonella typhimurium*, Gram-positive bacteria species *Bacillus substilis*, *Staphylococus aureus*. And antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *C. albicans*, *Fusarium oxysporium*. The antibiotics streptomycin and nystatin were used as standard drugs against bacteria and fungi species respectively. The experiments were carried out in triplicate; the results were taken as a mean of three determinations.

Antioxidant activity

The capacity to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the Blois method [11] using ascorbic acid as standard antioxidant. Samples dissolved in methanol (0-50 µg/mL; 0-5 µg/mL ascorbic acid) in 200 µL aliquot was mixed with 100 mM tris-HCl buffer (800 µL, pH 7.4) and then added 1 mL of 500 µM DPPH in ethanol (final concentration of 250 µM). The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The experiments were performed in triplicates; the results are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

***1-(3-(4-Fluorophenyl)-1,4-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone,* 3a**: Obtained as a light brown oil in 64% yield. IR (nujol): 1678, 1750 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.356 (s, 3H, CH_3), 4.122 (d, 1H, *J= 8.6Hz*, C_4-H), 4.390 (d, 1H, *J= 9.8Hz*, C_5-H), 6.708 (t, 1H, Ph-H), 7.222-7.561 (m, 9H, C_6H_5, Ph-H), 7.724 (dd, 2H, Ar-H), 7.812

(dd, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): 26.30 (1C, $\underline{C}H_3$), 42.22 (1C, 4-$\underline{C}$), 66.98 (1C, 5-$\underline{C}$), 121.56 (2C, Ph-$\underline{C}$), 122.31 (2C, Ar-$\underline{C}$), 124.77 (1C, Ph-$\underline{C}$), 127.26 (1C, Ar-$\underline{C}$), 128.66 (2C, Ar-$\underline{C}$), 129.12 (1C, C_6H_5-$\underline{C}$), 129.72 (2C, C_6H_5-$\underline{C}$), 130.13 (1C, C_6H_5-$\underline{C}$), 131.44 (2C, Ph-$\underline{C}$), 135.09 (1C, Ph-$\underline{C}$), 138.00 (1C, 3-$\underline{C}$), 141.03 (1C, C_6H_5-$\underline{C}$), 149.92 (1C, Ar-$\underline{C}$),170.06 (1C, $\underline{C}$=O). MS (relative abundance) m/z: 359 (MH^+, 29), 358 (M^+, 100), 315 (32), 302 (12), 235 (23), 213 (11), 198 (44), 137 (25). Anal. Cacld. for $C_{23}H_{19}FN_2O$: C, 77.08, H, 5.34, N, 7.82%; Found: C, 77.02, H, 5.37, N, 7.85%.

1-(3-(4-Chlorophenyl)-1,4-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone, **3b**: Obtained as a light brown oil in 78% yield. IR (nujol): 1688, 1744 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.372 (s, 3H, CH_3), 4.260 (d, 1H, *J= 8.0Hz,* C_4-H), 4.373 (d, 1H, *J= 7.6Hz,* C_5-H), 6.604 (t, 1H, Ph-H), 7.203-7.559 (m, 9H, C_6H_5, Ph-H), 7.703 (dd, 2H, Ar-H), 7.808 (dd, 2H, Ar-H). MS (relative abundance) m/z: 376 (MH^+, ^{37}Cl, 33), 374 (MH^+, ^{35}Cl, 28), 373 (M^+, ^{35}Cl, 100), 331 (08), 269 (06), 235 (08). Anal. Cacld. for $C_{23}H_{19}ClN_2O$: C, 73.69, H, 5.11, N, 7.47%; Found: C, 73.71, H, 5.14, N, 7.56%.

1-(1,4-Diphenyl-3-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)ethanone, **3c**: Obtained as a light brown oil in 80% yield. IR (nujol): 1680, 1740 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.125 (s, 3H, CH_3), 2.325 (s, 3H, CH_3), 4.222 (d, 1H, *J= 9.2Hz,* C_4-H), 4.430 (d, 1H, *J= 8.1Hz,* C_5-H), 6.728 (t, 1H, Ph-H), 7.236-7.568 (m, 9H, C_6H_5, Ph-H), 7.719 (dd, 2H, Ar-H), 7.892 (dd, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): 21.80 (1C, $\underline{C}H_3$), 26.54 (1C, $\underline{C}H_3$), 43.02 (1C, 4-$\underline{C}$), 67.08 (1C, 5-$\underline{C}$), 121.42 (2C, Ph-$\underline{C}$), 122.55 (2C, Ar-$\underline{C}$), 124.21 (1C, Ph-$\underline{C}$), 127.30 (1C, Ar-$\underline{C}$), 128.28 (2C, Ar-$\underline{C}$), 129.20 (1C, C_6H_5-$\underline{C}$), 129.88 (2C, C_6H_5-$\underline{C}$), 130.24 (1C, C_6H_5-$\underline{C}$), 131.10 (2C, Ph-$\underline{C}$), 135.02 (1C, Ph-$\underline{C}$), 137.78 (1C, 3-$\underline{C}$), 141.32 (1C, C_6H_5-$\underline{C}$), 149.82 (1C, Ar-$\underline{C}$), 170.01 (1C, $\underline{C}$=O). Anal. Cacld. for $C_{24}H_{22}N_2O$: C, 81.33, H, 6.26, N, 7.90%; Found: C, 81.31, H, 6.20, N, 7.80%.

1-(1,3,4-Triphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone, **3d**: Obtained as a light brown oil in 72% yield. IR (nujol): 1695, 1735 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.225 (s, 3H, CH_3), 4.214 (d, 1H, *J= 8.0Hz,* C_4-H), 4.446 (d, 1H, *J= 6.8Hz,* C_5-H), 6.733 (t, 1H, Ph-H), 7.216-7.571 (m, 14H, C_6H_5, Ph-H). MS (relative abundance) m/z: 341 (MH^+, 26), 340 (M^+, 100), 297 (14), 235 (30), 195 (22), 180 (14), 119 (30). Anal. Cacld. for $C_{23}H_{20}N_2O$: C, 81.15, H, 5.92, N, 8.23%; Found: C, 81.07, H, 5.90, N, 8.18%.

1-(3-(4-Methoxyphenyl)-1,4-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone, **3e**: Obtained as light brown oil in 68% yield. IR (nujol): 1690, 1742 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.205 (s, 3H, CH_3), 3.900 (s, 3H, OCH_3), 4.208 (d, 1H, *J= 8.0Hz,* C_4-H), 4.442 (d, 1H, *J= 7.8Hz,* C_5-H), 6.732 (t, 1H, Ph-H), 7.240-7.663 (m, 9H, C_6H_5, Ph-H), 7.701 (dd, 2H, Ar-H), 7.992 (dd, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): 26.26 (1C, $\underline{C}H_3$), 42.32 (1C, 4-$\underline{C}$), 55.64 (1C, O$\underline{C}H_3$), 66.14 (1C, 5-$\underline{C}$), 121.18 (2C, Ph-$\underline{C}$), 122.54 (2C, Ar-$\underline{C}$), 124.85 (1C, Ph-$\underline{C}$), 127.17 (1C, Ar-$\underline{C}$), 128.32 (2C, Ar-$\underline{C}$), 129.30 (1C, C_6H_5-$\underline{C}$), 129.84 (2C, C_6H_5-$\underline{C}$), 130.10 (1C, C_6H_5-$\underline{C}$), 131.36 (2C, Ph-$\underline{C}$), 134.98 (1C, Ph-$\underline{C}$), 137.44 (1C, 3-$\underline{C}$), 140.94 (1C, C_6H_5-$\underline{C}$), 149.33 (1C, Ar-$\underline{C}$), 169.86 (1C, $\underline{C}$=O). MS (relative abundance) m/z: 371 (MH^+, 18), 370 (M^+, 100), 327 (28), 314 (10), 265 (08), 235 (24), 225 (15), 210 (16), 149 (32). Anal. Cacld. for $C_{24}H_{22}N_2O_2$: C, 77.81, H, 5.99, N, 7.56%; Found: C, 77.74, H, 5.93, N, 7.50%.

1-(3-(3,4-Dimethoxyphenyl)-1,4-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone, **3f**: Obtained as a light brown oil in 75% yield. IR (nujol): 1688, 1755 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.250 (s, 3H, CH_3), 3.896 (s, 6H, OCH_3), 4.382 (d, 1H, *J= 8.1Hz,* C_4-H), 4.769 (d, 1H, *J= 8.8Hz,* C_5-H), 6.721 (t, 1H, Ph-H), 6.911 (d, 1H, Ar-H), 7.240-7.600 (m, 11H, C_6H_5, Ph-H). ^{13}C NMR ($CDCl_3$): 26.28 (1C, $\underline{C}H_3$), 42.48 (1C, 4-$\underline{C}$), 55.60 (2C, O$\underline{C}H_3$), 66.29 (1C, 5-$\underline{C}$), 121.02 (2C, Ph-$\underline{C}$), 123.76 (1C, Ar-$\underline{C}$), 124.50 (1C, Ar-$\underline{C}$), 125.15 (1C, Ph-$\underline{C}$), 127.67 (1C, Ar-$\underline{C}$), 128.56 (1C, Ar-$\underline{C}$), 129.22 (1C, C_6H_5-$\underline{C}$), 129.44 (2C, C_6H_5-$\underline{C}$), 130.02 (2C, C_6H_5-$\underline{C}$), 131.57 (2C, Ph-$\underline{C}$), 134.75 (1C, Ph-$\underline{C}$), 136.30 (1C, 3-$\underline{C}$), 139.77 (1C, C_6H_5-$\underline{C}$), 149.23 (2C, Ar-$\underline{C}$), 169.22 (1C, $\underline{C}$=O). MS (relative abundance) m/z: 401 (MH^+, 17), 357 (17), 346 (M-56, 100), 331 (58), 255 (16), 240 (14), 235 (24), 179 (12). Anal. Cacld. for $C_{25}H_{24}N_2O_3$: C, 74.98, H, 6.04, N, 7.00%; Found: C, 75.12, H, 5.91, N, 7.06%.

1-(3-(Furan-2-yl)-1,4-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone, **3g**: Obtained as a light brown oil in 76% yield. IR (nujol): 1684, 1745 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.212 (s, 3H, CH_3), 4.211 (d, 1H, *J= 8.3Hz,* C_4-H), 4.439 (d, 1H, *J= 9.9Hz,* C_5-H), 6.532 (d, 1H, Ar-H), 6.712 (t, 1H, Ph-H), 6. 912 (d, 1H, Ar-H), 7.258-7.610 (m, 10H, C_6H_5, Ph-H). Anal. Cacld. for $C_{21}H_{18}N_2O_2$: C, 76.34, H, 5.49, N, 8.48%; Found: C, 76.26, H, 5.52, N, 8.45%.

The physical and analytical data of the two isomeric minor products 4a and 4c were given below.

***1-(1-(4-Fluorophenyl)-3,4-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)ethanone,* 4a**: Obtained as a light brown oil in 64% yield. IR (nujol): 1678, 1750 cm^{-1}. ^1H NMR ($CDCl_3$): 2.356 (s, 3H, CH_3), 4.122 (d, 1H, *J= 8.6Hz*, C_4-H), 4.390 (d, 1H, *J= 9.8Hz*, C_5-H), 6.708 (t, 1H, Ph-H), 7.222-7.561 (m, 9H, C_6H_5, Ph-H), 7.724 (dd, 2H, Ar-H), 7.812 (dd, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): 26.30 (1C, $\underline{C}H_3$), 42.22 (1C, 4-$\underline{C}$), 66.98 (1C, 5-$\underline{C}$), 121.56 (2C, Ph-$\underline{C}$), 122.31 (2C, Ar-$\underline{C}$), 124.77 (1C, Ph-$\underline{C}$), 127.26 (1C, Ar-$\underline{C}$), 128.66 (2C, Ar-$\underline{C}$), 129.12 (1C, C_6H_5-$\underline{C}$), 129.72 (2C, C_6H_5-$\underline{C}$), 130.13 (1C, C_6H_5-$\underline{C}$), 131.44 (2C, Ph-$\underline{C}$), 135.09 (1C, Ph-$\underline{C}$), 138.00 (1C, 3-$\underline{C}$), 141.03 (1C, C_6H_5-$\underline{C}$), 149.92 (1C, Ar-$\underline{C}$),170.06 (1C, $\underline{C}$=O). MS (relative abundance) m/z: 359 (MH^+, 29), 358 (M^+, 100), 315 (32), 302 (12), 235 (23), 213 (11), 198 (44), 137 (25). Anal. Cacld. for $C_{23}H_{19}FN_2O$: C, 77.08, H, 5.34, N, 7.82%; Found: C, 77.02, H, 5.37, N, 7.85%.

***1-(3,4-Diphenyl-1-(4-methylphenyl)-4,5-dihydro-1H-pyrazol-5-yl)ethanone,* 4c:** Obtained as a light brown oil in 80% yield. IR (nujol): 1680, 1740 cm^{-1}. ^1H NMR ($CDCl_3$): 2.125 (s, 3H, CH_3), 2.325 (s, 3H, CH_3), 4.222 (d, 1H, *J= 9.2Hz*, C_4-H), 4.430 (d, 1H, *J= 8.1Hz*, C_5-H), 6.728 (t, 1H, Ph-H), 7.236-7.568 (m, 9H, C_6H_5, Ph-H), 7.719 (dd, 2H, Ar-H), 7.892 (dd, 2H, Ar-H). ^{13}C NMR ($CDCl_3$): 21.80 (1C, $\underline{C}H_3$), 26.54 (1C, $\underline{C}H_3$), 43.02 (1C, 4-$\underline{C}$), 67.08 (1C, 5-$\underline{C}$), 121.42 (2C, Ph-$\underline{C}$), 122.55 (2C, Ar-$\underline{C}$), 124.21 (1C, Ph-$\underline{C}$), 127.30 (1C, Ar-$\underline{C}$), 128.28 (2C, Ar-$\underline{C}$), 129.20 (1C, C_6H_5-$\underline{C}$), 129.88 (2C, C_6H_5-$\underline{C}$), 130.24 (1C, C_6H_5-$\underline{C}$), 131.10 (2C, Ph-$\underline{C}$), 135.02 (1C, Ph-$\underline{C}$), 137.78 (1C, 3-$\underline{C}$), 141.32 (1C, C_6H_5-$\underline{C}$), 149.82 (1C, Ar-$\underline{C}$), 170.01 (1C, $\underline{C}$=O). Anal. Cacld. for $C_{24}H_{22}N_2O$: C, 81.33, H, 6.26, N, 7.90%; Found: C, 81.31, H, 6.20, N, 7.80%.

The structures of the cycloadducts were provided by IR, ^1H NMR, ^{13}C NMR, MS studies and elemental analysis. For instance, in IR spectra, the cycloadducts **3a-g** gave the absorptions bands in the region 1735-1755 cm^{-1} for C=N (str) group of newly formed five membered ring, a strong absorption bands in the region 1678-1695 cm^{-1} for C=O (str). In ^1H NMR spectra, the cycloadducts showed the peaks due to aromatic and substituent protons at the expected region. The consistent pattern signals due to C_4-H and C_5-H appeared as doublet in the region δ 4.122-4.382 ppm., and δ 4.373-4.769 ppm. The coupling constant (*J*) values obtained were in range 6.8-9.9 *Hz;* indicating that both C_4- and C_5- protons are *cis* in the products.

In ^{13}C NMR, all products gave the signals due to aromatic and substituent carbons at the expected region. The consistent pattern signals observed for the carbon atom of the newly formed five membered rings. The signals appeared in the region δ_c 42.22-43.02 ppm, δ_c 66.14-67.08 ppm, and δ_c 136.30-138.00 ppm. for C_4-carbon, C_5-carbon, and C_3-carbon respectively supported the formation of cycloadducts. The cycloadducts **3a-g** gave significantly stable molecular ion peaks with a relative abundance ranging up to 40% and base peak at (MH^+). Further, all showed satisfactorily CHN analysis with a deviation of ±0.02% from the theoretically calculated values. All these observations strongly favor the formation of the cycloadducts.

The results of MICs) of the synthesized compounds **3a-g** against different bacterium were tabulated in table-1.

Table 1: MIC's of the synthesized compounds 3a-g against bacteria species

Compound	Minimum inhibitory concentration (MIC's) in µg/mL*			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
3a	50	100	100	50
3b	100	200	50	25
3c	50	50	100	200
3d	50	100	**	**
3e	25	50	200	100
3f	25	25	100	100
3g	50	100	**	**
streptomycin	25	50	50	25

** Values are expressed as mean of the three determinations (n=3).*
*** No inhibition even at a higher concentration of 200 µg/mL.*

The synthesized compounds exerted a wide range of *in vitro* antibacterial activity against the tested organisms. However, the compound **3g** failed to inhibit the growth against *S. typhimurium* and *E.coli* organisms tested and compound **3d** failed to show the inhibition against *S. Typhimurium* and *E. coli* organisms even at a higher concentration of 200 µg/mL. The compound **3f** having two electron donating methoxy substituents good activity particularly against *S. aureus* and *S. Pyogenes* with reference to the standard. The compound **3b** having chloro substitution exhibited good activity against *S. typhimurium* and *E. coli* species with reference to the standard.

The results of MICs) of the synthesized compounds **3a-g** against different fungi species were tabulated in table-2.

Table 2: MIC's of the synthesized compounds 3a-g against fungi species

Compound	Minimum inhibitory concentration (MIC's) in µg/mL*			
	A. niger	*A. flavus*	*C. albicans*	*F. oxysporium*
3a	50	100	100	50
3b	25	50	25	25
3c	50	50	100	200
3d	50	100	100	50
3e	25	50	50	50
3f	100	200	200	100
3g	**	**	**	**
nystatin	25	50	50	25

** Values are expressed as mean of the three determinations (n=3).*
*** No inhibition even at a higher concentration of 200 µg/mL.*

The synthesized compounds showed promising *in vitro* antifungal activity against the tested organisms. The compound **3b** having chloro and **3e** having methoxy substitution in the aromatic ring exerted remarkable activity against all the tested organicsm. The compounds **3a**, **3c**, **3d** and **3f** showed moderate to good inhibition. But the compound **3g** failed to inhibit the growth all the tested organisms.

The results of antioxidant activity of the synthesized compounds against different bacterium were tabulated in table-3.

Table-3: DPPH Radical Scavenging activity of the compounds 3a-g relative to the standard ascorbic acid

Samples	% Radical Scavenging activity				
	Concentration (µg/mL)				
	10	20	30	40	50
3a	14.46 ± 0.82	20.30 ± 0.89	28.81 ± 1.01	37.52 ± 0.98	40.73 ± 1.00
3b	13.12 ± 0.78	16.36 ± 0.92	24.42 ± 0.83	27.23 ± 0.78	31.92 ± 1.00
3c	9.22 ± 0.85	11.96 ± 0.89	22.61 ± 0.68	28.96 ± 0.98	32.67 ± 0.71
3d	22.76 ± 0.94	24.23 ± 0.88	36.90 ± 0.74	48.66 ± 0.98	59.17 ± 0.82
3e	13.16 ± 0.80	18.22 ± 1.00	29.90 ± 0.94	32.61 ± 0.95	36.18 ± 0.80
3f	18.32 ± 0.86	25.22 ± 0.76	30.26 ± 1.00	32.22 ± 0.90	37.96 ± 0.88
3g	11.12 ± 0.88	14.36 ± 0.98	19.80 ± 0.81	24.75 ± 0.78	26.80 ± 1.00
Control at Concentration 0 µg/mL 0.00 ± 0.00					
**Values are expressed as mean ± standard deviation (n=3)*					

The compounds **3a-g** showed promising free radical scavenging ability but of lesser activity compared with the standard antioxidant. No much significant variations in the free radical scavenging ability were observed at the initial concentrations of (10-20 µg/mL). However, at the higher concentrations (30-50 µg/mL) all showed a promising activity. Among the series of synthesized compounds **3d** showed radical scavenging ability up to 60%, where as **3a**, **3b**, **3c**, **3e** and **3f** showed radical scavenging ability up to 36-40% with reference to the standard antioxidant. The compound **3g** showed poorer antioxidant activity.

CONCLUSION

The divergence in the biological activity of synthesized new compounds by a simple 1,3-dipolar cycloaddition reactions validates the significance of this study. The study revealed that the most of the compounds tested showed moderate to good antimicrobial and antioxidant activities. However, the effect of compounds on the host cell and their mode of action remain to be studied.

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