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Antioxidant and Antimicrobial Activities of new Triazole-Linked Pyrazoline Hybrid Compounds

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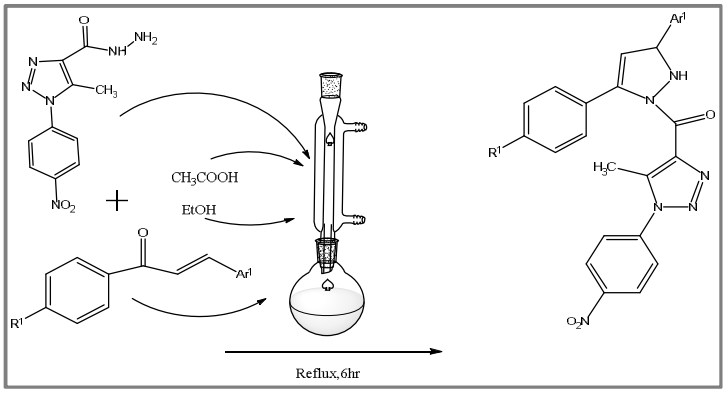
ABSTRACT

*In this study a series of 1, 2, 3-triazole containing pyrazoline **(11a-l)** derivatives were synthesized by the condensation of various substituted chalcones **(7a-f/10g-l)** with substituted 1, 2, 3-triazole carbohydrazide **(4)**. These compounds have been screened for their antioxidant, antibacterial and antifungal activities. The structure of newly synthesized pyrazoline derivatives are confirmed by analytical and spectral data.*

Graphical Abstract



Keywords: 1, 2, 3-Triazole, Pyrazoline, Propeonone, Antioxidant.

INTRODUCTION

Now a days the heterocyclic compounds and their derivatives are receiving considerable attention in the drug discovery field and thus been used by the industries to develop bioactive molecules. Among them triazoles paid significant contribution in the field of medicinal chemistry [1]. These triazoles exhibit variety of biological activity such as antituberculosis [2], antimalarial [3], antiepileptic [4], antiallergic [5], anticancer [6], anti-HIV [7], antioxidant, antifungal, antibacterial [8] etc.

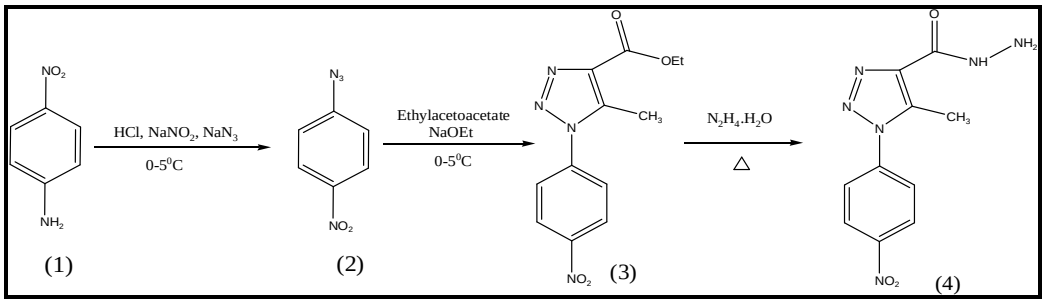
Pyrazoline is biologically accepted molecule in medicinal chemistry. Owing to the interesting activity and ease of synthesis, tremendous research has been done on the synthesis of this ring system [4]. Chalcones are one of the important precursors for the synthesis of pyrazolines [9]. The presence of triazole and pyrazoline nucleus in different organic structures leads to potent biological activities such as antimicrobial [10], anti-inflammatory [11], anti-cancer [12], antituberculosis [13], antiamoebic [14], antidiabetic, anti-oxidant [15], antiviral [16], antidepressant [17], anticonvulsant [18], antipyretic [19]. In addition to this, pyrazoline derivatives are also used in the field of dyestuffs, analytical reagents, agrochemicals [20]. In view of these observations and in continuation of our work on biologically active heterocycles [21, 22] we planned to incorporate these two bioactive entities into one compact structure and evaluated their biological potencies.

MATERIALS AND METHODS

The melting points of the newly synthesized compounds were recorded in open capillary tube and are uncorrected. Thin layer chromatography was used to monitor the progress of the reaction.The ^{1}H NMR spectra were recorded on a Bruker Avance- 400 MHz NMR spectrometer using $CDCl_3$ as solvent and TMS as internal standard.IR spectra were recorded in KBr pellet on a SHIMADZU FT-IR 157 spectrophotometer. Mass spectra were recorded on a Shimdzu LCMS-8030 Mass Spectrometer.

General procedure: The synthesis of novel triazole linked pyrazoline hybrid analogues were shown in Scheme IV.

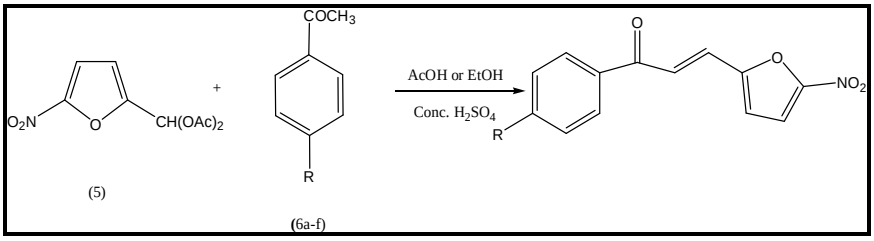
Synthesis of 5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide (4) : The ethyl-5-methyl-1-(*p*-nitrophenyl)-1*H*-1, 2, 3-triazole-4-carboxylate **(3)** was prepared by the reaction of 1-azido-4-nitrobenzene **(2)** with ethylacetoacetate in the presence of sodium ethoxide. The 1-azido-4-nitrobenzene was in turn obtained by the diazotization of p-nitroaniline **(1)** followed by the reaction with sodium azide. The 5-methyl-1-(p-nitrophenyl)-1H-1, 2, 3-triazole-4-carbohydrazide **(4)** was obtained in good yield by hydrazinolysis of ethyl-5-methyl-1-(*p*-nitrophenyl)-1*H*-1, 2, 3-triazole-4-carboxylate **(3)** [23].



Scheme I. Synthesis of 5-methyl-1-(p-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide.

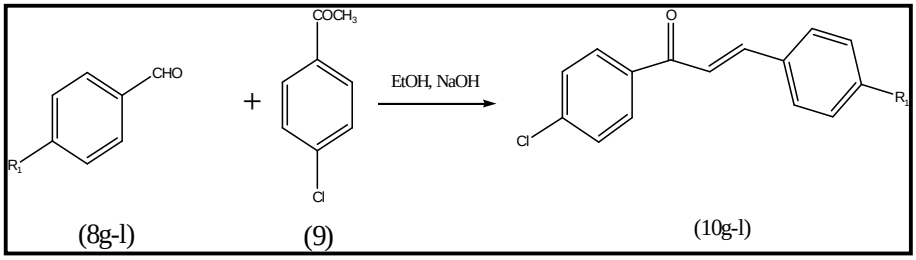
Synthesis of 1-aryl-3-(5-nitro-2-furyl)-2-propen-1-ones (7 a-f): A solution of 5-nitro-2-furfural diacetate (0.01 mol) **(5)** and an appropriate acetophenone (0.01) **(6a-f)** in glacial acetic acid (25 mL) was treated with 2 mL concentrated sulphuric acid. The mixture was allowed to stand at room temperature with stirring for 24 h. The precipitated crystals of propenones were collected by filtration, washed with ethanol and recrystallized from suitable solvents and characterized by reference to literature [24].

Synthesis of 1,3-Diaryl prop-2-en-1-ones (10 g-l): To a mixture of substituted acetophenone (0.01 mol) **(9)** and substituted aldehyde (0.01 mol) **(8)** in 25 mL ethanol,30% sodium hydroxide (5 mL) was added drop wise under ice bath and the mixture was stirred for 4 h. The solid obtained was filtered, washed thoroughly with water and recrystallized from ethanol-DMF solvent [25].



R=H, CH_3, OCH_3,Cl,Br, 2,4-Cl

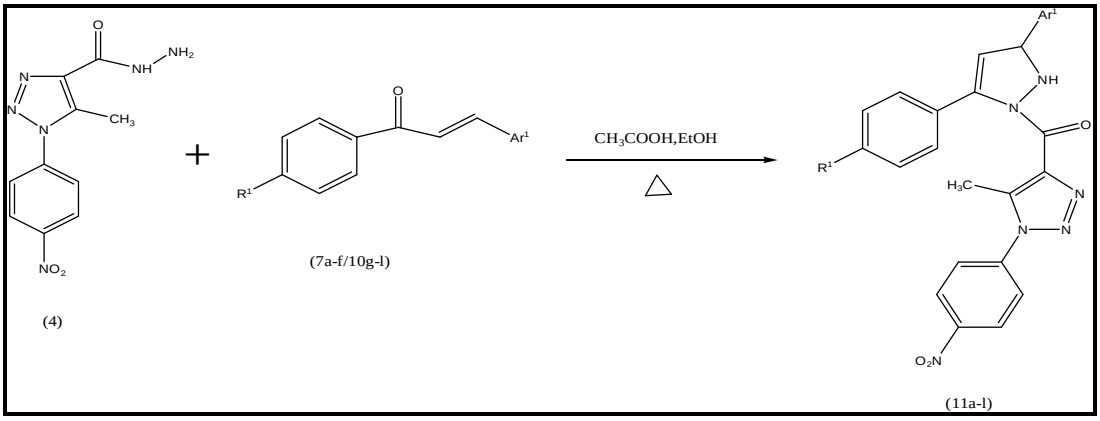
Scheme II. Synthesis of 1-aryl-3-(5-nitro-2-furyl)-2-propen-1-ones derivatives.



R_1= CH_3, OCH_3, H, Cl, 2,4-Cl, Br

Scheme III: Synthesis of 1, 3-Diaryl prop-2-en-1-ones.

Synthesis of novel triazoles-linked pyrazoline derivatives (11 a-l): To a solution of 1,2,3-triazole hydrazide (0.01 mol) **(4)** and substituted propenones (0.01 mol) **(7 a-f and 10 g-l)** in ethanol (25mL), 2mL glacial acetic acid was added drop wise and refluxed for 6 hour. The product which separated out was filtered, washed with water, dried and crystallized from ethanol [**6**].



Scheme IV. Synthesis of (5-methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl-(3-(5-nitrofuran-2-yl)-5-phenyl-2,3-dihydro-1*H*-pyrazol-1-yl)methanone derivatives.

Biological Activity

Antibacterial activity: The sterilized nutrient agar medium was distributed 100 mL each in two 250 mL conical flasks and allowed to cool to room temperature. To this media, 18-24 h grown bacterial sub-cultures were added and shaken thoroughly to ensure uniform distribution of organism's throughout the medium. Then, this agar medium was distributed in equal portions, in sterilized petridishes, ensuring that each petridish contains about 45-50 mL of the medium. The medium was then allowed for solidification. Then, cups were made with the help of a sterile cork borer (6 mm diameter) punching into the set of agar media. The solutions of required concentrations (50 µg mL^{-1})

Table 1. Characterization data of Pyrazoline derivatives **(11a-l)**

Compound No.	R^1	Ar^1	Yield (%)	M.P(^OC)	Molecular formula (Mol.Wt)
11a	H	5-nitro-2-furyl	77	204-206	$C_{23}H_{17}N_7O_6$ (487.43)
11 b	OCH_3	5-nitro-2-furyl	82	176-178	$C_{24}H_{19}N_7O_7$ (517.46)
11 c	CH_3	5-nitro-2-furyl	80	226-228	$C_{24}H_{19}N_7O_6$ (501.46)
11 d	Cl	5-nitro-2-furyl	61	234-236	$C_{23}H_{16}ClN_7O_6$ (521.87)
11 e	Br	5-nitro-2-furyl	75	216-218	$C_{23}H_{16}BrN_7O_6$ (566.33)
11 f	2,4-di-Cl	5-nitro-2-furyl	68	220-222	$C_{23}H_{15}Cl_2N_7O_6$ (556.32)
11 g	Cl	4-methylphenyl	72	190-192	$C_{26}H_{21}ClN_6O_3$ (500.94)
11 h	Cl	4-methoxyphenyl	70	200-202	$C_{26}H_{21}ClN_6O_4$ (516.94)
11 i	Cl	Phenyl	75	185-187	$C_{25}H_{19}ClN_6O_3$ (486.92)
11 j	Cl	4-chlorophenyl	66	165-67	$C_{25}H_{18}Cl_2N_6O_3$ (521.36)
11 k	Cl	2,5-dimethoxyphenyl	70	202-204	$C_{27}H_{23}ClN_6O_5$ (546.97)
11 l	Cl	4-bormophenyl	61	210-212	$C_{25}H_{18}BrClN_6O_3$ (565.81)

of test compounds were prepared by dissolving the compounds in DMSO and were filled into the cups with 1mL of respective solution. Then, the petridishes were kept for incubation in an inverted position for 24-48 h at 37° C in an incubator [26]. When growth inhibition zones were developed surrounding each cup, their diameter in mm was measured and compared with that of the standard drugs.

Antifungal activity: The compounds were dissolved in DMSO and antifungal activity was determined by well plate method at concentration of 50 µg mL^{-1}. The required amounts of each fungal strain were taken from the stock and suspended in 5ml of distilled water with 2 drops of tween 80. This suspension was uniformly spread on petri plates containing Potato dextrose agar media using sterile swabs. After applying samples into the wells, the plates were incubated at 25°C for 7 days [26]. The plates were then examined for the presence of zone of inhibition and the results were recorded. Ketoconazole was used as a positive control at a concentration of 50 µg mL^{-1}.

Antioxidant activity: Nitric oxide (NO) radical are generated from sodium nitropruside solution at physiological pH. Sodium nitropruside (1 mL of 10 mM) is mixed with 1ml of test compounds at different concentration (10-50 µg mL^{-1}) in phosphate buffer (pH 7.4). The mixture is incubated at 25°C for 150 min. To 1 ml of the incubated solution, 1 mL of Griess's reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) is added [27]. Absorbance is read at 546 nm. Ascorbic acid was used as standard.

$$\text{NO radical scavenging activity (\%)} = \frac{\mathbf{Abs_{control} - Abs_{sample}}}{\mathbf{Abs_{control}}} \times \mathbf{100}$$

RESULTS AND DISCUSSION

A series of substituted pyrazoline derivatives were synthesized according to the steps depicted in figure 1. The characterization data of the newly synthesized compounds are given in table 1. The structural elucidation of the compounds is achieved by FT-IR, Mass and ^{1}H- NMR spectral data. As a representive example the spectral data of compound **11a** is described here. Methyl protons of the

pyrazoline appeared as singlet at δ 2.67 ppm. The pyrazoline protons which are magnetically non-equivalent resonated as two doublets in the range of δ, 6.38-6.34(*J*=16.32Hz) and δ, 7.50-7.46 (*J*=16.28Hz). Protons from the furan ring appeared as a doublet at δ 7.03, *J*=3.88 and δ 7.61, *J*=3.92 Hz. N-H protons of pyrazoline ring resonated at δ 10.12 ppm as a broad singlet. In the IR spectra the stretching band at 3311 cm^{-1} clearly shows the presence of pyrazoline N-H. Aromatic C-H and C=C stretching frequency are observed at 3115 cm^{-1} and 1481 cm^{-1} respectively. A strong absorption band at 1693 cm^{-1} clearly indicates presence of carbonyl group. Asymmetric and symmetric stretching bands of nitro group are observed at 1571 $cm^{-1}$and 1386 cm^{-1} respectively. The mass spectrum of the compound showed molecular ion peak at m/z 488.01 consistence with the molecular formula $C_{23}H_{17}N_7O_6$.

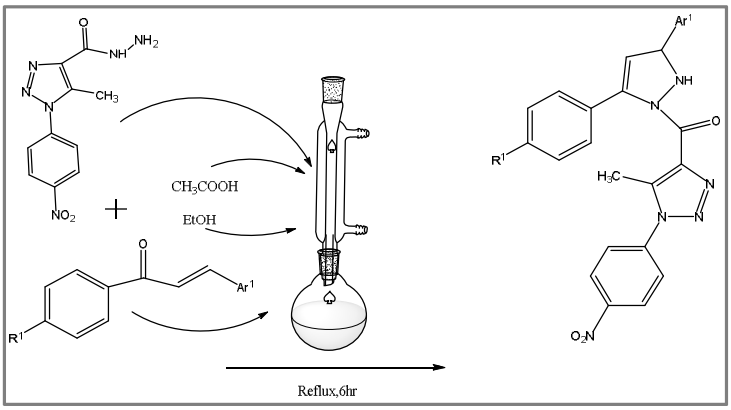


Figure 1. Synthesis of substituted pyrazoline derivatives.

Analytical and Spectral Data of synthesized compounds

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)(3-(5-nitrofuran-2-yl)- 5-(4-methoxyphenyl) -2,3-dihydro-1*H*-pyrazol-1-yl) methanone (11b): Pale yellow solid; Yield: 82%, m.p.: 176-178°C; IR(KBr,cm^{-1}): 3307 (N-H), 3099 (C-H), 1693 (C=O), 1571, 1386 (NO_2), 1487(C=C), 686(C-Br); ^{1}H NMR(400MHz,$CDCl_3$) δ: 2.76 (s, 3H, CH_3), 3.92(s, 3H, OCH_3), 6.34 (d,1H,16.28 Hz, pyrazoline -H), 6.59 (d,1H,3.8 Hz ,furan-3H), 7.14(d, 2H, 6.84 Hz, Ar-H), 7.27 (d,2H, 8.88 Hz, Ar-H), 7.31(d, 1H, 3.8Hz, furan-4H), 7.58 (d, 1H, 16.28 Hz,pyrazoline -H), 7.7(d, 2H, 7.0Hz, Ar-H), 8.46 (d, 2H, 6.92Hz, Ar-H), 10.25(s, 1H, NH); MS:m/z =518.20 (M^++1).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)(3-(5-nitrofuran-2-yl)-5-(*p*-tolyl)-2,3-dihydro-1*H*-pyrazol-1-yl)methanone (11c) : Light yellow solid; Yield: 80%, m.p.: 226-228°C; IR(KBr,cm^{-1}) :3307 (N-H), 3100(C-H), 1691(C=O), 1581 and 1390(NO_2), 1487(C=C), 540(C-Cl); ^{1}H NMR (400MHz,$CDCl_3$) δ: 2.49(s, 3H, CH_3), 2.76(s, 3H, CH_3), 6.32 (d,1H,16.28 Hz, pyrazoline -H), 6.58 (d,1H,3.8 Hz ,furan-3H), 7.22(d, 2H, 8.0 Hz, Ar-H), 7.32(d, 1H, 3.84Hz, furan-4H), 7.44 (d,2H, 7.88 Hz, Ar-H), 7.58 (d, 1H, 16.32 Hz, pyrazoline -H), 7.69(d, 2H, 7.0Hz, Ar-H), 8.46 (d, 2H, 6.96 Hz, Ar-H), 10.22(s, 1H, NH); MS:m/z =502.14 (M^++1).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) (3-(5-nitrofuran-2-yl)-5-(4-chlorophenyl) -2,3-dihydro-1*H*-pyrazol-1-yl)methanone (11d): Yellow green solid;Yield: 61%, m.p.: 234-246°C ; IR(KBr,cm^{-1}): 3315 (N-H), 3110 (C-H), 1695 (C=O), 1523,and 1321 (NO_2), 1489(C=C), 812(C-Cl)); ^{1}H NMR(400MHz,$CDCl_3$) δ: 2.77 (s, 3H, CH_3), 6.28 (d,1H,16.36 Hz, pyrazoline -H), 6.58 (d,1H,3.76 Hz ,furan-3H), 7.2(d, 2H, 7.27 Hz, Ar-H), 7.3(d, 1H, 3.52Hz, furan-4H), 7.58 (d, 1H, 16.32 Hz, pyrazoline -H), 7.64 (d,2H, 8.32 Hz, Ar-H), 7.69 (d,2H, 8.88 Hz, Ar-H), 8.46 (d,2H, 8.92 Hz, Ar-H), 10.13(s, 1H, NH); MS:m/z =522.9 (M^++1), 524.98 (M^++3).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)(3-(5-nitrofuran-2-yl)- 5-(4-bromophenyl)-2,3-dihydro-1*H*-pyrazol-1-yl)methanone (11e): Light brown solid; Yield:75%,m.p.: 216-218°C ; IR(KBr,cm^{-1}): 3321(N-H), 3125 (C-H), 1697(C=O), 1490(C=C), 1581and 1392 (NO_2), 569(C-Br); ^{1}H NMR(400MHz,$CDCl_3$) δ: 2.56 (s, 3H, CH_3), 6.26 (d,1H,16.36 Hz, pyrazoline-H), 6.58 (d,1H,3.72 Hz ,furan-3H), 7.22(d, 2H, 7.27 Hz, Ar-H), 7.36(d, 1H, 3.52Hz, furan-4H), 7.6 (d, 1H, 16.32 Hz, pyrazoline-H), 7.64 (d,2H, 8.34 Hz, Ar-H), 7.7 (d,2H, 8.86 Hz, Ar-H), 8.46 (d,2H, 8.91 Hz, Ar-H), 9.92(s, 1H, NH); MS:m/z =567.03 (M^++1), 569.4 (M^++3).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) (3-(5-nitrofuran-2-yl) -5-(3,5-dichlorophenyl) -2,3-dihydro-1*H*-pyrazol-1-yl)methanone(11f): Yellow solid; Yield:68%,m.p.:220-222°C; IR (KBr,cm^{-1}): 3277(N-H), 3111 (C-H), 1681(C=O), 1469(C=C), 1570,and 1380 (NO_2), 850 (C-Cl); ^{1}H NMR(400MHz,$CDCl_3$) δ: 2.76 (s, 3H, CH_3), 6.56 (d,1H,16.34 Hz, pyrazoline -H), 6.58 (d,1H,3.72 Hz ,furan-3H), 7.28(d, 2H, 7.27 Hz, Ar-H), 7.36(d, 1H, 3.52Hz, furan-4H), 6.92 (d, 1H, 16.33 Hz, pyrazoline -H), 7.63 (d,2H, 8.35 Hz, Ar-H), 7.7 (d,2H, 8.56 Hz, Ar-H), 8.2 (s,1H, 8.4 Hz, Ar-H) 10.13(s, 1H, NH); MS:m/z =557.05 (M^++1), 559 (M^++3), 561(M^++5).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl) (3-(p-tolyl)- 5-(4-chlorophenyl)-2,3-dihydro-1*H* pyrazol-1-yl)methanone (11g): Light yellow solid; Yield:72%, m.p.:190-192°C ; IR(KBr,cm^{-1}): 3313(N-H), 3105 (C-H), 1690(C=O), 1462(C=C), 1530,and 1324 (NO_2), 827(C-Cl); ^{1}H NMR (400MHz,$CDCl_3$) δ: 2.3 (s,3H,CH_3),2.62(s,3H,CH_3), 6.5 (d,1H,16.3 Hz, pyrazoline -H), 7.5 (d, 1H, 16.3 Hz, pyrazoline -H), 7.25(d,2H,8Hz,Ar-H), 7.32(d, 2H,8Hz,Ar-H),7.61 (d, 2H, 8.33 Hz, Ar-H), 7.68 (d, 2H, 8.55 Hz, Ar-H),8.1(d, 2H,8.5Hz,Ar-H) , 8.38(d, 2H,8.5 Hz, Ar-H) ,10.17(s, 1H, NH) ; MS:m/z =502.06 (M^++1), 504 (M^++3).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)(3-(4-methoxyphenyl)- 5-(4-chlorophenyl)-2,3-dihydro-1*H* pyrazol-1-yl)methanone (11h): Light brown solid;Yield:75%,m.p.:200-202°C; IR(KBr,cm^{-1}): 3309(N-H), 3100 (C-H), 1694(C=O), 1469(C=C), 1523,and 1321 (NO_2), 850(C-Cl); ^{1}H NMR(400MHz,$CDCl_3$) δ: 2.45(s, 3H, CH_3), 3.02(s, 3H, OCH_3), 6.52 (d,1H,16.23 Hz, pyrazoline -H), 7.51 (d, 1H, 16.31 Hz, pyrazoline -H), 7.26(d,2H,7.8Hz,Ar-H), 7.35(d,2H,7.8Hz,Ar-H),7.45(d, 2H, 8 Hz, Ar-H), 7.6 (d,2H, 8 Hz, Ar-H), 8.21(d, 2H, 8.2Hz, Ar-H), 8.46 (d, 2H, 8.5 Hz, Ar-H), 10.20(s, 1H, NH) ; MS:m/z =518.18 (M^++1), 520 (M^++3).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)(3-phenyl-5-(4-chlorophenyl)-2,3-dihydro-1*H* pyrazol-1-yl) methanone (11i): Light brown solid;Yield:72%,m.p.: 185-187°C ; IR(KBr,cm^{-1}): 3319(N-H), 3110 (C-H), 1681(C=O), 1473(C=C), 1532,and 1335 (NO_2), 816 (C-Cl); ^{1}H NMR (400MHz,$CDCl_3$) δ: 2.74 (s, 3H, CH_3), 6.4(d,1H,16.36 Hz, pyrazoline -H), 7.0 (d, 1H, 16.22 Hz, pyrazoline -H),7.3(d,2H, 8Hz , Ar-H),7.38(d,2H, 8Hz , Ar-H), 7.45-7.5(m,5H,Ar-H), 7.7(d, 2H, 8.5 Hz, Ar-H), 8 (d,2H, 8.5 Hz, Ar-H), 10.15(s, 1H, NH); MS:m/z =488.01 (M^++1), 490.05(M^++3).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)(3,5-bis(4-chlorophenyl)-2,3-dihydro-1*H* pyrazol-1-yl)methanone (11j): Yellow brown solid;Yield:77%,m.p.: 165-167°C ; IR(KBr,cm^{-1}): 3305(N-H), 3113 (C-H), 1676(C=O), 1483(C=C), 1516,and 1324 (NO_2), 820(C-Cl); ^{1}H NMR (400MHz,$CDCl_3$) δ: 2.54(s, 3H, CH_3), 6.5 (d,1H,16.36 Hz, pyrazoline -H), 7.1 (d, 1H, 16.32 Hz, pyrazoline -H), 7.23-7.41(m,8H,Ar-H) 7.7(d, 2H, 8.5 Hz, Ar-H), 8.0 (d,2H, 8.5Hz, Ar-H), 10.16(s, 1H, NH); MS:m/z =522.18 (M^++1), 524.2(M^++3), 526(M^++5).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)(3-(2,5-dimethoxyphenyl)- 5-(4-chlorophenyl)-2,3-dihydro-1*H* pyrazol-1-yl)methanone (11k): Yellow brown solid;Yield:75%,m.p.: 202-204°C ; IR(KBr,cm^{-1}): 3312(N-H), 3116 (C-H), 1685(C=O), 1483(C=C), 1571,and 1380 (NO_2), 806 (C-Cl); ^{1}H NMR(400MHz,$CDCl_3$) δ: 2.54(s, 6H, CH_3), 3.3-3.5(m, 6H, OCH_3), 6.52 (d,1H,16.32 Hz, pyrazoline -H), 6.9 (d, 1H, 16.32 Hz, pyrazoline -H),7.35(d,2H, 8Hz , Ar-H), 7.44(d,2H, 8Hz, Ar-H), 7.5-7.63(m,3H,Ar-H), 7.78(d, 2H, 8.5 Hz, Ar-H), 8.02 (d,2H, 8.5 Hz, Ar-H), 10.16(s, 1H, NH); MS:m/z =548.11 (M^++1), 550.2(M^++3).

(5-Methyl-1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl)(3-(4-bromophenyl)-5-(4-chlorophenyl)-2,3-dihydro-1*H* pyrazol-1-yl)methanone (11l): Light yellow brown solid; Yield:76%,m.p.: 210-212°C ; IR(KBr,cm^{-1}): 3310(N-H), 3115 (C-H), 1680(C=O), 1480(C=C), 1570 and 1380 (NO_2), 815 (C-C), 558(C-Br) ; ^{1}H NMR(400MHz,$CDCl_3$) δ: 2.54 (s, 3H, CH_3), 6.56 (d,1H,16.34 Hz, pyrazoline -H), 6.92 (d, 1H, 16.33 Hz, pyrazoline -H), 7.24-7.40 (m,8H,Ar-H), 7.8(d, 2H, 8.5Hz, Ar-H), 8 (d,2H, 8.5 Hz, Ar-H), 10.20(s, 1H, NH); MS:m/z =566.93 (M^++1).

APPLICATION

Antibacterial activity: The newly synthesized compounds were screened for their antibacterial activity against four bacterial strains namely *Staphylococcus aureus* (NCIM-2127), *Pseudomonas aeruginosa* (NCIM-2242), *Eschericia coli* (NCIM-2065), *Bascillus subtilis* (NCIM-2117) and *Xanthomonas campestris* (NCIM -5028) by cup-plate method at 50µg/mL concentration. Among the tested compounds **11a, 11b** showed prominent bacterial inhibition against *P. aeruginosa* and *X. campestris*. All other compounds showed moderate bacterial inhibition. The results are shown in table 2.

Table 2. Antibacterial activity of compounds (11a-l)

Sample code	Zone of Inhibition (mm) at 50 µg mL^{-1}				
	Gram negative bacteria			Gram positive bacteria	
	P.aeruginosa	*E.coli*	*X.campestris*	*B.subtilis*	*S.aureus*
11 a	12±.0.6	05±.0.12	04±.0.10	05±.0.29	05±.0.37
11 b	07±.0.28	06±.0.11	07±.0.09	04±.0.27	04±.0.21
11 c	05±.0.11	03±.0.17	03±.0.17	03±.0.14	03±.0.22
11 d	03±.0.14	05±.0.16	03±.0.24	03±.0.13	--
11 e	--		04±.0.34	--	03±.0.19
11 f	--		03±.0.44	--	--
11 g	--		--	--	--
11 h	--		--	--	--
11 i	--		--	07±.0.28	03±.0.08
11 j	--	03±.0.24	05±.0.54	--	02±.0.18
11 k	---		04±.0.11	--	03±.0.24
11 l	--		05±.0.21	03±.0.36	03±.0.28
Std (Ciprofloxacin)	**18±0.23**	**18±0.33**	**17±.0.13**	**17±0.58**	**18±0.45**

Antifungal activity: The newly synthesized compounds were also screened for their antifungal activity against *Aspergillus flavus*. Inhibitions obtained for the test compounds are tabulated in table 3**.** Results show that the compound **11g** showed moderate activity.

Table 3. Antifungal activity of compounds (11a-l)

Zone of Inhibition (mm) at 50µg mL^{-1}	
Compound No.	*A.flavus*
11 a	07.83
11 b	08.10
11 c	05.13
11 d	08.96
11 e	04.26
11 f	06.73
11 g	10.43
11 h	06.40
11 i	05.50
11 j	04.53
11 k	03.73
11 l	05.06
Std (Ketoconazole)	21.3

Antioxidant activity: The results are obtained from Nitric oxide radical scavenging assay is presented in table 4. Compounds **11 g, 11 h, 11 i, 11 j, 11 l** showed excellent radical inhibition activity as compared with Ascorbic acid.

Table 4. Antioxidant activity of compounds (11a-l)

Sample code	10µg	20 µg	30 µg	40 µg	50 µg	IC_{50}
11 a	21.32	30.15	42.15	50.18	58.11	39.71
11 b	24.52	30.25	38.24	45.24	50.24	45.25
11 c	27.25	35.24	42.12	48.22	54.11	40.96
11 d	21.21	27.54	35.24	42.21	48.21	48.5
11 e	20.11	26.24	34.11	41.18	48.11	49.39
11 f	18.24	25.11	31.21	37.41	44.12	54.54
11 g	27.25	37.25	48.25	59.24	66.35	33.2
11 h	29.65	39.88	47.57	55.58	68.35	33.06
11 i	28.35	39.54	48.27	62.31	67.54	32.03
11 j	27.85	40.21	52.36	61.28	74.18	30.33
11 k	22.21	30.11	37.42	44.21	52.11	44.91
11 l	29.36	38.59	47.25	56.24	66.24	33.65
Std (ascorbic acid)	35.85	43.25	49.57	54.58	66.25	32.49

CONCLUSION

In the present paper a new series of pyrazoline derivatives were synthesized and evaluated for their antibacterial, antifungal, antioxidant activity. Among this few compounds showed good antioxidant activity and moderate antimicrobial activity.

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