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Synthesis of Some Novel Pyrazole Derivatives and their Antibacterial and Antifungal Activity

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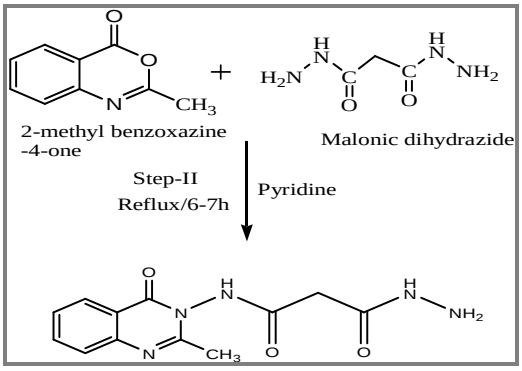
ABSTRACT

The series of synthesized compounds and substituted pyrazoles analogs are 2-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-N-(2-methyl-4-oxoquinazolin-3(4H)-yl)-2-oxoacetamide. The newly synthesized compounds are evaluated as anti-bacterial and anti-fungal activity. The antibacterial activity are shown the highest activity is 3k with reference as streptomycin. The antifungal activities are shown the highest activity is 3j with reference as Griesofulvin.

Graphical Abstract



Synthesis of Quinazoline malonic dihydrazide.

Keywords: Pyrazoline, Quinazoline, Anti-bacterial, Anti-fungal activity.

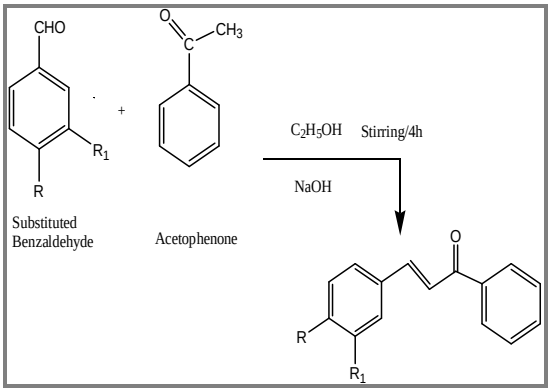
INTRODUCTION

Heterocyclic compounds have a wide range of medicinal application, the true utility of heterocyclic scaffolds is the ability to binds a variety of different receptors, it has several activities of pharmacological compounds. The fusion of several rings resulting in polycyclic structures it has interest to both organic and medicinal chemist. Hence, a practical method for the preparation of such compounds is of great interest in synthetic organic chemistry [**1**]. Pyrazoline and its derivatives, a class of well known nitrogen containing heterocyclic compounds, occupy an important position in medicinal and pesticide chemistry with having a wide range of bioactivities [**2**]. Pyrazole heterocyclic compounds represent important building blocks in organic and medicinal Chemistry [**3**]. Many

significant research activities were carried out towards this structure [4]. The heterocyclic pyrazole and its derivatives shows remarkable biological and pharmacological activities as antibacterial and anti-oxidant [2] antitumor [5, 7], anti-angiogenic [6] antimicrobial [8, 9], ACE-inhibitor [10], antioxidant [11] and soon.

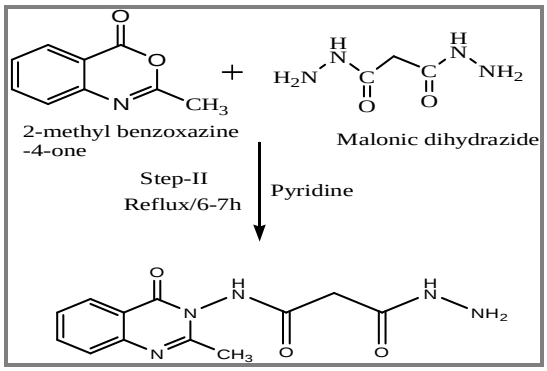
MATERIALS AND METHODS

Step I Synthesis of Chalcone: Condense acetophenone (0.01 mol) and different substituted benzaldehydes (0.01 mol) in ethanol (20 mL). Stir at room temperature by adding aqueous KOH (40% 10 mL) and keep over night in a bulboven at room temperature. After 14-16 h, the reaction mixture was filtered and crystallized from Ethanol to give corresponding chalcone derivatives (Scheme 1).



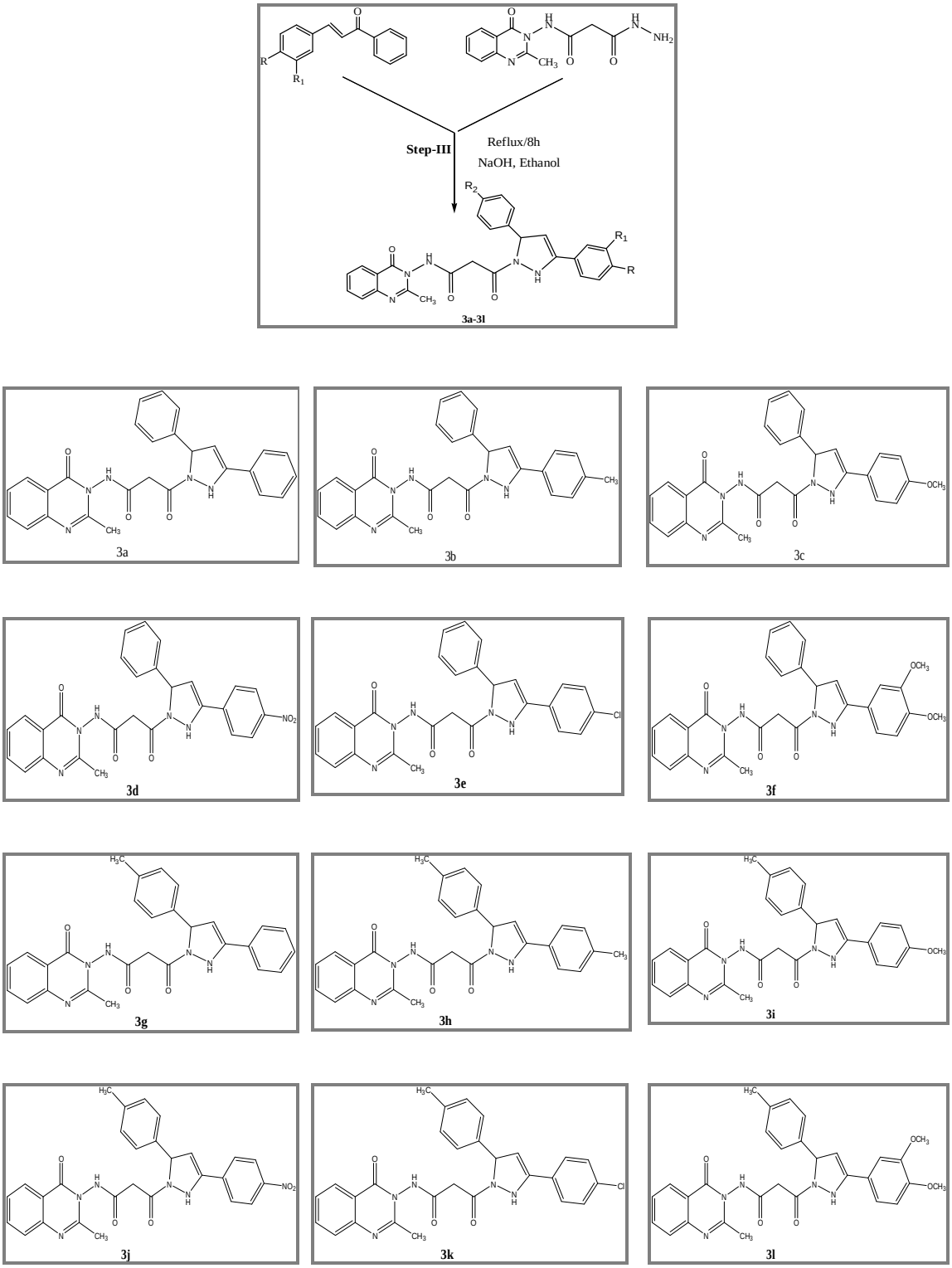
Scheme 1. Synthesis of Chalcone.

Step II Synthesis of Quinazoline malonic dihydrazide: A mixture of equimolar quantities of 2-methyl benzoxazine-4-one (0.01 mol) and Malonic dihydrazide (0.01 mol) was refluxed in pyridine for 7-8 h. One KOH pellet was added to this mixture. The progress of the reaction was monitored by TLC. After completion of the reaction resulting mass was poured into crushed ice and neutralized with dil. HCl. Precipitate was filtered, dried and the product was recrystallized form methanol or Ethanol (Scheme 2).



Scheme 2. Synthesis of Quinazoline malonic dihydrazide.

Step III Synthesis of Pyrazole derivatives (3a-3l): Pyrazole derivatives (3a-3l) was prepared by a mixture of Chalcone (0.01 mol) and malonic dihydrozido quinazolinone (0.01 mol) in ethanol (30 mL) aqueous KOH (40% 10 mL) then refluxed were refluxed for 8h on a water bath. The reaction mixture was concentrated, cooled and poured into ice-cold water. The resulting solid was filtered, dried and recrystallized from ethanol (Scheme 3).



Scheme 3. Synthesis of Pyrazole derivatives (3a-3l).

RESULTS AND DISCUSSION

Synthesis: The synthesis of the target molecule (3a-l)2-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-N-(2-methyl-4-oxoquinazolin-3(4H)-yl)-2-oxoacetamide were synthesized and it has been

characterized by FTIR, H^1,^{13}C-NMR and Mass spectral analysis. The yield of the synthesized compound was found to be in the range from70-87%.

3a: IR Cm^{-1} (KBr): 3412(-NH *Str*,pyrazole),3375(-NH *Str*), 3132(-CH *Str*, benzene), 2987(-CH $_3$*Str*, aliphatic),2892(-CH *Str*, aliphatic),1720 (C=O *Str*), 1705 (C=O *Str*), 1556(C=N *Str*), 12967(C-N *Str*).**^{1}H-NMR (DMSO δ ppm):**12.0 (1H, -NH in pyrazole),11.32(1H, -NH, amide), 8.4- 6.90(13H, Ar-H), 6.34 (1H, -CH in pyrole), 3.20 (1H, -CH_2 in diketone Alkyl), 2.43(3H, -CH_3 , Quinazolin). **Mass (EI-MS)**: 466(M+1, 100%).

3b: IR Cm^{-1} (KBr): 3450 (-NH *Str*,pyrazole),3303(-NH *Str*), 3103(-CH *Str*, benzene), 2956(-CH *Str*, aliphatic),1696 (C=O *Str*), 1671 (C=O *Str*), 1586(C=N *Str*), 1255(C-N *Str*) (Figure 1) **^{1}H-NMR (**DMSO δ ppm): 12.6 (1H, -NH, pyrazole),11.2(1H, -NH, amide), 8.1- 6.8(13H, Ar-H), 6.84 (1H, -CH in pyrole), 3.41 (1H, -CH_2 in diketone Alkyl), 2.32 (3H, -CH_3 , Quinazolin). 1.91 (3H, -CH_3 , Phenyl).)(Figure 2). **Mass (EI-MS)**: 478(M+1, 100%) (Figure 3).

3c: IR Cm^{-1} (KBr): 3398 (-NH *Str*,pyrazole),3353(-NH *Str*), 3143(-CH *Str*, benzene), 2983(-CH_3 *Str*, aliphatic), 2897(-CH *Str*, aliphatic),1714 (C=O *Str*), 1708 (C=O *Str*), 1542(C=N *Str*), 1282(C-N *Str*),1246(C-O-C*Str*-OCH_3). **^{1}H-NMR (**DMSO δ ppm): 11.89 (1H, -NH, pyrazole),10.22(1H, -NH, amide), 8.0- 6.98(13H, Ar-H), 6.56 (1H, -CH in pyrole), 3.41 (1H, -CH_2 in diketone Alkyl), 2.40 (3H, -CH_3 , Quinazolin). 2.32 (3H, -OCH_3 , Phenyl).**Mass (EI-MS)**: 496(M+1, 100%).

3d: IR Cm^{-1} (KBr): 3398 (-NH *Str*,pyrazole),3364(-NH *Str*), 3132(-CH *Str*, benzene), 2943(-CH $_3$*Str*, aliphatic),2899(-CH *Str*, aliphatic),1715 (C=O *Str*), 1709 (C=O *Str*), 1589(-NO_2 *Str*), 1556(C=N *Str*), 1289(C-N *Str*), **^{1}H-NMR (**DMSO δ ppm): 12.03 (1H, -NH, pyrazole),11.31(1H, -NH, amide), 8.43- 6.89(13H, Ar-H), 6.67(1H, -CH in pyrole), 3.01 (1H, -CH_2 in diketone Alkyl), 2.09 (3H, -CH_3 , Quinazolin). **Mass (EI-MS)**: 511(M+1, 100%).

3e: IR Cm^{-1} (KBr): 3410(-NH *Str*,pyrazole),3386(-NH *Str*), 3143(-CH *Str*, benzene), 2965(-CH $_3$*Str*, aliphatic),2889(-CH *Str*, aliphatic),1708(C=O *Str*),1696 (C=O *Str*), 1576(C=N *Str*), 1267(C-N *Str*),897(C-Cl *Str*). **^{1}H-NMR (DMSO δ ppm):**12.01(1H, -NH, pyrazole),11.24(1H, -NH, amide), 8.23- 6.24(13H, Ar-H), 6.24(1H, -CH in pyrole), 3.20 (1H, -CH_2 in diketone Alkyl), 2.32 (3H, -CH_3 , Quinazolin). **Mass (EI-MS)**: 500(M+1, 100%).

3f: IR Cm^{-1} (KBr): 3402 (-NH *Str*,pyrazole),3334(-NH *Str*), 3165(-CH *Str*, benzene), 2956(-CH $_3$*Str*, aliphatic),2887(-CH *Str*, aliphatic),1712 (C=O *Str*), 1704 (C=O *Str*), 1534(C=N *Str*), 1290(C-N *Str*),1254(C-O-C*Str*-OCH_3). **^{1}H-NMR (DMSO δ ppm):** 12.24(1H, -NH, pyrazole),11.42(1H, -NH, amide), 8.00- 6.65(13H, Ar-H), 6.24 (1H, -CH in pyrole), 3.02 (1H, -CH_2 in diketone Alkyl), 2.02(3H, -CH_3 , Quinazolin). 2.00 (6H, -OCH_3 , Phenyl).**Mass (EI-MS)**: 526(M+1, 100%).

3g: IR Cm^{-1} (KBr): 3379(-NH *Str*,pyrazole),3346(-NH *Str*), 3123(-CH *Str*, benzene), 2976(-CH $_3$*Str*, aliphatic),2854(-CH *Str*, aliphatic),1698 (C=O *Str*), 1692 (C=O *Str*), 1534(C=N *Str*), 1245(C-N *Str*).**^{1}H-NMR (DMSO δ ppm):**11.23(1H, -NH, pyrazole),10.24(1H, -NH, amide), 8.24-6.34(13H, Ar-H), 6.54 (1H, -CH in pyrole), 3.65(1H, -CH_2 in diketone Alkyl), 2.43 (3H, -CH_3 , Quinazolin), 1.68(3H, -CH_3 , Phenyl). **Mass (EI-MS)**: 478(M+1, 100%).

3h: IR Cm^{-1} (KBr): 3380 (-NH *Str*,pyrazole),3345(-NH *Str*), 3103(-CH *Str*, benzene), 2956(-CH $_3$*Str*, aliphatic), 2876(-CH *Str*, aliphatic),1721 (C=O *Str*), 1712 (C=O *Str*), 1566(C=N *Str*), 1234(C-N *Str*). **^{1}H-NMR (DMSO δ ppm):**12.24 (1H, -NH, pyrazole),11.34(1H, -NH, amide), 8.20-6.98(13H, Ar-H), 6.24 (1H, -CH in pyrole), 3.02 (1H, -CH_2 in diketone Alkyl), 2.04(3H, -CH_3 , Quinazolin), 1.84 (6H, -CH_3 , Phenyl). **Mass (EI-MS)**: 494(M+1, 100%).

3i: IR Cm^{-1} (KBr): 3423 (-NH *Str*,pyrazole),3389(-NH *Str*), 3143(-CH *Str*, benzene), 2923(-CH $_3$*Str*, aliphatic),2873(-CH *Str*, aliphatic),1712 (C=O *Str*), 1692 (C=O *Str*), 1568(C=N *Str*), 1292(C-N *Str*),1243(C-O-C*Str*-OCH_3). **^{1}H-NMR (DMSO δ ppm):**12.02(1H, -NH, pyrazole),11.08(1H, -NH,

amide), 8.01- 6.56(13H, Ar-H), 6.02 (1H, -CH in pyrole), 3.03 (1H, $-CH_2$ in diketone Alkyl), 2.45 (3H, $-CH_3$, Quinazolin), 2.02 (3H, $-OCH_3$, Phenyl),1.84(3H, $-CH_3$, Phenyl). **Mass (EI-MS)**: 510(M+1, 100%).

3j: IR Cm^{-1} (KBr): 3380 (-NH *Str*,pyrazole),3345(-NH *Str*), 3134(-CH *Str*, benzene), 2928(-CH_3*Str*, aliphatic),2887(-CH *Str*, aliphatic),1712 (C=O *Str*), 1709 (C=O *Str*), 1584(-NO_2*Str*), 1553(C=N *Str*), 1290(C-N *Str*). **^{1}H-NMR (DMSO δ ppm):**12.012(1H, -NH, pyrazole),11.22(1H, -NH, amide), 8.13-6.78(13H, Ar-H), 6.32 (1H, -CH in pyrole), 3.24 (1H, $-CH_2$ in diketone Alkyl), 2.23 (3H, $-CH_3$, Quinazolin), 1.48(3H, $-CH_3$, Phenyl). **Mass (EI-MS)**: 530(M+1, 100%).

3k: IR Cm^{-1} (KBr): 3378(-NH *Str*,pyrazole),3332(-NH *Str*), 3102(-CH *Str*, benzene), 2926(-CH_3*Str*, aliphatic),2856(-CH *Str*, aliphatic),1717(C=O *Str*), 1701 (C=O *Str*), 1567(C=N *Str*), 1282(C-N *Str*),896(C-Cl*Str*).**^{1}H-NMR (DMSO δ ppm):**12.34(1H, -NH, pyrazole),11.21(1H, -NH, amide), 8.51- 6.78(13H, Ar-H), 6.23 (1H, -CH in pyrole), 3.13 (1H, $-CH_2$ in diketone Alkyl), 2.54(3H, $-CH_3$, Quinazolin),1.96(3H, $-CH_3$, Phenyl). **Mass (EI-MS)**: 514(M+1, 100%).

3l: IR Cm^{-1} (KBr): 3384 (-NH *Str*,pyrazole),3356(-NH *Str*), 3123(-CH *Str*, benzene), 2934(-CH_3*Str*, aliphatic),2893(-CH *Str*, aliphatic),1702 (C=O *Str*), 1698 (C=O *Str*), 1556(C=N *Str*), 1289(C-N *Str*), 1232(C-O-C*Str*-OCH_3). **^{1}H-NMR (DMSO δ ppm):**12.29(1H, -NH, pyrazole), 11.24(1H, -NH, amide), 8.24- 6.89(13H, Ar-H), 6.12 (1H, -CH in pyrole), 3.23 (1H, $-CH_2$ in diketone Alkyl),2.67 (6H, $-OCH_3$, Phenyl),2.56 (3H, $-CH_3$ Quinazolin), 1.76(3H, $-CH_3$ Phenyl). **Mass (EI-MS)**: 540(M+1, 100%).

Biological Evaluation: A volume of 25 mL of sterile hot agar medium was poured in each plate and allowed to harden on a level surface. The agar plates were inoculated with 24 h test cultures by spreading uniformly with sterile cotton swabs. The plates were then allowed to dry in the inverted position in an incubator for 30 min. After wards they were removed and bore were made on the medium using sterile borer. A volume of 0.1 mL of test solution was added to respective bores. Streptomycin at a concentration of 100 µg 0.1 mL^{-1} was taken as standard reference. A control having only DMSO in the cup was maintained in each plate. The petri dishes were kept in the refrigerator at 4°C for 15 min for diffusion to take place. After wards they were incubated at 37°C for 24 h and zones of inhibition were observed and measured using a scale. Each experiment was carried out in triplicate and the mean diameter of inhibition zone was recorded. The various antibacterial results of synthesized compounds are shown in the table 1.

Table 1. Antibacterial activity of Pyrazole derivatives by zone of inhibition

Micro organism	Zone of Inhibition (in mm)												
	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	3l	Streptomycin
L.bacillus	10	22	19	20	12	23	20	--	24	17	**26***	24	31
Pseudomonas	16	25	12	12	10	20	19	--	20	15	25	20	30
E.coli	--	17	10	24	24	10	--	22	19	22	--	10	34
P.vulgares	22	19	23	15	22	10	10	20	10	24	**27***	22	34

All values are expressed as Zone of Inhibition in mm, Bore size = 6mm;*Compounds showed maximum activity against respective bacteria, Zone size 9-11=Poor activity; Zone size 12-18=Moderate activity, Concentration of test compounds is 50 µg mL^{-1}.

Antifungal activity: The sterilized potato dextrose agar media was melted on water bath and transferred to sterile properly labelled petri dishes and then allowed for solidification. After these in incubator, then the discs were carefully kept on the periphery of the agar using sterile forceps and these petridishes were kept for 1 h for diffusion at room temperature .These plates were incubated at 28°C for 48 h, for antifungal activity. After 48 h was measured as the zone of inhibition in mm. DMSO was used as solvent control. Standard drug used for comparison is Griseofulvin

Table 6. Antifungal activity of Pyrazole derivatives by Zone of Inhibition

Microorganism	Zone of Inhibition (in mm)												
	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	3l	Gresiofulvin
A. nagram	-	14	19	-	18	19	12	-	20	10	23	22	28
P. notatum	10	16	14	22	24	13	-	-	22	**26***	10	-	32
C. coffeanum	09	-	12	19	23	-	-	09	18	**27***	29	22	36

All values are expressed as Zone of Inhibition in mm, Bore size = 6mm, *Compounds showed maximum activity against respective Fungal; Zone size 9-11=Poor activity; Zone size 12-18=Moderate activity, Concentration of test compounds is 50 µg mL^{-1}.

APPLICATION

In the present study the derivatives which we have synthesized were screened for their antibacterial and antifungal activity, which are promising as active pharmacophore. Further studies are undergoing to explore the scope of the various biological activities.

CONCLUSION

The objective of the present work was to synthesize, purify, characterize and evaluate the biological activity of newly synthesized structural analogs of Pyrazoles. The yield of the synthesized compound was found to be in the range from 70-87%. All these molecules were characterized by FTIR, $H^{1,}$ ^{13}C-NMR and Mass spectral analysis along with physical data.

All the synthesized compounds were evaluated for their antibacterial activity against various gram positive and gram negative bacterial strains by measuring zone of inhibition by agar diffusion method. Streptomycin was used as a standard drug. The synthesized compounds 2-(3-(p-chloro)-phenyl-5-((p-methyl) phenyl-4,5-dihydro-1H-pyrazol-1-yl)-N-(2-methyl-4-oxoquinazolin-3(4H)-yl)-2-oxo acetamide (3k) have shown highest activity. The synthesized compounds (3a-3l) were also screened for antifungal activity by taking Gresiofulvin as standard. The synthesized compounds 2-(3-(p-nitro)-phenyl-5-((p-methyl) phenyl-4,5-dihydro-1H-pyrazol-1-yl)-N-(2-methyl-4-oxoquinazolin-3(4H)-yl)-2-oxoacetamide (3j) have shown highest activity.

The present study highlights the importance of Pyrazole derivatives having various heterocyclic moiety features responsible for the antibacterial, antifungal, anthelmintic and antioxidant activities and may serve as a lead molecule for further modification to obtain clinically useful novel entities.

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