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Synthesis, characterization and biological activity of certain Pyrazole derivatives

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ABSTRACT

*Synthesis of a new series of [5-(1H-indol-3-yl)-3-phenyl-4, 5-dihydropyrazol-1-yl]pyridin-3-yl methanone derivatives (3a-3g) by the reaction of nicotinic acid hydrazide with various indolyl chalcones (2a-2g) has been described. The indolyl chalcones have been synthesized from indole-3-carboxaldehyde and different substituted acetophenones. The structures of these compounds are confirmed by physico-chemical as well as IR, ^{1}H NMR, ^{13}C NMR and Mass spectral means. The synthesized compounds are screened for their in-vitro antibacterial potentials against various strains of bacterial organisms. Minimum inhibitory concentration (MIC) and minimum bactericidal count (MBC) have been determined for the compound **3c,** which showed good anti bacterial activity.*

Key words: Pyrazole, Chalcones, Nicotinic Acid Hydrazide, Anti bacterial activity

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INTRODUCTION

Indole derivatives have been recently the subject of great interest due to their interesting pharmacological activities such as anticancer[1], antioxidant[2], antirheumatoidal and anti HIV[3]. Literature survey revealed that many pyrazole derivatives have a broad spectrum of biological activities including antibacterial[4], antifungal[5], antiviral[6], antioxidant[7], anti- inflammatory[8], cytotoxic[9], A3 adenosine receptor *antagonists*[10], anti hypertensive[11] and etc. Pyrazolines of nicotinic acid hydrazide have been studied owing to their wide pharmacological activities which include antiHIV activity[12], antimalarial[13], antimicrobial activity[14] and etc. In the present study certain new pyrazole derivatives have been synthesized by the reaction of indolyl chalcones with nicotinic acid hydrazide. The structures of the various synthesised compounds are assigned on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data.

EXPERIMENTAL SECTION

All the chemicals and reagents were purchased from Alfa Aesar and MERCK. Melting points of the synthesised compounds are determined in open capillaries and are uncorrected. TLC was used to monitor the reaction and to check the purity. IR spectra were recorded on a Schimatzu 8201 pc (4000-400cm^{-1}). The NMR spectra were recorded on BRUKER AVANCE III 500 MHz multi nuclei solution NMR spectrometer. The mass spectra (EI) were recorded on a JEOL GCMATE II GC-MS Spectrometer operating at 70 eV. Elemental analyses were performed for C, H and N and were found to be within ± 0.5% of the theoretical values.

2.1. General procedure for the synthesis of derivatives of (E)-3-(1H-indol-3-yl)-1-phenylprop-2-en-1-one ***(2a – 2g)***
A mixture of indole-3-carboxaldehyde **1** (0.01 mol) and various substituted acetophenones (0.01mol) was refluxed in the presence of methanolic NaOH for 6 to 25 hrs. The reaction mixture was poured into crushed ice and neutralized with dil. HCl. The solid obtained was filtered and recrystallised from ethanol to obtain pure chalcones.

The purity of the product was checked on TLC by using the mixture of toluene and ethyl acetate as mobile phase. (Scheme 1)

2.1.1.(E)-3-(1H-indol-3-yl)1-phenylprop-2-en-1-one **(2a)**
Prepared by the above method from 1(0.01mol) and simple acetophenone (0.01mol).
Dark Brown crystalline solid. M.Pt.:182 ± 2°C. IR(KBr, cm^{-1}): 3145(NH), 2924(Ar-C-H), 1639(C=O), 1595(-CH=CH); ^{1}H NMR (DMSO-d6, δ (ppm)): 7.22-8.13(m,10H,Ar), 7.66(d,1H,J=15Hz, Hα), 8.06(d,1H,J=15Hz,Hβ), 11.82(s,1H,NH)

2.1.2.(E)-1-(4-chlorophenyl)-3-(1H-indol-3-yl)prop-2-en-1-one **(2b)**
Yellow crystalline solid. M.Pt.: 184± 2°C. IR(KBr, cm^{-1}): 3217(NH), 2924(Ar-C-H), 1631 (C=O), 1581 (-CH=CH), 738; ^{1}H NMR (DMSO-d6, δ (ppm)): 7.24-8.10(m,9H,Ar), 7.52(d,1H, J=15Hz, Hα), 8.03(d,1H,J=15Hz, Hβ), 11.77(s,1H,NH)

2.1.3.(E)-1-(4-bromophenyl)-3-(1H-indol-3-yl)prop-2-en-1-one **(2c)**
Brown crystalline solid. M.Pt.: 123 ± 2°C. IR (KBr, cm^{-1}): 3215(NH), 2924(Ar-C-H), 1633 (C=O), 1581 (-CH=CH), 599; ^{1}H NMR (DMSO-d6, δ (ppm)): 7.23-8.04(m,9H,Ar), 7.58(d,1H,J=15Hz, Hα), 8.08(d,1H,J=15Hz, Hβ), 11.85(s,1H,NH)

2.1.4.(E)-3-(1H-indol-3-yl)-1-(4-methoxyphenyl)prop-2-en-1-one **(2d)**
Orange crystalline solid. M.Pt.: 169 ± 2°C. IR (KBr, cm^{-1}): 3190(NH), 2924(Ar-C-H), 1633 (C=O), 1585 (-CH=CH), 1173; ^{1}H NMR (DMSO-d6, δ (ppm)): 7.08-8.14(m,9H,Ar), 7.66(d,1H,J=15.6Hz, Hα), 8.03(d,1H ,J=15.6Hz, Hβ), 11.86(s,1H,NH), 3.86 (s,3H,OCH_3).

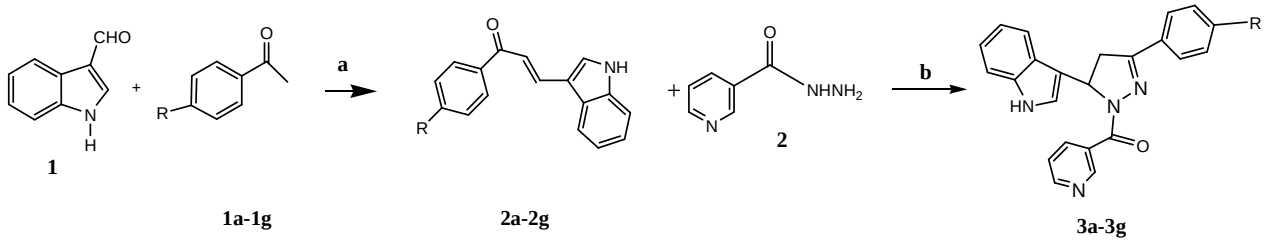
2.1.5.(E)-1-(4-hydroxyphenyl)-3-(1H-indol-3-yl)prop-2-en-1-one **(2e)**
Orange crystalline solid. M.Pt.: 147 ± 2°C. IR (KBr, cm^{-1}): 3430(OH), 3288(NH), 2927 (Ar-C-H), 1639 (C=O), 1556 (-CH=CH); ^{1}H NMR (DMSO-d6, δ (ppm)): 6.89-8.28 (m,9H,Ar), 7.64(d,1H,J=15Hz, Hα), 7.99(d,1H,J=15Hz, Hβ), 11.83(s,1H,NH),3.39(s,1H,OH).

2.1.6.(E)-3-(1H-indol-3-yl)-1-(4-nitrophenyl)prop-2-en-1-one **(2f)**
Red crystalline solid. M.Pt.: 210±2°C. IR (KBr, cm^{-1}): 3437(NH), 2924(Ar-C-H), 1653 (C=O), 1560 (-CH=CH), 1340; ^{1}H NMR (DMSO-d6, δ (ppm)): 7.22-8.37(m,9H,Ar), 7.51(d,1H,J=15Hz, Hα), 8.14(d,1H,J=15Hz, Hβ), 12.06(s,1H,NH)

2.1.7.(E)- 3-(1H-indol-3-yl)-1-(4-methylphenyl)prop-2-en-1-one **(2g)**
Yellow crystalline solid. M.Pt.: 174±2°C. IR (KBr, cm^{-1}): 3174(NH), 2924(Ar-C-H), 1600 (C=O), 1514(-CH=CH); 1H NMR (DMSO-d6, δ (ppm)): 7.22-8.10(m,9H,Ar), 7.64(d,1H,J=15.5Hz, Hα), 8.05(d,1H,J=15.5Hz, Hβ), 11.87(s,1H,NH), 2.40(s,3H, CH_3)

2.2. General procedure for the synthesis of derivatives of [5-(1H-indol-3-yl)-3-phenyl-4,5-dihydropyrazol-1-yl] pyridin-3-yl methanone*) **(3a-3g)***

To the solution of the appropriate chalcone (0.01mol) (**2a-2g**) in 30 ml ethanol, nicotinic acid hydrazide **2** (0.01mol) and catalytic amount of glacial acetic acid were added and the reaction mixture was refluxed for 11-36 hrs. The excess of solvent was removed under reduced pressure and the reaction mixture was poured into ice cold water. The product obtained was filtered, washed with water and recrystallised with suitable solvents. The purity of the product was checked on TLC by using mixture of acetone and petroleum ether as mobile phase.



Scheme -1. Synthesis of Pyrazole derivatives

R→	a	b	c	d	e	f	g
	H	Cl	Br	OCH_3	OH	NO_2	CH_3

Reagents and conditions: (a) Solid NaOH, MeOH, reflux 20h (b) Gl. AcOH, reflux 16h

2.2.1. [5-(1H-indol-3-yl)-3-phenyl-4, 5-dihydropyrazol-1-yl] pyridin-3-yl methanone ***(3a)***
IR (KBr, cm^{-1}): 3124(NH), 2912(Ar-C-H), 1641(C=O), 1591(C=N), 1097(C-N); ^{1}H NMR (500 MHz, DMSO-d6, δ (ppm)): 3.51(dd,1H, Ha, J=17.6Hz, 4.8Hz), 3.80 (dd,1H, Hb,J=17.6Hz, 12Hz), 6.14 (dd,1H, Hx, J=12Hz, 4.8Hz), 7.34-7.78(m, 11H, Ar), 8.25(d,1H, Py-H-6), 8.39 (s,1H, Ind-H-2), 8.67 (d,1H, Py-H-2), 9.25 (s,1H, NH); ^{13}C NMR, δ (ppm): 164.50(C=O), 156.18(C=N), 151.25(C-3), 137.56-125.79(Ar-C), 55.13(CH), 40.04(CH2); MS m/z=365.8 (M^+)

2.2.2. [3-(4- cholorophenyl) 5-(1H-indol-3-yl)-4, 5-dihydropyrazol-1-yl]pyridin-3-yl methanone ***(3b)***
IR (KBr, cm^{-1}): 3120(NH), 2962(Ar-C-H), 1645(C=O), 1593(C=N), 1093(C-N); ^{1}H NMR (500 MHz, DMSO-d6, δ (ppm)): 3.39(dd,1H, Ha, J=17.7Hz, 4.8Hz), 3.70(dd,1H, Hb,J=17.7Hz, 12Hz), 6.14(dd,1H, Hx, J=12Hz, 4.8Hz), 7.05-7.63(m,10H, Ar), 8.13(d,1H, Py-H-6), 8.59 (s,1H, Ind-H-2), 9.11 (d,1H, Py-H-2), 9.32 (s,1H, NH); ^{13}C NMR, δ (ppm): 162.40(C=O), 152.08(C=N), 146.45(C-3), 132.24-124.21(Ar-C), 52.13(CH), 24.89(CH2); MS m/z=400.7 (M^+)

2.2.3. [3-(4 bromophenyl)- 5-(1H-indol-3-yl) 4, 5-dihydropyrazol-1-yl]pyridin-3-yl methanone ***(3c)***
IR (KBr, cm^{-1}): 3120(NH), 2964(Ar-C-H), 1641(C=O), 1591(C=N),1099(C-N); ^{1}H NMR (500MHz, DMSO-d6, δ (ppm)): 3.48(dd,1H, Ha, J=17.6Hz, 5.2Hz), 3.77(dd,1H, Hb, J=17.6Hz, 12Hz), 6.14(dd,1H, Hx, J=12Hz, 5.2Hz), 7.03-7.64(m,10H, Ar), 8.21(d,1H, Py-H-6), 8.30 (s,1H, Ind-H-2), 8.68 (s,1H, Py-H-2), 9.22 (s,1H, NH); ^{13}C NMR, δ (ppm): 164.50(C=O), 155.06(C=N), 151.34(C-3), 137.50-124.83(Ar-C), 55.27(CH), 39.85(CH2); MS m/z=445.5 (M^+)

2.2.4. [5-(1H-indol-3-yl)-3-(4- methoxyphenyl)-4, 5-dihydropyrazol-1-yl]pyridin-3-ylmethanone ***(3d)***
IR (KBr, cm^{-1}): 3446(NH), 2920(Ar-C-H), 1622(C=O), 1560(C=N), 1033(C-N); ^{1}H NMR (500MHz, DMSO-d6, δ (ppm)): 3.53(dd,1H, Ha, J=18.0Hz, 5.2Hz), 3.81(dd,1H, Hb, J=18.0Hz, 11.6Hz), 6.14(dd,1H, Hx, J=11.6Hz, 5.2Hz), 6.98-8.71(m,13H, Ar), 9.36 (s,1H, NH), 3.89(s,3H,OCH_3); ^{13}C NMR, δ (ppm): 161.82(C=O), 156.23(C=N), 149.95(C-3), 136.86-123.79(Ar-C), 55.45(CH), 40.12(CH2), 54.45(OCH_3); MS m/z=395.8 (M^+)

2.2.5. [3-(4 hydroxyphenyl)- 5-(1H-indol-3-yl)-4, 5-dihydropyrazol-1-yl]pyridin-3-yl methanone ***(3e)***
IR (KBr, cm^{-1}): 3334(OH), 2926(Ar-C-H), 1620(C=O), 1564(C=N), 1107(C-N); ^{1}H NMR (500MHz, DMSO-d6, δ (ppm)): 3.29(dd,1H, Ha, J=18.0Hz, 5.0Hz), 3.87(dd,1H, Hb, J=18.0Hz, 12Hz), 6.03(dd,1H, Hx, J=12Hz, 5.0Hz), 6.85-8.18(m,11H, Ar), 8.98(s,1H, Py-H-2), 8.66 (s,1H, Ind-H-2), 10.03(s,1H,OH), 11.03(s,1H, NH); ^{13}C NMR, δ (ppm): 163.00(C=O), 159.75(C=N), 156.48(C-3), 150.94-124.89(Ar-C), 54.23(CH), 40.36(CH2); MS m/z=382.8 (M^+)

2.2.6. [5-(1H-indol-3-yl)-3-(4 nitrophenyl)-4, 5-dihydropyrazol-1-yl]pyridin-3-yl methanone ***(3f)***
IR (KBr, cm^{-1}): 3217(NH), 2922(Ar-C-H), 1615(C=O), 1566(C=N), 1107(C-N); ^{1}H NMR (500MHz, DMSO-d6, δ (ppm)): 4.01(dd,1H, Ha, J=18.0Hz, 12Hz), 4.38(m,1H, Hb, J=18.0Hz, 5Hz), 6.13(dd,1H, Hx, J=12Hz, 5.0Hz), 6.95-8.96(m,11H, Ar), 8.70(d,1H, Ind-H-2), 8.96(s,1H, Py-H-2), 11.09(s,1H, NH); ^{13}C NMR, δ (ppm): 163.79(C=O), 154.69(C=N), 151.35(C-3), 148.17-123.96(Ar-C), 63.07(CH), 40.00(CH2); MS m/z=410.8 (M^+)

2.2.7. [5-(1H-indol-3-yl)-3-(4 methylphenyl)-4, 5-dihydropyrazol-1-yl]pyridin-3-yl methanone ***(3g)***
IR (KBr, cm^{-1}): 3120(NH), 2966(Ar-C-H), 1639(C=O), 1589(C=N), 1105(C-N); ^{1}H NMR (500MHz, DMSO-d6, δ (ppm)): 3.49(dd,1H, Ha, J=18.0Hz, 5.2Hz), 3.78(dd,1H, Hb, J=18.0Hz, 12Hz), 6.11(dd,1H, Hx, J=5.2Hz, 12Hz), 7.01-7.67(m,10H, Ar), 8.33(d,1H, Py-H-6), 8.55 (s,1H, Ind-H-2), 8.67 (d,1H, Py-H-2), 9.29(s,1H, NH), 2.41(s,3H,CH_3); ^{13}C NMR, δ (ppm): 163.00(C=O), 156.68(C=N), 150.19(C-3), 149.99-124.83(Ar-C), 55.09(CH), 40.08(CH2), 21.55(CH_3); MS m/z=379.8 (M^+).

Table-1: Physico-chemical characterization of the synthesized compounds

Compound	R	M.P. ^oC ±2	M.W.	Yield	M.F.	Elemental Analysis Calculated(found) %
3a	H	238	366	68	$C_{23}N_4H_{18}O$	C75.41(75.35);H4.92(4.84); N15.30(15.25)
3b	Cl	248	401	75	$C_{23}N_4H_{17}ClO$	C68.83(68.90);H4.24(4.19); N13.97(13.80)
3c	Br	262	445	70	$C_{23}N_4H_{17}OBr$	C62.02(62.16);H3.82(3.72); N12.58(12.60)
3d	OCH_3	252	396	64	$C_{24}N_4H_{20}O_2$	C75.79(75.89);H5.26(5.60); N14.74(14.47)
3e	OH	264	382	52	$C_{23}N_4H_{18}O_2$	C72.25(72.36);H4.71(4.65); N14.66(14.21)
3f	NO_2	226	411	48	$C_{23}N_5H_{20}O$	C67.45(67.45);H4.14(4.58); N17.03(17.24)
3g	CH_3	254	380	65	$C_{24}N_4H_{17}O_3$	C75.79(75.39);H5.26(5.23); N14.74(14.36)

1.3. IN-VITRO ANTIBACTERIAL ASSAY
In-vitro antimicrobial activity was examined for the series of synthesized compounds against clinical strains of multidrug resistant. Amongst four microorganisms investigated, three Gram-negative bacteria were *Klebsiella*

pneumoniae, *Psuedomonas aeruginosa & Shigella* sp and one gram-positive bacteria was Methicillin resistant *Staphylococcus aureus*. All the microorganisms were maintained at 4˚C on nutrient agar slants.

2.3.1. Media Preparation and Antibacterial Activity: The antimicrobial assay was performed by agar well diffusion method for synthesized compounds. The test microorganisms were seeded into Mueller Hinton agar by spread plate 10µl (10^6). For agar well diffusion method, the well (0.7 cm) was loaded with 50µl of the test compound on the seeded agar plate. The plates were incubated overnight at 37˚C. Microbial growth was determined by measuring the diameter of zone of inhibition. The result was obtained by measuring the zone diameter (Table 2).

2.3.2. Minimum Inhibitory Concentration (MIC) of Compound-3c: The minimum inhibitory concentration (MIC) was performed by the serial dilution technique using 96-well microplates[15]. The 12 wells of each row were filled with 0.5 ml sterilized Mueller Hinton broth. Sequentially, wells 3–12 received an additional 100µl of a mixture of culture medium and Compound-3c. The active compound-3c dissolved in Dimethyl Sulfoxide (DMSO) against *K. pneumoniae*, *P. aeruginosa* and *Shigella* sp serially diluted to create a concentration sequence from 50 to 1000µg/ml. Well 1 served as growth control and well 2 as solvent control. The deep-wells were incubated for 24h at 37°C. The resulting turbidity was observed, and after 24h MIC was determined to be where growth was no longer visible by assessment of turbidity by optical density readings at 600nm with a micro plate reader (Table. 3).

2.3.3. Minimum Bactericidal Count (MBC) of Compound-3c: The MBC of the compound-3c, taken as the lowest concentration that could kill 99.9% of the initial inoculum within 24 h, was determined using a spread plate method. To determine the MBCs, the loopful culture taken from deep-wells were incubated for 24h at 37°C. The concentration of each inoculum was determined using viable counts on nutrient agar (NA) plates for bacteria (Table. 3).

Table.2. Antibacterial activity of synthesized compound:

Clinical isolates	Zone of inhibition by series of compound in diameter						
	3a	3b	3c	3d	3e	3f	3g
MRSA	BS	-	-	BS	-	BS	-
K. pneumoniae	BS	11mm	14mm	BS	BS	BS	BS
P. aeruginosa	-	-	12mm	-	11mm	-	11mm
Shigella sp	-	-	10mm	BS	BS	-	BS

Note: BS (Bacteriostatic), - (No zone)

Table.3 MIC and MBC value of compound-3c against clinical isolates

Clinical isolates	MIC value (3c)	MBC value
Klebsiella pneumoniae	350 µg ml^{-1}	650µg ml^{-1}
Pseudomonas aeruginosa	350 µg ml^{-1}	650 µg ml^{-1}
Shigella sp	350 µg ml^{-1}	650 µg ml^{-1}

Fig.1 MIC determination of compound 3c

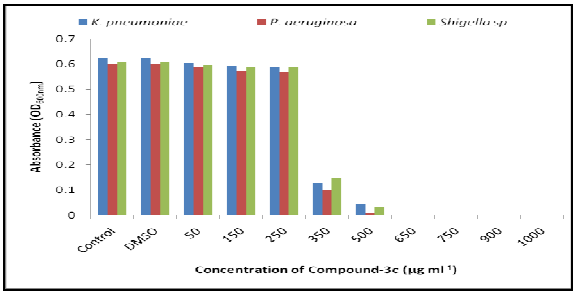


Fig.2. MBC of compound-3c against MDR clinical strains

RESULTS AND DISCUSSION

The present study describes the synthesis, characterization and antibacterial evaluation of certain pyrazole derivatives **3a – 3g**. Assignment of selected characteristic IR bands[16,17] provides significant indications for the formation of cyclized pyrazoline analogues of nicotinic acid hydrazide **3a – 3g**. The IR spectra of the compounds showed $\upsilon_{C=N}$ stretching at 1566-1595 cm^{-1} and υ_{C-N} stretching vibration at 1093 - 1107 cm^{-1} respectively. In addition, the absorption bands at 1419-1440cm^{-1} were attributed to υ_{N-N} stretching vibration, which also confirms the formation of pyrazole derivatives.

In the ^{1}H NMR spectra of these compounds , the three hydrogen atoms attached to the C-4 and C-5 carbon atoms of the pyrazoline ring gave an ABX spin system[18]. The CH_2 protons of the pyrazoline ring resonated as a pair of doublet of doublets around δ 3.41ppm (Ha) and δ 3.79 ppm (Hb). The CH (Hx) proton appeared as a doublet of doublets around δ 6.14 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the C-4 carbon of pyrazoline ring. The J values were calculated for these signals and found to be around 18 Hz and 5 Hz for signal around δ 3.41 ppm and 18 Hz and 12 Hz for signals around δ 3.79 ppm respectively. The 'dd' pattern of Hx proton (5-H, δ around 6.14 ppm) of pyrazoline ring showed J value around 12 Hz and 5 Hz. The –NH proton of indole ring appeared as a singlet near δ 9.25 ppm and other aromatic protons were observed at the expected regions. The ^{13}C NMR spectra of all the compounds were recorded in DMSO and spectral signals are in good agreement with the given structure. The C-4 and C-5 carbons of pyrazolines (3a-3g) resonated at δ 40.36- 39.85ppm and 52.13-55.27 ppm respectively. The characteristic peaks were observed in the mass spectra of all compounds, which followed the similar fragmentation pattern.

The results of antibacterial screening showed that compound-3c were active against all the three gram negative bacteria *K. pneumoniae, Pseudomonas aeruginosa* and *Shigella* sp (Table.1) The compounds 3g & 3e were found to be active against *P. aeruginosa* while the compound 3b was active against *K. pneumoniae*. The compounds 3a, 3d and 3f were showed Bacteriostatic effect on *K. pneumoniae* and the gram positive bacteria *Methicillin Resistant Staphylococcus aureus*. With this panel, none of the synthesized compound was active against *MRSA.* For *Shigella* sp, the compounds 3d, 3g and 3e were bacteriostatic, only compound-**3c** showed moderate activity. The graph shows the MIC of the compound 3c (fig.1 and table.1)**.** The MBC of compound-3c against MDR strains of *K. pneumoniae, Pseudomonas aeruginosa* and *Shigella* sp was performed in nutrient agar plates and bactericidal effect was found to be one fold increased concentration of MIC value. The results of bactericidal concentration was listed in (table.2) The antibacterial results of present study indicated that presence of electron releasing groups (compounds 3d, 3e & 3g OCH_3, OH & CH_3) on phenyl ring improved the antibacterial activity of the synthesized compounds against *K. pneumoniae* & *Shigella* sp. This is in accordance with the results obtained by Nandagokula et al[19]. Presence of electron with drawing (p-Br) substituent on phenyl ring increased the antibacterial activity of the compound 3c against all the three gram negative bacteria. The role of electron with drawing groups in improving antibacterial activities is supported by the studies of Mustafa et al[20] and Konda et al[21]. The behavior of the pyrazole compound 3a with the unsubstituted phenyl ring when compared to other compounds with the substituents in the phenyl ring towards the microorganisms led to the conclusion that the antimicrobial activity of such compounds may increase with the introduction of a specific group.

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