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Synthesis and Characterisation of Some New Pyrazole Analogues for Antimicrobial Activity

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ABSTRACT

Several pharmacological activities like antitubercular, analgesic, anti-cancer, anti-inflammatory, antiasthmatic, antioxidant and antibacterial activities have been attributed to pyrazoles. The above observations prompted us to synthesize some novel pyrazole derivatives as possible antimicrobial agents. A series of novel 1,3,5-trisubstituted pyrazole derivatives (P_1-P_{15}) have been synthesized by the reaction of substituted chalcones (C_1-C_{15}) with succinichydrazide. The starting material, chalcones were prepared by claisen Schmidt condensation of acetophenone with aldehydes in the presence of sodium hydroxide in ethanol. Succinichydrazide was synthesized by condensing succinic acid with hydrazine hydrate. The cycloaddition of chalcones with succinichydrazide gives 1,3,5-trisubstituted pyrazole derivatives. The structures of synthesized derivatives were confirmed by IR, 1HNMR and Mass spectrum. The synthesized compounds were screened for their antibacterial and antifungal activity. The antibacterial activity data of the synthesized derivatives revealed that the compound P_4, P_{13} and P_7, P_{14} were effective against gram positive and gram negative organisms respectively. The antifungal activity data revealed that the compound P7 and P8 showed good activity against tested fungi.

Keywords: 1, 3, 5-trisubstituted Pyrazoles, Antimicrobial activity, Antifungal activity.

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INTRODUCTION

Pyrazoles are unique in their chemical behaviour not only among heterocyclic compounds in general, but also among related azoles. This is because pyrazole possesses the typical properties of the aromatic system, which are in fact rather pronounced in these derivatives, together with the high liability of the ring under certain condition. Although pyrazole derivatives have been known for more than 80 years, the investigation of their chemistry commended rather slowly. Earlier studies were mainly devoted to the development of synthetic methods. Recently the attention was focused on the investigation of chemical properties and in particular on the peculiarities of the behaviour of pyrazole derivatives and the elucidation of their physicochemical characteristics. This enabled new datas to be obtained that were considerable importance. Pyrazole derivatives have a long history of application in agrochemicals as herbicides and insecticides and in pharmaceutical industry as antipyretic and anti-inflammatory. Antipyrine is one of the earliest synthetic drug.

Now a days vast number of compounds with pyrazole nucleus have been reported to show a broad spectrum of biological activity including antimicrobial, antifungal, antioxidant, antiamoebic, analgesic, antitubercular, neuroprotective, anticancer, antiproliferative, antiviral, anticonvulsant, muscle relaxant, anti-inflammatory activities. Due to its wide range of biological activity, pyrazoles ring constitutes a relavent synthetic route in pharmaceutical industry. In fact, such a heterocyclic moiety represents the core structure for number of drugs.

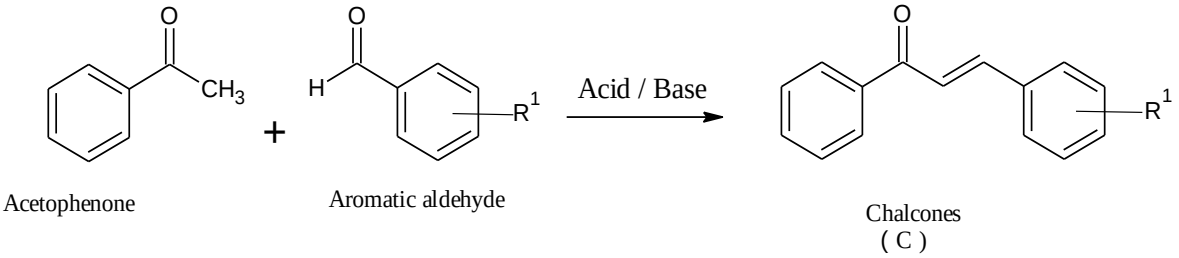
MATERIALS AND METHODS:

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on SHIMADZU FT-IR 8400 with KBr Pellets. ^{1}H-NMR spectra were recorded on 300 MHz–Bruker DPX 200. The chemical shifts are reported as parts per million down fields from tetramethylsilane. Mass spectra were recorded on GCMS-QP5050 SHIMADZU instrument. The purity of the compounds was checked by TLC on precoated SiO2 gel (604 GF 254) aluminium plates (E Merck).

General procedure for the synthesis of chalcones:

The synthetic strategy leading to the target compounds are illustrated in figure1 scheme and number of substitution is mentioned in Table 1 and 2. Equimolar quantities of acetopenone (0.01 mol) and aromatic aldehydes (0.01 mol) in ethanol was cooled to 10-15°C in ice bath. To the cooled solution 40% sodium hydroxide was added drop wise with continuous stirring for 30 min using magnetic stirrer and then left overnight. The reaction mixture was poured into crushed ice

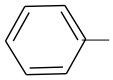
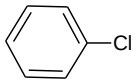
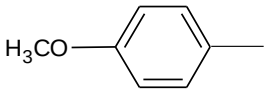
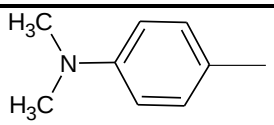
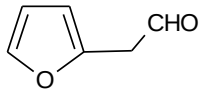
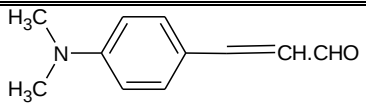
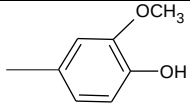
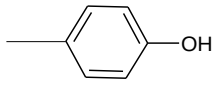
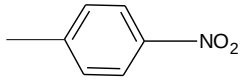
and acidified carefully using dilute hydrochloric acid. The solid was filtered, washed with ice-cold water, dried and recrystallised using ethanol to give compound (C_1-C_{15}). The reaction progress was monitored by TLC using chloroform: petroleum ether (8:2) as mobile phase.



Acetophenone Aromatic aldehyde Chalcones (C)

Figure 1:reaction

Table 1: Substitutions of Chalcones

Compound	Structure
C_1	
C_2	
C_3	
C_4	
C_5	
C_6	
C_7	
C_8	
C_9	
C_{10}	

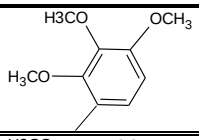
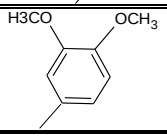
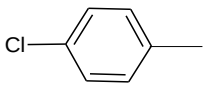
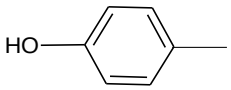
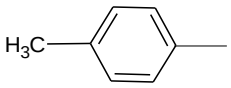
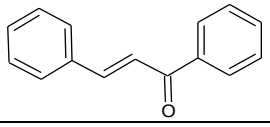
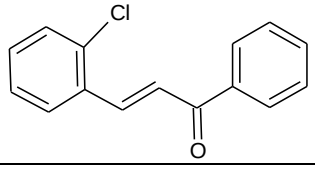
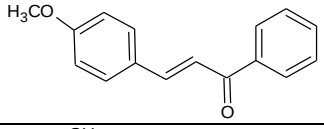
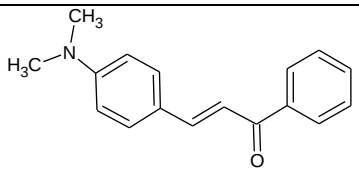
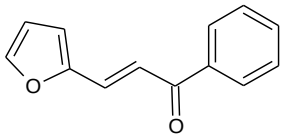
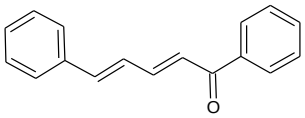
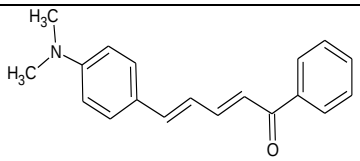
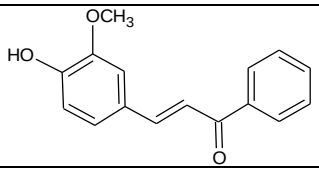
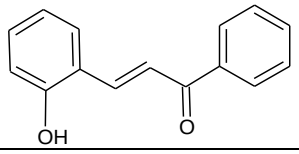
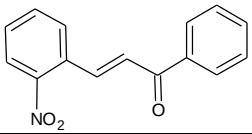
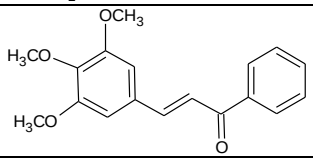
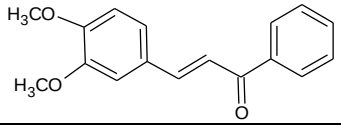
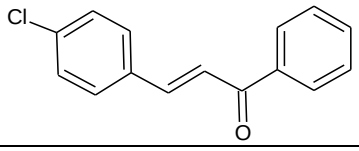
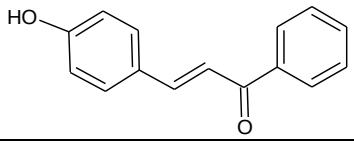
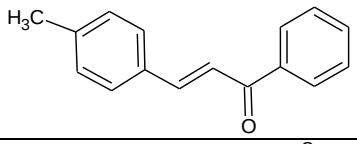
C_{11}	
C_{12}	
C_{13}	
C_{14}	
C_{15}	

Table 2: Physical characterisation data of synthesized chalcones (C_1-C_{15}).

SL NO	Comp.	Chemical structure	Chemical name	Mol. Formula	Yield (%)	Colour	R_f Value
1.	C_1		(2E)-1,3-diphenylprop- 2-en-1-one	$C_{15}H_{14}O$	86.31%	Yellow	0.81
2.	C_2		(2E)-3-(2-chlorophenyl)- 1-phenylprop-2-en-1-one	$C_{15}H_{13}OCl$	82.29%	Yellow	0.65
3.	C_3		2E)-3-(4-methoxphenyl)-1-phenylprop-2-en-1-one	$C_{16}H_{16}O_2$	68.00%	Yellow	0.75
4.	C_4		(2E)-3-[4-(dimethylamino)phenyl]- 1-phenylprop-2-en-1-one	$C_{17}H_{19}ON$	50.00%	Yellow	0.68
5.	C_5		(2E)-3-(2-furyl)-1-phenylprop-2-en-1-one	$C_{13}H_{12}O_2$	67.5%	Brown	0.71
6.	C_6		(2E,4E)-1,5-diphenylpenta-2,4-dien- 1-one	$C_{17}H_{14}O$	94.44%	Orange	0.72
7.	C_7		(2E,4E)-5-[4-(dimethylamino)phenyl]- 1-phenylpenta-2,4-dien- 1-one	$C_{19}H_{19}ON$	83.47%	Orange	0.66

8.	C_8		2E)-3-(4-hydroxy-3-methoxyphenyl)-1-phenylprop-2-en-1-one	$C_{16}H_{16}O_3$	64.34%	Colorless	0.57
9.	C_9		(2E)-3-(2-hydroxyphenyl)-1-phenylprop-2-en-1-one	$C_{16}H_{14}O_2$	54.93%	Brown	0.62
10	C_{10}		(2E)-3-(2-nitrophenyl)-1-phenylprop-2-en-1-one	$C_{15}H_{13}O_3N$	44.32%	Blue	0.86
11	C_{11}		2E)-1-phenyl-3-(3,4,5-trimethoxyphenyl)prop - 2-en-1-one	$C_{18}H_{18}O_4$	64.83%	Yellow	0.69
12	C_{12}		2E)-3-(3,4-dimethoxyphenyl)-1-phenylprop-2-en-1-one	$C_{17}H_{16}O_3$	71.81%	Yellow	0.63
13	C_{13}		(2E)-3-(4-chlorophenyl)- 1-phenylprop-2-en-1-one	$C_{15}H_{13}OCl$	81.03%	Yellow	0.77
14	C_{14}		(2E)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one	$C_{15}H_{12}O_2$	64.83%	Yellow	0.83
15	C_{15}		(2E)-3-(4-methylphenyl)-1-phenylprop-2-en-1-one	$C_{16}H_{14}O$	64.83%	Yellow	0.79

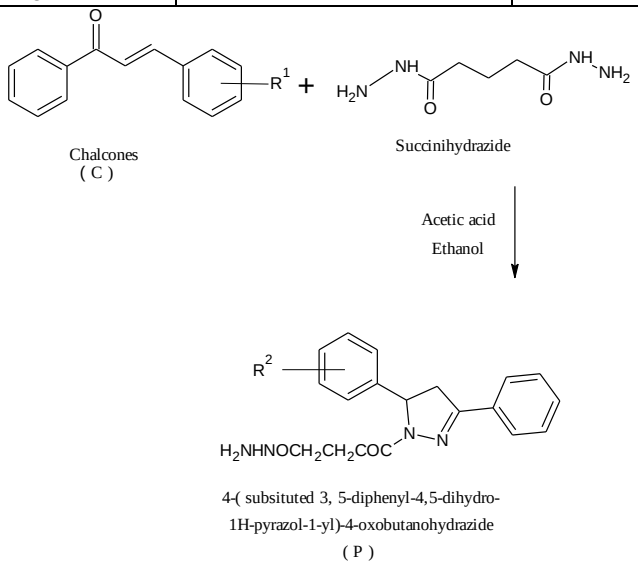
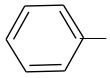
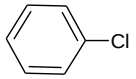
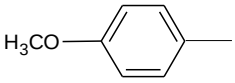
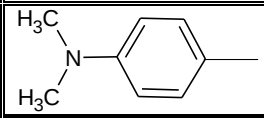
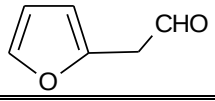


Figure 2: reaction scheme

Table 3: Substitutions of 1, 3, 5-trisubsituted pyrazole:

Compound	Structure
P_1	
p_2	
P_3	
P_4	
P_5	
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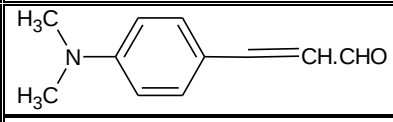
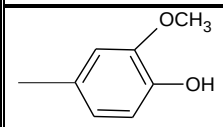
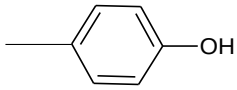
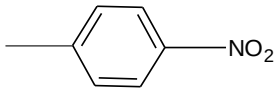
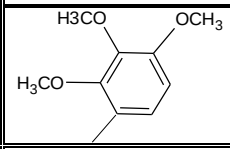
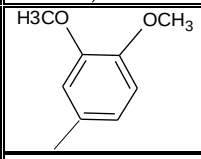
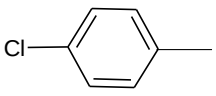
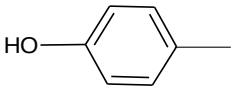
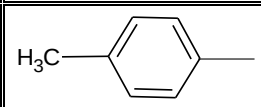
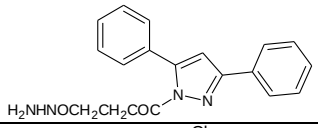
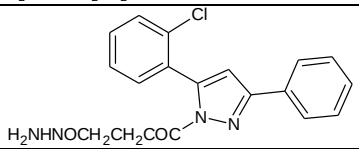
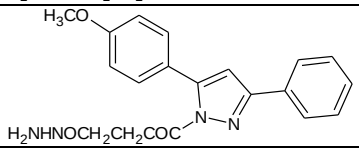
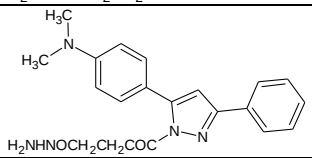
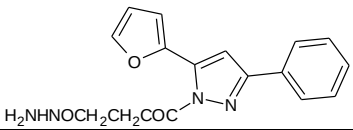
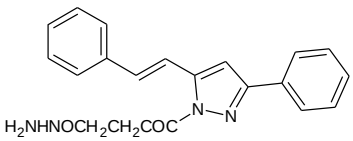
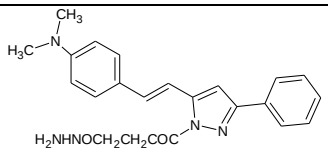
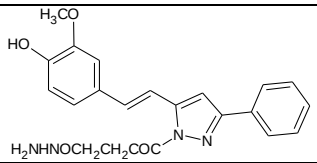
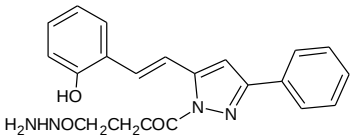
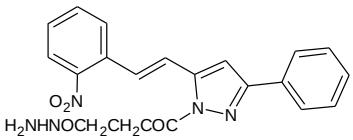
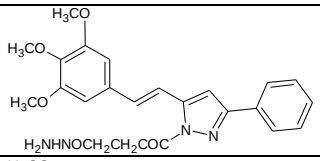
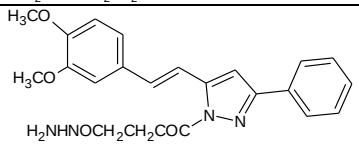
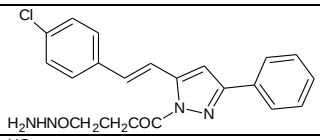
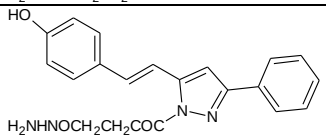
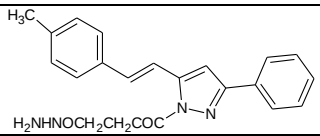
P_7	
P_8	
P_9	
P_{10}	
P_{11}	
P_{12}	
P_{13}	
P_{14}	
P_{15}	

General procedure for synthesis of 1,3,5-trisubstituted pyrazole.

The synthetic strategy leading to the target compounds are illustrated in figure 2 scheme and number of substitution is mentioned in Table 3and 4 .A mixture of chalcone (C_1-C_{15}) (0.01mol), succinichydrazide (0.01mol) and acetic acid (5ml) in ethanol was refluxed for 8 hrs. The reaction mixture was cooled and poured over ice water. The solid separated was filtered, washed with water to give compound (P_1-P_{15}). The completion of reaction was monitored by TLC.

Table 4: Physical Characterization data of synthesized 1,3,5-trisubsituted pyazole (P1-P15).

Sr No	Comp	Chemical structure	Chemical name	Mol. Formula	Yield (%)	Colour	R_f Value
1.	P_1		4-3,5-diphenyl-4,5-dihydro-1*H*-pyrazol- 1-yl)-4- oxobutanohydrazide	$C_{19}H_{20}N_4O_2$	25.44	White	0.82
2.	P_2		4-[5-(2- chlorophenyl)-3-phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide	$C_{19}H_{17}ClN_4O_2$	37.58	Yellow	0.91
3.	P_3		4-[5-(4- methoxyphenyl)-3-phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide	$C_{20}H_{22}N_4O_3$	41.25	Pale yellow	0.88
4.	P_4		4-[5-(4-dimethylaminophenyl)-3-phenyl-1*H*-pyrazol-1-yl]-4-oxobutanohydrazide	$C_{12}H_{25}N_5O_2$	44.85 %	Yellow	0.79
5.	P_5		4-[5-(2-furyl)-3- phenyl-1*H*-pyrazol-1- yl]-4-oxobutanohydrazide	$C_{17}H_{18}N_4O_3$	46.31 %	Pale brown	0.88
6.	P_6		4-oxo-4-{3-phenyl-5- [(E)-2-phenylvinyl]- 1*H*-pyrazol-1-yl}butanohydrazide	$C_{21}H_{22}N_4O_2$	58.01 %	Yellow	0.86
7.	P_7		4-oxo-4-{3-dimethylaminophenyl -5-[(E)-2- phenylvinyl]-1*H*-pyrazol-1-yl}butanohydrazide	$C_{23}H_{27}N_5O_2$	20.00 %	Brown	0.92
8.	P_8		4-[5-(4-hydroxy-3-methoxyphenyl)-3- phenyl-1*H*-pyrazol-1- yl]-4-oxobutanohydrazide	$C_{20}H_{20}N_4O_4$	17.36 %	Dark brown	0.89

9	P_9		4-[5-(2- hydroxyphenyl)-3-phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide	$C_{19}H_{18}N_4O_3$	38.28 %	White	0.80
10	P_{10}		4-[5-(2-nitrophenyl)- 3-phenyl-1*H*-pyrazol- 1-yl]-4- oxobutanohydrazide	$C_{19}H_{17}N_5O_4$	33.24 %	Blue	0.93
11	P_{11}		4-[5-(3,4,5-trimethoxyphenyl)-3-phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide	$C_{22}H_{24}N_4O_5$	52.45 %	Yellow	0.72
12	P_{12}		4-[5-(4,5-dimethoxyphenyl)-3-phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide	$C_{21}H_{22}N_4O_4$	24.57 %	Yellow	0.69
13	P_{13}		4-[5-(4- chlorophenyl)-3-phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide	$C_{19}H_{17}ClN_4O_2$	34.67 %	Yellow	0.75
14	P_{14}		4-[5-(4- hydroxyphenyl)-3-phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide	$C_{19}H_{18}N_4O_3$	26.87 %	White	0.84
15	P_{15}		4-[5-(4- methylphenyl)-3-phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide	$C_{20}H_{20}N_4O2$	23.62 %	Yellow	0.79

Antibacterial activity & antifungal activity:

Antibacterial activity and Antifungal activity assessment: All Standard cultures of *Staphylococcus aureus, Staphylococcus faecalis, Bacillus subtilus , Escherichia coli, klebsiella penumonia* and *Saceharomyces cerevisiae, Aspergillus niger* species were obtained from Department of Microbiology, CFTRI, Mysore. All the newly synthesized compounds were dissolved in Dimethylsulphoxide (DMSO) or ethanol to prepare chemicals stock solution of 100 mg/ ml.

Agar-well diffusion method: The petri plate were washed thoroughly and sterilized in hot air oven at 160°C for one hr 30 ml of sterile nutrient agar medium was poured into sterile Petri dishes and allow to solidify. The petri plates were incubated at 37°C for 24 hrs to check for sterility. The medium was seeded with the organism by spread plate method using sterile cotton swabs. Bores were made on the medium using sterile borer and 0.1 ml of the Ciprofloxacin at a concentration of 1µg/ml was taken as standard reference. A control having only DMSO in the cup was maintained in each plate. The petri plates were kept in refrigerator at 4°C for 15 min,

allowing diffusion to take place. Agar diffusion, the petri plate were incubated at 37°C for 24 hrs and zone of inhibition were observed.

RESULTS AND DICUSSION:

Spectral data of synthesized chalcones:

1. (2E)-1,3-diphenylprop- 2-en-1-one(C_1)

IR data : 3046cm^{-1} (Ar C-H), 1661cm^{-1} (C=O), 1600 cm^{-1} (C=C), 761 cm^{-1} (mono substituted Ar- ring) ^{1}H-NMR (DMSO)(d/ppm): δ 8.05(1H,d,=CH-Ar), δ 7.60(1H,d,-CO-CH=), δ 7.14-7.80(10H,m,Ar-H) , Mass spectroscopy data: m/z 208 (M^+).

2. (2E)-3-(2-furyl)-1- phenylprop-2-en-1-one (C_5)

IR data: 3130cm^{-1} (Ar C-H) 1661cm^{-1} (C=O), 1600cm^{-1} (C=C), 1165cm^{-1} (C-O-C), 752 cm^{-1} (mono substituted Ar-ring), Mass spectroscopy data: m/z 200 (M^+).

3. (2E)-3-(2- hydroxyphenyl)-1- phenylprop-2-en-1-one (C_9)

IR data : 1647 cm^{-1} (C=O), 1579 cm^{-1} (C=C), 3456 cm^{-1} (OH), ^{1}H-NMR (DMSO) (d/ppm): δ 8.08(1H,s,=CH-Ar), δ 7.50(1H,s,-CO-CH=), δ 4.4(1H,s,-OH), δ 6.4-7.7(9H,m,Ar-H), Mass spectroscopy data: m/z 224 (M^+).

4. (2E)-3-(4-chlorophenyl)- 1-phenylprop-2-en-1-one (C_{13})

IR data : 1656cm^{-1} (C=O), 1595 cm^{-1} (C=C), 825 cm^{-1} (Ar-Cl), Mass spectroscopy data: m/z 244(M^+).

Spectral data of synthesized 1,3,5-trisubstiuted pyrazoles:

1. 4-3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-oxobutanohydrazide (P_1).

IR data : 3205.69 cm^{-1} (C-H stretch aromatic), 3047.53 cm^{-1} (C-H stretch) 1660.77 cm^{-1} (C=N) 1489.75 cm^{-1} (C=C aromatic), 3313.62 cm^{-1} (-NH2 stretch) 3501.89 cm^{-1} (-NH stretch) 750.33 cm^{-1} (Mono substituted phenyl ring) , ^{1}H-NMR (DMSO) (d/ppm): δ 1.26-1.68(4H,d,methyene of pyrazoline), δ 5.30 (1H,s,methane of pyrazoline) δ 4.81(4H,d,methylene side chain) δ 6.82-7.37(10H,m,Ar-H) δ 1.53(2H,s,NH2), Mass spectroscopy data: m/z 336 (M^+).

2. 4-[5-(2-chlorophenyl)-3-phenyl-1*H*-pyrazol-1-yl]-4-oxobutanohydrazide (P_2).

IR data : 3026.69 cm^{-1} (C-H stretch aromatic) , 2926.01 cm^{-1} (C-H stretch) 1666.50 cm^{-1} (C=O)amide, 1552.75 cm^{-1} (C=N) 1602.85 cm^{-1} (C=C aromatic), 3474.64 cm^{-1} (-NH2 stretch),3501.89 cm^{-1} (NH stretch), ^{1}H-NMR (DMSO) (d/ppm): δ 3.7(1H,s,Ha,pyrazoline ring), δ 3.8(1H,s,Hb,pyrazoline ring), δ 5.10 (1H,s,methane of pyrazoline), δ 2.35-2.49(4H,d,methylene side chain), δ 6.96-7.76 (9H,m,Ar-H), δ 1.58 (2H,s,NH_2), δ 8.95(1H,s,NH), Mass spectroscopy data: m/z 368 (M^+).

3. 4-[5-(4- methoxyphenyl)-3- phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide(P_3)

IR data : 3064.99 cm^{-1} (C-H aromatic stretch), 2993.62 cm^{-1} (C-H stretch) 1658.84 cm^{-1} (C=O amide) 1575.89 cm^{-1} (C=N) 1458.23 cm^{-1}(C=C aromatic) 3362.23 cm^{-1} (-NH2 stretch) 3479.89 cm^{-1} (-NH stretch) 1213.27 cm^{-1} (Ar-O-C) 1276.92 cm^{-1} (Ar-O-CH3) 779.27 cm^{-1} (Mono substituted ring), Mass spectroscopy data: m/z 345 (M^+).

4. 4-[5-(2-furyl)-3- phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide (P_5)

IR data : 3064.99 cm^{-1} (Ar C-H), 2984.63 cm^{-1} (C-H), 1660 cm^{-1} (C=O), 1600.97 cm^{-1} (C=N), 1585.54 cm^{-1} (C=C), 3362.23 cm^{-1} (-NH2), 3479.89 cm^{-1} (-NH), 1069.82 cm^{-1} (C-O-C), 779.27 cm^{-1} (substituted aryl or hetero aryl ring). Mass spectroscopy data: m/z 326 (M^+).

5. 4-[5-(2- hydroxyphenyl)-3- phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide(P_9)

IR data : 3047.63 cm^{-1} (Ar C-H), 2924.18 cm^{-1} (C-H), 1666.69 cm^{-1} (C=O), 1616.47 cm^{-1} (C=N), 1456.23 cm^{-1} (C=C), 3498.63 cm^{-1} (-NH2), 3548.52 cm^{-1} (-NH), 3317.28 cm^{-1} (-OH), 744.55 cm^{-1} (mono substituted aromatic ring). Mass spectroscopy data: m/z 350 (M^+).

6. 4-[5-(4,5- dimethoxyphenyl)-3- phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide (P_{12})

IR data : 3072.71 cm^{-1} (Ar C-H), 2926.11 cm^{-1} (C-H), 1600.77 cm^{-1} (C=O), 1552.75 cm^{-1} (C=N), 1598.75 cm^{-1} (C=C), 3424.50 cm^{-1} (-NH2), 3498.45 cm^{-1} (-NH),1168.90 cm^{-1} (Ar-O-CH3), 750.33 cm^{-1} (mono substituted aromatic ring). ^{1}H-NMR (DMSO) (d/ppm): δ 1.23-1.54(2H,d, methylene of pyrazoline), δ 3.04(9H,m,methoxy), δ 5.16(1H,d,methane of pyrazoline), δ 6.52-7.66(7H,m,Ar-H), δ 2.43(4H,d,methylene side chain). Mass spectroscopy data: m/z 394 (M^+).

7. 4-[5-(4- hydroxyphenyl)-3- phenyl-1*H*-pyrazol-1- yl]-4- oxobutanohydrazide (P_{14})

IR data : 3102.87 cm^{-1} (Ar C-H), 2963.59 cm^{-1} (C-H), 1708.99 cm^{-1} (C=O), 1660.77 cm^{-1} (C=N), 1516.10 cm^{-1} (C=C), 3473.74 cm^{-1} (-NH2), 3421.83 cm^{-1} (-NH), 3396.74 cm^{-1} (-OH), 748.48 cm^{-1} (mono substituted aromatic ring). Mass spectroscopy data: m/z 348 (M^+).

Antibacterial activity & antifungal activity:

The Zone of inhibition of the derivatives are as shown in below table no.5,6 &7 All the synthesized compounds (P_1-P_{10}) were purified, characterized and screened for their antimicrobial activity and antifungal activity. They were tested against three gram positive (*Staphylococcus aureus, Staphylococcus faecalis* and *Bacillus subtilis*) and two gram negative (*Escherichia coli* and *Klebsiella penumoniae)* organisms. The antifungal activity tested against *Saceharomyces cerevisiae* and *Aspergillus niger.* The activity of the derivatives were performed by cup plate method at different concentration level. Ketoconazole was used as standard drug at concentration remains same.

Table 5: Antibacterial activity of synthesized pyrazole derivatives against gram positive bacteria

Compound	Zone of inhibition in mm					
	Staphylococcus aureus		***Staphylococcus faecalis***		***Bacillus substilis***	
	50µg	**100 µg**	**50µg**	**100 µg**	**50µg**	**100 µg**
P_1	3	5	4	5	3	6
P_2	4	5	5	6	5	8
P_3	4	4	4	5	7	10
P_4	6	9	4	4	3	5
P_5	4	4	5	6	6	12
P_6	3	5	4	4	4	7
P_7	4	5	4	5	4	8
P_8	5	6	4	6	7	10
P_9	5	5	5	6	7	11
P_{10}	4	4	4	6	4	5
P_{11}	4	8	3	7	6	9
P_{12}	3	5	4	7	5	7
P_{13}	3	6	5	8	5	8
P_{14}	4	9	4	6	3	6
P_{15}	5	6	5	7	5	7
DMSO	-	-	-	-	-	-
Ciprofloxacin	7	10	8	12	9	13

Table 6: Antibacterial activity of synthesized pyrazole derivatives against gram negative bacteria.

Compound	Zone of inhibition in mm			
	Escherichia coli		***Klebsiella penumoniae***	
	50µg	**100 µg**	**50µg**	**100 µg**
P_1	3	5	4	5
P_2	5	7	5	6
P_3	4	4	4	5
P_4	5	6	4	4
P_5	4	4	5	6
P_6	3	5	4	4
P_7	5	8	6	10
P_8	5	5	5	6
P_9	6	8	3	7
P_{10}	4	4	4	6
P_{11}	6	8	3	7
P_{12}	3	5	4	7
P_{13}	3	6	5	8
P_{14}	6	9	4	6
P_{15}	5	6	5	7
DMSO	-	-	-	-
Ciprofloxacin	7	10	8	12

Table 7: Antifungal activity of synthesized pyrazole derivatives.

Compound	Zone of inhibition in mm			
	Saceharomyces Cerevisia		***Aspergillus niger***	
	50µg	100 µg	50µg	100 µg
P_1	5	9	5	9
P_2	4	8	4	8
P_3	4	7	4	7
P_4	7	10	4	7
P_5	5	9	7	10
P_6	9	14	8	11
P_7	6	11	9	11
P_8	8	12	8	12
P_9	6	10	5	10
P_{10}	4	7	4	9
P_{11}	5	8	5	8
P_{12}	6	8	4	6
P_{13}	4	9	6	9
P_{14}	8	12	5	7
P_{15}	5	8	6	9
DMSO	-	-	-	-
Ketoconazole	7	10	8	12

CONCLUSION:

1, 3, 5-trisubstituted pyrazoles are therapeutically important class of heterocyclic compounds. The method used in the present study is one of the best method for introducing substitution at 1, 3, 5 positions of the pyrazole ring. The cycloaddition reaction of chalcones with hydrzides to obtain 1, 3, 5-trisubstituted pyrazole derivatives (P_1-P_{15}) was attempted by employing various reagents and reaction conditions. However the desired cycloaddition was successful only when the reaction was carried out by using acetic acid as a catalyst and ethanol as a solvent. The desired 1,3,5-trisubstituted pyrazole derivatives (P_1-P_{15}) were obtained in a good yield by conventional method. The antibacterial activity, of the synthesized 1,3,5-trisubstituted pyrazole derivatives revealed that the compounds P_4, P_5 and P_7, P_{14} were effective against gram Positive and gram negative organisms respectively. The antifungal activity, of the synthesized 1,3,5-trisubstituted pyrazole derivatives revealed that the compound P_7 and P_8 showed good activity against tested fungi.

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