

## **Antimicrobial Activity of Some Novel Pyrazoline Derivatives**

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### **ABSTRACT:**

Different Pyrazoline derivatives were synthesized by cyclization of substituted chalcone derivatives in presence of hydrazine hydrate (P1-P6). All the synthesized compounds were characterized by spectral analysis (IR, MS and NMR). These compounds were screened for their antibacterial activity against Gram-positive bacteria and Gram negative bacteria. Antifungal activity was also performed using agar cup plate method. The minimum inhibitory concentrations (MICs) of the compounds were also determined by agar streak dilution method. Among the synthesized compounds; (P1) was found to exhibits the most potent *in-vitro* antimicrobial activity with the MICs of 3.121, 1.5, 22 µg/ml against *E. coli*, *P. aeruginosa*, *B. pumilus* respectively. Compound (P6) was found to exhibit the most potent *in-vitro* anti-fungal activity with MICs 0.83 and 0.093 µg/ml against *A. niger* and *P. chrysogenum*.

**Keywords:** - Antimicrobial, MIC, Pyrazoline, Heterocyclic-compound

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### **INTRODUCTION:**

Considerable attention has been focused on Pyrazolines and substituted Pyrazolines due to their interesting biological activities. They have found to possess anti-fungal, anti-depressant, anti-convulsant, anti-inflammatory, anti-bacterial, anti-cancer, antioxidant, anti-pyretic, anti-neoplastic activities, anti-viral, anti-amoebic, acaricidal agro chemical fungicides or insecticides, anti-cholinergic, antidiabetic, anti-HIV, anti-malarial, anxiolytic, antiparasitic, anti-allergic, anti-microbial, anti-tuberculosis, tyrosinase inhibitor, hypoglycemic, hypotensive, immuno suppressive, anti-tumor [1-15]. Pyrazoline have usually been prepared by starting from aldehydes or ketones. The purpose of this study was to develop new pyrazoline derivatives as potent anti-microbial agents.

### **EXPERIMENTAL**

Melting points were determined by Thieles tube method (Table 1) and were uncorrected. <sup>1</sup>H NMR spectra were recorded on Avance II 400 (Make; Bruker, France) NMR Spectrometer. FT-IR spectra were recorded on MB 3000 (Make; ABB Bomem, Canada) spectrometer and Mass spectra were recorded on Q-TOF Micro (Make; Waters, Massachusetts) spectrometer.

### **Synthesis of substituted pyrazoline derivatives:**

#### **1. Procedure for synthesis of substituted chalcone derivative:**

A solution of sodium hydroxide (40%) in water and rectified spirit was placed in a flask provided with a mechanical stirrer. The flask was immersed in a bath of crushed ice. Substituted acetophenone (0.005M) was poured with constant stirring, Substituted benzaldehydes (0.005M) was added to the solution. The temperature of the mixture was kept at about 25°C and stirred vigorously until the mixture was thick enough to retard the stirring (4 hr). The stirrer was removed and the reaction mixture was kept at 8 °C overnight. The product was filtered with suction on a buchner funnel, washed with cold water until the washings were neutral to litmus and then with ice cold ethanol. The crude product was recrystallized from ethanol.

#### **2. Procedure for synthesis of substituted pyrazoline derivatives:**

##### **Addition and cyclization:**

In a mixture of substituted chalcone in ethanol, hydrazine hydrate was added drop wise in a round bottom flask. The reaction mixture was heated under reflux for 6 hrs on a water bath followed with addition of ice cold water at room temperature. The mixture was kept overnight at 8 °C. The precipitates were filtered, washed with distilled water and dried. The product was recrystallized with ethanol to get final product.

##### **Biological evaluation:**

##### **In-vitro Antimicrobial Screening:**

The *in vitro* antibacterial screenings of synthesized compounds were performed against the following standard bacterial strains: *Bacillus pumillus* (MTCC 1456), *Pseudomonas fluorescens* (MTCC 2421), *Micrococcus luteus* (MTCC 1538), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 1573), *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 1430). For antifungal screening *Penicillium chrysogenum* (MTCC 161), *Aspergillus niger* (MTCC 2546) were used.

**Cylinder plate method:**

A definite volume of the microbial suspension (inoculums) was poured into the sterilized nutrient agar media (cooled at 40°C) and mixed thoroughly. About 20 ml of this suspension was poured aseptically in the petri plates and kept till the solidification. The surface of agar plates was pierced using a sterile cork borer. The prepared wells were filled with equal volume of a solution of synthesized compounds and standard drugs; separately. After a period of pre-incubation diffusion, the plates were incubated face up for a definite time under specified conditions. The zones of inhibition were measured as a parameter of antimicrobial properties of synthesized derivatives.

**Minimum inhibitory concentration (MIC)**

A series of glass tubes containing different concentrations of the synthesized compounds (In Dimethyl Sulphoxide) with Mueller Hinton broth was inoculated with the required amount of the inoculum to obtain a suspension of microorganism which contains  $10^5$  colony forming units per milliliter. Growth control tube was prepared with the addition of the compound and blank was prepared without the addition of microorganism. The tubes were incubated at 37 °C for 24 h. The turbidity produced in each tube was recorded by using a UV-visible spectrometer.

**RESULTS AND DISCUSSION:**

Novel pyrazoline derivative were synthesized by cyclization of substituted chalcone derivatives in presence of hydrazine hydrate (Figure 1). Physiochemical properties of synthesized compounds were determined in terms of melting point & % yield (Table 1). Synthesized compounds were also characterized using FT-IR and  $^1\text{H-NMR}$ . The IR spectrum of the synthesized compounds revealed the presence of C=O stretching at  $1615\text{-}1655\text{ cm}^{-1}$ ,  $3402\text{ cm}^{-1}$  (O-H str. of alcohol),  $1528\text{ cm}^{-1}$  (N=O str. of nitro) and C=N (aromatic) stretching at  $1515\text{-}1655\text{ cm}^{-1}$ . In  $^1\text{H-NMR}$  spectra  $\delta$  value of various synthesized compounds was found in the range of 1.29-1.68 for methyl proton and 6.14-7.87 for benzyl proton (Table 2). The synthesized derivatives showed the presence of aromatic ring which was also evident in the  $^1\text{H NMR}$  spectra. Antifungal & antibacterial activities were also performed as *in-vitro* antimicrobial screening against fungal strains & bacterial strain respectively (Table 3 & 4). The minimum inhibitory concentrations (MICs) values for all active compounds were determined by agar streak dilution method.

**Table: 1 Physical constant of synthesized Pyrazoline derivatives.**

<b>Compound Code</b>	<b>Melting range (°C)</b>	<b>% Yield (Mean±S.D.)</b>	<b>R<sub>f</sub>-value (Mean±S.D.)</b>
P1	110-115	60±0.1	0.67±0.01
P2	111-115	65±0.2	0.78±0.02
P3	115-118	70±0.4	0.71±0.06
P4	101-104	83±0.5	0.64±0.03
P5	104-108	78±0.1	0.84±0.04
P6	105-109	60±0.3	0.81±0.05

**Table: 2 Spectral analysis of the synthesized derivatives.**

<b>Comp. code</b>	<b>IR spectra (cm<sup>-1</sup>)</b>	<b><sup>1</sup>H NMR Spectra (δ) in ppm</b>
P1	3402 (O-H str. of alcohol), 3339 (N-H str.), 3198 (aromatic C-H str.), 1624 (C=C str.), 1000 (C=C bend. aliphatic), 800 (C-H bend), 603 (C-Br str.).	7.40 (r, 4H, aromatic ring), 6.15-6.78 (s, 4H, aromatic ring), 4.58 (r, 2H, OH), 3.64 (m, 1H), 1.25-2.59 (r, 2H, CH <sub>2</sub> in ring).
P2	3285 (N-H str.), 2930 (C-H str.), 1682 (C=C str.), 1531 (aromatic C=C str.), 1048 (C=C bend. aliphatic), 927 (C-H bend.), 1528 (N=O str. of nitro).	6.62-7.39 (r, 4H, aromatic ring), 6.14-6.62 (s, 3H, aromatic ring), 4.0 (r, 3H), 3.7 (m, 1H), 1.5-2.1 (r, 2H, CH <sub>2</sub> in ring).
P3	3425 (O-H str. of alcohol), 3350 (aromatic O-H str.), 2922 (C-H str.), 1576 (aromatic C=C str.), 1092 (C=C bend.), 925 (C-H bend.).	7.27-7.43 (r, 4H, aromatic ring), 6.15-6.78 (s, 3H, aromatic ring), 4.5 (r, 2H, OH), 3.9 (m, 1H), 1.2-2.5 (r, 2H, CH <sub>2</sub> in ring).

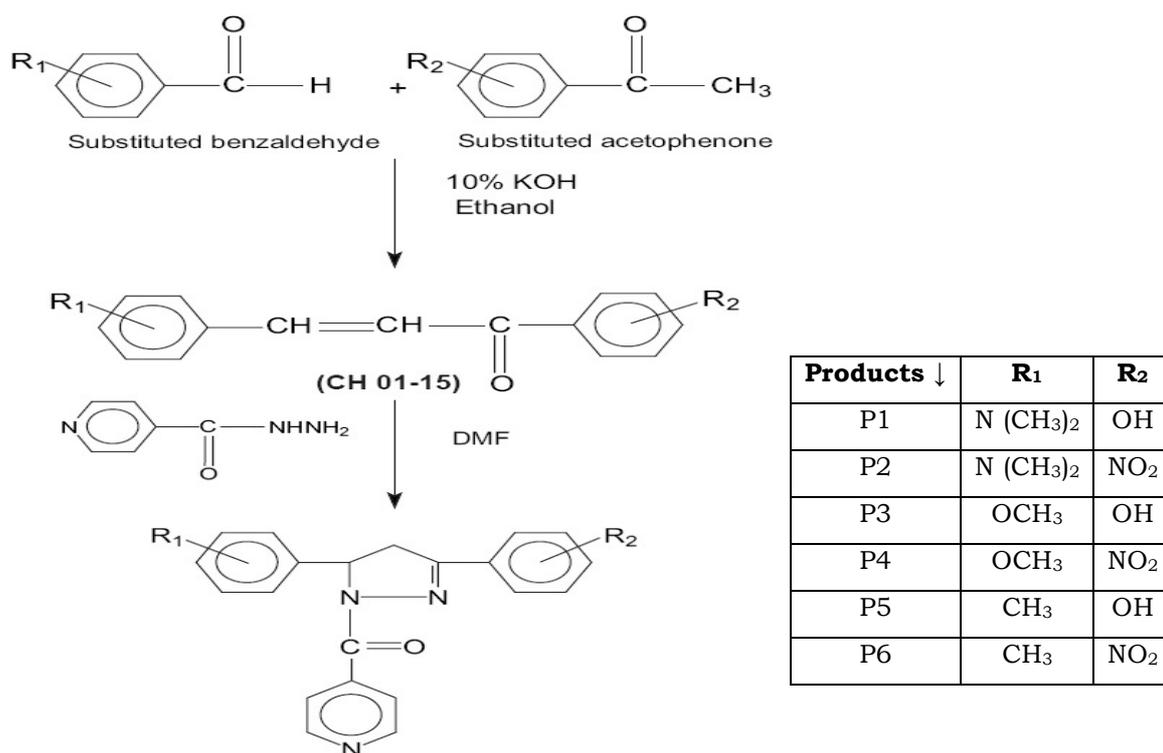
P4	2955 (aromatic C-H str.), 2839 (aliphatic C-H str.), 1528 (N=O str. of nitro), 1504 (aromatic C=C str.), 1043 (C=C bend.), 849 (C-H bend.).	6.6 (r, 2H, aromatic ring), 6.18-6.75 (s, 3H, aromatic ring), 3.7 (m, 1H), 3.73 (s, 9H, CH <sub>3</sub> ), 1.8-2.0 (r, 2H, CH <sub>2</sub> in ring).
P5	3335 (O-H str. of alcohol), 2916 (C-H str.), 1582 (aromatic C=C str.), 1366 (C-H bend. of aliphatic), 1065 (C=C bend.), 997 (C-H bend.).	6.6-7.4 (r, 4H, aromatic ring), 6.15-6.78 (r, 3H, aromatic ring), 5.0 (r, 2H, OH), 3.6 (m, 1H), 2.85 (s, 6H), 1.3-2.1 (r, 2H, CH <sub>2</sub> in ring).
P6	2920 (C-H str.), 1647 (C=C str.), 1587 (aromatic C=C str.), 1009 (C=C bend.), 887 (C-H bend.), 1528 (N=O str. of nitro).	7.5 (r, 4H, aromatic ring), 7.0-7.01 (s, 4H, aromatic ring), 3.9 (m, 1H), 2.35 (s, 3H, CH <sub>3</sub> ), 1.8-2.3 (r, 2H, CH <sub>2</sub> in ring).

**Table 3. Antifungal Activity (Paper Disc Diffusion Method)**

Compounds	Antifungal Activity (Paper Disc Diffusion Method)			
	Zone of Inhibition (mm)		Minimum Inhibitory Concentration ( $\mu\text{g mL}^{-1}$ )	
	Fungal Strain			
	<i>Penicillium chrysogenum</i> (MTCC 161)	<i>Aspergillus niger</i> (MTCC2546)	<i>Penicillium chrysogenum</i> (MTCC 161)	<i>Aspergillus niger</i> (MTCC 2546)
P1	24	29	37	84
P2	24	31	51	61
P3	21	19	62	108
P4	27	18	21	92
P5	21	22	61	91
P6	32	31	0.83	0.093
Fluconazole	-	-	0.8	0.07

**Table 4. Antibacterial Activity (Minimum Inhibitory Concentration)**

Compounds	Zone of Inhibition (Mm)						
	Gram negative bacteria			Gram positive bacteria			
	<i>Escherichia coli</i> (MTCC 1573 )	<i>Pseudomonas aeruginosa</i> (MTCC 424)	<i>Pseudomonas fluorescens</i> (MTCC 2421)	<i>Staplococc aureus</i> (MTCC 1430 )	<i>Bacill subtilis</i> (MTCC 441)	<i>Bacillus pumillus</i> (MTCC 1456)	<i>Microoccus luteus</i> (MTCC 1538)
P1	3.121	1.5	144	92	191	22	60
P2	63	52	77	126	185	41	137
P3	54	42	108	201	160	48	-
P4	35	-	40	163	104	82	21
P5	118	124	91	113	85	31	56
P6	81	66	-	194	29	158	66
Norfloxacin	2.91	1.19	3.6	13	13	11	3.2



**R<sub>1</sub>** = N (CH<sub>3</sub>)<sub>2</sub>, OCH<sub>3</sub>, CH<sub>3</sub>

**R<sub>2</sub>** = OH, NO<sub>2</sub>

**Fig. 1: Scheme for the synthesis of substituted pyrazoline derivatives**

According to preliminary antibacterial screening by paper disc method all compounds were found to have comparable antibacterial activity against *S. aureus*, *B. subtilis*, *B. pumillus*, *E. coli* compared to Norfloxacin as a standard drug, and for antifungal screening all compounds were found to active against *A. niger* and *P. chrysogenum* using Fluconazole as a standard drug. The antimicrobial screening revealed that the compound P1 & compound P6 exhibited potent antibacterial & antifungal activity respectively as compared to other derivatives. Compound (P1) was found to exhibit potent *in-vitro* antimicrobial activity with the MICs of 3.121, 1.5, 22 µg/ml against *E. coli*, *P. aeruginosa*, *B. pumilus* respectively while compound (P6) was found to exhibit potent *in-vitro* anti-fungal activity with MICs 0.83 and 0.093 µg/ml against *A. niger* and *P. chrysogenum*. (Table 3, Table 4).

#### **CONCLUSION:**

Present research work involves synthesis of novel pyrazoline derivative to explore their antimicrobial activity. Compound P1 exhibited highest antibacterial activity against *E. coli*, *P. aeruginosa* and *B. Pumillus*. Compound A6 exhibited highest antifungal activity against *A. niger* and *P. Chrysogenum*. Hence, it is concluded that there is ample scope for further study in developing these as good lead compounds for the treatment of bacterial strain as well as fungal strain.

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