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Synthesis, Characterization and Biological Evaluation of Some New Pyrazoline Derivatives

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Pyrazoline derivatives have been found to possess appreciable antitubercular activity against *Mycobacterium tuberculosis*. The present study aimed to synthesize some potent antitubercular pyrazoline derivatives. Different substituted pyrazoline derivatives were synthesized by cyclization of substituted chalcone derivatives in presence of hydrazine hydrate. Synthesized derivative were characterized by melting point, TLC, FT-IR, ^{1}H MR and MS spectrometry. Synthesized derivatives were evaluated for their *in vitro* antitubercular activity against *M. tuberculosis*. Derivatives JA_1, JA_2, JA_3 and JA_5 were found to be most potent and showed greater zone of inhibition than control which is comparable to standard drug rifampicin.

Key Words: Pyrazoline, Antitubercular activity, Chalcone, Hydrazine hydrate.

INTRODUCTION

Tuberculosis, an infectious disease caused by *Mycobacterium tuberculosis*, is the primary cause of mortality in the world. Mycobacteria are ubiquitous organisms that are becoming increasingly important intracellular pathogens that establish an infection in oxygen-rich macrophage of the lung. Resistance of *M. tuberculosis* strains to antimycobacterial agents is an increasing problem worldwide. However, powerful new anti TB drugs with new mechanism of action have not been developed in the last 40 years. In spite of severe toxicity on repeated dosing of isoniazid (INH); it is still considered to be a first line drug for chemotherapy of tuberculosis[1]. Recent chemotherapy of tuberculosis, suggested isoniazid and rifampin are the key components of first-line treatment of tuberculosis[2]. Pyrazoline derivatives are active against many mycobacteria. Pyrazoline are well known and important nitrogen containing 5-membered heterocyclic compounds and various methods have been worked out for their synthesis. Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field. Pyrazoline derivatives were found to have potential analgesic and antiinflammatory[3], antiamoebic[4], antimicrobial[5], antidepressant[6], antitubercular[7] and antimalarial activity[8]. They also found to exhibit a cytoxic activity, inhibitory activity of platelet aggregation. 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-tubercular agents against *M. tuberculosis*.

EXPERIMENTAL

Melting points were determined by Thieles tube method (Table-1) and were uncorrected. ^{1}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

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 (0.005 M) was poured with constant stirring, Substituted benzaldehydes (0.005 M) was added to the solution. The temperature of the mixture was kept at *ca.* 25 ºC and stirred vigorously until the mixture was thick enough to retard the stirring (4 h). The stirrer was removed and the reaction mixture was kept at 8 ºC 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 (Figs. 1 and 2).

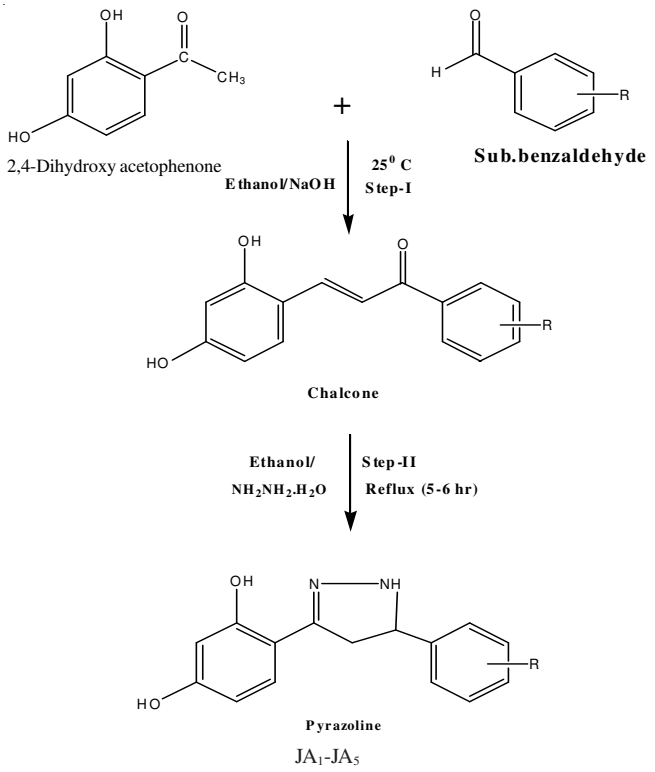
Procedure for synthesis of substituted pyrazoline derivatives

Addition and cyclization: In a mixture of substituted chalcone in ethanol, hydrazine hydrate was added drop wise

TABLE-1
PHYSICAL CONSTANTS OF SYNTHESIZED PYRAZOLINE DERIVATIVES

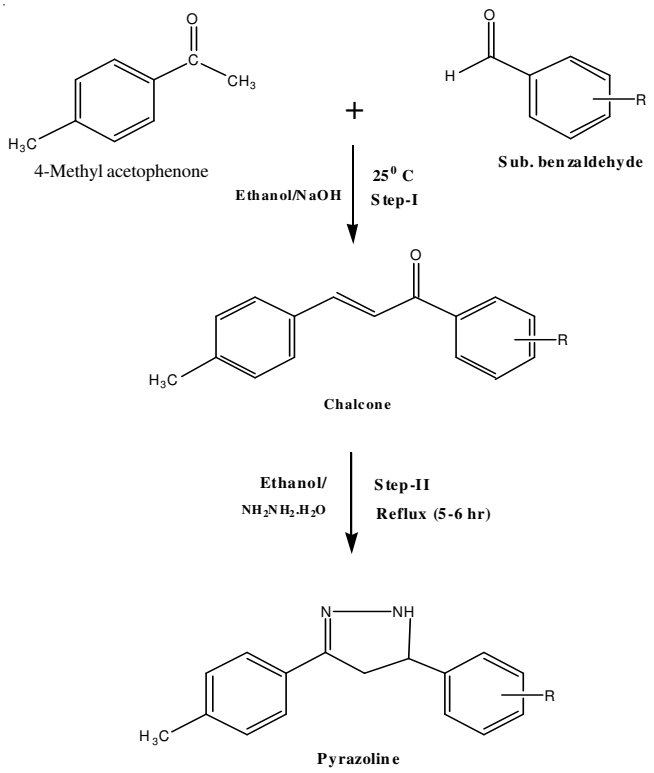
Compound code	m.f.	m.w. (g/mol)	Melting range (ºC)	Yield (%)	R_f value (Mean ± SD)*
JA_1	$C_{15}H_{13}N_2O_2Br$	333 ± 1.2	170-175	80 ± 0.1	0.68 ± 0.02
JA_2	$C_{15}H_{14}N_2O_3$	270 ± 1.3	190-195	75 ± 0.2	0.79 ± 0.01
JA_3	$C_{15}H_{13}N_2O_2Cl$	288 ± 1.5	185-190	80 ± 0.4	0.72 ± 0.04
JA_4	$C_{18}H_{20}N_2O$	344 ± 1.3	120-125	73 ± 0.5	0.65 ± 0.02
JA_5	$C_{17}H_{19}N_3O_2$	297 ± 1.1	110-115	68 ± 0.1	0.88 ± 0.03
JB_1	$C_{16}H_{15}N_2Br$	315 ± 1.6	105-110	60 ± 0.3	0.83 ± 0.01
JB_2	$C_{16}H_{16}N_2O$	352 ± 1.2	175-180	71 ± 0.2	0.74 ± 0.05
JB_3	$C_{16}H_{15}N_2Cl$	270 ± 1.4	160-165	80 ± 0.4	0.66 ± 0.02
JB_4	$C_{19}H_{22}N_2O_3$	326 ± 1.5	180-185	77 ± 0.6	0.89 ± 0.06
JB_5	$C_{18}H_{21}N_3$	279 ± 1.2	195-200	70 ± 0.5	0.47 ± 0.03

*(Mean ± SD), (n = 6).



JA_1-JA_5

Fig. 1. Scheme for the synthesis of 2,4-dihydroxy substituted pyrazoline derivatives. R = Br, OH, Cl, tri-OCH_3, $N(CH_3)_2$



JB_1-JB_5

Fig. 2. Scheme for the synthesis of 4-methyl substituted pyrazoline derivatives. R = Br, OH, Cl, tri-OCH_3, $N(CH_3)_2$

in a round bottom flask. The reaction mixture was heated under reflux for 6 h on a water bath followed with addition of ice cold water at room temperature. The mixture was kept overnight at 8 ºC. The precipitates were filtered, washed with distilled water and dried. The product was recrystallized with ethanol to get final product (Figs. 1 and 2).

Biological evaluation

***In vitro* antitubercular activity of synthesized derivatives:** All derivatives were dissolved in DMSO (100 mg/mL) and derivatives were filled in each well *i.e.*, 2.5, 5, 7.5 and 10 mg. DMSO was used as a control. Kirby-Bauer disk diffusion method for antibacterial stability test:

The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial activity. The *M. tuberculosis* strain were inoculated in nutrient broth and incubated at 37 ºC for overnight. After overnight incubation, the bacterial culture was swapped in solidified Muller hinton agar plate and wells of 6 mm diameter were punched in the plate. Then different concentration of derivatives were dispensed in separate wells such as 2.5, 5, 7.5 and 10 mg and 100 µL of DMSO to centre well was added as control. The plates were incubated at 37 ºC for 24 h. After incubation, the diameter of zone of inhibition around the wells were measured and recorded.

RESULTS AND DISCUSSION

Substituted pyrazoline derivatives were synthesized through cyclization of substituted chalcones. Ten such compounds were synthesized. They were evaluated for their *in vitro* antitubercular activity against *M. tuberculosis*. They showed potent antitubercular activity than control which is comparable to standard drug Rifampicin.

Structure of all the synthesized derivatives was established on the basis of their consistent IR, ^{1}H NMR and mass spectral data (Fig. 3). The synthesized derivatives showed the hydroxy and amine functional group along with the presence of

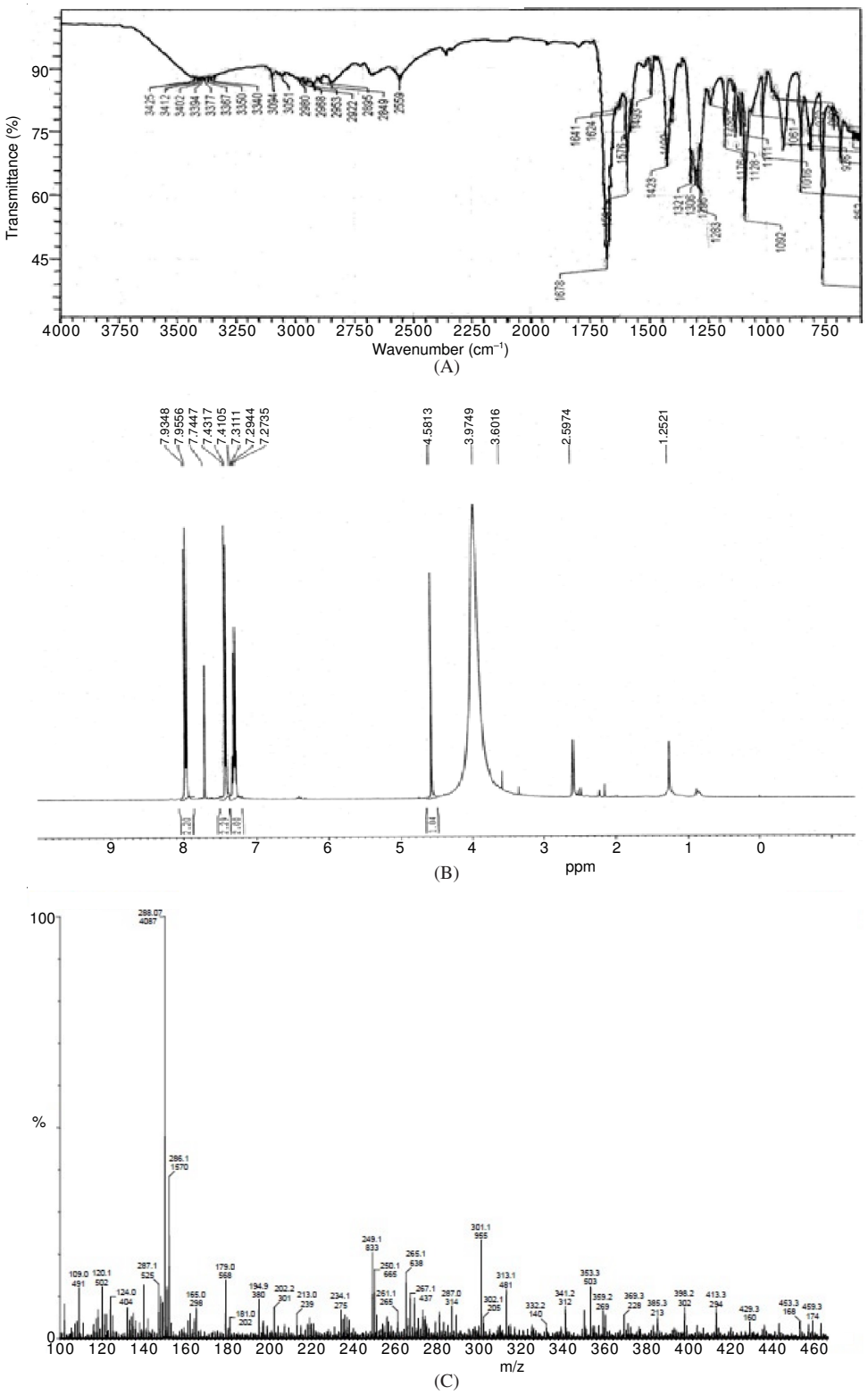


Fig. 3. (A) FT-IR, (B) ^{1}H NMR and (C) Mass spectra of the synthesized JA_3 derivatives

aromatic ring which was also evident in the ^{1}H NMR spectra (Table-2).

Derivatives JA_1, JA_2, JA_3 and JA_5 were found to be most potent antitubercular activity and derivatives JA_3 showed greater zone of inhibition than control which is comparable with the standard drug rifampicin at similar concentration (Table-3).

Conclusion

On the basis of the results, it may be concluded that derivatives with electron releasing groups such as N-dimethyl amine and electron widrawing groups such as hydroxy, chloro and bromo showed potent *in vitro* antitubercular activity against *M. tuberculosis* than control which is comparable to standard drug rifampicin. Derivative having groups such as

TABLE-2
SPECTRAL ANALYSIS OF THE SYNTHESIZED DERIVATIVES

Comp. code	IR spectra (cm^{-1})	Mass spectra (molecular ion peak)	^1H NMR spectra (δ) (ppm)
JA_1	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.).	332.02	7.40 (r, 4H, aromatic ring), 6.15-6.78 (s, 4H, aromatic ring), 7.25 (r, NH), 4.58 (r, 2H, OH), 3.64 (m, 1H), 1.25-2.59 (r, 2H, CH_2 in ring).
JA_2	3285 (N-H str.), 3259 (O-H str. of alcohol), 2930 (C-H str.), 1682 (C=C str.), 1531 (aromatic C=C str.), 1048 (C=C bend. aliphatic), 927 (C-H bend.).	270.10	6.62-7.39 (r, 4H, aromatic ring), 6.14-6.62 (s, 3H, aromatic ring), 7.1 (r, NH), 4.0 (r, 3H, OH), 3.7 (m, 1H), 1.5-2.1 (r, 2H, CH_2 in ring).
JA_3	3425 (O-H str. of alcohol), 3394 (N-H str.), 3350 (aromatic O-H str.), 2922 (C-H str.), 1641 (N-H bend.), 1576 (aromatic C=C str.), 1092 (C=C bend.), 925 (C-H bend.), 759 (C-Cl bend.).	288.07	7.27-7.43 (r, 4H, aromatic ring), 6.15-6.78 (s, 3H, aromatic ring), 7.2 (r, NH), 4.5 (r, 2H, OH), 3.9 (m, 1H), 1.2-2.5 (r, 2H, CH_2 in ring).
JA_4	2955 (aromatic C-H str.), 2839 (aliphatic C-H str.), 1622 (N-H bend.), 1504 (aromatic C=C str.), 1043 (C=C bend.), 849 (C-H bend.).	344.14	6.6 (r, 2H, aromatic ring), 6.18-6.75 (s, 3H, aromatic ring), 7.0 (r, NH), 5.0 (r, 2H, OH) 3.7 (m, 1H), 3.73 (s, 9H, CH_3), 1.8-2.0 (r, 2H, CH_2 in ring).
JA_5	3335 (O-H str. of alcohol), 2916 (C-H str.), 1649 (N-H bend.), 1582 (aromatic C=C str.), 1366 (C-H bend. of aliphatic), 1065 (C=C bend.), 997 (C-H bend.).	297.15	6.6-7.4 (r, 4H, aromatic ring), 6.15-6.78 (r, 3H, aromatic ring), 7.02 (r, NH), 5.0 (r, 2H, OH), 3.6 (m, 1H), 2.85 (s, 6H, CH_3), 1.3-2.1 (r, 2H, CH_2 in ring).
JB_1	2920 (C-H str.), 1684 (N-H bend.), 1647 (C=C str.), 1587 (aromatic C=C str.), 1009 (C=C bend.), 887 (C-H bend.), 614 (C-Br str.).	314.04	7.5 (r, 4H, aromatic ring), 7.0-7.01 (s, 4H, aromatic ring), 7.1 (r, NH), 3.9 (m, 1H), 2.35 (s, 3H, CH_3), 1.8-2.3 (r, 2H, CH_2 in ring).
JB_2	3329 (O-H str. of alcohol), 3319 (N-H str.), 1661 (C=C str.), 1516 (aromatic C=C str.), 1013 (C=C bend.), 868 (C-H bend).	252.13	6.90-6.92 (r, 4H, aromatic ring), 7.73-7.75 (s, 4H, aromatic ring), 7.7 (r, NH), 5.2 (r, H, OH), 3.60 (m, 1H), 2.32 (s, 3H, CH_3), 1.2-2.5 (r, 2H, CH_2 in ring).
JB_3	2955 (C-H str.), 1680 (C=C str.), 1593 (N-H bend.), 1506 (aromatic C=C str.), 1013 (C=C bend.), 816 (C-Cl bend.).	270.09	7.3-7.6 (r, 4H, aromatic ring), 7.00-7.01 (s, 4H, aromatic ring), 7.0 (r, NH), 3.9 (m, 1H), 2.35 (s, 3H, CH_3), 2.0 (r, 2H, CH_2 in ring).
JB_4	2922 (C-H str.), 1676 (C=C str.), 1591 (N-H bend.), 1508 (aromatic C=C str.), 1005 (C=C bend.), 922 (C-H bend.).	326.16	6.6 (r, 2H, aromatic ring), 7.1-7.4 (s, 4H, aromatic ring), 7.02 (r, NH), 3.9 (m, 1H), 3.73 (s, 9H, CH_3), 2.35 (s, 3H, CH_3), 1.8-2.0 (r, 2H, CH_2 in ring).
JB_5	2912 (C-H str.), 1595 (C=C str.), 1551 (N-H bend.), 1520 (aromatic C=C str.), 1063 (C=C bend.), 862 (C-H bend.).	279.17	6.6-7.6(r, 4H, aromatic ring),6.69-7.25(s,4H, aromatic ring),7.2(r,NH), 3.0 (m,1H), 2.85 (s, 6H, CH_3),2.35(s,3H,CH_3),1.2-2.0 (r, 2H,CH_2 in ring).

TABLE-3
IN VITRO ANTI-TUBERCULAR ACTIVITY OF SYNTHESIZED PYRAZOLINE DERIVATIVES

Compounds	Concentration of derivatives (zone of inhibition in mm)				Inhibition (%)
	2.5 mg	5.0 mg	7.5 mg	10 mg	
JA_1*	12	14	16	19	105.56
JA_2*	15	16	18	20	111.11
JA_3*	12	16	19	21	116.67
JA_4	–	–	11	12	66.67
JA_5*	11	14	18	20	111.11
JB_1	–	–	–	–	–
JB_2	–	11	13	15	83.33
JB_3	–	11	13	15	83.33
JB_4	–	–	13	15	83.33
JB_5	–	–	–	10	55.56
Standard (rifampicin) 10 mg			18		
Control (DMSO) 100 µL			–		

(–): Sign indicate as negative (no zone). *Indicates potent derivatives.

chloro showed potent antitubercular activity than the other derivatives. These results suggest that the pyrazoline derivatives have excellent scope for further development as commercial antituberculosis activity.

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