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# SYNTHESIS AND STUDY OF SOME PYRAZOLE DERIVATIVES AS ANTI TUBERCULAR AGENT.

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## ABSTRACT

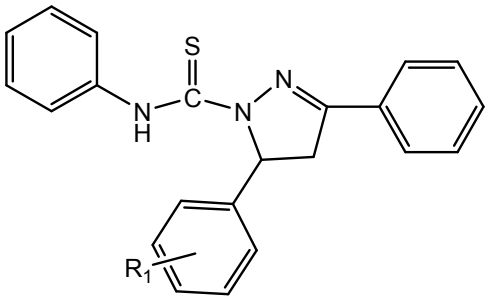
The present work deals with evaluation of anti-tubercular activity of various aldehyde derivatives synthesized by Claisen-Schmidt condensation method. The formation of pyrazole derivatives by reaction with phenyl isothiocyanate was also attempted. The synthesized derivatives were screened for anti-tubercular activity and the compounds. All the Compounds characterized on the basis of their IR, MASS, 1H NMR spectroscopic data analysis.

## KEYWORDS

Synthesis,condensation,Pyrazolines,Antimycobacterial.

## INTRODUCTION

**DEFINITION:** **Tuberculosis** or **TB** is a common and often deadly infectious disease caused by mycobacteria, usually *Mycobacterium tuberculosis* in humans. Tuberculosis usually attacks the lungs but can also affect other parts of the body. It is spread through the air, when people who have the disease cough, sneeze, or spit Tuberculosis (TB) is the most prevalent communicable infectious disease on earth and remains out of control in many developing nations. These nations require medical and financial assistance from developed nations in order to control the spread of TB globally.Because of long duration of therapy, we have to design such type of molecules having minimum adverse effects,high potency & low cost.Mycobacteria posses a thick and highly lipophilic cell wall. So a drug to be more effective towards mycobacterium should posses an effective pharmacophore. We have plan to synthesised different substitutions on pyrazole moiety at 5-position and to evaluate their activity against mycobacteriae.




## EXPERIMENTAL SECTION:

Melting points were determined in open capillary tubes and are uncorrected. IR spectra carried out on Perkin-Elmer FTIR spectrophotometer ($cm^{-1}$, in KBr). $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker spin spectrometer (400 MHz) in $CDCl_3$ and TMS was used as internal standard. Peak values are shown in ppm, in the d scale. Mass spectra were recorded on a Waters LC-MS. Elemental analyses were carried out on Perkin-Elmer analyzer.

***General procedure for 3-(substitutedphenyl)-1-phenylprop-2-en-1-one (3a-c).***

A mixture of acetophenone (1.5017 g, 0.01 mmol), appropriate aldehyde(0.01 mmol) in ethanol and sodium hydroxide (30%,5 mL) in presence of 10 mL of petroleum ether was stirred under room temperature for 4 h. The resulting solution was allowed to stand overnight and poured into ice-cold water, and then it was neutralized with hydrochloric acid. The solid so obtained was filtered, dried and crystallized from ethanol.

***General procedure for 5-(substituted phenyl-3-phenyl-4,5-dihydro-1H-pyrazole(4a-c).***

To a solution of chalcone (**3a-c**) in ethanol,hydrazine hydrate (99%) was added dropwise. The reaction mixture was heated under reflux for 7 h and then cooled and poured onto crushed ice. The solid pyrazoline product was filtered and recrystallized from ethanol.

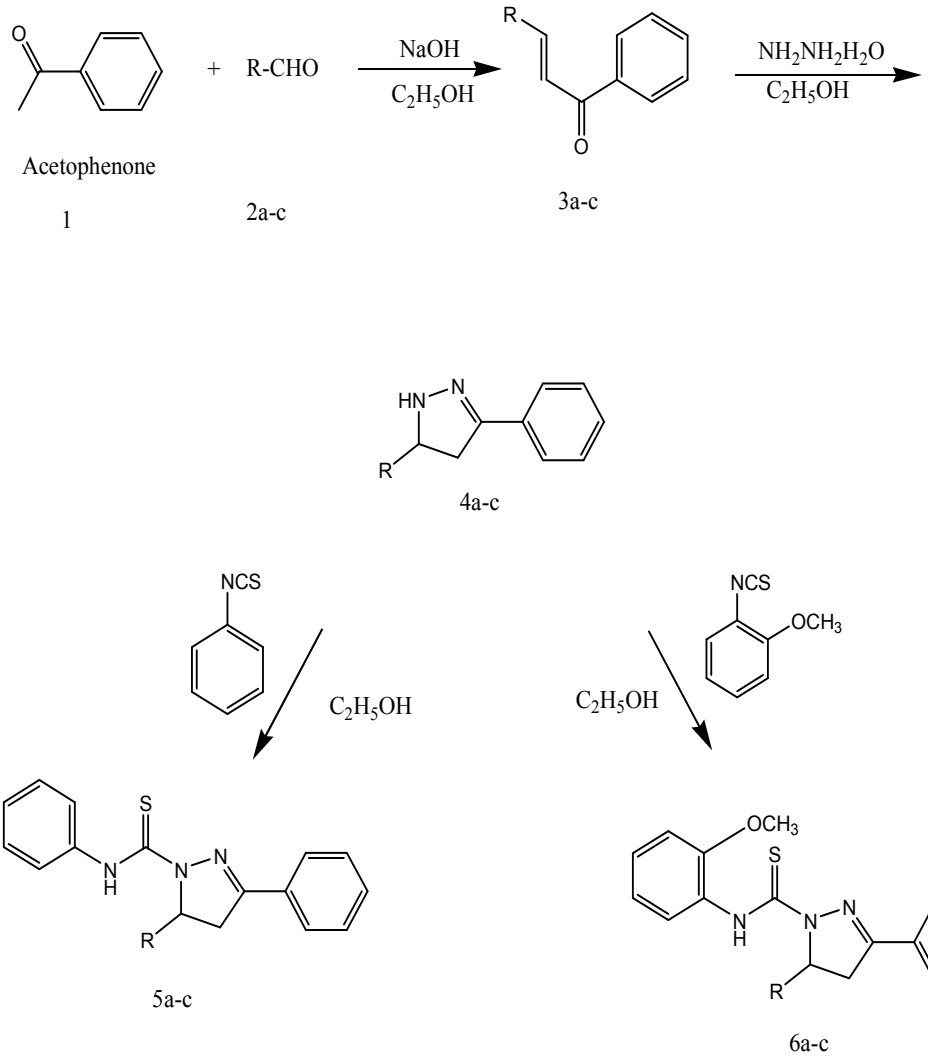
***General procedure for 5-(substituted phenyl)-N-(2-methoxyphenyl)-3-phenyl-4,5-dihydropyrazole-1-carbothioamide(5a-c).***

1-isothiocyanatobenzene (0.01 mol) was added to a solution of pyrazoline (**4a-c**) (0.01 mol) in ethanol (20 mL). The reaction mixture was refluxed for 4 h and after cooling it was poured onto crushed ice. Then, the separated solid mass was filtered, washed with water and crystallized from ethanol.

***General procedure for 5-(substituted phenyl)-N, 3-diphenyl-4, 5-dihydropyrazole-1-carbothioamide (6a-c).***

To a solution of pyrazoline **(4a-c)** in ethanol (20 mL) 1-isothiocyanato-2-methoxybenzene (0.01 mol) was added and the reaction mixture was refluxed for 4 h. Then, after cooling, the reaction mixture was poured onto crushed ice and the separated solid mass was filtered, and crystallized from ethanol.

## Scheme




## RESULT AND DISCUSSION

***Physical characteristics of synthesised compounds:***

| Compound No. | R | Yield (%) | M.P. ($^0$C) | Mol.formula | Mol.weight | $R_f$ |
|---|---|---|---|---|---|---|
| 5a | 2-Cl phenyl | 85 | 58-60 | $C_{22}H_{18}ClN_3S$ | 391.92 | 0.4 |
| 5b | p-hydroxy phenyl | 77 | 178-180 | $C_{22}H_{19}N_3OS$ | 373.47 | 0.6 |
| 5c | cinnamaldehyde | 82 | 128-130 | $C_{24}H_{21}N_3S$ | 383.51 | 0.4 |
| 6a | 2-Cl phenyl | 90 | 58-60 | $C_{23}H_{20}ClN_3OS$ | 421.94 | 0.4 |
| 6b | p-hydroxy phenyl | 75 | 138-140 | $C_{22}H_{20}N_3O_2S$ | 390.48 | 0.5 |
| 6c | cinnamaldehyde | 76 | 150-152 | $C_{25}H_{23}N_3OS$ | 413.53 | 0.5 |

### *Spectral data of synthesised compounds:*

| Compound Code | IR($cm^{-1}$,KBr) | Mass (m/e) | $^1$H NMR ($CDCl_3$,ppm) |
|---|---|---|---|
| 5a | ~3220(NH), ~1590(C=N), ~1320(C-N), ~1130(C=S), ~748(C-Cl) | 390(M+) 392(M+2) | 1.2(3H,s),2.1(1H,s),6.8(2H,t), 7.0(1H,t),7.5(5H,m), |
| 5b | ~3220(NH), ~1590(C=N), ~1320(C-N), ~1130(C=S), ~3307(OH) | 372.1(M+) 374.6(M+2) | 1.2(3H,S),2.1(1H,S),6.9(2H,d) 7.2(1H,t),7.5(5H,q)3.4(2H,d) |
| 5c | ~3220(NH), ~1590(C=N), ~1320(C-N), ~1130(C=S), ~1600(C=C) | 382.3(M+) 384.1(M+2) | 3.9(1H,s),3.8(1H,t),7.7(2H,d), 7.4(2H,t),2.1(1H,s) |
| 6a | ~3220(NH), ~1590(C=N), ~1320(C-N), ~1130(C=S), ~748(C-Cl) | 420.2(M+) 422.1(M+2) | 7.5(5H,m),7.3(3H,t),7.2(2H,m), 3.2(2H,d),3.8(1H,q) |
| 6b | ~3220(NH), ~1590(C=N), ~1320(C-N), ~1130(C=S), ~3307(OH) | 389.2(M+) 391.1(M+2) | 3.8(1H,q),3.2(2H,d),7.5(5H,m), 3.2(1H,d),3.9(1H,s) |
| 6c | ~3220(NH), ~1590(C=N), ~1320(C-N), ~1130(C=S), ~1600(C=C) . | 412.8(M+) 414.1(M+2) | 7.8(4H,m),3.8(1H,t),3.2(2H,d), 7.5(5H,m),6.8(1H,S) |

## ANTITUBERCULAR ACTIVITY

### Evalution techniques:-

Three well-known measures of sensitivity test are available:

(1) The minimal inhibitor concentration or the MIC,

These tests are set up on solid media.

***(1) The minimal inhibitor concentration:***

MIC is defined as the minimal concentration of the drug required to inhibit the growth of the organisms, where growth is defined as 20 colonies or more .This definition of growth is chosen so that only a small proportion (e.g. 1%) of wild strains would be classified as resistant by its use. This method is simple and be carried out with a single drug

containing slope although it is preferable to use more than one slope.

We have used the **minimal inhibition concentration** to evaluate the anti-tuberculosis activity. It is one of the non automated in vitro bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in bottle.

***DETERMINATION OF MINIMAL INHIBITION CONCENTRATIONS BY L.J SLOPE METHOD***
***MATERIALS AND METHOD:-***

| SR.NO | CODE NO | M.TUBERCULOSIS [µg/ml] |
|---|---|---|
| 1 | 5a | 25 |
| 2 | 5b | 62.5 |
| 3 | 5c | 250 |
| 4 | 6a | 500 |
| 5 | 6b | 6.25 |
| 6 | 6c | 25 |

1 All the Synthesized Drugs Were Used For Anti-tubercle Test Procedures
2 All Necessary Controls Like:

- Drug Control
- Vehicle Control
- Agar Control
- Organism Control
- Known Antibacterial Drugs Control
- M.tuberculosis H37 RV Cultures Were Tested Against Above Mentioned Known and Unknown Drugs.
- L.J Was Used As Nutrient Medium To Grow And Dilute The Drug Suspension For The Test.
- Inoculum Size for Test Strain Was Adjusted to 1mg/ml.
- Following common standard strain is used for screening of antitubercle activities: The strains were procured from Institute of Microbial Technology, Chandigarh.
- Mycobacterium tuberculosis H37Rv [Acid Fast Bacilli] MTCC – 200

DMSO was used as diluents / vehicle to get desired concentration of drugs to test upon Standard bacterial strains.

## METHODS USED FOR PRIMARY AND SECONDARY SCREENING

Each synthesized drug was diluted obtaining 2000 microgram /ml concentration, as a stock solution.

***Primary screen:*** In primary screening 500 micro/ml, 250 micro/ml, and 125 micro/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

***Secondary screen:*** *The* drugs found active in primary screening were similarly diluted to obtain 100 micro/ml, 50 micro/ml, 25 micro/ml, 12.5 micro/ml, 6.250 micro/ml, 3.125 micro/ml and 1.5625 micro/ml concentrations.

***Reading Result:-*** The highest dilution showing at least 99 % inhibition is taken as MIC.The result of this is much affected by the size of the inoculum. The test mixture should contain $10^8$ organism/ml.

***The Standard Drugs:-***

The Standard strain M.tuberculosis, $H_{37}$ RV is tested with each new batch of medium. The recommended drug concentrations are 4 mg/l for streptomycin, 0.2 mg/l for isoniazide, 40 mg/l for Rifampicin and 2 mg/ l for ethambutol.

## CONCLUSION

Compound 6b is good activity compare to other compound and compound 6a is better yield compare to other synthesized compounds.

## ACKNOWLEDGEMENT

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