Spectrum of Engineering Sciences
ISSN (e) 3007-3138 (p) 3007-312X Volume 3, Issue 9, 2025

SYNTHESIS AND CHARACTERIZATION OF VARIOUS ACETYL PYRAZOLINE (5a-b)

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DOI: https://doi.org/10.5281/zenodo.17130342

Keywords
Pyrazolines, N-acetyl derivatives, Chalcones, Antioxidant activity, FRAP, DPPH

Article History
Received: 14 June 2025
Accepted: 24 August 2025
Published: 16 September 2025

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Abstract
Background: Pyrazolines, a significant class of nitrogen-containing heterocycles, are widely recognized for their diverse pharmacological properties. N-acetylated pyrazolines, in particular, have attracted interest due to their potential antioxidant and antimicrobial activities. Materials and Methods: Chalcones were synthesized via Claisen–Schmidt condensation of acetophenone with substituted benzaldehydes, followed by oxidative cyclization using hydrazine hydrate to yield pyrazolines. Subsequent acetylation with glacial acetic acid under reflux afforded acetyl pyrazoline derivatives (5a–b). The compounds were characterized through UV/Vis, IR spectroscopy, and melting point analysis. Antioxidant potential was evaluated using DPPH radical scavenging and ferric reducing power assays, while antimicrobial activity was determined by agar disk diffusion method. Results: Compound 5a exhibited superior free radical scavenging at lower concentrations, reaching 70.59% at 1 mg/ml, whereas compound 5b showed moderate activity at low concentrations but comparable efficacy at higher doses. In FRAP assay, 5b demonstrated greater reducing power (42.8%) than 5a (33.5%). Conclusion: The synthesized N-acetyl pyrazolines (5a–b) exhibit promising antioxidant properties with structural variations influencing activity, supporting their potential as pharmacologically valuable scaffolds.

INTRODUCTION

Pyrazolines are an interesting class of organic compounds with various pharmacological activities (Sridhar S., Rajendarparasad Y. 2011). Pyrazoline is a heterocyclic five membered moiety having two adjacent nitrogen atoms in the same ring with an endocyclic double bond and its basic nature (Ganesh A. 2013). Pyrazolines are famous as they are intermediate for the synthesis of various bio-active compounds, like pyrimidine, isoxazolines and thiazolines. These are synthesized by oxidative cyclization of Chalcone, these are are very novel because of the synthesis of various bioactive molecules and their therapeutic values (Khalil M. O. 2011).

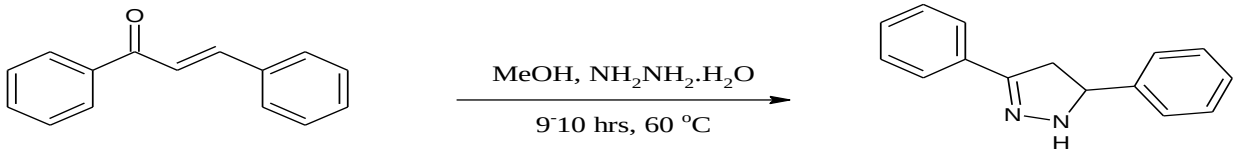


Fig. 1.1 Preparation of Pyrzoline

Pyrazolines can be obtained in good yields by treating chalcones with different reagents in the presence of different catalyst such as; phenylhydrazine, hydrazine, hydrazine hydrate, in hot pyridine or in glacial acetic acid (Sridhar S., Rajendarparasad, Y. 2011). Several routes have been used to synthesize pyrazolines in lab as well as on industrial levels. A most familiar procedure for preparation of pyrazolines is; reaction of α, β unsaturated ketone with phenylhydrazine (Bashir R. et al., 2011), by ultrasonic method pyrazolines can be synthesized (Gupta G.*et al.,* 2010)

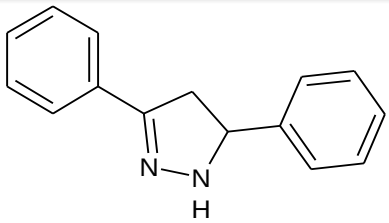
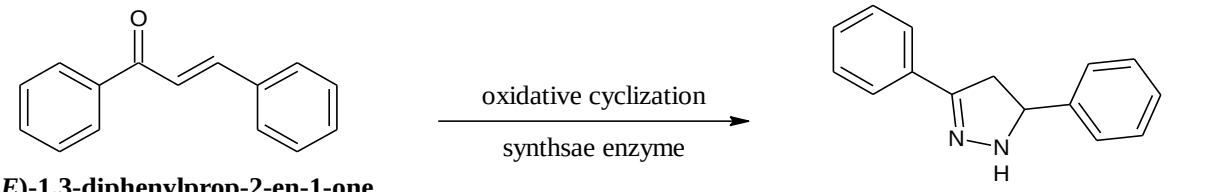


Fig. 1.2: 3,5-diphenyl-4,5-dihydro-1H-Pyrazole

In plants, pyrazolines naturally forms by simple rearrangement as well as cyclization in the presence of synthase enzyme(Nakayama et al., 2000).



(2*E*)-1,3-diphenylprop-2-en-1-one

3,5-diphenyl-4,5-dihydro-1*H*-pyrazole

Fig. 1.3: Oxidative Cyclization

Pyrazolines have captured the attention of medicinal chemist because of their heterocyclic chemistry and pharmaceutical activities, for that different methods have been used among the world for their preparation. Because of their interesting biological activities, consider able attention has been focused on substituted pyrazolines and simple pyrazolines.

Intensive scientific significance have been developed on the structure of pyrazoline because of its wide range of natural activities such as antiamoebic (Abid M. *et al.*, 2006) (Abid M. *et al.*, 2009), antimicrobial (Abdel Wahab A. F. *et al.*, 2009), monoamine oxidase inhibitors (Sahoo A. et al., 2010), antimycobacterial (Ali A. A. *et al.*, 2007), anti-depressant (Prasaad Y. R. *et al.,* 2005), Anti convulsant (Ozidemir Z. *et al.,* 2007) and anti-inflammatory (Rathish I. G. et al., 2009) activities. It is significant to discuss that morphine derivatives have been assumed to show antiinflammatory (Verma M. *et al.,*1984) and Anti-microbial (Panneerselvam, P. et al., 2005) activities. Beside these biological activities, pyrazolines are used for the cure of Parkinson's, Alzheimers disease and cerebral edema (Kawazura H. et al., 1997).. Pyrazolines are also used for organic synthesis of various novel compounds (Padmavathi V. et al., 1999). Hence, chalcones and its pyrazolines derivatives have precious and essential biological activities which made these compounds useful moiety in pharmacological research.

Acetyl Pyrazolines derivatives are the significant class of nitrogen-containing 5-membered heterocyclic compounds. Series of N-acetyl pyrazolines have been investigated for their antimicrobial ability.

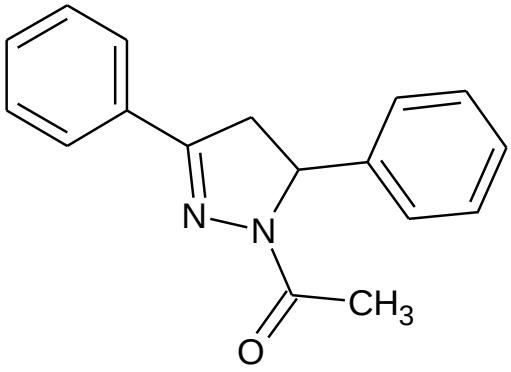


Fig. 1.4: N-acetyl Pyrazoline

N-acetyl pyrazolines are prepared by the reaction of chalcones with phenylhydrazine hydrate in the occurrence of pyridine. (Levai, A. & Jeko, J. 2009). Different acidic media and catalysts have been used for the synthesis of N-acetyl pyrazolines in recent decades, such as chalcones react with hydra*zine*

hydrate in occurrence of Glacial acetic acid to get N-acetylated pyrazolines (Suman et al., 2011).

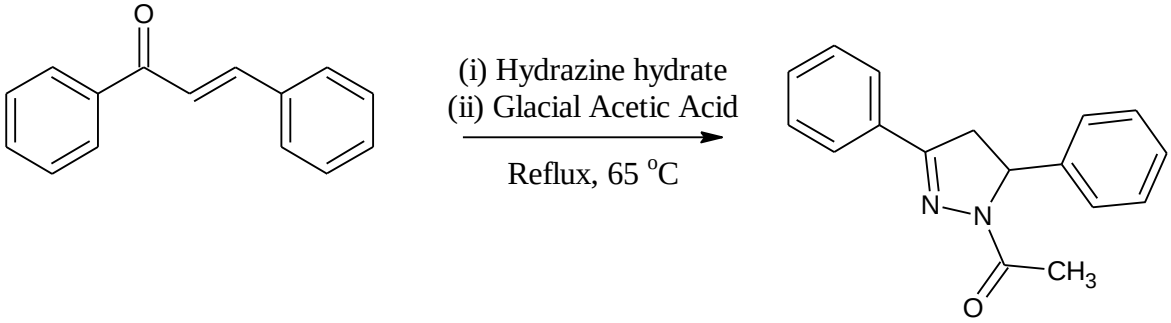


Fig. 1.5: Preparation of Acetyl Pyrazoline

1.1 Research Objective

- Investigate more routes to synthesize acetyl pyrazolines in labs by various synthetic methods as pyrazolines have played vital role in diverse pharmacological applications.
- create acetyl pyrazoline derived from pyrazoline
- Characterized the pyrazolines and its derivatives by using UV/Vis, IR, and Mass Spectra.

2. Materials and Methods

All the required chemicals, reagents and solvents were purchased from the local suppliers of Alfa-Aesar, sigma Aldrich & Merck from Urdu bazar at Aabqari road. All the chemicals were of analytical grade and not polluted with any kind of impurity. In research laboratory of Chemistry at University of Education, Division of science and Technology Township campus Lahore, all the experimental work was done for the synthesis of acetyl pyrazoline; while compounds were characterized from LUMS university Lahore, Government College University (GCU), HEJ Research Institute Karachi.

2.1 Matrix of Materials Required

Category	Items
Apparatus	Pipette, Measuring flask, Round bottom flask, Spatula, UV lamp for TLC, TLC tank, TLC plate, Filter paper, Vials, Separating funnel, Magnetic stirrer, Iron stand with clamp, Lab syringes, Beaker dropper, Petri dish, Hot plate, Funnel, Lab coat, Melting point apparatus, Condenser with pipes, Weighing balance, TLC tweezer, Conical flask, Capillary tubes
Chemicals	2-methoxy benzaldehyde, Acetophenone, 2,4,5-trimethoxy benzaldehyde, 3,4,5-trimethoxy benzaldehyde, 2,4-dimethoxy benzaldehyde, Benzaldehyde, Dichloromethane, Acetic acid, Methanol, n-hexane, Sodium hydroxide, Ethanol, Hydrochloric acid
Instruments	Magnetic hotplate, HNMR Spectroscopy, Mass spectroscopy, FTIR spectroscopy, Weighing balance

2.2 Method

Synthesis of acetyl pyrazolines comprises of three different steps. First one is Claisen Schmidt reaction of acetophenone and different substituted benzaldehyde to yield Chalcones. Second step involves oxidative cyclisation of Chalcones in the presence of hydrazine hydrate to synthesize pyrazolines. In third step, acetyl group is introduced on pyrazoline ring by the reaction of glacial acetic acid by respective pyrazolines.

2.3 Experimental Work

Different Chalcones derivatives, pyrazolines and their acetyl pyrazoline derivatives were synthesized and characterized for their biological activity. The experimental work was done in tow portion;

- Synthesis of Acetyl pyrazoline derivatives
- Biological screening of synthesized compounds

2.4 Methodology for the Preparation of Various Acetyl Pyrazolines (5a-E)

2.4.1 1-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl) ethenone

A mixture of pyrazoline derivative (0.01moles) and glacial acetic acid (10 ml) was heated under gentle reflux for 3 hrs. The reaction progress was monitored time to time with the help of thin layer chromatography (TLC) and formation of product was examined under UV lamp. Then it was cooled with the crushed ice water bath until 0^{O}C. By using solvent extraction mechanism, synthesized compound was separated. Dichloromethane was used as solvent. After separation organic solvent was evaporated and pure crystals of acetyl pyrazoline were obtained and stored for biological screening.

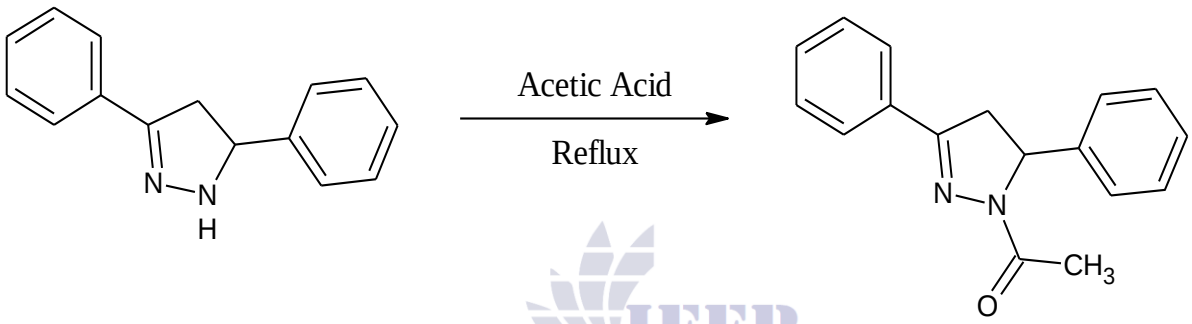


Fig. 2.1: Synthesis of 1-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl) ethenone

2.4.2 1-[3-phenyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl] ethenone

0.01moles of pyrazoline derivative and 10 ml glacial acetic acid was heated under gentle reflux for 3 hrs. The reaction progress was monitored time to time with the help of thin layer chromatography (TLC) and formation of product was examined under UV lamp. Then it was cooled with the crushed ice water bath until 0^{O}C. By using solvent extraction mechanism, synthesized compound was separated. Dichloromethane was used as solvent. After separation organic solvent was evaporated and pure crystals of acetyl pyrazoline were obtained and stored for biological screening.

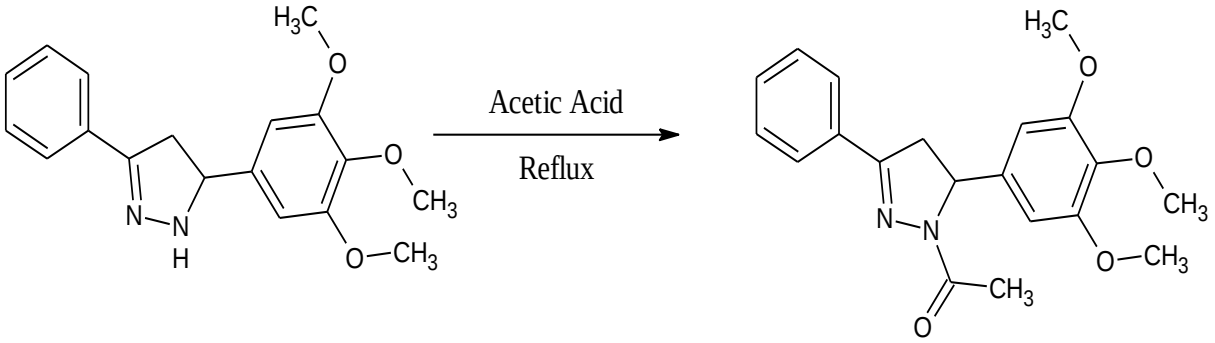


Fig. 2.2: 1-[3-phenyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl] ethenone

2.5 Biological Screening of Synthesized Compounds

2.5.1 Antioxidant Activity

To evaluate antioxidant activity of synthesized pyrazoline and chalcone derivatives, reducing power assay and free radical scavenging activity was done.

- **Free DDPH Radical Scavenging Activity**

For antioxidant activity of synthesized compounds, DDPH test was carried out. By adding Gallic acid and DCM to all synthesized compounds 0.25, 0.5, 0.75, 1.0 and 1.5 dissolutions were prepared. About 0.02 mM (4ml) DDPH was poured in each reaction mixture and for 20 min were stored in dark , absorbance was recorded at 720nm. (Zhaung *et al.,* 2012)

The scavenging activity was calculated by using following formula;

Scavenging Activity (%) = (C observed – C sample) /C observed x 100

C observed= Absorbance of the Solvent

C sample= Absorbance of Test Samples

- **The Ferric Reducing Power Activity**

By applying Oyaizu method, Reducing power of prepared compounds was calculated. Each acetyl pyrazoline and chalcone derivatives were dissolved in (0.175 mg/ml) ethanol. Then 0.2M Sodium phosphate buffer (pH 6.5, 2.5 ml) and 1% potassium ferricyanide (2.5 ml) were added to each dissolution. At 50°C all the solutions were incubated for 20 min and 10% w/v trichloroacetic acid (2.5ml)was added in to the reaction mixture. Then were centrifuged for 10 min at speed of 10,000 rpm, and to the supernatant 0.1% ferric chloride (0.5ml) and distilled water (2.5ml) were added. In a spectrophotometer, absorbance was recorded at 700nm and was related with that of control. By using following formula, for each compound reduction potential was calculated;

% Reducing power activity= (Ac – As) / Ac x 100

- Ac= absorbance of control
- As= absorbance of test sample

2.5.2 Antimicrobial Activity

An antimicrobial activity that is antibacterial activity of N-acetyl pyrazoline derivatives were measured by using Agar Diffusion method.

- **Agar Disk Diffusion Method**

For *in vitro* investigation of antibacterial activity of synthesized compounds against E. coli, P. aeruginosa, S. aureus and S. epidermis agar disk diffusion method and minimum inhibition concentration was measured in µg/ml in DMSO (it's a control solvent). For antibacterial activity Penicillin G. was used as standard drug. All activities were checked three times while mean value of all three experimental result was recorded.

About 6mm in diameter agar paper disks are used, carrying the test samples of fix concentrations placed on the agar surface. The petri dishes are incubated at 35-37°C temperature for about 16-18 hrs. During the incubation period, antimicrobial agent inhibits the growth of test microorganism by diffusing into the agar. After incubation, the diameter of inhibition zones is measured.

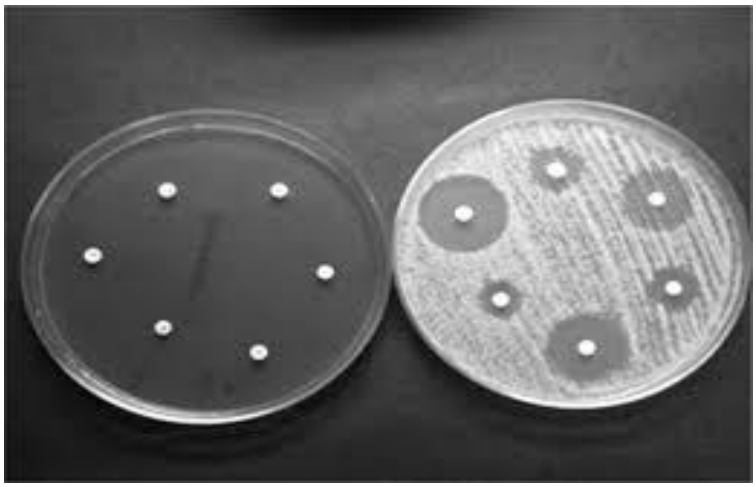


Fig. 2.3: Agar Disk Plate after 16 hrs. Incubation

3. Results

3.1 Characterization Of 1-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)ethenone

Chemical formula: $C_{17}H_{16}N_2O$
Molecular weight: 264.3g/mol
IR Stretching: C=N 1667, C=O 1590, N-H 3053
M.P: 136^0
Uv/Vis : Crescent Peak

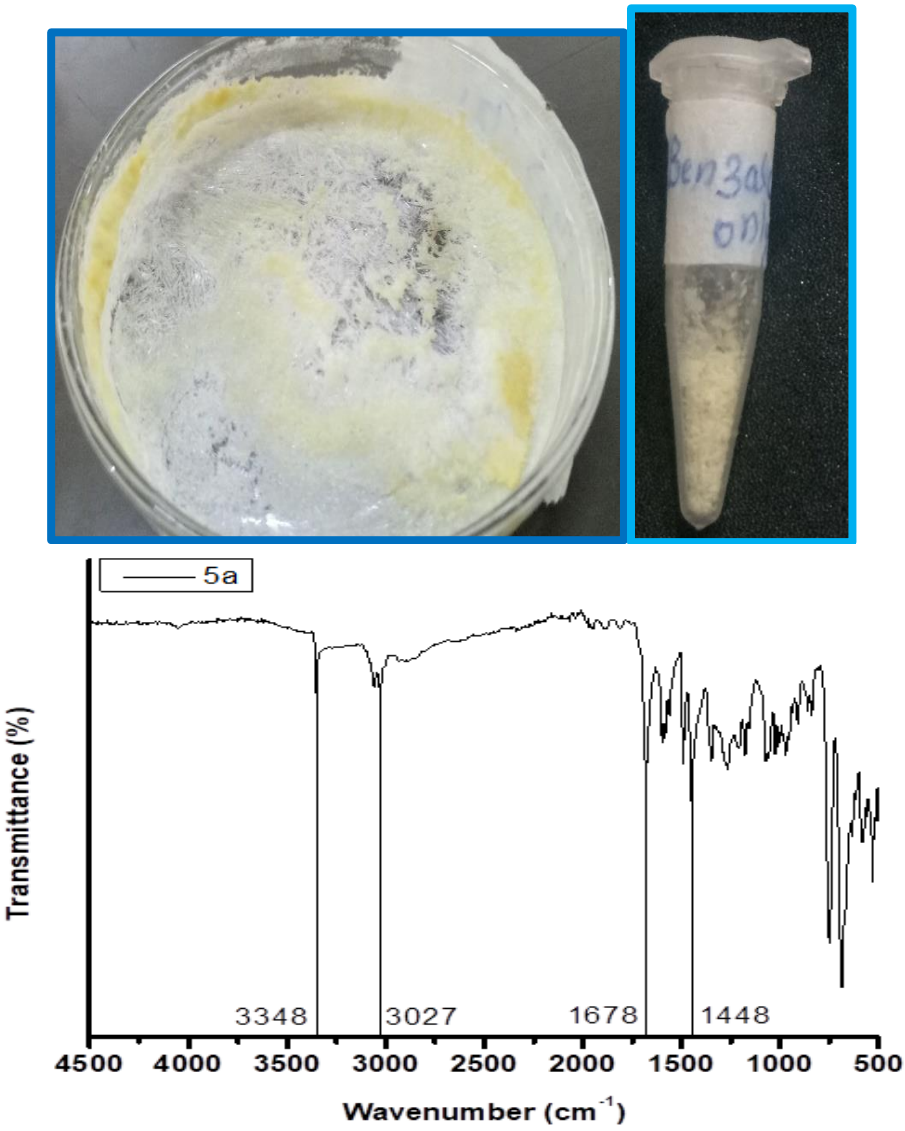
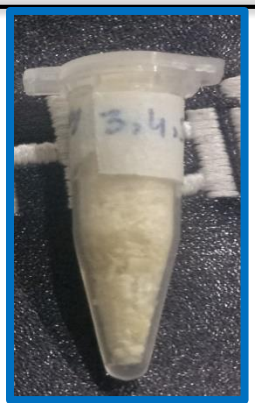
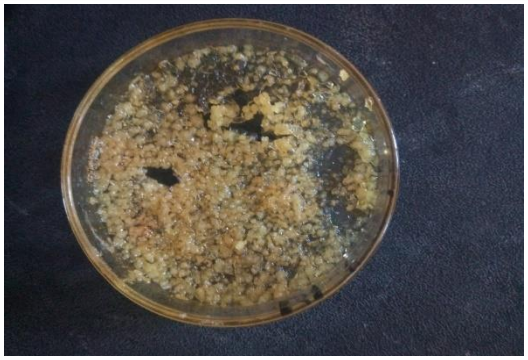


Fig. 3.1: IR spectra for compound 5a

3.2 Characterization Of 1-[3-phenyl-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl] ethanone

Chemical formula: $C_{20}H_{22}N_2O_4$
Molecular weight: 354
IR Stretching: 1661 C=N, 1579 C=C, 1125 C-N, 2934 C-H
M.P: 140^0
Uv/Vis : Crescent Peak, UV visible





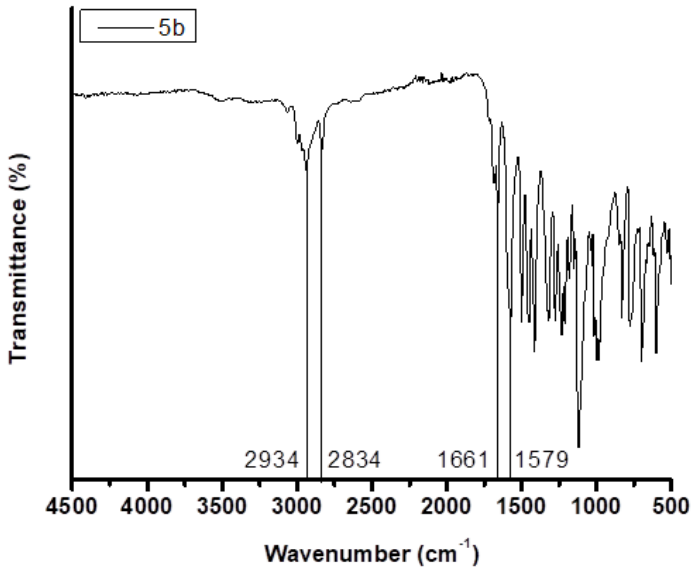


Fig. 4.2: IR spectra for compound 5b

3.3 Results of Antioxidant activities of N-acetylated pyrazolines

3.3.1 Free radical scavenging activity (DDPH)

The antioxidant potential of synthesized pyrazoline derivatives (5a and 5b) was evaluated using the DPPH free radical scavenging assay at concentrations ranging from 0.25 to 1.0 mg/ml.

- Compound 5a demonstrated a consistently higher scavenging activity compared to 5b across all concentrations. At the lowest tested concentration (0.25 mg/ml), it already exhibited 55.08% scavenging, which increased gradually with concentration, reaching 70.59% at 1 mg/ml. This indicates a dose-dependent increase in activity.
- Compound 5b showed moderate activity at lower concentrations, with 25.75% at 0.25 mg/ml and 30.56% at 0.75 mg/ml. However, a marked increase was observed at the highest concentration, where scavenging activity rose sharply to 67.54% at 1 mg/ml.

Overall, both derivatives displayed antioxidant activity in a concentration-dependent manner, but 5a was more potent at lower concentrations, whereas 5b required higher concentrations to exhibit comparable activity.

Table 3.1: Percentage radical scavenging of synthesized pyrazolines derivative

Sr. No.	Concentration of samples (mg/ml)	0.25	0.5	0.75	1
1.	5a	55.08	55.35	57.34	70.59
2.	5b	25.75	28.43	30.56	67.54

3.3.2 Ferric Reducing Power Assay (FRAP)

The ferric reducing power of the synthesized N-acetyl pyrazoline derivatives was assessed at a concentration of 0.1 mg/ml, measuring absorbance at 700 nm.

- Compound 5a exhibited an absorbance of 0.81, corresponding to a reducing power of 33.5%. This indicates a moderate ability to donate electrons and reduce ferric ions.
- Compound 5b showed a slightly lower absorbance value (0.73) but surprisingly demonstrated a higher reducing power of 42.8%. This suggests that despite the lower optical density at 700 nm, 5b possessed comparatively stronger electron-donating and reducing capacity than 5a at the tested concentration.

Overall, both compounds revealed notable reducing potential, with compound 5b being more effective in FRAP assay, while 5a showed higher absorbance but relatively lower reducing percentage.

Table 3.2: Reduction Potential of Synthesized N-acetyl Pyrazolines

Sr. No.	Acetyl pyrazoline (0.1 mg / ml)	Absorbance of sample (700nm)	Reducing power (%)
1.	5a	0.81	33.5%
2.	5b	0.73	42.8%

4. Summary and Conclusion

3.1 Summary

The present study focused on the synthesis, characterization, and biological screening of acetyl pyrazoline derivatives (5a–b). Chalcones were initially prepared through Claisen–Schmidt condensation, followed by oxidative cyclization to yield pyrazolines. Subsequent acetylation of pyrazolines in the presence of glacial acetic acid under reflux conditions afforded the target compounds. The synthesized derivatives were characterized by UV/Vis spectroscopy, IR spectroscopy, and melting point determination, confirming the expected functional groups and structural moieties.

Biological screening emphasized antioxidant evaluation through DPPH radical scavenging and FRAP assays. Results revealed that both compounds exhibited concentration-dependent free radical scavenging ability, with compound 5a showing superior activity at lower concentrations while compound 5b matched comparable activity only at higher doses. Conversely, FRAP assay demonstrated that compound 5b possessed stronger reducing power (42.8%) than compound 5a (33.5%), highlighting differences in their electron-donating potential. These findings indicate that the synthesized derivatives possess noteworthy antioxidant activity, with complementary mechanisms of action.

3.2 Conclusion

The successful synthesis and characterization of acetyl pyrazoline derivatives (5a–b) highlight their potential as pharmacologically valuable heterocyclic compounds. Compound 5a demonstrated higher radical scavenging activity, whereas compound 5b showed superior ferric reducing capacity, suggesting that structural modifications influence antioxidant behavior. Overall, the study confirms that N-acetyl pyrazolines are promising scaffolds for further exploration in medicinal chemistry, particularly in the development of therapeutic agents with antioxidant and antimicrobial potential.

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