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DESIGN& SYNTHESIS OF ISOXAZOLE DERIVATIVES FROM CHALCONES FOR ANTI-MICROBIAL ACTIVITY

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Abstract:

Isoxazole derivatives are important heterocyclic compounds known for their diverse pharmacological activities, including antimicrobial, anti-inflammatory, anticancer, and analgesic properties. The incorporation of an isoxazole ring into organic molecules often enhances their biological potential. In the present study, a series of chalcone intermediates were synthesized through condensation reactions and subsequently converted into corresponding isoxazole derivatives using hydroxylamine reagents.

METHODOLOGY: Five chalcone derivatives (AB-1 to AB-5) were synthesized by condensation reactions between benzaldehyde and various active methylene compounds or ketones in the presence of sodium hydroxide under controlled conditions. The reactions were carried out in ethanol medium with continuous stirring and temperature control. The formation of chalcones was monitored by TLC using ethyl acetate-based mobile phases. The obtained chalcone intermediates were further reacted with hydroxylamine or hydroxylamine hydrochloride in ethanolic medium under alkaline conditions. Cyclization reactions were performed under reflux at temperatures ranging from 70–80°C for 2–4 hours to obtain the corresponding isoxazole derivatives. The products were isolated by precipitation, filtration, washing with water, drying, and recrystallization. TLC analysis was employed throughout the synthesis to confirm completion of reactions and assess purity.

RESULTS: All five chalcone intermediates were successfully synthesized and converted into their respective isoxazole derivatives. TLC analysis revealed distinct spots with improved purity after recrystallization, confirming successful product formation. The cyclization of chalcones with hydroxylamine proceeded efficiently under alkaline reflux conditions, resulting in satisfactory yields of isoxazole derivatives. Among the synthesized compounds, AB-4 (3,5-diphenyl-4,5-dihydroisoxazole) showed the most efficient conversion and highest product purity, while AB-2 and AB-3 also exhibited good reaction completion and product formation. The synthesized derivatives displayed characteristic physical properties such as crystalline appearance, stability, and reproducible TLC profiles, indicating successful synthesis of the target heterocyclic compounds.

CONCLUSION: The present study successfully established an efficient synthetic route for the preparation of isoxazole derivatives via chalcone intermediates. The use of hydroxylamine-mediated cyclization provided a simple, economical, and reproducible method for isoxazole synthesis. TLC studies confirmed the successful formation and purity of the products. These synthesized isoxazole derivatives may serve as promising scaffolds for further pharmacological and biological investigations.

Keywords: Isoxazole Derivatives, Chalcones, Cyclization, Hydroxylamine Hydrochloride, Thin Layer Chromatography (TLC), Heterocyclic Compounds, Condensation Reaction

1. INTRODUCTION

Chalcone scaffolds are considered one of the most important and versatile chemical frameworks in medicinal chemistry due to their wide range of biological and pharmacological activities. These naturally occurring compounds belong to the class of plant secondary metabolites and are characterized by the presence of an α, β-unsaturated carbonyl system that links two aromatic (aryl) rings. This structural feature generally exists in a thermodynamically stable trans-configuration, which contributes significantly to the chemical stability and biological effectiveness of chalcone molecules. Owing to their unique molecular architecture, chalcones serve as key intermediates in the synthesis of numerous heterocyclic compounds and are recognized as valuable building blocks in drug discovery and development. Chalcone and its derivatives exhibit a broad spectrum of biological properties, including antimicrobial, antifungal, anti-inflammatory, antioxidant, anticancer, antimalarial, antiviral, and antitubercular activities, making them highly attractive for pharmaceutical research. Furthermore, these compounds occur naturally in a variety of medicinal plants and have been isolated from several botanical sources, including Dracaena cinnabari, Medicago sativa, and Angelica keiskei. The abundance of chalcones in nature, combined with their structural diversity and remarkable biological potential, has stimulated extensive scientific interest in their synthesis, characterization, and evaluation for the development of novel therapeutic agents. Chalcone scaffolds are considered one of the most important and versatile chemical frameworks in medicinal chemistry due to their wide range of biological and pharmacological activities. These naturally occurring compounds belong to the class of plant secondary metabolites and are characterized by the presence of an α, β-unsaturated carbonyl system that links two aromatic (aryl) rings. This structural feature generally exists in a thermodynamically stable trans-configuration, which contributes significantly to the chemical stability and biological effectiveness of chalcone molecules.

Isoxazoles are an important class of five-membered aromatic heterocyclic compounds characterized by the presence of adjacent oxygen and nitrogen atoms within the ring structure. Due to their unique chemical properties and structural stability, isoxazole derivatives have attracted considerable attention in medicinal and pharmaceutical chemistry. The isoxazole ring system occurs naturally in a wide variety of biologically active molecules and naturally occurring compounds, demonstrating its significance as a valuable pharmacophore in drug design and development. These compounds exhibit a broad range of biological activities, including antimicrobial, antifungal, anti-inflammatory, anticancer, antiviral, analgesic, and antioxidant effects, making them highly useful in therapeutic applications. Isoxazole-containing compounds are particularly important in medicine because several clinically significant drugs possess the isoxazole nucleus as a key structural component. Among these, sulfisoxazole and sulfamethoxazole are well-known bacteriostatic sulfonamide antibiotics that inhibit bacterial growth and are widely used either alone or in combination with other antimicrobial agents for the treatment of various bacterial infections. The versatility of the isoxazole scaffold, together with its presence in numerous pharmacologically active molecules, has encouraged extensive research into the synthesis, characterization, and biological evaluation of novel isoxazole derivatives. Consequently, isoxazole chemistry continues to play a crucial role in the discovery and development of new drugs aimed at combating infectious diseases and other health-related disorders.

The project involves the rational design and synthesis of isoxazole derivatives using chalcones as key intermediates. Various aromatic substitutions will be introduced into the chalcone framework to obtain structurally diverse isoxazole analogues. Appropriate synthetic methodologies will be employed to achieve efficient cyclization and formation of the isoxazole ring.

Evaluation of Antimicrobial Activity

The synthesized isoxazole derivatives will be screened for antimicrobial activity against selected Gram-positive bacteria, Gram-negative bacteria, and fungal strains. The antimicrobial potential of the compounds will be assessed using standard microbiological methods, and the results will be compared with those of standard antimicrobial agents.

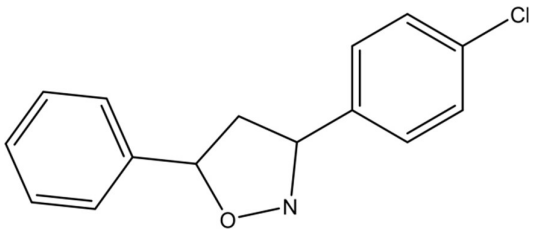
Structure–Activity Relationship (SAR) Analysis

The biological activity data obtained from antimicrobial screening will be analyzed to establish structure–activity relationships. This analysis will help identify the structural features responsible for enhanced antimicrobial activity and provide guidance for future optimization of isoxazole-based antimicrobial agents. Further studies may be conducted on the most active compounds to understand their possible mechanism of action through molecular docking studies, enzyme inhibition studies, or advanced biological evaluations. The scope of this project encompasses the design, synthesis, characterization, and antimicrobial evaluation of novel isoxazole derivatives prepared from chalcone intermediates. The work includes organic synthesis, purification, structural confirmation through spectroscopic techniques, antimicrobial screening, and interpretation of biological data.

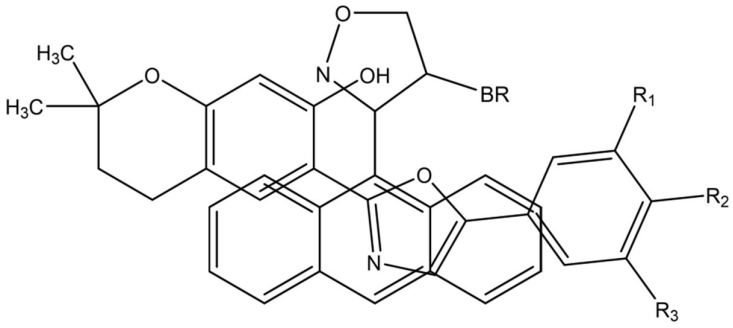
The project is limited to laboratory-scale synthesis and preliminary antimicrobial investigations. It does not include extensive in vivo studies, toxicity assessments, pharmacokinetic evaluations, or clinical investigations. However, the findings of this study may provide valuable information for the development of new antimicrobial agents based on the isoxazole scaffold. The successful completion of this work is expected to contribute to medicinal chemistry research by identifying promising isoxazole derivatives with potential antimicrobial activity and establishing a foundation for further drug discovery and development studies.

2. LITERATURE REVIEW

- **Archana pathak** et, al was synthesized and antimicrobial study of isoxazole derivatives

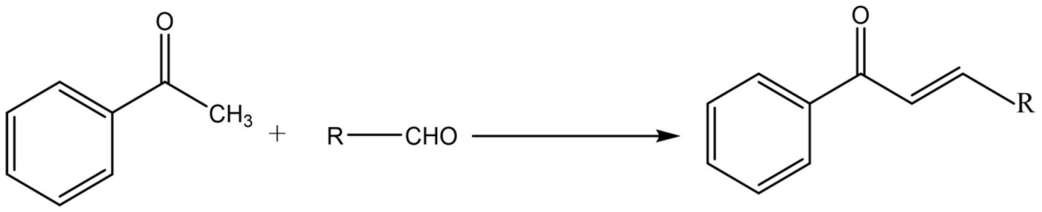


- **Sarbast M. Ahmed** et, al was synthesized characterization and biological activity of some isoxazole derivatives via 1,3-dipolar cycloaddition reaction of nitrile oxide.

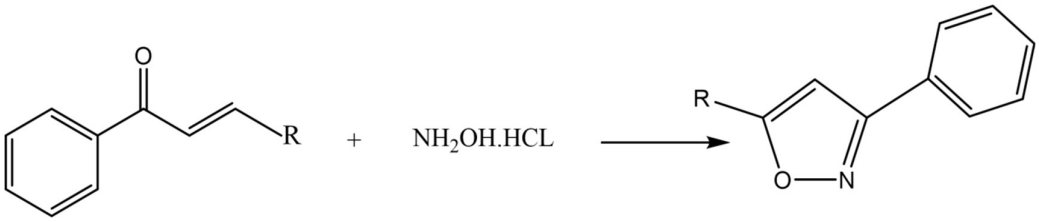


3. SCHEME OF WORK

STEP I: SYNTHESIS OF CHALCONE DERIVATIVES FROM DIFFERENT ALDEHYDES WITH KETONE(ACETOPHENONE)



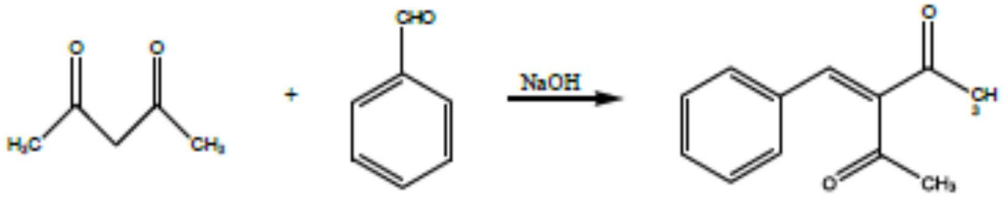
STEP II: SYNTHESIS OF ISOXAZOLE DERIVATIVES FROM CHALCONES



4. EXPERIMENTAL WORK

SYNTHETIC METHOD: (AB – 1)

I. PREPARATION OF CHALCONE



Acetylacetone + Benzaldehyde → 3-Benzylidene-2,4-pentanedione

STEP 1 METHOD OF PREPARATION

- Take an RBF and add 5.14 mL of acetylacetone.
- Add 5.1 mL of benzaldehyde.
- Add 1 mL of 10% dil. NaOH solution slowly, dropwise.
- Place the flask in an ice bath.
- Add 10 mL of ethanol and stir2 to 3hours to ensure a homogeneous solution.
- Maintain the temperature at 0–5°C.
- The reaction will progress to a deep yellow coloured heavy oily product.

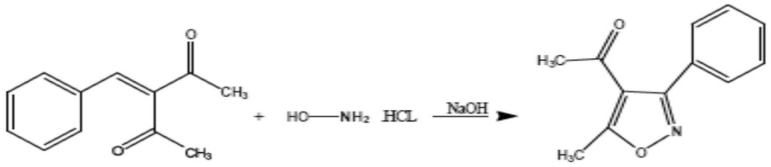
STEP-2-PREPARATION OF MOBILE PHASE: Take a beaker 80 ml and 20 ml ethyl acetate 8:2

STEP-3: PREPARATION OF TLC PLATE: Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

STEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate.

STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product

I. PREPARATION OF ISOXAZOLE



3-Benzylidene-2,4-pentanedione + NH_2OH + NaOH → 4-Benzylidene-3,5-dimethyl Isoxazole

STEP 1: METHOD OF PREPARATION

- Take an RBF and add 1.081 g of 3-benzylidene-2,4-pentanedione.
- Add 2.68 g of hydroxylamine dissolved in 15 mL of 95% ethanol.
- Add 10 mL of NaOH dropwise with constant stirring for 3 hours.
- The reaction will progress to a deep yellow coloured heavy oily product.

STEP-2-PREPARATION OF MOBILE PHASE

Take a beaker 80 ml and 20 ml ethyl acetate 8:2

STEP 3: PREPARATION OF TLC PLATE

Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

STEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate

STEP 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative .

STEP 1: METHOD OF PREPARATION

- Take an RBF and add 22.0 g of cyclohexanone and 22.0 g of benzaldehyde.
- Dissolve 4.6 g of NaOH in 100 mL water and add it slowly with constant stirring.
- Maintain the temperature at about 30°C for 3 hours.
- The reaction will progress to a deep yellow coloured heavy oily product.

STEP-2-PREPARATION OF MOBILE PHASE

Take a beaker 80 ml and 20 ml ethyl acetate 8:2

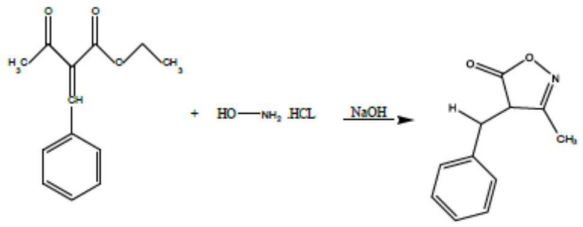
STEP 3: PREPARATION OF TLC PLATE

Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

STEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate

STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product.

I. PREPARATION OF ISOXAZOLE



2-Benzylidene Cyclohexanone + Hydroxylamine Hydrochloride + NaOH → 3-Phenyl-4,5,6,7-Tetrahydrobenzisoxazole

STEP 1: METHOD OF PREPARATION

- Dissolve 10 mmol of 2-benzylidene cyclohexanone in 20 mL of 95% ethanol in a 100 mL flask.
- In a separate beaker, dissolve 12 mmol of hydroxylamine hydrochloride and 15 mmol of sodium acetate in 10 mL solvent.
- Heat the reaction mixture at 70–80°C under reflux with stirring for 2 to 3 hours.
- The reaction will progress to a deep yellow coloured heavy oily product.

STEP-2-PREPARATION OF MOBILE PHASE

Take a beaker 80 ml and 20 ml ethyl acetate 8:2.

STEP 3: PREPARATION OF TLC PLATE

Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

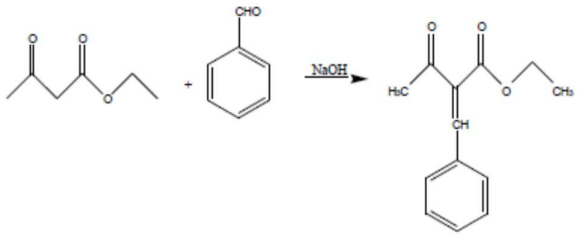
STEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate

STEP 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and

dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative .

SYNTHETIC METHOD: (AB-3)

I. PREPARATION OF CHALCONE



Ethyl Acetoacetate + Benzaldehyde →Ethyl 2-benzylidene-3-oxobutanoate

STEP 1: METHOD OF PREPARATION

- Take a RBF and add 6.5 g (6.4 mL) of ethyl acetoacetate.
- Add 6.3 g of benzaldehyde and 100 mL of ethanol.
- Stir with a magnetic bar until the solid completely dissolves.
- Heat the reaction mixture on an aluminium heating block or water bath at a gentle reflux for 40 minutes.
- The reaction will progress to a deep yellow coloured heavy oily product.

STEP-2-PREPARATION OF MOBILE PHASE

Take a beaker 80 ml and 20 ml ethyl acetate 8:2.

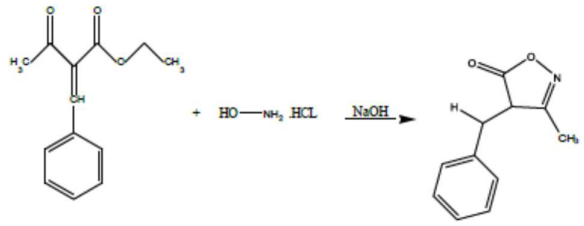
STEP 3: PREPARATION OF TLC PLATE

Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

STEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate

STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product.

II. PREPARATION OF ISOXAZOLE



Ethyl 2-benzylidene-3-oxobutanoate + Hydroxylamine (NaOH) → 4 benzylidene 3-methyl isoxazol-5-one

STEP 1: METHOD OF PREPARATION

- Take an RBF and add 2.12 g of ethyl 2-benzylidene-3-oxobutanoate.
- Add 0.76 g of hydroxylamine and dissolve it in 15 mL ethanol in a 50 mL RBF.
- Add 0.76 g hydroxylamine hydrochloride and 0.98 g sodium acetate dissolved in 10 mL ethanol.
- Fit the flask with a reflux condenser and heat under stirring at 78–80°C for 2–4 hours.
- The reaction will progress to a deep yellow coloured heavy oily product.

STEP-2-PREPARATION OF MOBILE PHASE

Take a beaker 80 ml and 20 ml ethyl acetate 8:2.

STEP 3: PREPARATION OF TLC PLATE

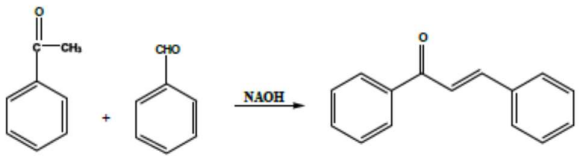
Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

STEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate

STEP 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative.

SYNTHETIC METHOD: (AB-4)

I. PREPARATION OF CHALCONE



Acetophenone + Benzaldehyde → 1,3-Diphenylprop-2-en-1-one

STEP 1: METHOD OF PREPARATION

- Take a round-bottom flask (RBF) and add 11.7 mL of acetophenone into the cooled solution while stirring continuously.
- Immediately add 10.6 g (10.1 mL) of freshly distilled benzaldehyde to the mixture.
- Dissolve 5.0 g of NaOH pellets in 50 mL distilled water in a 250 mL conical flask.
- Add 30 mL of 95% ethanol to the alkaline solution as a co-solvent. Place the flask in an ice bath and allow the solution to cool to 15–20°C.
- Stir the reaction mixture magnetically for 2–3 hours until it becomes thick and turbid.

STEP-2-PREPARATION OF MOBILE PHASE

Take a beaker 80 ml and 20 ml ethyl acetate 8:2

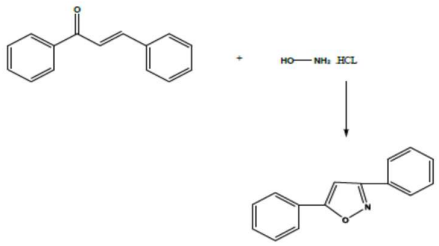
STEP 3: PREPARATION OF TLC PLATE

Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

STEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate

STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product.

II. PREPARATION OF ISOXAZOLE



1,3-Diphenylprop-2-en-1-one + Hydroxylamine Hydrochlorid (EtOH / NaOH, Heat) ⟶ 3,5-Diphenyl-4,5-dihydroisoxazole

STEP 1: METHOD OF PREPARATION

- Take a Round Bottom Flask (RBF) and place 2.23 g of 3,5-diphenyl-4,5-dihydroisoxazole in 30 mL absolute ethanol and dissolve it.
- In a separate beaker, dissolve 1.04 g of hydroxylamine hydrochloride and 0.80 g of NaOH in 20 mL distilled water.
- Add the hydroxylamine solution to the RBF containing the chalcone solution.
- Assemble the reflux setup with a heating mantle and heat gently under reflux at 80°C with continuous stirring for 3–4 hours.
- The reaction will progress to a deep yellow coloured heavy oily product.

STEP-2-PREPARATION OF MOBILE PHASE

Take a beaker 80 ml and 20 ml ethyl acetate 8:2

STEP 3: PREPARATION OF TLC PLATE

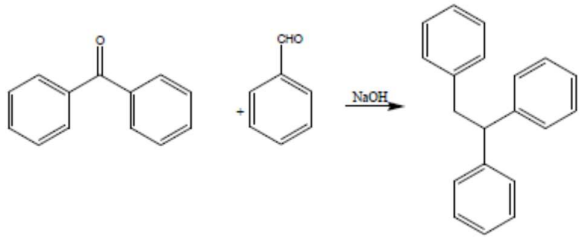
Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

STEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate

STEP 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative .

SYNTHETIC METHOD: (AB-5)

I. PREPARATION OF CHALCONE



Benzophenone + Benzaldehyde NaOH → 1,1,2-Triphenyl-2-hydroxy ethene

STEP 1: METHOD OF PREPARATION

- take care RBF and add these 10 grams of benzophenone at the 5.8 grams of benzaldehyde and dissolve the mixture in 40 ml of ethanol
- And the NaoH 10 to 5 ml of solutions slowly with continuous stirring.
- Stirr the reaction at the room temperature and poor if the reaction mixture into ice cold water to ppt the product,And filter the solid using vaccum filtration
- -wash the ppt with cold water to remove impurities
- -Dry the product and recrystalline from ethanol to obtained pure 1,1,2 triphenyl ethene.

STEP-2-PREPARATION OF MOBILE PHASE

Take a beaker 80 ml and 20 ml ethyl acetate 8:2

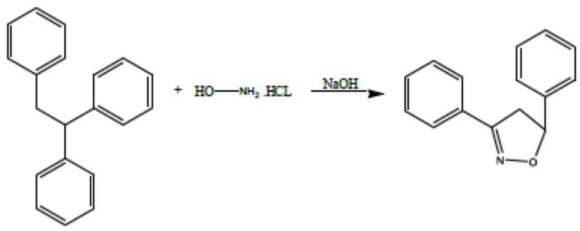
STEP 3: PREPARATION OF TLC PLATE

Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

STEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate

STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product.

II. PREPARATION OF ISOXAZOLE



1,1,2-Triphenyl-2-hydroxy ethene + Hydroxylamine → 3,5-Diphenyl isoxazoline

STEP 1: METHOD OF PREPARATION

- Take a round-bottom flask (RBF) and add 10 g of chalcone and 20 mL of ethanol. Stir until completely dissolved.
- Add 0.45 g of hydroxylamine hydrochloride.
- Dissolve 5.0 g of NaOH in 50 mL of distilled water to prepare a 10% NaOH solution.
- Reflux the reaction mixture for 4–6 hours.
- After completion, cool the reaction mixture and pour it into ice-cold water (80–100 mL).
- The reaction will progress to a deep yellow coloured heavy oily product.

STEP-2-PREPARATION OF MOBILE PHASE

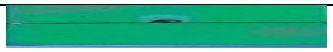
Take a beaker 80 ml and 20 ml ethyl acetate 8:2.

STEP 3: PREPARATION OF TLC PLATE

Take TLC Plate cut the TLC plate with required length of 6cm and to this plate make a mark of 1cm and draw a line.

TEP 4: Place the reaction on TLC Plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate reaches above 70% remove it from beaker and allow it for drying, after the drying of TLC plate completely, spray the 2,4 DNP solution on TLC plate by using spray nozzle (or) injection, allow it for dry, the dried TLC plate is placed under the UV cabinate and observe the spots presents on TLC plate

STEP 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative.

COMPUND CODE	TLC PLATES	PRODUCT
AB-1		
AB-2		
AB-3		
AB-4		
AB-5		

5. RESULTS AND DISCUSSIONS

5.1 Anti-bacterial activity results

5.1.1. Table 5: zone of inhibition of synthesized compoundsAnti-bacterial activity of synthesized compounds on

Comp No	Mean zone of inhibition (*in mm*)											
	ANTI-BACTERIAL ACTIVITY											
	Bacillus Subtilis			*Staphylococcus Aureus*			*K. pneumonia*			*E. coli*		
	25 µg/ml	50 µg/ml	100 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
STD	12mm	18mm	17mm	14mm	15mm	16mm	15mm	16mm	16mm	13mm	17mm	17mm
AB 1	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm
AB 2	5mm	9mm	8mm	5mm	7mm	7mm	-	9mm	6-3mm	5mm	-	4.3mm
AB 3	1mm	1mm	1mm	-	1mm	1mm	1mm	1mm	1mm	1mm	1mm	4.8mm
AB 4	9mm	11mm	11mm	8mm	11mm	10mm	8mm	11mm	6.6mm	6mm	7mm	7.6mm
AB 5	8mm	10mm	10mm	7mm	9mm	9mm	9mm	10mm	10.3mm	7mm	9mm	9mm
CONT	6mm	8mm	7.6mm	6mm	7mm	7mm	7mm	11mm	10mm	7mm	9mm	8.6mm

Escherichia coli

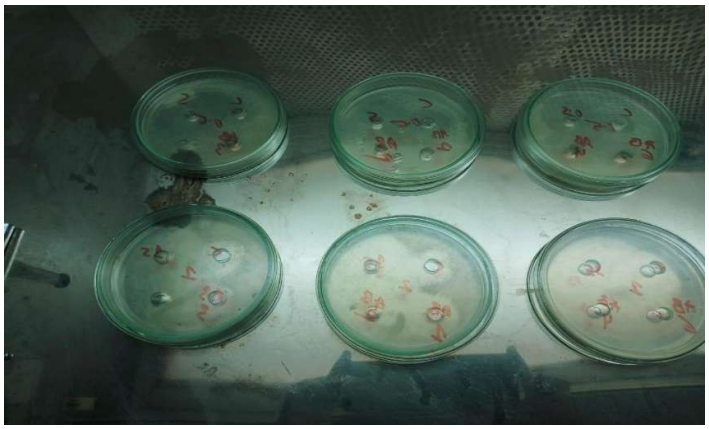
Zone of inhibition of synthesized compounds against Escherichia coli bacteria.

Compounds : AB1-AB5

Concentrations: 25,50, and 100 microgram/ml

Control : DMSO

Standard : Ciprofloxin.



5.1.2. Anti-bacterial activity of synthesized compounds on Bacillus subtilis

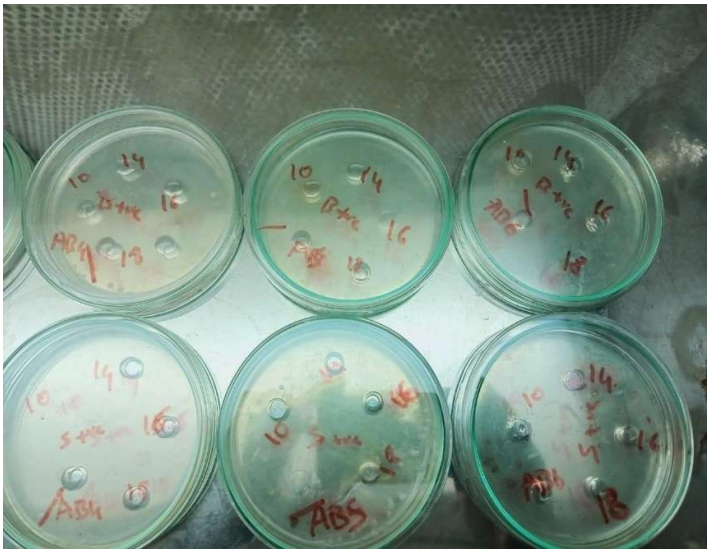
Zone of synthesized Compounds against *Bacillus Subtilis* bacteria.

Compounds : AB1-AB5

Concentrations : 25,50, and 100 microgram/ml

Control :DMSO

Stanard : Ciprofloxin.



5.1.3. Anti-bacterial activity of synthesized compounds on Staphylococcus Aureus

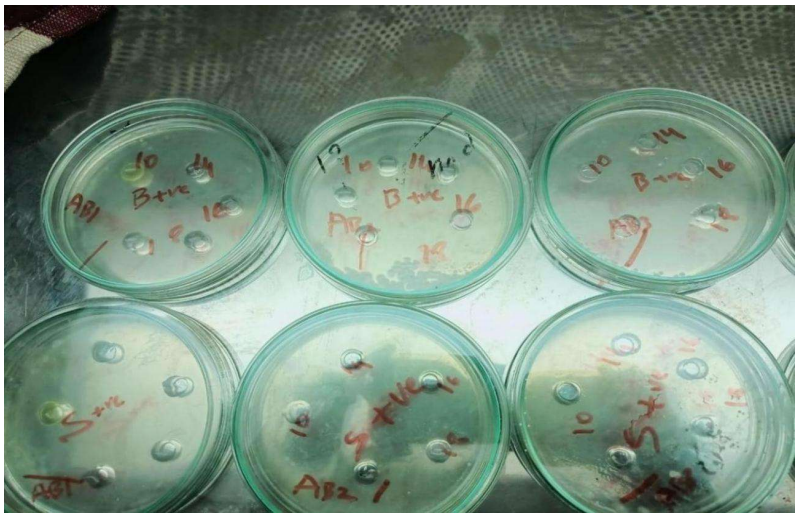
Zone of inhibition of synthesized compounds against Staphylococcus Aureus bacteria.

Compounds : AB1-AB5

Concentrations: :25,50, and 100microgram/ml

Control : DMSO

Standard : Ciprofloxin.



5.1.4. Antibacterial activity of synthesized compounds on Klebsiella pneumonia

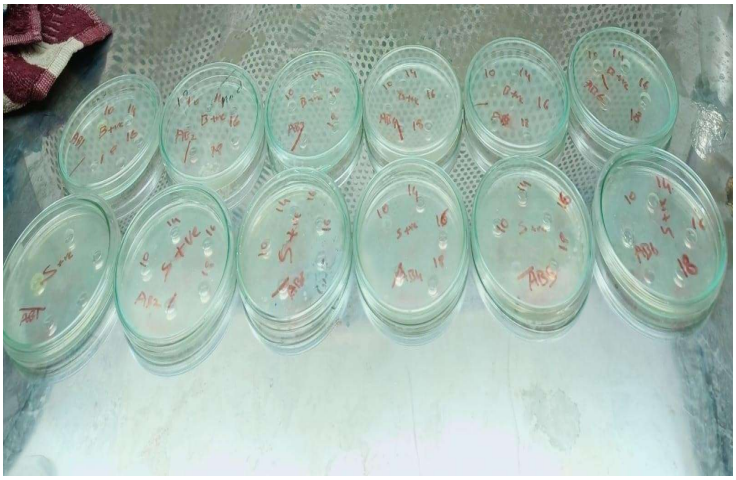
Zone of inhibition of synthesized compounds against Klebsiella pneumonia bacteria.

Compounds : AB1-AB5

Concentrations : 25,50, and 100 microgram/ml

Control : DMSO

Standard : Ciprofloxin.



The synthesized compounds (AB1–AB5) were evaluated for their antibacterial activity using the **disc diffusion method** at concentrations of **25, 50, and 100 µg/mL**. The activity was tested against both **Gram-positive** and **Gram-negative** bacteria, including *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. **Ciprofloxacin** was used as the **standard drug**, and **DMSO** was used as the **negative control**.

The **zone of inhibition** (in mm) was measured to assess the antibacterial effectiveness of each compound.

AB1 and AB3 showed **very weak activity**, with minimal inhibition zones (1–1.5 mm) across all test organisms and concentrations.

AB2 showed **moderate antibacterial activity**, especially against *Klebsiella pneumoniae* and

E. coli, with inhibition zones ranging from **4.3 to 9 mm**.

AB4 and AB5 demonstrated **comparatively better activity**, particularly at higher concentrations (100 µg/mL), with inhibition zones ranging from **6 mm to 11 mm**, especially against *Bacillus subtilis* and *Staphylococcus aureus*.

The **standard drug ciprofloxacin** showed significantly higher zones of inhibition (**12–18 mm**) across all bacterial strains, indicating strong antibacterial activity.

The results indicate that the synthesized compounds exhibited **mild to moderate antibacterial activity** in comparison to the standard. Among them:

AB4 and AB5 showed the **best activity**, especially at higher concentrations.

AB2 showed **selective moderate**

AB1 and AB3 were the **least effective**.

Overall Activity Order: AB4 > AB5 > AB2 > AB1 ≈ AB3

6. SUMMARY AND CONCLUSION

In the present research work, five chalcone derivatives (coded as AB1 to AB5) were successfully synthesized using the **Claisen-Schmidt condensation method**, which involved the reaction of various substituted aromatic aldehydes with acetophenone in ethanolic sodium hydroxide medium. The resulting compounds were purified through recrystallization and confirmed by **Thin Layer Chromatography (TLC)** and further characterized using spectral methods (IR, NMR), ensuring the correctness of the synthesized structures.The synthesized chalcone derivatives were screened for **antibacterial activity** against selected Gram-positive and Gram-negative bacterial strains, including *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. The **disc diffusion method** was employed for antibacterial screening at concentrations of **25 µg/mL, 50 µg/mL, and 100 µg/mL**. **Ciprofloxacin** was used as the **standard drug**, and **DMSO** served as the negative control.

Observations: AB4 and AB5 exhibited notable antibacterial activity, with maximum zones of inhibition observed at 100 µg/mL, especially against *Bacillus subtilis* and *Staphylococcus aureus*.

AB2 showed moderate activity, particularly against *Klebsiella pneumoniae* and *Escherichia coli*.

AB1 and AB3 displayed negligible or very weak activity against all tested bacterial strains.The standard drug ciprofloxacin showed higher zones of inhibition in all tested strains, confirming the sensitivity of the assay.

Conclusion: From the results, it can be concluded that certain chalcone derivatives, especially AB4 and AB5, possess promising antibacterial properties and could serve as lead compounds for further development. The antibacterial activity varied depending on the nature and position of substituents on the aromatic rings, indicating a structure-activity relationship (SAR). The findings support the potential of chalcone scaffolds in designing new antimicrobial agents, especially in the context of rising antibiotic resistance. Further research involving mechanistic studies, SAR analysis, and chemical modification may enhance the activity and therapeutic value of these derivatives.

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