Journal of Innovations in Applied Pharmaceutical Science [JIAPS]

Content available at: www.saap.org.in ISSN: 2455-5177

# SYNTHESIS, CHARACTERIZATION AND ANTITUBERCULAR ACTIVITY OF NOVEL CHALCONE DERIVATIVES BY INHA TARGETING

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**Article History**

Received: 04-05-2025
Revised: 19-05-2025
Accepted: 16-06-2025

***Keywords:*** Chalcones, InhA, H37RV strain, microplate Alamar Blue assay, Anti-tubercular activity

**Abstract**

Background: The authors targeted InhA to estimate the therapeutic potential of novel chalcones against tuberculosis. Methods: 07 synthesized compounds were tested for in vitro antimycobacterial activity against Mycobacterium tuberculosis (H37RV; American Type Culture Collection number: 27294) using the microplate Alamar Blue assay. Results: The most potent compounds, trans-1-(2-napthyl)-3-phenyl-2-propen-1-one showed in vitro activity, with a minimum inhibitory concentration (MIC) of 1.16 µg/ml. Conclusion: Compound code 1e i.e. trans-1-(2-napthyl)-3-phenyl-2-propen-1-one showed strong in vitro antimycobacterial activity at a concentration of 1,16 µg/ml.



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https://doi.org/10.37022/jiaps.v10i2.705

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## Introduction

Heterocyclic compounds have attracted significant interest due to their various important medical and biological applications. The research focus on heterocyclic compounds is increasing rapidly because of extensive studies on their synthesis and functional uses. They are present in over 90% of new drugs and bridge the domains of biology and chemistry, where many scientific advancements and applications take place. In our review, we introduce a new phase of potential therapies, including antifungal, anti-inflammatory, antibacterial, antiviral, antioxidant, anticonvulsant, anthelmintics, antipyretics, anti-allergic, antihistamines, herbicides, anticancer agents, anti-tubercular drugs treatments.

### General Introduction of Chalcones

Chalcones typically consist of α,β-unsaturated ketones formed by two aromatic rings connected by a three-carbon alkenone unit; however, they may also include certain saturated ketones known as dihydrochalcones, which have a three-carbon unit instead. The term “chalcone” comes from the Greek word “chalaco’s”, which translates to “bronze”, reflecting the colors of most natural chalcones. Chalcone compounds feature a shared chemical framework of 1,3-diaryl-2-propen-1-one, also referred to as chalconoid, which appears in both trans and cis forms, with the trans configuration being more stable thermodynamically [1,10-12].

### General Introduction of Tuberculosis

Tuberculosis (TB), an chronic infectious bacterial disease caused by Mycobacterium tuberculosis, remains a major cause of mortality, claiming around 5,000 lives daily worldwide and is more prevalent in males than females. Currently, poor adherence to medication, the emergence of multi-drug-resistant strains, and the HIV/AIDS epidemic are some of the factors contributing to the resurgence of TB. Resistance to

medication arises from insufficient adherence, and individuals with HIV/AIDS, who have a compromised immune system, are particularly vulnerable to Mycobacterium tuberculosis, thus significantly increasing their risk of death. The interplay between Mycobacterium tuberculosis and the human immunodeficiency virus (HIV) exacerbates the situation, as they are more harmful in conjunction than when they act alone. Approximately 70-80% of individuals infected with HIV are also co-infected with Mycobacterium tuberculosis leading to the development of active TB in 60-70% of those who are HIV positive. Today, the global situation regarding TB has become critical due to the rising prevalence of "multi-drug-resistant tuberculosis" (MDR-TB), "extensively drug-resistant tuberculosis" (XDR-TB), and "totally drug-resistant tuberculosis" (TDR-TB). Additionally, the HIV pandemic poses a significant challenge to disease management. The escalation of drug-resistant tuberculosis alongside HIV could substantially affect TB treatment strategies. This situation underscores the urgency to discover new, more effective anti-TB medications. The primary focus of this thesis is to investigate innovative approaches in modern drug discovery aimed at creating new drugs to effectively overcome MDR/XDR/TDR-TB. To provide context, the following sections will outline a brief history, examine the epidemiology and pathogenesis of tuberculosis, and discuss aspects of modern drug discovery and their relevance to medicinal chemistry [2-6]. Chalcones are known for their varied pharmacological effects, including their antimicrobial and anti-tubercular properties. In this research, a range of chalcone derivatives were synthesized and tested for their in vitro anti-tubercular activity against the Mycobacterium tuberculosis H37Rv strain using the Microplate Alamar Blue Assay (MABA). These results indicate that chalcones hold potential as promising frameworks for the development of new anti-tubercular agents, and further refinement may yield effective treatments against drug-resistant TB strains. The findings showed that several chalcone derivatives had considerable inhibitory effects, with minimum inhibitory concentration (MIC) values between 3.12 to 50 µg/mL. Particularly, derivatives containing electron-withdrawing groups like halogens (Cl, Br) or nitro groups at the para position of the phenyl ring exhibited improved antimycobacterial activity. This study validates the potential of chalcoane frameworks as lead compounds for continued progress in antitubercular drug discovery. Optimizing these compounds through rational design and combining them with known pharmacophores may result in novel agents that effectively tackle resistant strains of M. tuberculosis. Future research will concentrate on assessing in vivo efficacy, evaluating cytotoxicity, and investigating mechanisms of action to push these promising candidates toward clinical use. The goal of the current research is to create novel anti-tubercular molecules, particularly trans-1-(2-naphthyl)-3-phenyl-2-propen-1-one, which targets the enzyme InhA. Moreover, this research article aims to illustrate how chalcone derivatives can offer a new chemical framework for drug discovery, thereby validating the title of this article [7-9].

## Materials and methods

### Instrumentation

All the chemicals and reagents were purchased from ahAlddrich. All the solvents were dried and distilled before use. The melting points of the derivatives were determined by capillary tubes using melting point apparatus and are uncorrected. The synthesized compounds were purified by Column chromatography using Chloroform: DMSO: Ethanol (7:3 :1) is as solvent system. Reactions were monitored by T.L.C. The visualization was achieved by Staining with I2 or U.V light. IR spectrum of derivatives was recorded on FT-IR(Model:schimadzu) using potassium bromide. The 1H NMR were recorded in DMSO 400MHZ BRUKER
ADVANCE 1HNMR. Chemical shifts are reported by using Tetramethylsilane (TMS) as a internal standard.

## Scheme of work

### Scheme-I

Chalcones 1a–g were constructed by treatin, naphthalene with benzaldehyde and/or substituted benzaldehyde in methanol and potassium hydroxide, and the constructed derivatives exhibited anti-tubercular, antibacterial and antifungal activities (Scheme 1)

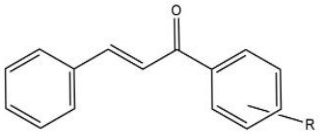



Figure 1:Chalcone derivatives

## Experimental Procedure

### Scheme-1

Detailed procedure for Chalcone Synthesis: Aldol Condensation

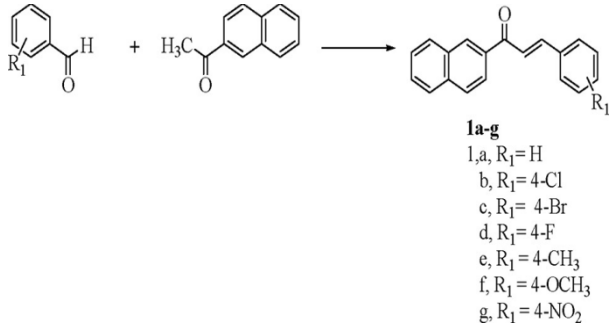



Figure 2: Chalone synthesis

Chalcone synthesis is typically achieved via the Claisen-Schmidt condensation, a type of aldol condensation between an aromatic aldehyde and an aromatic ketone in the presence of a base. The following procedure was followed for synthesis of chalcone:

- Dissolve 20gm 2-acetyl naphthalene in 32ml water and take 20ml from the solution.
- To the 20ml solution add 20ml Benzaldehyde of equimolar amount.
- Slowly add an aqueous solution of KOH(60%) dropwise while stirring.
- Maintain the temperature between 25-30°C with the help of ice bath and stir the reaction mixture slowly for 30min.
- After completion pour the reaction mixture into cold water

- Acidify with HCl to neutralize the base pH- 5-6
- Filter the solid and wash with cold water.
- Recrystallize the product by 40ml Methanol to obtain Pure Chalone Product.

## Biological Evaluation

5.1 Anti-Tubercular Activity

All the synthesized compounds (1a, 1b ,1c ,1d, 1e, 1f, 1g) were subjected to in-vitro antimycobacterial assay so as to detect their in-vitro potency of inhibiting tuberculosis. The desired in-vitro anti-tubercular activity study was performed by the "Microplate Alamar Blue Assay" (MABA) method.

## Materials

- The in-vitro anti-mycobacterial assay of the synthesized products was performed using Micro plate Alamar Blue Assay (MABA) against Mtb (H37RV)
- Pyrazinamide, Ciprofloxacin and Streptomycin were used as reference drugs. The results were based upon the minimum inhibitory concentration (MIC)

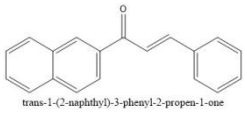
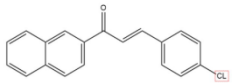
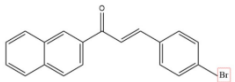
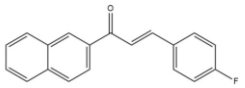
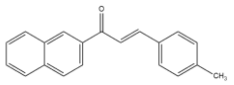
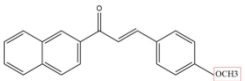
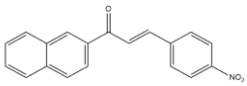
## Experimental

All the synthesized compounds were assessed for the anti - mycobacterial activity against M. tb using 'Micro Plate Alamar Blue Assay" (MABA). MABA is a non-toxic method.In order to reduce evaporation of medium in the test wells throughout incubation two hundred microliter of sterile deionized water added to all outer perimeter wells of sterile 96 wells plate. Additionally, the 96 wells plate received hundred micro liter of the Middle brook 7H9 broth. Sequential dilution of compounds was made directly on plate. The final drug concentrations tested were 100to 0.8 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 were added to the plate and incubated for 24 hrs. Bacterial growth in the well was interpreted. Blue color was recorded as no growth, and pink color was recorded as indication of bacterial growth. The Minimum inhibitory concentration was defined as lowest drug concentration which prevented the color change from blue to pink.

Tabulation-1: Synthesized Chalcone Derivatives

| COMPOUND | R1(Aromatic aldehyde) |
|---|---|
| 1a | H |
| 1b | 4-Cl |
| 1c | 4-Br |
| 1d | 4-F |
| 1e | 4-CH3 |
| 1f | 4-OCH3 |
| 1g | 4-NO2 |

*Tabulation-2: Synthesized compounds with their IUPAC Name*:

| Compounds | Structures | IUPAC Name |
|---|---|---|
| **1a** |   | (E)-1-(2-napthyl)-3-phenyl-2-propen-1-one |
| **1b** |   | (E)-1-(2-napthyl)-3-(4-chlorophenyl)-2-propen-1-one |
| **1c** |   | (E)-1-(2-napthyl)-3-(4-bromophenyl)-2-propen-1-one |
| **1d** |   | (E)-1-(2-napthyl)-3-(4-fluorophenyl)-2-propen-1-one |
| **1e** |   | (E)-1-(2-napthyl)-3-(4-methylphenyl)-2-propen-1-one |
| **1f** |   | (E)-1-(2-napthyl)-3-(4-methoxyphenyl2-propen-1-one |
| **1g** |   | (E)-1-(2-napthyl)-3-(4-nitrophenyl)-2-propen-1-one |

Tabulation-3: Physical Characterization of synthesized compound

| Sample Code | Mo. Wt. | Mol. Formula | Color | Solubility | Melting Point | % of Yield | Rf Value |
|---|---|---|---|---|---|---|---|
| 1a | 258.10 | C19H14O | Yellowish | CHCl3 DMSO, Methanol | 75°C | 90 % | 0.52 |
| 1b | 292.07 | C19H13ClO | Brownish | CHCl3 DMSO, Methanol | 51°C | 88 % | 0.57 |
| 1c | 336.02 | C19H13BrO | Black | CHCl3 DMSO, Methanol | 139°C | 75 % | 0.64 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1d | 276.31 | C19H13FO | Yellowish brown | CHCl3 DMSO, Methanol | 81°C | 79 % | 0.48 |
| 1e | 272.1 | C20H16O | Brownish | CHCl3 DMSO, Methanol | 122°C | 80 % | 0.41 |
| 1f | 288.1 | C20H16O2 | Yellowish | CHCl3 DMSO, Methanol | 51°C | 93 % | 0.53 |
| 1g | 287.1 | C19H13 NO2 | Brownish Black | CHCl3 DMSO, Methanol | 251°C | 87 % | 0.59 |

## Results and discussion

trans-1-(2-napthyl)-3-phenyl-2-propen-1-one
yellowish solid, yield,90%,M.P.75ºC
IR (KBr): 3037.80cm-1 aromatic ch stretching, 1326.10cm-1-C=C- stretching in ring, 1880.80cm-1-C=O- stretching in Carbonyl
1HNMR(400MHZ,CDCl3): δ 8.28(S,1H),8.0(D,J=15.21H),7.26(D,J=7.2Hz,1H),2.1(S,3H).
FTIR Investigation of the synthesized Chalcone Product

Figure no 3 : FTIR Graph 1a –(E)-1-(2-napthyl)-3-phenyl-2-propen-1-one:

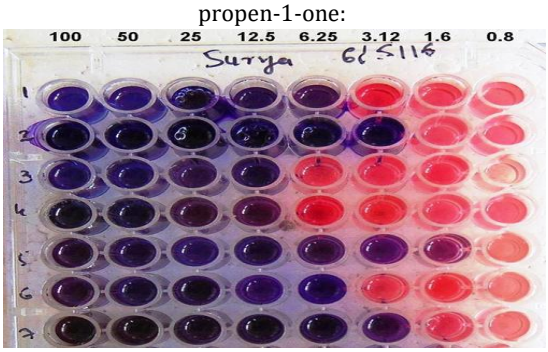



Figure no 4: Anti-Tubercular Activity of synthesized compounds by MABA

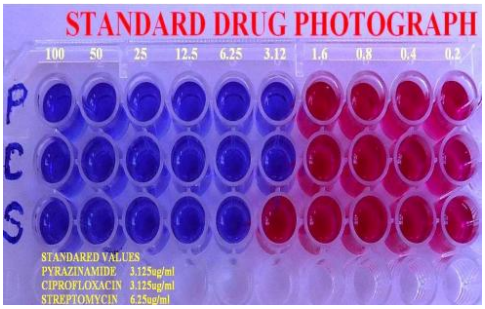



Figure no5: Anti-Tubercular Activity of standard drugs by MABA

Table No 4. Ranking of the compounds based on the in-vitro antimycobacterial activity

| Rank Compound | MIC(µg/ml) |
|---|---|
| 1a | 6.25 |
| 1b | 3.12 |
| 1c | 12.5 |
| 1d | 12.25 |
| **1e** | **1.6** |
| 1f | 6.25 |
| 1g | 3.12 |

All the Seven synthesized compounds were screened for their in-vitro antitubercular activity against MTB(H37RV) using Alamar Blue Assay (MABA). Pyrazinamide, Ciprofloxacin and Streptomycin were used as reference drugs. The results as minimum inhibitory concentration (MIC) (Figure 13) are presented in table .

When screened against mycobacterium tuberculosis (MTB) the synthesized compounds (1a, 1b, 1c, 1d, 1e, 1f, 1g) showed moderate to potent in-vitro activity against MTB with MIC range 0.8-100µg/ml. Compound 1e exhibited most potent in-vitro activity with MIC 1.6µg/ml concentrations. While the other compounds showed moderate to good anti-tubercular activity. Based on the in-vitro anti-tubercular results, it is clear that the synthesized 4-methyl derivatives i.e. "**(E)-1-(2-napthyl)-3-(4-methylphenyl)-2-propen-1-one"** exhibit rearkable activity when compared with standard drugs.

## Summary and Conclusion

All the seven molecules (1a, 1b, 1c, 1d, 1e, 1f) were synthesized. The synthesized compounds were purified and characterized. The synthesized compounds were characterized by FT-IR.All the synthesized compounds were investigated for their in-vitro anti-tubercular potential using Microplate Alamar Blue Assay, (MABA). All the compounds showed moderate to potent in-vitro activity against MTB with MIC range 0.8-100µg/ml concentrations. Compound 1e i.e "**(E)-1-(2-napthyl)-3-(4-methylphenyl2-propen-1-one"** exhibited most potent in-vitro activity with MICs 1.6 µg/ml concentrations compared to MICs 3.12 µg/ml of standard drugs Pyrazinamide & Ciprofloxacin.

## Funding

This research work received no external funding.

## Acknowledgement

Thank our co-authors for supporting my research work.

## Conflict of Interest

No conflict of interest.

## Informed consent

The goal of research work is to develop effective, safe, novel drug, low cost, and decreasing the burden of tuberculosis worldwide.

## Ethical Statement

Increasing the credibility and validity of research work

## Author Contribution

Santanu Kumar Hotta devloped the methodology, data analysis, write up throughout my research work

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