International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 8s,2025
https://www.theaspd.com/ijes.php

New Insights into 1,2,4-Triazole-3-Thiol Chemistry: Synthesis and Tuberculostatic Activity

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Abstract

Background: Tuberculosis (TB), a formidable global health crisis, relentlessly claims countless lives annually, perpetuating widespread morbidity and mortality. The dire need for groundbreaking treatments has ignited fervent research into innovative therapeutic paradigms. Materials and Methods: This pioneering study embarked on synthesizing an array of benzimidazole derivatives, rigorously evaluated for their antimicrobial prowess through the cutting-edge Microplate Alamar Blue Assay. This sophisticated method meticulously determined the Minimum Inhibitory Concentration (MIC) against the formidable Mycobacterium tuberculosis H37Rv strain, setting a new benchmark for precision in anti-tubercular research. Results: From this ambitious endeavour, 1,2,4-triaole derivatives 3 emerged as titans, showcasing unparalleled potency against M. tuberculosis. Their remarkable anti-tubercular activity signals a monumental leap forward in combating this relentless pathogen. Conclusion: This trailblazing study emphatically validates the transformative power of synthetic chemistry in sculpting novel anti-tubercular agents. The extraordinary efficacy of compounds 3 heralds their potential as revolutionary therapeutic frontrunners, poised to redefine TB treatment landscapes. These findings galvanize further exploration and clinical scrutiny, promising to alleviate the global TB burden and usher in a new era of hope for millions afflicted by this devastating disease.

Keywords:- TB, Minimum Inhibitory Concentration (MIC), 1,2,4-triazoles, Microplate Alamar Blue Assay.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is a contagious bacterial infection primarily affecting the lungs but capable of impacting other organs. Spread through airborne droplets from coughing or sneezing, TB remains a leading cause of infectious disease-related mortality worldwide. In 2020, the World Health Organization (WHO) reported approximately 10 million new TB cases and 1.5 million deaths, underscoring its persistent global burden[1]. TB manifests as active disease or latent infection, with about 10% of latent cases progressing to active TB if untreated[2]. The disease disproportionately affects vulnerable populations, including those with HIV, malnutrition, or compromised immune systems, exacerbating its socio-economic impact[3]. TB poses significant challenges due to its prolonged treatment duration, typically 6–9 months, which often leads to poor patient adherence and increased risk of drug resistance[4]. Multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) complicate treatment, requiring longer regimens (up to 24 months) with toxic second-line drugs, resulting in lower cure rates and higher costs[5]. Side effects of first-line drugs, such as hepatotoxicity from rifampin and isoniazid, further hinder treatment success[6]. The emergence of drug-resistant strains, coupled with inadequate diagnostic tools in low-resource settings, amplifies the global TB burden[7]. Governments and international organizations have implemented robust strategies to

combat TB. The WHO's End TB Strategy aims to reduce TB mortality by 95% and incidence by 90% by 2035[8]. Key measures include the Directly Observed Treatment, Short-course (DOTS) program, which ensures treatment adherence, and the promotion of rapid molecular diagnostics like GeneXpert for early detection of drug-resistant TB[9]. National TB programs, such as India's Revised National Tuberculosis Control Programme (RNTCP), provide free diagnostics and treatment, with initiatives like Nikshay Poshan Yojana offering nutritional support to patients[10]. Globally, BCG vaccination programs target children to prevent severe forms of TB, though their efficacy against pulmonary TB in adults is limited[11]. Despite these efforts, the COVID-19 pandemic disrupted TB services, potentially increasing TB incidence in 2022–2023[1]. According to the WHO Global Tuberculosis Report 2020, an estimated 10 million people developed TB, with 5.6 million men, 3.3 million women, and 1.1 million children affected. The report highlighted 1.5 million TB-related deaths, including 214,000 among HIV-positive individuals. Drug-resistant TB accounted for 465,000 cases, with 78% being MDR-TB. Treatment success rates were 85% for drug-susceptible TB but only 57% for MDR-TB, emphasizing the need for novel therapeutics[1]. Triazole-based compounds have emerged as promising anti-tubercular agents due to their favorable pharmacological properties, including hydrogen bonding, moderate dipole moment, and enhanced water solubility, enabling effective binding to *M. tuberculosis* biomolecular targets[^12]. Triazole scaffolds, particularly 1,2,3- and 1,2,4-triazoles, exhibit broad-spectrum biological activities, including antimicrobial, anti-inflammatory, and anti-tubercular effects[^13]. Their ability to inhibit key enzymes in *M. tuberculosis*, such as those involved in siderophore biosynthesis, disrupts bacterial survival, making them potent candidates for TB treatment[^14]. Recent studies (2011–2021) have highlighted triazole hybrids with in vitro and in vivo anti-tubercular activity, offering improved efficacy and reduced toxicity[^12].The 1,2,4-triazole moiety, a five-membered heterocyclic ring containing three nitrogen atoms and two carbon atoms, is a versatile pharmacophore in medicinal chemistry. As an isostere of imidazole, it enhances drug stability and binding affinity due to its nitrogen-rich structure[15]. This moiety is integral to numerous clinically approved drugs, such as fluconazole, and exhibits diverse activities, including anti-tubercular effects through inhibition of *M. tuberculosis* enzymes and cell wall synthesis[16]. Its synthetic accessibility via click chemistry has accelerated the development of 1,2,4-triazole-based anti-tubercular agents, making it a cornerstone of modern drug discovery[17].The 5-(substituted)-4-phenyl-4H-1,2,4-triazole-3-thiol moiety is a potent anti-tubercular scaffold due to its ability to disrupt *M. tuberculosis* metabolic pathways. The thiol group enhances reactivity, facilitating interactions with bacterial enzymes, while the phenyl and substituted groups at the 5-position optimize lipophilicity and target specificity[18]. Studies have demonstrated that derivatives, such as 4-(2-chlorobenzylidene amino)-5-(4-isopropylthiazol-2-yl)-4H-1,2,4-triazole-3-thiol, exhibit significant anti-tubercular activity against *M. tuberculosis* H37Rv, with MIC values comparable to standard drugs like rifampicin[19]. The moiety's efficacy is attributed to its ability to inhibit bacterial growth by targeting essential proteins, offering a promising avenue for developing novel therapeutics with reduced resistance profiles[20].

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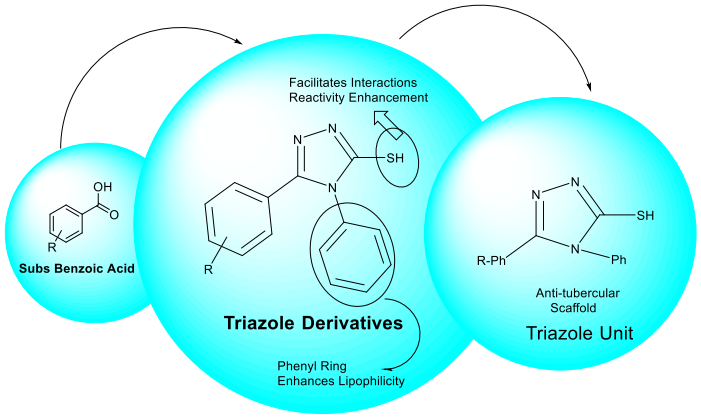
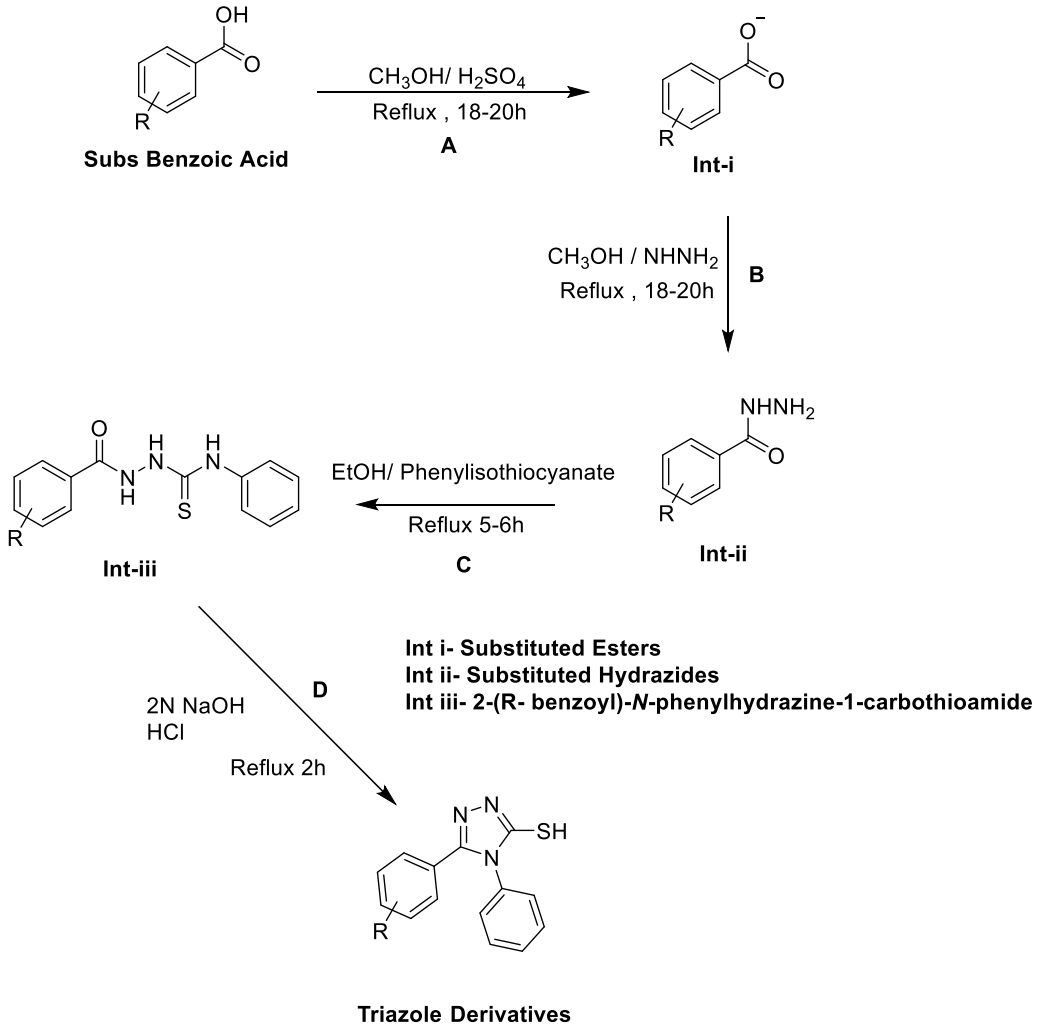
GRAPHICAL ABSTRACT



SYNTHETIC SCHEME:



EXPERIMENTAL

The initial material, reagents and solvents were purchased from Nice, SDFL, Molychem and glasswares were obtained from Borosil. The raw material was weighed on calibrated weighing balance. The synthetic scheme was drawn via ChemDraw 8.03. The confrmation of reaction at every step was done by TLC (thin layer chromatography). Melting point of the synthesized compounds was depicted by melting point equipment. For spectral characterizations of the compounds, Bruker 12060280, spectrometer using ATR for IR spectra (cm−1) and Bruker Avance III at 500 NMR and 150 MHz for 1 H($CDCl_3$) and 13CNMR (DMSO-d6, δ ppm) were used.

Procedure for synthesized 1,2,4-triazole derivatives

Step 1- In the first step of the synthesis of substituted 1,2,4-triazole derivatives, substituted benzoic acid (0.1mol) was reacted with concentrated sulfuric acid (2 ml,98.079 g/mol) in 150 mL of methanol as the solvent. The reaction mixture was stirred continuously and maintained at 80–90°C for 18–20 hours, yielding either a solid or liquid oily esters. After completion of reaction,the mixture was cooled at a room temperature and poured onto a crushed ice and the product was separated accordingly . The progress of reaction was monitored by TLC using ethylacetate:hexane(1:4) as eluent.

Step 2- The ester product thus obtained was reacted with hydrazine hydrate (0.5mol and 50 g/mol), respectively, in 1:5 molar ratio using methanol as a solvent and refluxed for18-20hrs, 80-90°C . After the completion of reaction, obtained solution was poured on to a crushed ice. The product obtained was flitered and dried. The progress of the reaction was monitored by TLC using acetone:hexane(6:4).

Step 3- The intermediate product thus obtained(0.1 mol), phenylisothiocyanate was refluxed in ethanol(absolute) for 4Hrs, 80-90°C . The solution was cooled and a white solid appeared. This was filtered and recrystallised from ethanol to afford the desired product. And then product obtained is mixed in 2N NaOH and was refluxed for 3hrs, 100-110°C. The resulting solution was cooled to room temperature and acidified to pH 3-4 with 37% HCl. The precipitate obtained was filtered, washed with water and recrystallised from ethanol/water(1:1) to afford the desired compounds

BIOLOGICAL STUDIES:

The anti-tubercular activity of the synthesized compounds was evaluated against Mycobacterium tuberculosis H37Rv using the Microplate Alamar Blue Assay (MABA). This method is advantageous due to its non-toxic nature, use of a stable reagent, and good correlation with both the proportional method and the BACTEC radiometric technique. To minimize medium evaporation, 200 µL of sterile deionized water was added to the outer perimeter wells of a sterile 96-well plate. Each test well received 100 µL of Middlebrook 7H9 broth, and the test compounds were serially diluted directly on the plate, with concentrations ranging from 100 to 0.2 µg/mL. The plates were then covered, sealed with parafilm, and incubated at 37°C for five days. Following the incubation, 25 µL of a 1:1 mixture of Alamar Blue reagent and 10% Tween 80 was added to each well, and the plates were further incubated for 24 hours. A colorimetric change indicated the results: pink wells signified bacterial growth, while blue wells indicated inhibition of growth. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compound that prevented a color change from blue to pink.

RESULTS AND DISCUSSION

Synthesized 1,2,4-triazole derivatives

[T-1] 5-(2-Chlorophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol :

Amorphus yellow solid, M.P: 190-193°C; Yield (%): 70%; Rf: 0.77; Mobile phase: Chloroform:Methanol (3.5:1.5) Molecular formula: $C_{14}H_{10}ClN_3S$; Molecular weight: 287.77g/mol IR (KBr, cm-1): 1491.51 (Ar C=C) , 2767.30 (S-H), 761.47 (C-Cl) , 1551.52 (C=N) , 1030.06 (N-N), 691.47 (C-S) 1H-NMR (CDCl3, δ ppm): 12.11 (s,1H,SH) , 7.26 (1H ,s ,H-3',J=8 Hz) , 7.27(1H ,s ,H-4',J=8 Hz) , 7.28(1H ,s ,H-5',J=7 Hz) , 7.3(1H ,s ,H-6',J=8 Hz) , 7.4–7.6 (m, 5H , Ph-H (phenyl ring))

[T-2] 5-(3-Flourophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol :

Amorphus yellow solid, M.P: 180-187°C; Yield (%): 70%; Rf: 0.81; Mobile phase: Chloroform:Methanol (3.5:1.5)

Molecular formula: $C_{14}H_{10}F\ N_3S$; Molecular weight: 271.31g/mol IR (KBr, cm-1): 1494.37(Ar C=C) , 2781.59 (S-H), 1238.64 (C-F) , 1585.81 (C=N) , 1020 (N-N), 694.33 (C-S) 1H-NMR (CDCl3, δ ppm): 14.13 (1H, s , SH) , 7.5(1H,d,H-3',J=8Hz) , 7.49(1H,d,H-3',J=8Hz) , 7.37(1H,d,H-3',J=8Hz) , 7.5 (1H,d,H-3',J=8Hz) , 7.2-7.3(5H,m,Ar-H).

[T-3] 5-(3-Nitrophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol :

Amorphous yellow solid, M.P: 200-207°C; Yield (%): 98%; Rf: 0.96; Mobile phase: Chloroform:Methanol (3.5:1.5)

Molecular formula: $C_{14}H_{10}O_2N_4S$; Molecular weight: 298.32g/mol IR (KBr, cm-1): 3092.17 cm-1(C-H), 1598.65 cm-1(C=N), 1322.99 cm-1(C-N), 694.33 cm-1(C-S), 1695.81 cm-1(C=C),2738 (S-H), 1520 (NO_2 asymm) and 1340 (NO_2 symm). 1H-NMR (DMSO, δ ppm): SH (Thiol) (11.2 s,1H,SH) (broad),H-2', H-6' (NO_2-Ph)8.16 d, 2H, J=8.9Hz (doublet),H-3', H-5' (NO_2-Ph)7.52 d,2H,J=8.9Hz (doublet),Ph-H (phenyl) 7.40–7.60 m,5H (multiplet)

[T-4] 5-(4-Flourophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol :

Amorphous yellow solid, M.P: 185-189°C; Yield (%): 70%; Rf: 0.98; Mobile phase: Chloroform:Methanol (3.5:1.5)

Molecular formula: $C_{14}H_{10}F\ N_3S$; Molecular weight: 271.31g/mol IR (KBr, cm-1): 1494.37(Ar C=C) , 2781.59 (S-H), 1238.64 (C-F) , 1585.81 (C=N) , 1020 (N-N), 694.33 (C-S) 1H-NMR (CDCl3, δ ppm): 14.13 (1H, s , SH) , 7.5(1H,d,H-3',J=8Hz) , 7.49(1H,d,H-3',J=8Hz) , 7.37(1H,d,H-3',J=8Hz) , 7.5 (1H,d,H-3',J=8Hz) , 7.2-7.3(5H,m,Ar-H) 13C NMR (500 MHz, DMSO-d6)151.36,131.84,130.71,130.49,129.32,116.82,116.64,39.92,39.75,39.58,39.42,39.25,39.08,38.91, MS, ES+(ToF): m/z—[M+ +1]: 272.0613 m/z

[T-5] 5-(2-Bromophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol :

Amorphous yellow solid, M.P: 178-184; Yield (%): 69%; Rf: 0.76; Mobile phase: Chloroform:Methanol (3.5:1.5)

Molecular formula: $C_{14}H_{10}BrN_3S$; Molecular weight: 332.22g/mol IR (KBr, cm-1): Characteristics peak at 1508 (Ar C=C) , 2933.03 (S-H), 612 (C-Br) , 1590.09 (C=N) , 1010 (N-N), 694.33 (C-S) 1H-NMR (CDCl3, δ ppm): 11.99(1H , s , SH) , 8.10 (d,1H,J_1 =7.5 Hz, H-3' (Br-Ph) ,7.57(d,1H,J_1 =7.5 Hz), H-4' (Br-Ph),8.2 (d,1H,J=7.5 Hz,H-5' (Br-Ph)), 8.2(d,1H,J = 7.5 Hz,H-6' (Br-Ph), 7.30–7.60 (m,5H,Ph-H (phenyl ring))

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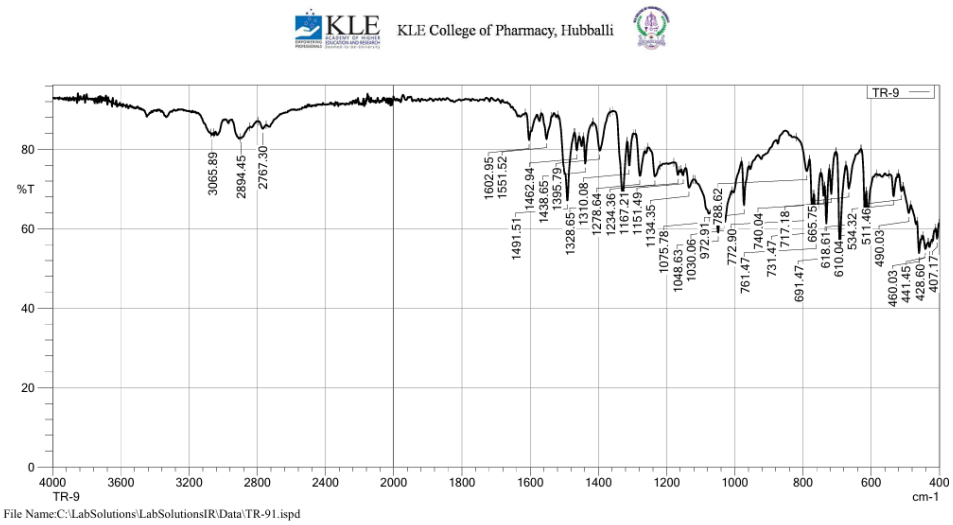
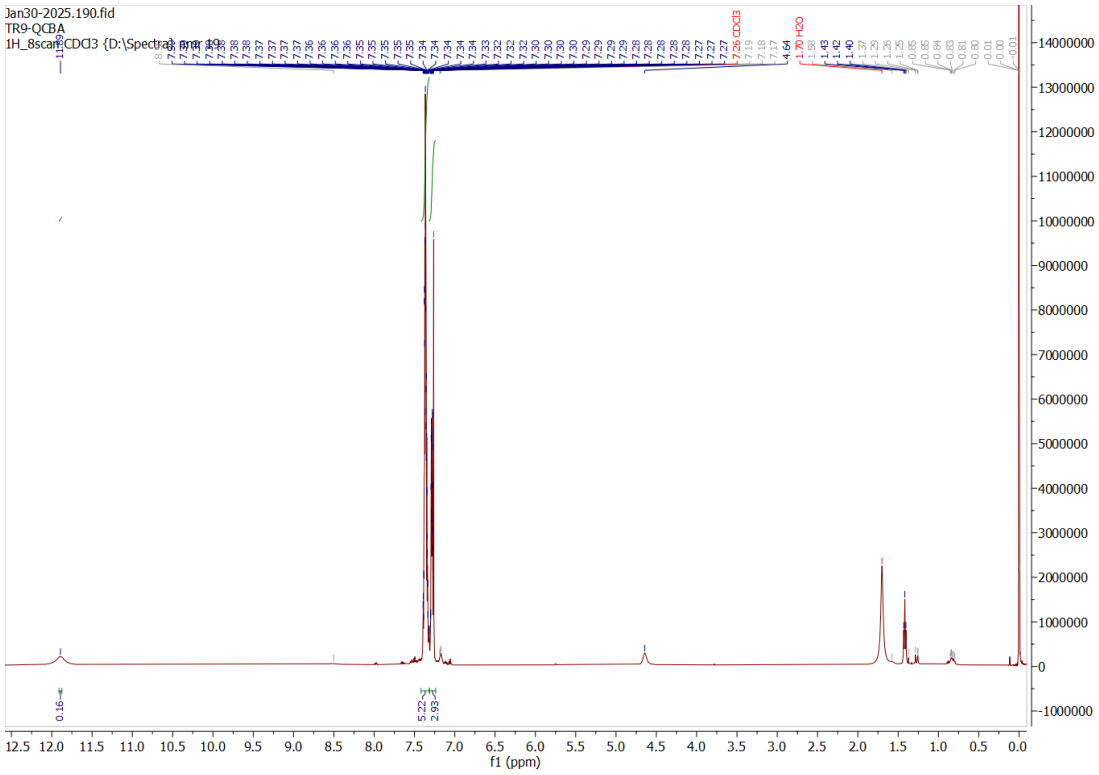
[T-6] 5-(4-Chlorophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol :

Amorphous yellow solid, M.P: 80-90°C; Yield (%): 84%; Rf: 0.74; Mobile phase: Chloroform:Methanol (3.5:1.5)Molecular formula: $C_{14}H_{10}ClN_3S$; Molecular weight: 287.77g/mol IR (KBr, cm-1): 1494.37 (Ar C=C) , 2748.73 (S-H), 742.90 (C-Cl) , 1598 (C=N) , 1015 (N-N), 695.75 (C-S) 1H-NMR (CDCl3, δ ppm): 12.11 (s,1H,SH) , 7.26 (1H ,s ,H-3',J=8 Hz) , 7.27(1H ,s ,H-4',J=8 Hz) , 7.28(1H ,s ,H-5',J=7 Hz) , 7.3(1H ,s ,H-6',J=8 Hz) , 7.4–7.6 (m, 5H , Ph-H (phenyl ring))



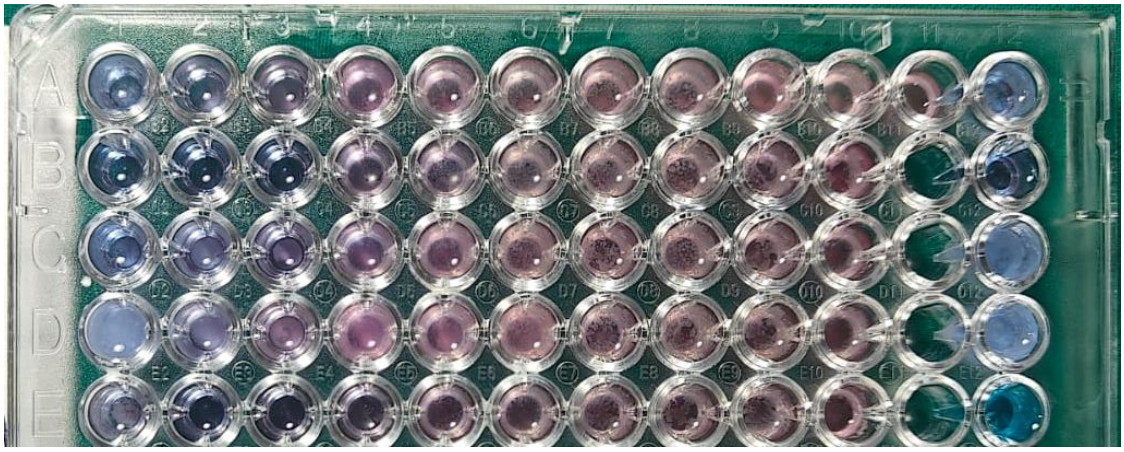
Spectrum 01:- IR of 5-(2-Chlorophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol



Spectrum no-02:- ^{1}H NMR of 5-(2-Chlorophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol

BIOLOGICAL ACTIVITY

Compound	MIC mcg/ml
T-1	25
T-2	25
T-3	6.25
T-4	25
T-5	12.5
T-6	25



CONCLUSION

All the compounds were synthesized according to synthetic scheme under appropriate experimental conditions and analysed by elemental analysis, IR, mass, and 1 H/13CNMR. The pharmacological potential was evaluated to study the effect of different substituents on antitubercular activities. From the outcomes of the pharmacological studies it can be concluded that introduction of nitro (T3) group at meta position enhances the antitubercular activity.. The substitution of o-Flouro (T3) groups on the aromatic ring may enhance the activity against MTB .

ABBREVIATIONS

IR: Infrared; NMR: Nuclear magnetic resonance; MIC: Minimum inhibitory concentration; SAR: Structure activity relationship; TLC: Thin layer chromatography; ATR: Attenuated total refection; DMSOd6: Di-methyl sulfoxide;$CDCl_3$-Chloroform;MABA: Microplate Alamar Blue Assay.

ACKNOWLEDGEMENTS

The authors are thankful to Head, Department of Pharmaceutical Chemistry, KLE College of Pharmacy, KLE University, Karnataka, for providing necessary facilities to carry out this research work.

Authors' contributions

Authors Pooja Kogonole , Sagar Shiraguppi have designed synthesized and carried out the anti tubercular activity and also carried out the spectral analysis, interpretation and anti tubercular evaluation of synthesized compounds. All authors read and approved the fnal manuscript.

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