Design, Synthesis & Biological Evaluation of Isoniazid Derivatives with Potent Antitubercular Activity

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Abstract

Isoniazid remains a key treatment for tuberculosis (TB) due to its effectiveness in inhibiting the production of mycolic acids in Mycobacterium tuberculosis. This research focuses on the synthesis of isoniazid and its derivatives, analyzing their physical and chemical characteristics using UV, IR, and mass spectroscopy. The antitubercular efficacy of these synthesized compounds was evaluated using the micro broth dilution method. Compounds 1(a) and 1(b) demonstrated significant potency with minimum inhibitory concentrations (MIC) of less than 7.8 µg/mL, whereas Compound 1(c) showed lower effectiveness with an MIC of 15.6 µg/mL. These findings suggest the potential of these derivatives in improving TB treatment and highlight the need for ongoing research and development of antitubercular agents.

Index terms: Isoniazid, Mycolic acid synthesis, Antitubercular activity, Minimum inhibitory concentration (MIC), Micro broth dilution method

Introduction

Isoniazid, a key medication in the treatment of tuberculosis, has been a standard part of therapy. This drug is highly effective due to its ability to target and inhibit the production of mycolic acids, which are vital components of the cell walls in Mycobacterium tuberculosis, the pathogen responsible for TB. By disrupting the synthesis of these acids, isoniazid damages the bacterial cell wall, leading to the elimination of the bacteria. It is a crucial element of TB treatment protocols, especially when used alongside other antitubercular medications like rifampin, pyrazinamide, and ethambutol.

Isoniazid is particularly notable for its role in preventing the progression of latent TB infections to active disease. This preventive capability makes it an indispensable tool in public health initiatives focused on controlling and eventually eliminating TB. Administered orally, isoniazid is efficiently absorbed through the gastrointestinal tract and distributes effectively throughout the body. Although generally well-tolerated, it can cause side effects such as liver toxicity and nerve damage, particularly in individuals with specific risk factors or during extended use.

Despite its effectiveness, the broad use of isoniazid has led to the development of resistant strains of Mycobacterium tuberculosis. Resistance typically arises due to genetic mutations that affect mycolic acid production, posing significant treatment challenges and necessitating alternative therapies. Nevertheless, isoniazid continues to play a pivotal role in TB management, highlighting its importance in both clinical practice and public health efforts.

Materials and Methods

Chemistry

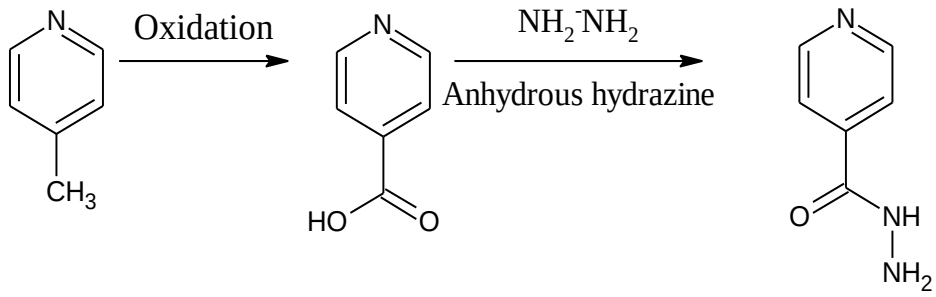
Isoniazid (INH) is a highly effective and widely utilized first-line drug for treating tuberculosis (TB). Synthesized in the early 1950s, it has been pivotal in the global TB treatment effort.

Isoniazid, chemically known as isonicotinic acid hydrazide, has the formula $C_6H_7N_3O$. Its structure includes a pyridine ring, which is a six-membered ring containing one nitrogen atom, attached to a hydrazide group (-$CONHNH_2$). Isoniazid functions as a prodrug, requiring activation within the bacterial cell to be effective. Inside Mycobacterium tuberculosis, isoniazid is activated by an enzyme called KatG, which is a catalase-peroxidase. This activation converts isoniazid into an active form, likely an isonicotinic acyl radical. The active form of isoniazid binds to NADH (nicotinamide adenine dinucleotide), creating an isoniazid-NAD complex. This complex inhibits the enzyme InhA, an enoyl-ACP reductase necessary for mycolic acid synthesis.

Mycolic acids are essential components of the mycobacterial cell wall. By blocking their production, isoniazid weakens the cell wall and leads to bacterial death. Isoniazid is effective in killing actively dividing Mycobacterium tuberculosis. It disrupts cell wall synthesis, halting bacterial replication and reducing the bacterial load in the infected person. Resistance to isoniazid poses a major challenge in treating TB. It can develop through mutations in the KatG Gene, these mutations can reduce or prevent the activation of isoniazid, making it ineffective. Alterations in InhA can diminish the binding of the isoniazid-NAD complex, leading to resistance. Increased production of InhA can counteract the inhibitory effects of isoniazid.

Synthesis Of Isoniazid

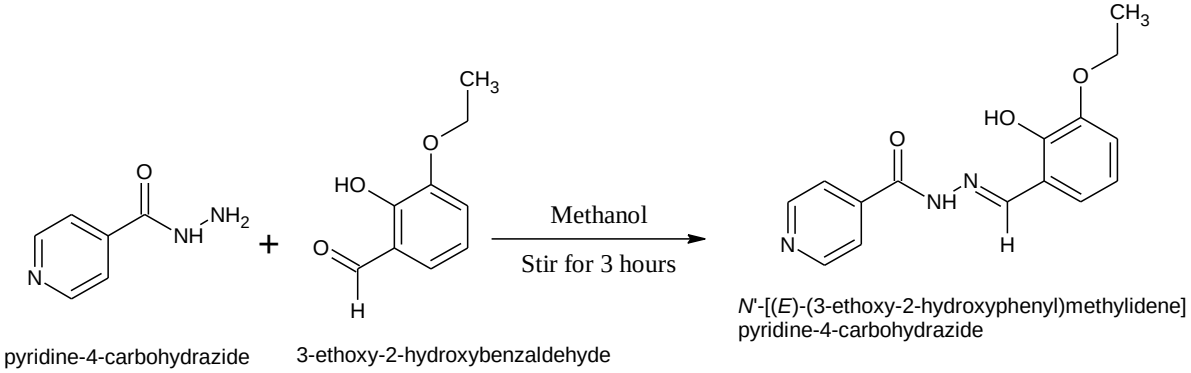
4-methylpyridine is oxidized to obtain Isonicotinic acid. Isonicotinic acid upon heating with anhydrous hydrazine form Isoniazid.



Scheme-1: Synthesis of Isoniazid

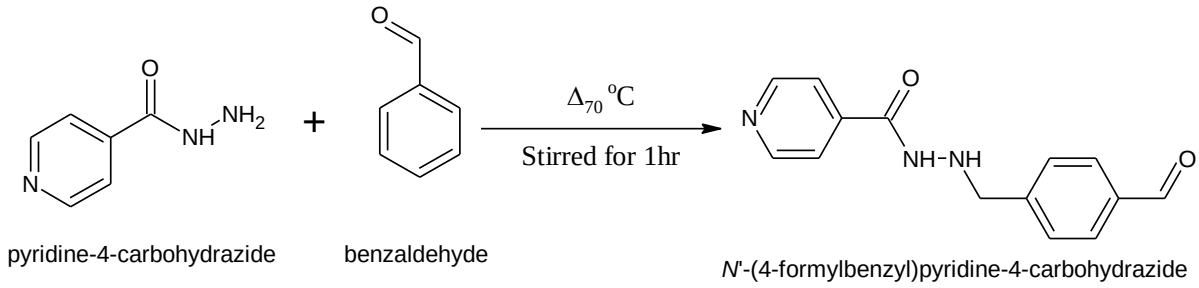
Synthesis Of Isoniazid Derivatives

Synthesis of 1.6g of 3-ethoxysalicyaldehyde was added to a solution of 1.3g of isoniazid in 30 ml of methanol and stirred for 3 hours. The pale yellowish solid separated was filtered, washed repeatedly with methanol, dried in air and recrystallized from ethanol.



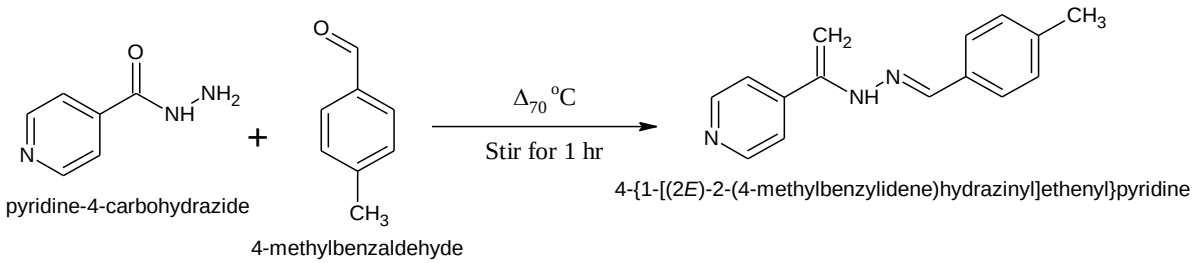
Scheme-2: Synthesis of Compound 1(a)

A mixture of 1.4g of isoniazid, 1ml of benzaldehyde and 10 ml of isopropyl alcohol was stirred thoroughly in a magnetic stirrer. The reaction mass was stirred at 70^0C for 1 hour. The obtained product was cooled and the solid was filtered, recrystallized by ethanol.



Scheme-3: Synthesis of Compound 1(b)

A mixture of 1.4g of isoniazid, 1.2 ml of 4-methylbenzaldehyde and 10ml of isopropyl alcohol. The reaction mass was stirred at 70^0C for 1 hour. The obtained product was cooled and the solid was filtered, recrystallized by ethanol.



Scheme 4: Synthesis of Compound 1(c)

Results And Discussion

Physical Characterization

The physical characterization of synthesized compounds is shown in the Table 1.

Compound	Molecular Formula	Molecular Weight (g/mol)	Colour	R_f value	Solubility	Percentage Yield (%)
1(a)	**$C_{15}H_{15}N_3O_3$**	285.30	Pale yellowish solid	0.9	Moderate soluble in ethanol, dimethyl sulfoxide, Limited soluble in hexane	93%
1(b)	**$C_{14}H_{11}N_3O_2$**	253.26	White solid	0.84	Soluble in methanol, ethanol, dimethyl sulfoxide, Limited solubility in water	69%
1(c)	**$C_{14}H_{13}N_3O$**	239.27	White solid	0.8	Soluble in methanol, ethanol, dimethyl sulfoxide, Limited solubility in water	71%

Table 1: Physical Characterization of Synthesized Compounds

Thin Layer Chromatography

Samples was analyzed using thin-layer chromatography on a silica gel F_{254} with a mobile phase comprising ethyl acetate, acetone, methanol and hexane in a 5:2:2:1 (v/v) ratio. Then the R_f value was calculated.

R_f = Distance travelled by solute / Distance travelled by solvent

UV spectroscopy

In UV Spectroscopy, there was a slight variation in the λmax of the compounds 1(a),1(b), 1(c) & the results are given in the Table 2

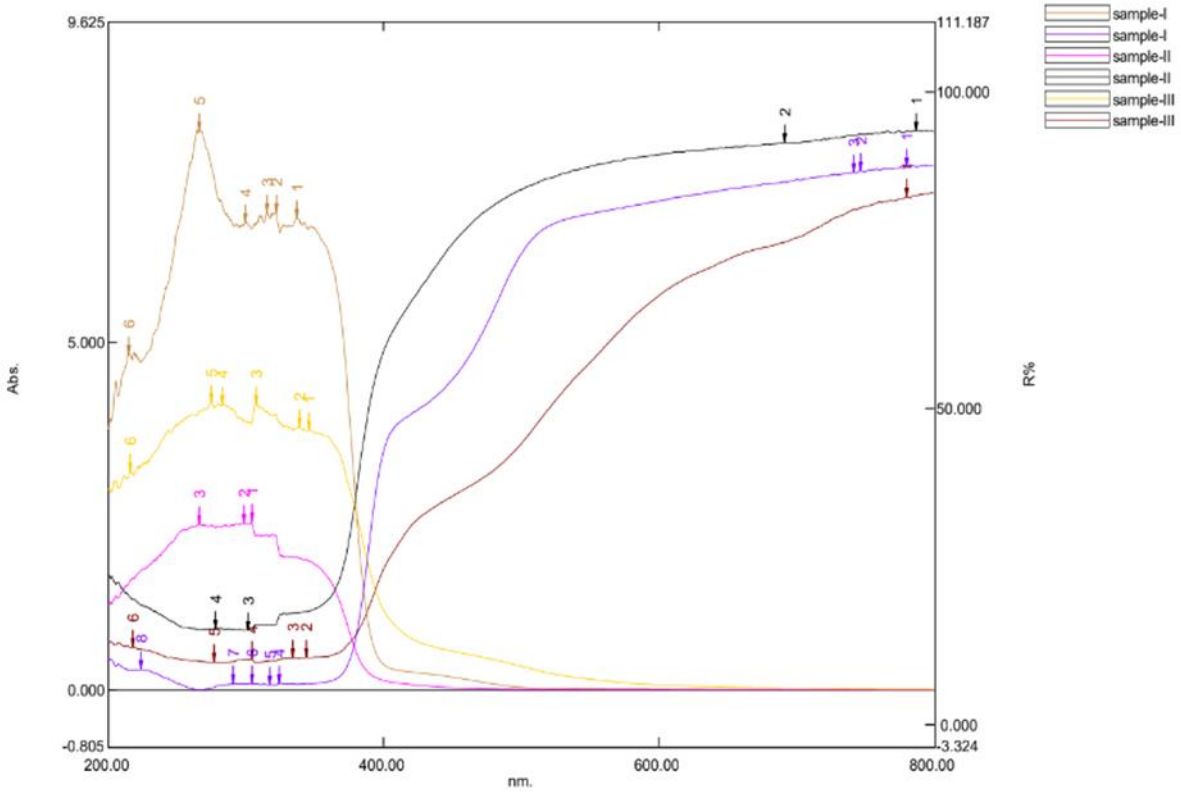


Figure 1: UV Spectrum of Compounds

Table 2:λ_{max} of Compounds

IR spectroscopy

Infrared (IR) spectroscopy is an absorption method widely used in both qualitative and quantitative analysis. Spectrum include electromagnetic radiation that can alter the vibrational and rotational states of covalent bonds in organic molecules.

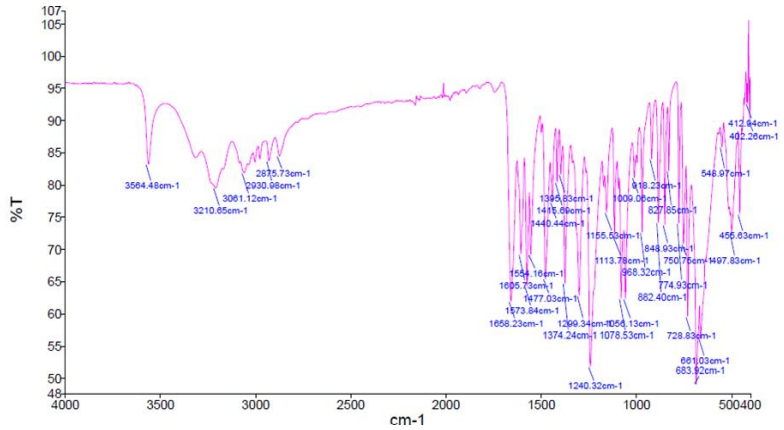


Figure 2: IR Spectrum of Compound 1(a)

IR Spectrum Interpretation of compound 1(a) : Phenolic aromatic ring : 3564.48 cm^{-1} , Amide : 1605.7 cm^{-1} , C=N : 1658.23 cm^{-1}, C-O : 1240.32 cm^{-1} , Aromatic ring : 2930.98 cm^{-1}

Compounds	λmax
1(a)	266nm
1(b)	299nm
1(c)	275nm

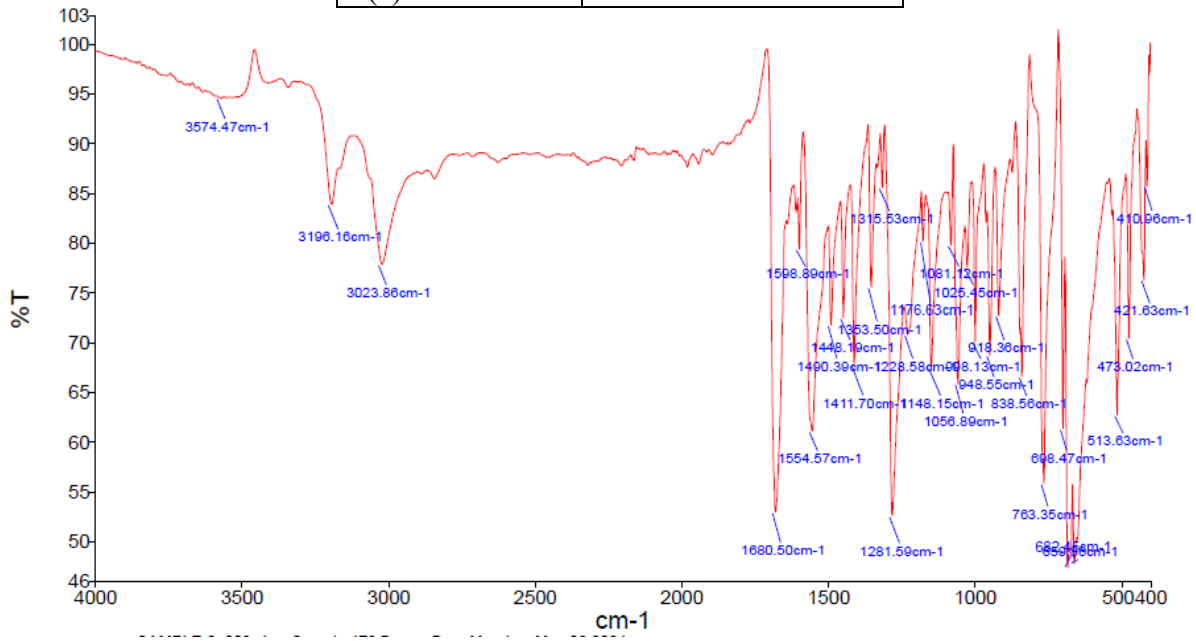


Figure 3: IR Spectrum of Compound 1(b)

IR Spectrum Interpretation of compound 1(b): C=N: 1680.50 cm^{-1}, Aromatic-aldehyde: 3023.86 cm^{-1}, N-H3196.16 cm^{-1}, CH_3: 1315.53 cm^{-1}

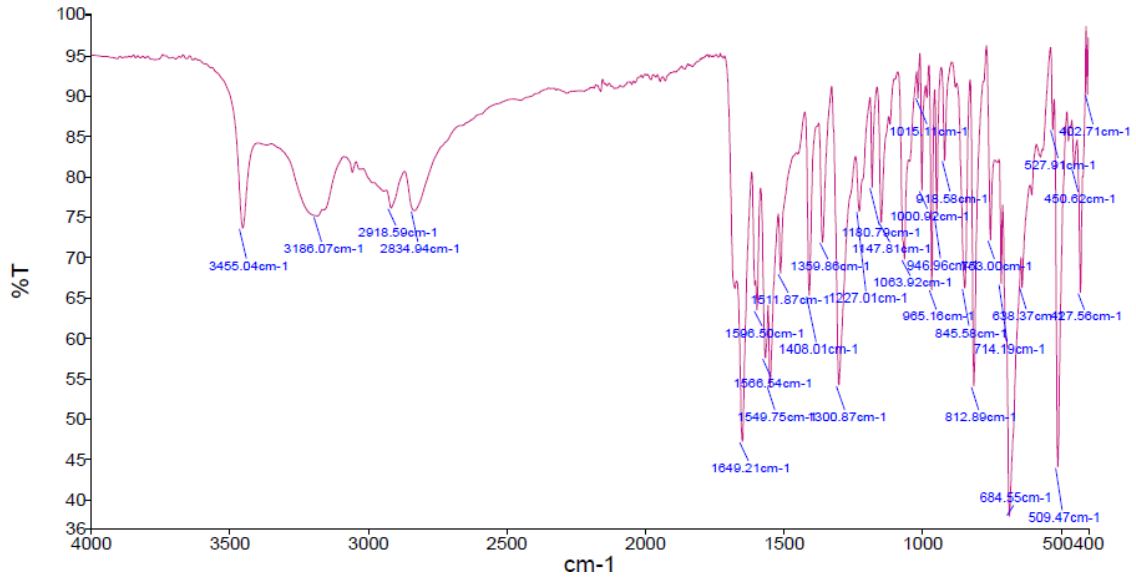


Figure 4: IR Spectrum of Compound 1(c)

IR Spectrum Interpretation of compound 1(c): Aromatic ring: 2918.59 cm^{-1}, C=N: 1649.21 cm^{-1}, Amide :1596.50 cm^{-1}, C=O:1015.11 cm^{-1}, CH_3: 1359.86 cm^{-1}

Mass spectroscopy

In mass spectrum, which represents the distribution of ions as a function of their mass-to-charge ratio, would provide information about the molecule's mass and fragmentation pattern under ionization. The mass spectrum of isoniazid typically shows peaks corresponding to the molecular ion and its fragments, allowing researchers to identify and characterize the compound.

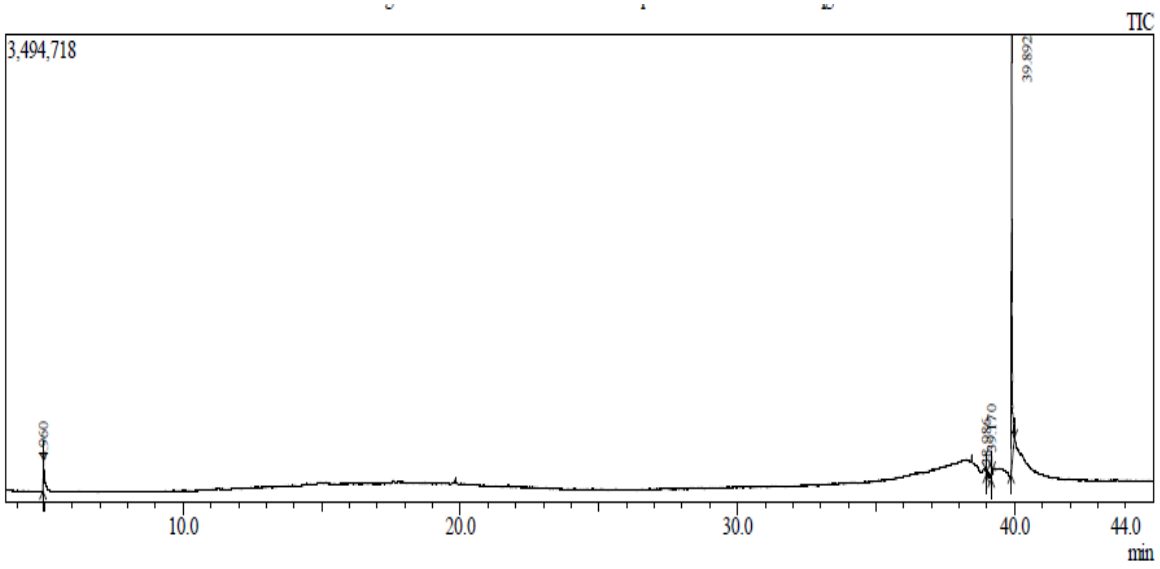


Figure 5: Mass Spectroscopy of Compound 1(a)

Antituberculosis Activity

Micro Broth Dilution Method: The micro broth dilution method is a widely used approach for determining the minimum inhibitory concentration (MIC) of an antimicrobial agent against a specific microorganism. The MIC represents the lowest concentration of the antimicrobial that effectively prevents the visible growth of the microorganism.

principle: The micro broth dilution method involves subjecting the microorganism to a range of two-fold dilutions of the antimicrobial agent within a liquid growth medium. After incubation, bacterial growth is evaluated either visually or using a spectrophotometer. The MIC is identified as the lowest concentration of the antimicrobial agent that completely inhibits visible bacterial growth.

materials: Bacterial strain: - *Mycobacterium tuberculosis* H37Rv**,** Antibiotic/drug: - The drug being tested at concentrations of 7.8, 15.6, 31.25, 62.5, 125, 250, 500 µg/mL**,** Solvent: - 0.2% DMSO (Dimethyl sulfoxide), Drug control: - 1 µg/mL of Rifampicin (RIF)**,** 96-well microtiter plate**,** Mueller-Hinton broth or another suitable medium for the microorganism**,** Sterile pipettes and tips, Incubator set to 37°C.

procedure: Prepare stock solutions of the drug in 0.2% DMSO. Perform two-fold serial dilutions in the growth medium to achieve final concentrations of 7.8, 15.6, 31.25, 62.5, 125, 250, 500 µg/mL. Standardize the bacterial inoculum of the M. tuberculosis H37Rv strain by adjusting the suspension to match a 0.5 McFarland standard. Further dilute the suspension to achieve the desired inoculum concentration in each well, typically $1x10^5$ CFU/mL. Add 100 µL of each drug dilution to the wells of a 96-well microtiter plate.[14] Introduce 100 µL of the prepared bacterial inoculum into each well containing the drug dilutions, resulting in a final volume of 200 µL per well. Include a positive control (inoculum without drug) and a negative control (medium without inoculum). Dispense 100 µL of the drug control (1 µg/mL RIF) into its designated well. Incubate the microtiter plate at 37°C for 7-14 days, depending on the growth rate of the microorganism. After incubation, examine the wells for any signs of bacterial growth (turbidity). Identify the MIC as the lowest drug concentration at which no visible growth is detected. Validate the test by comparing the results with those of the drug control (RIF). Record the MIC as the lowest concentration that effectively inhibits the microorganism's growth. Compare the MIC to clinical breakpoints to determine if the microorganism is susceptible or resistant to the antimicrobial agent.

Note: Ensure that the solvent (0.2% DMSO) is used at a concentration that does not inhibit bacterial growth. Perform the test in duplicate or triplicate to ensure reliable results. Use aseptic techniques throughout the procedure to avoid contamination.

Results

Compounds	MIC of Concentration(µg/ml)
1(a)	< 7.8
1(b)	< 7.8
1(c)	15.6

Table 3: Table for antituberculosis activity

Conclusion

Isoniazid continues to be a fundamental drug in the fight against tuberculosis (TB) due to its ability to inhibit the production of mycolic acids, crucial components of the bacterial cell wall. By disrupting this synthesis, isoniazid weakens the cell wall, leading to the destruction of *Mycobacterium tuberculosis*. It also plays a key role in preventing the advancement of latent TB to active disease, which is essential for effective public health measures.

Chemically, isoniazid is derived from 4-methylpyridine through oxidation to form isonicotinic acid, which is then converted to isoniazid using anhydrous hydrazine. The synthesis of isoniazid derivatives yielded products with varied solubility and yield, and these derivatives were analyzed using UV, IR, and mass spectroscopy.

The physical properties of these synthesized compounds differed, as evidenced by their solubility and Rf values. The micro broth dilution method revealed that Compounds 1(a) and 1(b) were highly effective, with MIC values below 7.8 µg/mL, whereas Compound 1(c) had a higher MIC of 15.6 µg/mL. These findings suggest that Compounds 1(a) and 1(b) are more potent against *Mycobacterium tuberculosis*, underscoring the importance of ongoing research in TB treatment development.

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