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Research Article

Synthesis of a triazole derivative and evaluation of their antituberculer activity

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Abstract

Tuberculosis (TB) is one of the major health issue which needs an immediate attention. All over the world, 18 % of people who develop TB die annually as per WHO report. A lot of research has been done since the finding of Streptomycin for treatment of this disease. Literature review suggests that 4-amino-5-(pyridin-4-yl)-4*H*-1, 2, 4-triazole-3-thiol can show anti TB activity. Hence, in the present study, we have targeted to synthesize the desired compound and evaluate for its anti-TB activity.

Keywords: Anti TB activity, Mycobacterium tuberculosis, Triazole derivative

1. Introduction

Tuberculosis is a chronic granulomatous disease which is a major health problem in developing countries. About 1/3rd of the world's population is infected with *Mycobacteria tuberculiosis* [1]. As per WHO, 9 million people having TB along with 1.7 million die annually. In India, every year nearly 2 million people develop this disease and about 0.5 million die [2].

Remarkable progress has been made in the last 60 years since the introduction of Streptomycin in the year of 1947 for the treatment of tuberculosis. It has full therapeutic potential and could be utilized only after 1942s when the isoniazid was produced. Since 1970s the efficacy of short course (6-9months) and domiciliary regimens has been demonstrated and treatment guidelines have been formulated. According to their clinical utility the anti-TB drugs [3] are divided into:

First Line: These classes of drugs have high antitubercular efficacy as well as low toxicity; used routinely. Example – Ionized, Rifampicin, Ethambutol.

Second line: These classes of drugs have either low antitubercular efficacy or high toxicity or both. Example - Para amino salicycic acid (PAS), Cycloserine (Cys), Amikacin (Am) [4].

The increasing resistance of *Mycobacteria tuberculosis* strains to the most effective (first line) anti-TB drugs are a major factor. Drug-resistant strains caused mainly due to incomplete or improper treatment of TB patients. Resistance of *M. tuberculosis* to anti-TB drugs is caused by chromosomal mutations in genes which encodes drug targets. Multidrug-resistant [5] (resistant, at least to rifampin and isoniazid) of M. tuberculosis (MDR-TB) evolve due to the accumulation of mutations in target genes. The MDR-TB is also a life threatening World Health Organization's target of tuberculosis. Minimum treatment of about 18-24 months is also longer, which makes it difficult for health care providers to ensure adherence to treatment [6].

1.1 Pathogenesis of TB

Mycobacterium tuberculosis is an obligatory aerobic, intracellular pathogen, having tendency of the lung tissue, rich in oxygen supply in which the bacilli mainly enters to the respiratory route [7]. This bacilli spreads to the site of initial infection in the lung through the blood to other parts of the body, the apex of the lung and lymph nodes [8]. This bacilli remains forever within the granuloma which gets re-activated or may gets discharged into the airways, finally the exponential growth of the bacilli is checked. Macrophages- Mycobacteria interactions in the host

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can be summarized by their surface binding of *M. tuberculosis* to macrophages; phagosome-lysosome [9-10] fusion, mycobacterial growth inhibition/killing; presentation of antigens to T cells for the development of acquired immunity.

1.2 Types of tuberculosis

1.2.1 Primary Tuberculosis

It refers to infection process which eventually eliminates the pathogen between the Mycobacteria and the immune system. With most TB infections, the immune system contains, although not eliminate, the mycobacteria within the tubercle, which ultimately prevents the spread of bacteria and progression of the disease

1.2.3 Secondary or Reactivated Tuberculosis

In this type the infection becomes reactivated if the Mycobacteria able to rupture the tubercle and spread through the lungs. This reactivation typically happens to those with a weakened or suppressed immune system.

1.2.4 Disseminated Tuberculosis

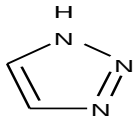
Here the spread of the disease is within the body which may result if there is an infected macrophage moving through the blood and lymph transport to other sites. Once infected, symptoms of disseminated TB correspond to the location being infected.

1.3 Triazole ring

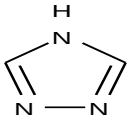
Triazoles are the class of heterocyclic compounds. Its diversity shows pharmacological activities and is well known by the medicinal chemists. Triazole, with many compounds incorporates with the other heterocyclic nucleus hydrazides, substituted triazoles, β-agonist or incorporated with antibiotics are some of the great uses of it [11-18]. The pharmacological properties are shown by its moiety which includes Phosphodiesterases enzyme inhibitor, hepatitis C, anti-inflammatory [19], antimicrobials [20], β-lactamase inhibitors, fungicidal [21], insecticidal, antitumor [22].

1.3.1 Chemistry of triazole

Molecular formula-$C_2H_3N_3$ Molecular weight-69.03



1*H*-1,2,3-triazole



4*H*-1,2,4-triazole

Triazole plays an important role among the classes of heterocycles. Generally, mixtures of tautomers are formed by the compounds having free NH group. It shows that alkylation and acylation of 1,2,4-triazole leads to 1-substituted compounds and in the absence, the tautomeric mixtures are represented by the 1-H form in both 1,2,3-triazoles and 1,2,4-triazoles [23].

Triazole refers to a pair of isomeric chemical compounds with the molecular formula $C_2H_3N_3$, and has a five membered ring containing two carbon and three nitrogen atoms. Triazoles have two isomeric forms, i.e., 1,2, 3-triazole and 1,2,4-triazole.

Fig 1: Isomeric Forms of Triazole

Triazoles have basic aromatic heterocyclic compounds, whereas certain triazoles are relatively easy to cleave by ring–chain tautomerism [24].

2. Materials and Methods

2.1 Chemicals used

The chemicals which are used Nicotinic acid, Isonicotinic acid, Benzoic acid, Hydrazine Hydrate, Sodium chloride, Carbon disulfide, Methanol, Concentrated Hydrochloric acid, Distilled water.

2.2 Synthesis of Pyridine-4-carbohydrazide

Transfer 0.1Mole of Nicotinic acid in a Round Bottom Flask, where 25ml of distilled water was added. Mix it thoroughly; pour 0.4g of sodium hydroxide till it gets completely solubilised in the round bottom flask. Shake it vigorously. Reflux it for 7-8 hours, evaporate the solvent and finally allow it for drying in the desiccators [25,26].

Similarly, in the place of Nicotinic acid, Isonicotinic acid and Benzoic acid were taken to get the compound named 4-pyridine carboxylic acid and benzene carboxylic acid.

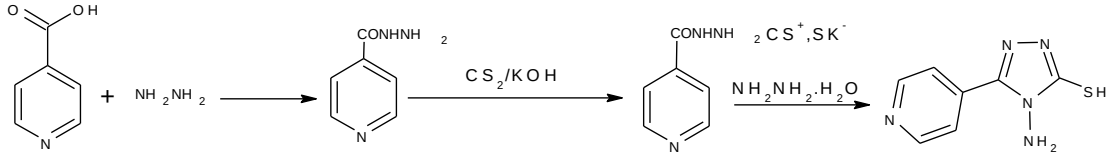
2.3 Synthesis of Pyridine potassium dithiocarbazate

From the synthesized product 1 mole of acid was taken in a small beaker, add 1.5m mol potassium hydroxide pellets in 10 ml methanol, stir the mixture continuously and then kept it in a water bath. To that mixture slowly add 1.15m mol of carbon disulfide and maintain the temperature of about 0-5°C.Stirr it overnight at room temperature so that products get separated and then filtered [27]. Washed it with chilled methanol. Keep it drying for some time. To above product 8 ml of water and 2m mol of hydrazine hydrate is added, again, it was refluxed for 4-5 hours to get the reaction mixture turned to green with the evolution of H2S gas which becomes homogenous. Dilute it with cold water. Add little amount of concentrated hydrochloric acid to above product to get the white precipitate. Filter and washed it with cold water. Recrystallize with aqueous methanol [28].

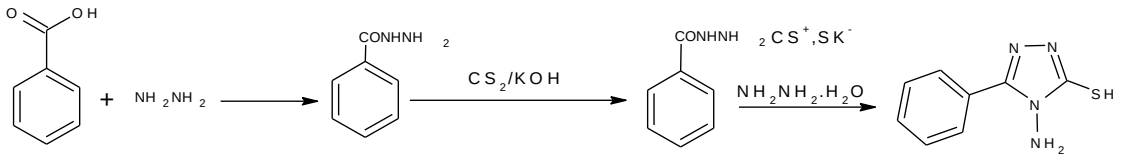
Similarly, the above procedure is followed for Isonicotinic acid and Benzoic acid to get the final product.

3. Result

Scheme-I [Synthesis of Compound I]



Scheme-II [Synthesis of Compound II]



Scheme-III [Synthesis of Compound III]

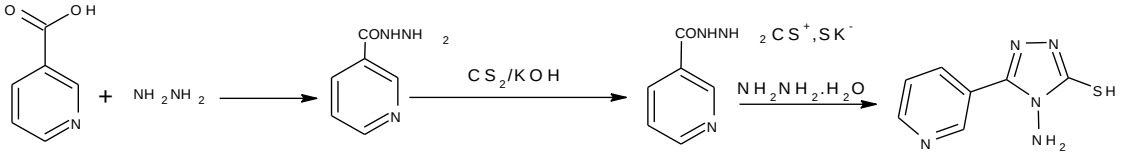
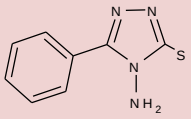


Table 1: Physiochemical Properties

Compound	Molecular Weight	Yield	Melting Point	Rf Value
I	193.22	75.68%	190°	0.71
II	193.24	81.02%	185°	0.82
III	193.22	63.83%	120°	0.65

Solvent System for TLC- CH_3OH**:** $CHCL_3$**:** $CH_3COC_2H_5$ = 1: 2 : 7

Table 2: Spectral Analysis

Compound	IR	NMR	Mass
	Ar-H- 3087 NH- 2980 SH- 2586	Ar-H- 7.3 δ NH_2- 5.6 δ SH- 13.89 δ	Base Peak- 77^+ Mol. Ion Peak- $194(M+2)^+$

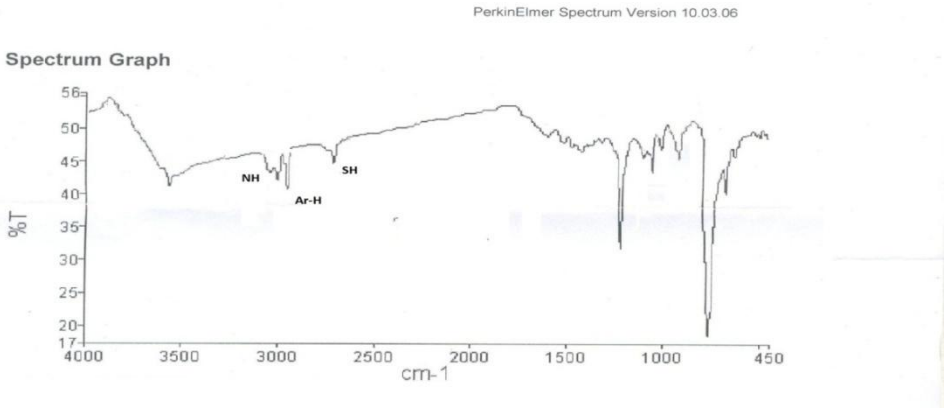


Figure 2: IR spectra of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol

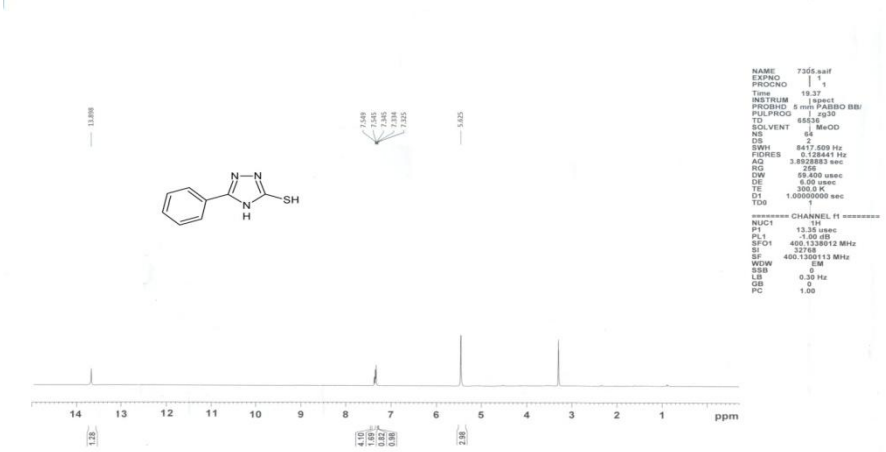


Figure 3: NMR spectra of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol

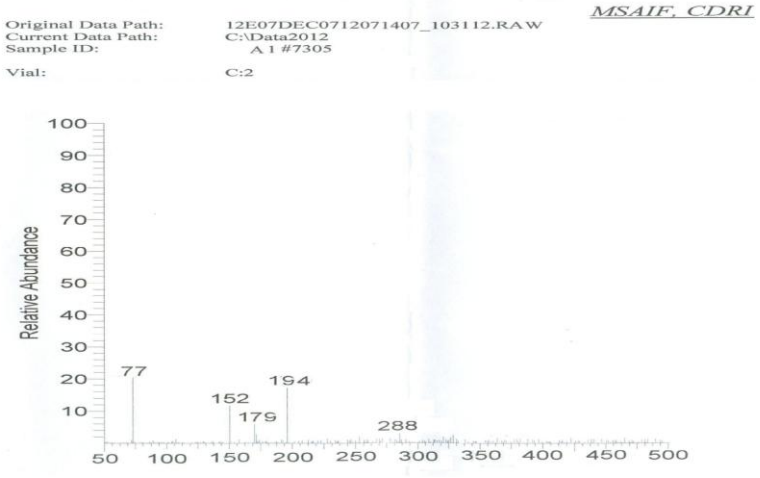


Figure 4: Mass spectra of 4-amino-5-phenyl-4*H*-1,2,4-triazole-3-thiol

Anti-tuberculosis activity procedure

The Anti-tuberculosis activity of fractions of synthesized compound were screened against *M. tuberculosis* [29] using MABA. Microplate Almar Blue Assay (MABA) is a non- toxic method, uses a thermally stable reagent which shows outstanding correlation with propotional and BACTEC radiometric assay. Briefly 96 sterile wells plates were taken followed by the addition of 200 µL sterile deionized water to all the outer perimeter wells to reduce the evaporation of medium in the test walls throughout incubation. Middlebrook 7H9 broth (100 µL) was mixed in all the well plates and serial dilution of test sample was made directly on the plate. The final fraction concentrations were tested 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100µg/ml. Each plate was covered and sealed with parafilm and incubated mixture of the Almar Blue reagent was mixed after the incubation period, 10% tween 80(1:1) was mixed to the plate and then again incubated for 24h [30-35]. A blue color in the well interpreted as no bacterial growth, while the pink color represents bacterial growth. The MIC was determined as minimal sample concentration which prevented the color changes from blue to pink.

Table 3: Anti-TB Activity of Triazole Derivatives

Treatment	Concentration (µg/mL)								MIC (µg/mL)
	100	50	25	12.5	6.25	3.125	1.6	0.8	
Compound I	S	S	S	R	R	R	R	R	25
Compound II	S	S	R	R	R	R	R	R	50
Compound III	S	S	S	S	R	R	R	R	12.5
Pyrazinamide	S	S	S	S	S	S	R	R	3.125
Streptomycin	S	S	S	S	S	R	R	R	6.25

S = Sensitive; **R** = Resistant

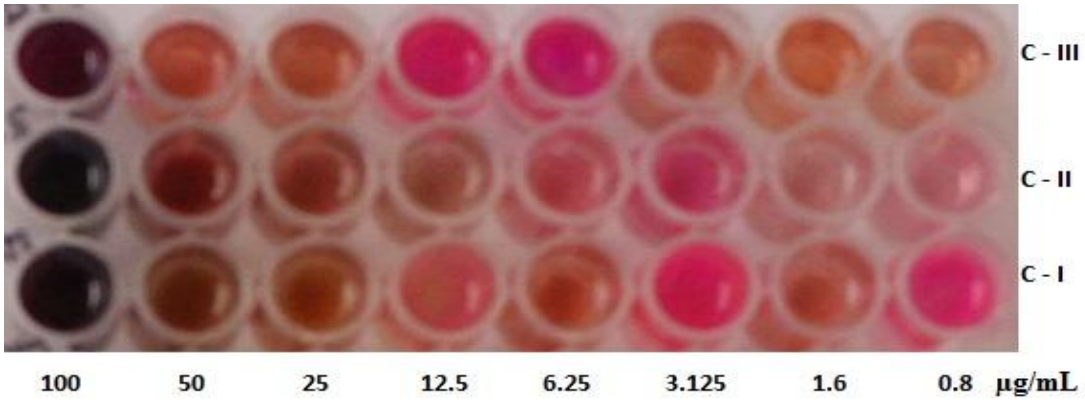


Figure 3: Anti-TB Activity of Triazole Derivative

4. Conclusion

Our finding suggests that this synthetic procedure can be a suitable method as we got a good yield for all the synthesized derivatives and the spectral data reveals that the target compound has been synthesized. So we can conclude that the synthetic methods we have followed are suitable for synthesizing targeted compounds.

The screening of anti-TB activity of the synthesized compounds was found to be good as one of the compounds showed MIC of 12.5µg/ml. Further spectral modification may require getting more potent anti-TB molecule.

5. Discussion

Triazole derivatives are well known for its antitubercular activity. Isoniazid is widely used marketed anti-TB drug is also pyridine-2-carboxylic acid derivative. In our present study, we tried to synthesize some compounds and to screen for their anti-TB activity.

Synthesis: Pyridine-3-carboxylic acid was combined with several aromatic ring systems by suitable synthetic procedure. In first step pyridine-3-carboxylic acid was prepared by reacting Hydrazine Hydrate with Sodium

chloride. In a second step the synthesized pyridine-3-corboxylic acid was reacted with KOH pellets and methanol in the presence of CS_2, stirred overnight to get the get the desired product.

All the compounds were purified and the purity was confirmed by TLC by suitable solvent system. Among all the three products Compound-II shared highest yield (81%) followed by Compound-I (75%) Compound-III (65%). The solvent system for TLC was CH_3OH:$CHCl_3$: $CH_3COOC_2H_5$ (1**:**2**:**7). The Rf value was observed 0.71, 0.82, 0.65 for compound I, II, III, respectively. The melting point of the synthesized compound was performed by open capillary method and was uncorrected. The melting point was 190°C, 185°C, 120°C for compound I, II, III respectively.

Spectral Study: The spectral analysis was performed for Compound-II. The IR, 1HNMR, MS spectroscopy study confirm the successful synthesis of the compound. The IR spectral study shared peak at 3087 cm^{-1}, 2980 cm^{-1}, 2586 cm^{-1} for Ar-H, NH, SH, respectively. 1HNMR spectral study shared δ value at 7.325 - 7.549 (Ar-H, m, 5H), 5.625 (NH, s, 2H) and 13.898 (SH, s, 1H). The mass spectral study shared base peak at 77 and $[M+2H]^+$ peak at 194.

Anti-Tb Activity: The anti-TB activity was performed in the synthesized compound the study shared that compound-III showed highest activity [12.5 µg/ml MIC value] followed by Compound I [25 µg/ml MIC value] and Compound II [50 µg/ml MIC value].

The result showed that introduction of the triazole ring in pyridine-3-carboxylic acid posses anti-TB activity, but it is less than that of standard drugs.

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