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Synthesis and Docking Study of Substituted Triazole Derivatives as Anti-Tubercular Agent

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ABSTRACT

A series of *N*- (4-Substituted Benzylidine)-amino-5-pyridine-3-yl-1,3,4-triazol-2-thiol (3a-f) were synthesized from Isoniazid. In literature survey revealed that, isoniazid was found to interfere with Nicotinamide adenine dinucleotide (NAD)-utilizing enzymes, primarily the enoyl-ACP reductase encoded by the *InhA* gene, leading to the arrest of mycolic acid synthesis, which is essential to M. tuberculosis. *InhA* enzyme was chosen based upon its hydrophobic properties that favorably interact with thioamide or thiourea moieties. All the synthesized compounds are having sulphahydryl group which shows better interaction with active site of *M. Tuberculosis*enoyl reductase enzyme encoded with the gene *InhA*. Synthesized compounds were characterized and evaluated for *in-vitro* anti-TB activity against *M.TbH*37Rv strain by Alamar-Blue assay. The results expressed as MIC (minimum inhibitory concentration) in mg/ml. Antitubercular assay has been carried out at Micropharm Diagnosis Center, Gandhinagar. Among the six compounds 3f has shown highest docking and gliding score (-7.172, -7.306) and highest activity (MIC 6.25 mg/ml).

Keywords: Triazole derivatives, Molecular docking study, Anti-tubercular activity, MIC

Abbreviations: MIC: Minimum Inhibitory Concentration, Enoyl ACP Reductase: Enoyl Acyl Carrier Protein Reductase, TB: Tuberculosis, NADH: Nicotinamide Adenine Dinucleotide, FAS: Fatty Acid Synthesis.

INTRODUCTION

Tuberculosis (TB), a bacterial infection caused by *Mycobacterium tuberculosis* (MTB), is one of the leading causes of death in the world due to a single infectious disease, which is second only to human immunodeficiency virus (HIV) acquired immunodeficiency syndrome (AIDS). MTB is an obligate, intracellular, non-motile bacillus that primarily infects humans. Bacterium is also known for its lipid-rich cell wall, which is impermeable to most dyes. MTB also divides at an incredibly slow pace, taking 15 to 20 hours [1]. *Mycobacteria* can be classified into non-pathogenic organisms, such as *Mycobacterium smegmatis*, which is fast growing and most often used as a laboratory model for MTB research, and pathogenic organisms, which cause diseases in humans and animals, such as MTB and *Mycobacterium bovis* [2].

TB has been one of the deadliest diseases over the past few decades affecting nearly one third of the world's population with new infection occurring at 1% of population each year. According to world health organization (WHO) studies, in 2016, there were 10.4 million new cases of TB (13% co-infected with HIV) and 2.4 million people died from TB including one million HIV negative people [1,2].

Enoyl acyl carrier protein reductase is the enzyme encoded with *InhA* gene, which is essential for the survival of mycobacterium through the biosynthesis of mycolic acid. In the biosynthesis *InhA* plays an important role of chain elongation by catalyzing the hydride transfer to long-chain enoylthioester substrates that is final essential enzymatic step in fatty acid elongation in the FAS-II pathway, converting 2-trans-enoyl-ACP to acyl-ACP via a hydride transfer from the 4S hydrogen of NADH to the C3 position of the 2-trans-enoyl-CoA (ACP) substrate. Mt*InhA* is a member of the *mycobacteria*l type II dissociated fatty acid biosynthesis system, and is the bonafide target for isoniazid, the most prescribed drug for tuberculosis treatment [3].

EXPERIMENTAL PROCEDURE

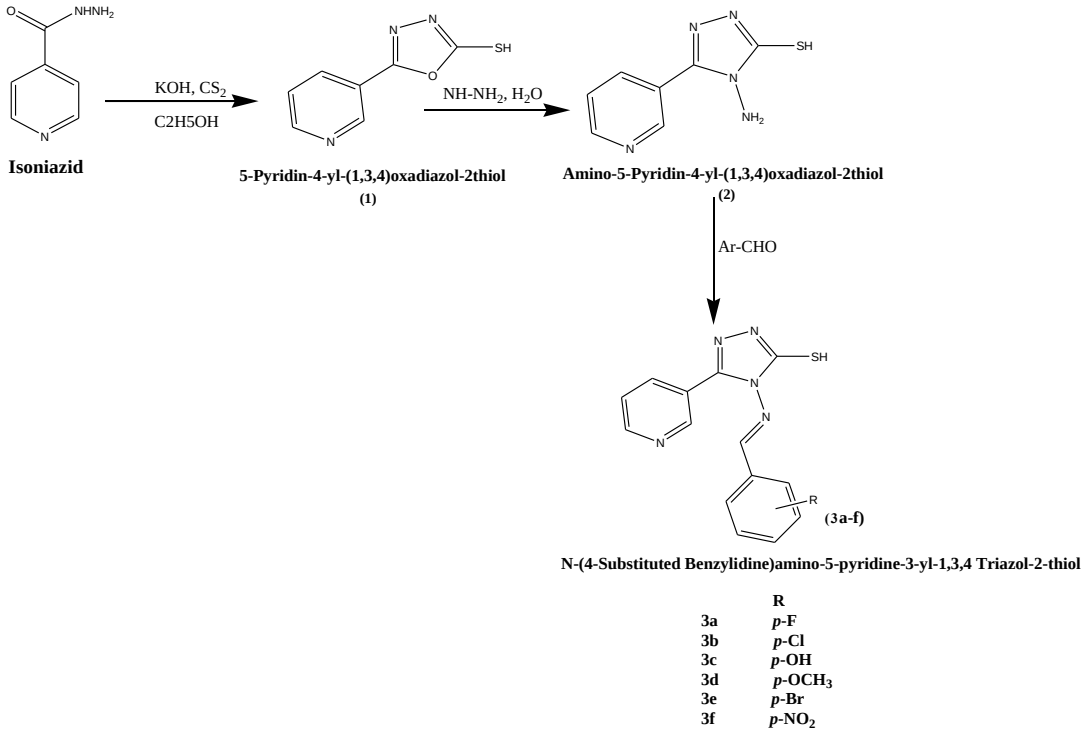
Material and methods

Melting points was determined using a VEEGO make microprocessor based melting point apparatus having silicone oil bath and are uncorrected. IR spectra (wave numbers in cm^{-1}) were recorded on a BRUKER ALPHA FT-IR spectrophotometer using Potassium bromide discs. NMR spectra were recorded on BRUKER AVANCE II 400 MHz instrument in $CDCl_3$ with TMS as internal standard for ^1H NMR. Chemical shift values are mentioned inδ, ppm. Mass spectra were recorded on Advion Expression, CMS, USA at SynZeal Research Solutions, Gandhinagar.

Chromatographic separations were performed on columns using silica gel 100–200 mesh. The progress of all reactions was monitored by TLC on 2 cm × 5 cm pre-coated silica gel 60 F_{254} (Merck) plates of thickness of 0.25 mm. The chromatograms were visualized under UV 254 nm and/or exposure to iodine vapours. All reagents used were of analytical reagent grade, obtained from LOBA chemicals, SDFCL and Spectrochem. Chemicals and solvents were purified by general laboratory techniques before use. All moisture free operations were performed in oven dried glasswares and under nitrogen atmosphere.

Synthesis and spectral data

Scheme of synthesis (Figure 1)



Isoniazid

5-Pyridin-4-yl-(1,3,4)oxadiazol-2thiol (1)

Amino-5-Pyridin-4-yl-(1,3,4)oxadiazol-2thiol (2)

(3a-f)

N-(4-Substituted Benzylidine)amino-5-pyridine-3-yl-1,3,4 Triazol-2-thiol

	R
3a	***p*-F**
3b	***p*-Cl**
3c	***p*-OH**
3d	***p*-OCH_3**
3e	***p*-Br**
3f	***p*-NO_2**

Figure 1: Scheme for the synthesis of N- (4-Substituted Benzylidine)-amino-5-pyridine-3-yl-1,3,4-Triazol-2-thiol (3a-f).

5- (Pyridine-4-yl)-1,3,4-oxadiazole-2-thiol (1): A mixture of isonicotinic acid hydrazide (0.1g, 0.0007 mole), potassium hydroxide (0.039g, 0.0007 mole) and carbon disulfide (0.7 ml) in ethanol (15 ml) was refluxed on a boil bath for 10-12 hrs. The solution was then concentrated, cooled and acidified with dil. HCL. The solid mass separated out was filtered, washed with ethanol, dried recrystallized from ethanol. [4-6].

Yield 0.110 g. (84.61%), m.p. 162-164°C, R_f.0.68 (Chloroform: Methanol, 9:1), IR (KBr, cm^{-1}) 1336 (C=S), 1598 (C=N str), 1009 (C-S str), 2579 (SH str), 3134 (NH str).

4-amino-5- (pyridine-4-yl)-4H-1,2,4-triazole-3thiol (2) : A mixture of 5- (Pyridine-4-yl)-1,34-oxadiazole-2-thiol (0.5 g, 0.0027 mole) and 99% hydrazine hydrate (0.39 ml, 0.0081 mole) in absolute ethanol (25 ml) was refluxed for 8-9 hr. The solvent and excess hydrazine hydrate was removed under reduced pressure; the residue was washed with ether, recrystallized from ethanol to give the product [4-6].

Yield 0.350 g. (64.93%), m.p. 190-193°C, R_f.0.55 (Chloroform: Methanol, 9.1), IR (KBr, cm^{-1}) 1607 (C=N str), 1002 (C-S str), 2579 (SH str), 3215 (NH str).

4- (4-Fluorobenzylideneamino)-5- (pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (3a): Equimolar of 4-amino-5- (pyridine-4-yl)-4H-1,2,4-triazole-3thiol (0.2 g, 0.001 mole) and p-Fluoro benzaldehyde (0.128 g, 0.001 mole) were taken in methanol. The mixture was heated in water bath for about 10-14 hrs. The solution was poured in ice cold water and the resulting solid was filtered, washed and recrystallized from ethanol [3-5].

Yield 0.222 g (71.84%), m.p. 237-239°C, R_f 0.33 (Hexane: Ethyl Acetate, 3:7), IR (KBr, cm^{-1}) 1604 (C=N str), 1007 (C-S str), 2710 (SH str), 1153 (C-F str). NMR (δ ppm) 7.79-786 (d, 2H,C_2 and C_6Ar-H), 7.61-7063 (d, 2H, C_3 and C_5 and C_6-H), 7.89-7.90 (d, 2H, pyridine-C_3 and C_5-H), 2.98 (s, 1H, aromatic C-SH) MASS (*m/z*) Molecular ion peak 299.8 (M+).

4- (4-chlorobenzylideneamino)-5- (pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (3b): Equimolar of 4-amino-5- (pyridine-4-yl)-4H-1,2,4-triazole-3thiol (0.2 g, 0.001 mole) and p-Chloro benzaldehyde (0.14 g, 0.001 mole) were taken in methanol. The mixture was heated in water bath for about 10-14 hrs. The solution was poured in ice cold water and the resulting solid was filtered, washed and recrystallized from ethanol [4-6].

Yield 0.180 g. (58.25%), m.p. 266-270 °C, R_f 0.51 (Hexane: Ethyl Acetate, 4.6), IR (KBr, cm^{-1}) 1647 (C=N str), 1027 (C-S str), 2600 (SH str), 781 (C-Clstr). NMR (δ ppm) 7.79-786 (d, 2H,C_2 and C_6Ar-H), 7.61-763 (d, 2H, C_3 and C_5 and C_6-H), 7.89-7.90 (d, 2H, pyridine-C_3 and C_5-H), 2.98 (s, 1H, aromatic C-SH) MASS (*m/z*) Molecular ion peak: 318 (M+1).

4-(4-Hydroxybenzylideneamino)-5- (pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (3c): Equimolar of 4-amino-5- (pyridine-4-yl)-4H-1,2,4-triazole-3thiol (0.2 g, 0.001 mole) and p-hydroxy benzaldehyde (0.122 g, 0.001 mole) were taken in methanol. The mixture was heated in water bath for about 10-14 hrs. The solution was poured in ice cold water and the resulting solid was filtered, washed and recrystallized from ethanol [4-6].

Yield 0.201 g. (65.04%), m.p. 302-305°C, R_f 0.61 (Hexane: Ethyl Acetate, 5:5), IR (KBr, cm^{-1}) 1644 (C=N str), 1092 (C-S str), 2571 (SH str), 3464 (-OH). NMR (δ ppm) 5.12 (s, 1H, -OH), 6.82-6.93 (d,2H,C_2 and C_6Ar-H), 7.39-7.42 (d, 2H, C_3 and C_5-H), 8.14 (s, 1H, N=CH), 8.57-8.63 (d, 2H, pyridine-C_2 and C_6-H), 7.80-7.84 (d, 2H, pyridine-C_3 and C_5-H), 3.11 (s, 1H, aromatic C-SH), MASS (*m/z*) Molecular ion peak: 296 (M-1).

4- (4-Methoxybenzylideneamino)-5- (pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (3d): Equimolar of 4-amino-5- (pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (0.2 g, 0.001 mole) and p-Methoxy benzaldehyde (0.136g, 0.001mole) were taken in methanol. The mixture was heated in water bath for about 10-14 hrs. The solution was poured in ice cold water and the resulting solid was filtered, washed and recrystallized from ethanol [4-6].

Yield 0.167 g (54.04%), m.p. 199-201°C, R_f 0.57 (Hexane: Ethyl Acetate, 5:5), IR (KBr, cm^{-1}) 1629 (C=N str), 1095 (C-S str), 2510 (SH str), 1225 (C-O-C). NMR (δ ppm) 3.75 (s, 3H, -OCH_3), 5.91-5.99 (d,2H,C_2 and C_6Ar-H), 6.99-7.02 (d, 2H, C_3 and C_5-H), 8.33 (s, 1H, N=CH), 8.44-8.55 (d, 2H, pyridine-C_2 and C_6-H), 7.66-7.72 (d, 2H, pyridine-C_3 and C_5-H), 3.33 (s, 1H, aromatic C-SH) MASS (*m/z*) Molecular ion peak: 311.2 (M+).

4-(4-Bromobenzylideneamino)-5- (pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (3e): Equimolar of 4-amino-5- (pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (0.2 g, 0.001 mole) and p-Bromo benzaldehyde (0.184 g, 0.001 mole) were taken in methanol. The mixture was heated in water bath for about 10-14 hrs. The solution was poured in ice cold water and the resulting solid was filtered, washed and recrystallized from ethanol [4-6].

Yield 0.214 g. (69.25%), m.p. 281-284°C, Rf-0.39 (Hexane: Ethyl Acetate, 3:7), IR (KBr, cm^{-1}) 1640 (C=N str), 1086 (C-S str), 2586 (SH str), 662 (C-Br str) NMR (δ ppm) 7.39-7.41 (d, 2H, C_2 and C_6Ar-H), 7.02-7.16 (d, 2H, C_3 and C_5-H), 8.11 (s, 1H, N=CH), 8.69-8.73 (d, 2H, pyridine-C_2 and C_6-H), 7.90-7.92 (d, 2H, pyridine-C_3 and C_5-H), 3.23 (s, 1H, aromatic C-SH) MASS (*m/z*) Molecular ion peak: 362.4 (M+2).

4- (4-Nitrobenzylideneamino)-5- (pyridine-4-yl)-4H-1,2,4-triazole-3-thiol (3f): Equimolar of 4-amino-5- (pyridine-4-yl)-4H-1,2,4-triazole-3thiol (0.2 g, 0.001 mole) and p-Nitro benzaldehyde (0.14 g, 0.001 mole) were taken in methanol. The mixture was heated in water bath for about 10-14 hrs. The solution was poured in ice cold water and the resulting solid was filtered, washed and recrystallized from ethanol [4-6].

Yield 0.245 g. (79.28%), m.p. 311-321°C, R_f0.49 (Hexane: Ethyl Acetate, 2:8), IR (KBr, cm^{-1}) 1607 (C=N str), 1103 (C-S str), 2653 (SH str), 1536 (-NO_2 str). NMR (δ ppm) =, 9.47-9.51 (d, 2H, C_2 and C_6Ar-H), 7.79-8.74 (d, 2H, C_3 and C_5-H), 8.91 (s, 1H, N=CH), 8.82-8.91 (d, 2H, pyridine-C_2 and C_6-H), 7.65-7.72 (d, 2H, pyridine-C_3 and C_5-H), 2.99 (s, 1H, aromatic C-SH) MASS (*m/z*) Molecular ion peak: 327.3 (M+1).

Molecular docking

Schrodinger software was used to perform all docking simulations. A set of new Triazole derivatives were subjected to docking with Enoyl acyl carrier protein Reductase (PDB ID 5JFO) from the Protein Data Bank (RCSB) (http://www.rcsb.org/pdb) [7-10]. To carry out in docking studies, the 2D structures of the synthesized ligands (3a-3f**)** were drawn and converted to energy minimized 3D, By removing the hetero atoms, water molecule and cofactors, the target protein file was prepared by leaving the associated residue with protein by using Auto Dock 4.2 (MGL tools-1.5.6). Preparation of target protein file Auto Dock 4.2 (MGL tools-1.5.6) tool has been done, which involves the assign of Gasteiger charges for all the atoms of molecules converting into AD4 type. Docking simulations for the compounds **(**3a-3f) were performed against *InhA* the active site of *Enoyl acp reductase*. Docking results tabulated in Table 1 & Figures 2 and 3 [11-13].

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Table 1: Result of the docking study.

Sr. No.	Conformer Code	Docking Score	Glide Score
1	Compound 3a	-6.902	-7.05
2	Compound 3b	-6.421	-6.569
3	Compound 3c	-6.794	-6.942
4	Compound 3d	-6.925	-7.179
5	Compound 3e	-6.507	-6.655
6	Compound 3f	-7.172	-7.306
7	Streptomycin	-6.857	-7.029

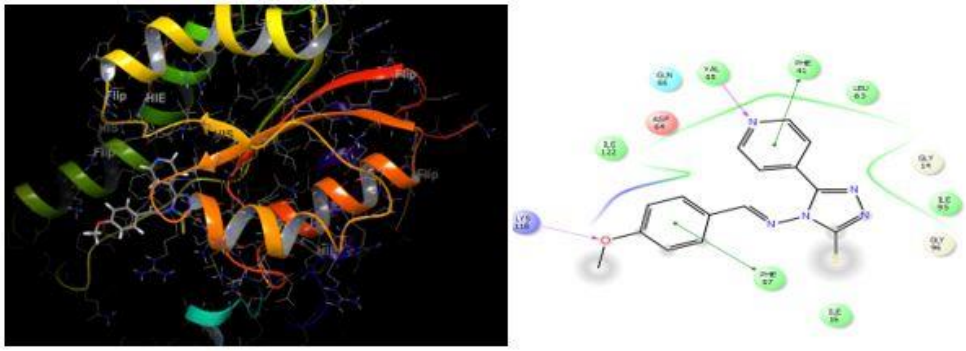

Figure 2: Docking and 2D interactions of compound 3f with the active amino acids of 5JFO.

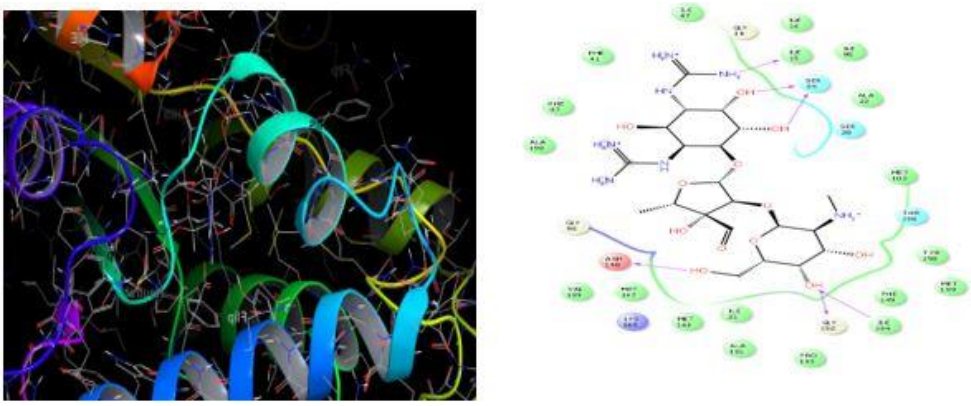

Figure 3: Docking and 2D interactions of Streptomycin with the active amino acids of 5JFO.

Biological activity

The anti-mycobacterial activity of compounds was assessed against mycobacterium strain H37Rv using the microplate Alamar blue assay (MABA).The 96 well plates received 100 µl of the Middlebrook 7H9 broth (having loopful inoculum of bacteria-108 CFU).Different dilutions of the respective compounds were made directly on the plate. The maximum concentration of the compounds tested was 50 mg/ ml. Plates were covered and sealed with parafilm and incubated at 37 C for five days. After this time, 25 µl of a freshly prepared 1:1 mixture of Alamar blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth (anti-mycobacterial activity) and a pink color was scored as growth susceptibility tests of the compounds and standard was carried out by well diffusion method. The sensitivity of the strains to the compounds was determined by measuring the diameter of the zone of inhibition surrounding the well by using millimeter scale. The compounds which were found to be satisfactory at a maximum concentration of 50 mg/ml were diluted further to concentrations viz. 25, 12.5, 6.25, 3.125 and 1.56 mg/ml respectively. Similarly, streptomycin was further diluted to 25 mg/ml in order to check the anti-tubercular activity. The MIC of the potent compounds was performed in microtiters plates by Alamar blue assay MIC is defined as the lowest drug concentration which prevented a color change from blue to pink [8-10] (Table 2).

Table 2: Anti-mycobacterial activity, MIC and MLC of synthesized compounds against *M. tuberculosis* H37Rv.

Code	Diameter of zone of inhibition (mm)	MIC in mg/ml	MLC in mg/ml
3a	>20	12.5	25
3b	>20	12.5	25
3c	> 20	12.5	25
3d	>20	12.5	25
3e	>20	12.5	25
3f	>25	6.25	12.5
Streptomycin	>25	6.25	12.5

RESULT AND DISCUSSION

All the derivatives were synthesized as per the designed scheme which has been started by isonicotinic acid hydrazide, potassium hydroxide and carbon disulfide after getting a docking score and gliding score. Molecular docking was performed by Schrodinger software. All the designed compounds and standard drug streptomycin were docked against Enoyl acyl carrier protein reductase enzyme encoded with *InhA* gene on PDB Id 5JFO. Compound 3f has shown the highest docking score when compared with standard and other compounds have shown almost equal interaction with active site of *InhA*. All the compounds were synthesized with satisfied yield, and characterized by IR, MASS and H'NMR [11-13].

Anti-tubercular activity of the entire compound was taken by microplate Alamar blue assay, at Micropharm Diagnosis Center, Gandhinagar, and result were expressed as MIC in mg/ml. In the study resistance of the *Mycobacterium* for the standard streptomycin was found at a Minimum concentration of 6.25 mg/ml and the resistance of *mycobacterium* for compounds 3f was found to be at concentration i.e., 6.25 mg/ml. and for the compound 3a-3e *mycobacterium* resistance was found at 12.5 mg/ml.

APPLICATION

The Triazoles which are prepared in this study may be useful in the treatment of Tuberculosis after further biologicaland toxicity studies.

CONCLUSION

A series of *N*- (4-Substituted Benzylidine)-amino-5-pyridine-3-yl-1,3,4-Triazol-2-thiol (3a-3f) were synthesized from Isoniazid. Docking result shown that all the compounds shown good interaction with the active site of enoyl reductase enzyme encoded with the gene *InhA*. From the result of Alamar blue assay for anti TB activity against *M.TbH*37Rv strain compounds 3f has shown highest docking and gliding score (-7.172, -7.306) and highest activity (MIC 6.25 mg/ml).

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