Screening and Evaluation of Multi Drug Resistance Activity of Various 1,2,4-Triazoles Derivatives through in Vitro Anti tubercular

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Abstract: Isoniazid (Isonicotinic acid hydrazide, INH) has been significantly used to treat Mycobacterium tuberculosis. Introduction of drugs like INH, Rifmapicin, Pyrazinamide and Streptomycin resulted in rapid decline in TB cases worldwide. Several factors lead to the emergence of resistant strains of Mycobacterium tuberculosis. HIV infection also contributed to the escalating burden of tuberculosis. In the present examination 1,2,4-triazole subordinates were planned, incorporated and exposed to in vitro antitubercular screening against Mycobacterium tuberculosis H37Rv.Lipophilicity (log P) of the compounds were also determined to establish a correlation ship between physicochemical properties and antitubercular activity. Mtb CYP121 and CYP125 are considered to be potential targets for drug design. Binding study of azoles with these enzymes have also been reported. However, enough reports are not available on Mtb CYP-ligand binding requirements to improve the MIC of Azole based antitubercular agents. Hence we conducted the docking study of our synthesized triazoles against both Mtb CYP 121 and CYP125 to establish a correlationship between antitubercular activity and receptor binding interactions. In this paper we discuss about the molecular docking studies of the synthesized mercapto and benzthio 1,2,4-triazole compounds 13-18 with different enzyme target which we have employed.

Keywords- 1,2,4-triazoles , Mycobacterium tuberculosis, GOLD, GLIDE CYP121 and CYP125.

I. INTRODUCTION

Tuberculosis (TB) will be a overpowering ailment achieved Eventually Tom's perusing the bacillus mycobacterium tuberculosis (Mtb). Following contamination, m. Tuberculosis pathogenesis happens On two periods. Those central phase will be an asymptomatic express that could perseveration to quite a while in the host, known as inactive tbilisi. Thus there is an urgent need for new drugs to treat tuberculosis with special emphasis on shortening the regimen than the current drugs and as well as novel pathway for mechanism of action to treat MDR-TB.[1-4] Recent studies have shown that nitrogen heterocyclic compounds act as anti tubercular agents by inhibiting P-450 mono-oxygenases.[5]

The genome of Mycobacterium tuberculosis encodes 20 distinctive cytochrome P450 compounds. This recommended the human genome encodes 57 P450s, though Mtb encodes 20 P450s.6-7 Greater CYP in Mtb shows that the life form depends vigorously on P450 catalysts for its survival. Recently reported that the CYP P450s are not involved in the mechanism of resistance.[10-21]

Auto Dock Vina is a generally new open-source program for prescription disclosure, nuclear docking and virtual screening, offering multi-focus capacity with unrivaled and precision.

II. MATERIALS AND METHOD

Docking accepts a huge employment in the target prescription structure to the regular furthermore pharmaceutical significances. Evaluation of newly synthesized compounds 13-18 against MDR strain by using *Mycobacterium tuberculosis (*H_{37}Rv).

Bacterial strain Modified Middlebrook 7H9 Broth, BACTEC MGIT 960 tubes, BACTEC MGIT 960 instrument (BD Biosciences Pvt. Ltd.). Middlebrook 7H9 broth (Difco), Bacto casitone (pancreatic digest of casein; Becton Dickinson), BBL Middlebrook OADC enrichment [(oleic acid, albumin, dextrose, catalase) Becton Dickinson], glycerol (Difco), polysorbate 80 (Difco), sterile water, Alamar blue (Invitogen) and deionised water (MilliQ, Millipore). The suspension prepared was used within 20 minutes for the study.

Antitubercular vulnerability testing was performed utilizing clear level bottomed 96-well microplates. 100 µL of media B was added to each well. At that point 100 µL of inoculum was added to each well. The examples were 100, 50, 25, 12.5, 6.25 and 3.125 µg/mL. DMSO was utilized as clear and extra control wells (positive and negative control) were additionally kept to limit the test blunder. Plates were secured with sterile breath seals and kept for hatching at 370C for seven days.And kept for brooding at 37oC for 24 h. After hatching for 24 hours, the microplates were imagined to identify the adjustment in shade of the wells. No adjustment in shading (blue) in the wells demonstrated the affectability of Mycobacterium tuberculosis to the test mixes and pink shading showed opposition of living being to them.

Media (A): The media was prepared addition of Glycerol (0.2 %), Casitone (0.1 %), Tween 80 (0.05 %) and 7H9 broth (0.47g/100 mL) in MilliQ water and made up to the desired volume and sterilized.**Media (B):** The media was prepared by mixing Glycerol (0.2 %), Casitone (0.1 %) and 7H9 broth (0.47g/100 mL) in MilliQ water and made up to desired volume and sterilized.

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Human Liver Microsomes (20 mg protein/mL, BD ultimo Pool tm HLM 150) What's more NADPH recovering framework results a and b (1. 3 mm NADP, 3. 3 mm glucose 6-phosphate 0. 4 U/mL glucose 6-phosphate dehydrogenase What's more 3. 3 mm MgCl2, bd Biosciences) were defrosted to 37 0C water shower What's more kept once wet ice in the recent past utilize. Will An 1. 5mL micro axis tube, 713 µL purified water, 50 µL NADPH recovering framework result A, 10 µL NADPH recovering framework result b What's more 2 µL about 5mM test compound (10 µM last concentration) were included Also blended completely by shutting those tube. The parts in the smaller scale rotator were warmed to 37^0C for 5 minutes in a hatchery. After brooding, the metabolic response was started by the expansion of 25 µL (0.5mg) Human Liver Microsomes. The blend was reversing the topped cylinder twice and kept in the hatchery at 37^0C. Following 0, 30 and an hour, response was ceased by pulling back 200 µL from the hatched cylinders and added to miniaturized scale rotator tubes (1.5mL) containing 200 µL of cold acetonitrile containing 0.2 µM propranolol as inner standard. The substance were blended and centrifuged at 12000 rpm for 5 minutes. The supernatant was expelled for investigation and pellet was put away at -200C. The examples at 0, 30 and an hour interim were broke down by HPLC utilizing RP-HPLC Microsorb-MV 100 C18 segment (250 x 4.6 mm) having molecule estimate 5µm. Acetonitrile: oxidan (0.1% HCOOH) in slope stream is utilized as portable stage. HPLC top territories were incorporated and communicated and a mean pinnacle zone an incentive for each time point was determined from the copies:

Cytotoxicity Screening

The cytotoxicity of the synthesized compounds were assessed by Micro culture Tetrazolium Assay. Reagents are Minimum Essential Medium Eagle (MEM, AT047-10x1L), Fetal Bovine Serum (FBS, RM9970-500mL), Trypsin Phosphate Versene Glucose, Phosphate Buffered Saline, pH 7.1 (PBS, TS1099-10x1L), Antibiotic Antimycotic solution 100x Liquid (A002-20mL), Thiazolyl Blue Tetrazolium Bromide reagent (MTT, AR, RM1131-500mG)and DMSO (AR, Spectrochem) and sterile deionised water (MilliQ, Millipore). Cell cultures are used namely Vero cells (African Green monkey kidney epithelial cells) and HepG2 cells (Human Hepato carcinoma cells)

III RESULTS AND DISCUSSIONS

The Compounds 13a-m were procured by showing pyrazine ring at fifth position and diverse alkyl/aryl substitutions by methods for an amide linkage at fourth position of 1,2,4-triazole ring. Ten mixes showed MIC against Mycobacterium tuberculosis at 50 µg/mL. Remaining mixes required MIC values ≥ 100 µg/mL. These mixes similarly shown extraordinary wellbeing profile (CC50 ≥ 300 µg/mL) against Vero in the same way that HepG2 units. When these compounds were docked against mycobacterial CYP 121, the –SH group of triazole derivatives showed hydrogen bonding interactions with Thr 77 and Ala 167 through a water bridge (H_2O 2584) and resulted in good docking score. In order to enhance the lipophilicity of the triazole derivatives, a 5-methyl group was substituted at the 5^{th} position of the pyrazine ring to afford compounds **14a-r**. Although the LogP of the projected compounds improved but the antitubercular activity was significantly attenuated. Only compound **14f** could manage to have MIC at 50 µg/mL.

It was also found that the amide bond of compounds drifted away from the plane by an angle of 119-120^0 and oriented themselves towards H_2O-2761. This was the major change observed in case of series (**14a-r**) compounds. Also –SH group of the triazole ligands displayed H-bond interaction with H_2O-2761. Although this change in geometry facilitated them to interact with H_2O 2761, they lost their interaction with H_2O 2584, **14f** which led to a weaker docking score.

Pyrazine ring was replaced with comparatively hydrophobic 3-phenoxy phenyl moiety at 5^{th}position of the triazole ring, to afford compounds **16a-d**. Among these derivatives, **16d** has shown antitubercular activity at MIC 25 µg/mL, but unable to show sound safety profile against human hepatocytes (CC_{50} 93 µg/mL) which could be attributed to the presence of hydrophobic diphenyl ether ring. Although compounds **17d** and **18c** displayed moderate to week safety profile (CC_{50} 90-300 µg/mL) they could manage to have SI ≥ 10. Introduction of diphenyl ether ring in these compounds improved their hydrophobic interactions but could not form any H-bonds both in CYP 121 and CYP 125. Compound **18c** formed H-bond interaction with Gly 202 in CYP 125 and antitubercular activity.

I. *N*-(3-Mercapto-5-(substituted pyrazin-4-yl)-4*H*-1,2,4-triazol-4-yl)amides 12 and 13

13a. R= H, R^1= CH_3, **13b.** R= H, R^1= C_2H_5, **13c.** R= H, R^1= C_3H_7, **13d.** R= H, R^1= C_4H_9, **13e.** R= H, R^1= C_5H_{11}, **13f.** R= H, R^1= C_6H_{13}, **13g.** R= H, R^1= 2-CH_3Ph, **13h.** R= H, R^1= 3-OCH_3Ph, **13i.** R= H, R^1= 4-OCH_3Ph, **13j.** R= H, R^1= 4-F Ph, **13k.** R= H, R^1= 4-C_2H_5 Ph, **13l.** R= H, R^1= 4-OC_2H_5 Ph, **13m.** R= H, R^1= 3,4-OCH_3 Ph

II. *N*-(3-Mercapto-5-aryl-4H-1,2,4-triazol-4-yl) (substituted pyrazin-4-yl)-2-caroxamides 14 and 15

14a. R= CH_3, R^1= CH_3, **14b.** R=CH_3, R^1= C_2H_5, **14c.** R=CH_3, R^1= C_3H_7, **14d.** R=CH_3, R^1= C_4H_9, **14e.** R=CH_3, R^1= C_5H_{11}, **14f.** R= CH_3, R^1= C_6H_{13}, **14g.** R= CH_3, R^1= Ph, **14h.** R= CH_3, R^1= 2-CH_3Ph, **14i.** R= CH_3, R^1= 4-CH_3Ph, 1**4j.** R= CH_3, R^1= 3-OCH_3Ph, 1**4k.** R= CH_3, R^1= 4-OCH_3Ph, 1**4l.** R= CH_3, R^1= 2-Cl Ph, 1**4m.** R= CH_3, R^1= 4-Cl Ph, 1**4n.** R= CH_3, R^1= 4-F Ph, 1**4o.**R= CH_3, R^1= -CH_2O Ph, 1**4p.** R= CH_3, R^1= 4-C_2H_5 Ph, 1**4q.** R= CH_3, R^1= 4-OC_2H_5 Ph, 1**4r.** R= CH_3, R^1= 3,4-OCH_3 Ph. **15a.** R= H, **15b.** R= 2-CH_3, **15c.** R= 4-CH_3, **15d.** R= 3-OCH_3, **15e.** R= 4-OCH_3, **15f.** R= 2-Cl, **15g.** R= 4-Cl.

III.
3-(Benzylthio)-4-(2,4-dichlorophenyl)-5-(3-phenoxyphenyl)-4*H*-1,2,4-triazole 16d

IV.
3-Methoxy-*N*-(3-(5-methylpyrazine-2-yl)-5-oxo-1*H*-1,2,4-traizol- 4-(5*H*) yl)benzamide 17c

V.
4-({[3-Substituted-1H-pyrazol-4-yl]methylidene}amino)-5- [(substituted)methyl]-1,2,4- triazole-3-thiols (Schiff bases) 18

Compd	Docking Score [a]		Residue Interaction [b]	
	CYP 125	CYP 121	CYP 125	CYP 121
13a	-5.73	-5.61	SH-597 H_2O	SH-2584 H_2O
13b	-5.3	-5.01	Nil	Amide-NH-2584 H_2O
13c	-5.31	-5.22	SH-597 H_2O	Tz-NH-2584 H_2O
13d	-5.17	-5.03	Nil	Nil
13e	-5.14	-5.5	Nil	Tz-NH-2584 H_2O
13f	-5.14	-5.5	Nil	Nil
13g	-5.74	-7.53	Nil	Nil
13h	-5.82	-5.31	Nil	Nil
13i	-5.82	-7.56	SH-597 H_2O	Nil
13j	-5.97	-7.61	Nil	Nil
13k	-5.79	-7.79	SH-597 H_2O	Nil
13l	-5.89	-7.66	Nil	Nil
13m	-6.31	-4.97	SH-597 H_2O	Nil
14a	-5.73	-6.24	Nil	SH-2761 H_2O
14b	-3.83	-6.18	Nil	SH-2761 H2O
14c	-5.43	-5.2	Nil	SH-2761 H2O
14d	-5.46	-5.15	Nil	SH-2761 H2O
14e	-5.41	-3.62	Amide-597H2O and Pyz-Gly202	SH-2761 H2O
14f	-5.65	-3.23	SH-597 H2O	Tz-NH-2584 H2O
14g	-5.14	-6.06	Nil	SH-2761 H2O
14h	-6.05	-6.56	Nil	SH-2761 H2O
14i	-6.20	-5.96	Nil	SH-2761 H2O
14j	-6.08	-5.67	Methoxy-Gly202	SH-2761 H2O
14k	-5.7	-5.85	Amide-597H2O	SH-2761 H2O
14l	-6.37	-7.02	SH-597 H2O	SH-2761 H2O
14m	-5.71	-6.56	Nil	SH-2761 H2O
14n	-5.49	-5.69	SH-597 H2O	SH-2761 H2O
14o	-6.3	-6.76	SH-597 H2O	SH-2761 H2O
14p	-6.16	-5.95	SH-597 H2O and Pyz-Gly202	SH-2761 H2O
14q	-6.04	-6.07	SH-597 H2O	Tz-NH-2761 H2O
14r	-6.18	-4.41	Methoxy-Gly202	Amide-C=O-2761 H2O
15a	-4.34	-4.85	Nil	Pyz- NH-2761 H2O, SH-Gly-385
15g	-6.05	-6.58	SH-597 H2O	SH-2761 H2O
16a	-5.97	-4.7	PhenylIle97,Met264 ; SH-Gly202	Nil
16b	-6.77	-6.34	Nil	Nil
16c	-6.95	-5.94	S-Val11	Nil
16d	-7.9	-6.71	S-Val11	Nil
17d	-8.46	-3.64	-S-Gly202,Ile97	Nil
18c	-5.92	-6.38	Tz-C=O-597 H2O	Tz-C=O- H2O 2584,Amide-C=O-Gly-385

Table-I: Molecular docking data of synthesized selective compounds in CYP 125 and CYP 121 enzyme

1. [a]Molecular docking studies with binding score in Schrodinger, [b]Interactions of molecules with the amino acids in receptor CYP125 and CYP121 through hydrogen bonding.Tz=triazole.

Table-II: Biological activities of synthesized compounds

Compd	Anti Tubercular activity MIC [a] (µg/mL)	Vero (CC50) [b] (µg/mL)	HepG2(CC50) [c] (µg/mL)
13a	50	>300	>300
13b	50	>300	>300
13c	50	>300	>300
13d	>100	>300	>300
13e	>100	>300	>300
13f	50	>300	>300
13g	100	>300	>300
13h	>100	>300	>300
13i	50	>300	>300
13j	>100	>300	>300
13k	50	>300	>300
13l	100	>300	>300
13m	>100	>300	>300
14a	>100	>300	>300
14b	>100	>300	>300
14c	>100	>300	>300
14d	>100	>300	>300
14e	>100	>300	>300
14f	50	>300	>300
14g	100	>300	>300
14h	>100	>300	>300
14i	100	>300	>300
14j	>100	>300	>300
14k	100	>300	>300
14l	100	>300	>300
14m	50	>300	>300
14n	50	>300	>300
14o	100	>300	>300
14p	100	>300	>300
14q	50	>300	>300
14r	100	>300	>300
15a	>100	>300	>300
15g	20	>300	>300
16a	25	>300	>300
16b	50	>300	>300
16c	50	>300	>300
16d	>100	>300	>300
17d	50	<50	<50
18c	25	>300	>300

[a]Antitubercular activity against $H_{37}Rv$ strain, [b]Cytotoxicity evaluation by MTT assay method using Vero (African green monkey epithelial cells), [c]cytotoxicity evaluation by MTT assay method using HepG2 (hepatic carcinoma cells).

Table- III: Log P of compounds with anti TB MIC ≤ 25 µg/mL

Compd	Log P[1]
15b	0.228
15g	2.010
16d	3.692
17d	2.140
18c	0.920

[1] Calculated from the capacity factors of compounds at various % of organic phase used.

Fig-1: Typical plot of antitubercular activity Vs Log P of compounds with MIC ≤ 25 µg/mL µg/mL and SI ≥ 10

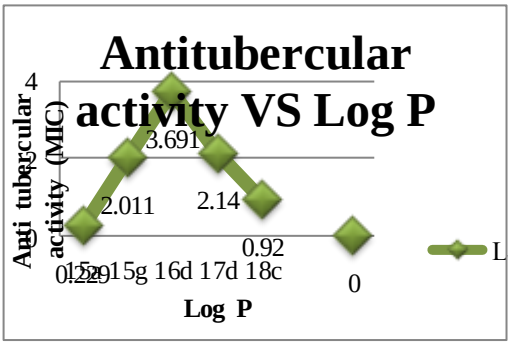
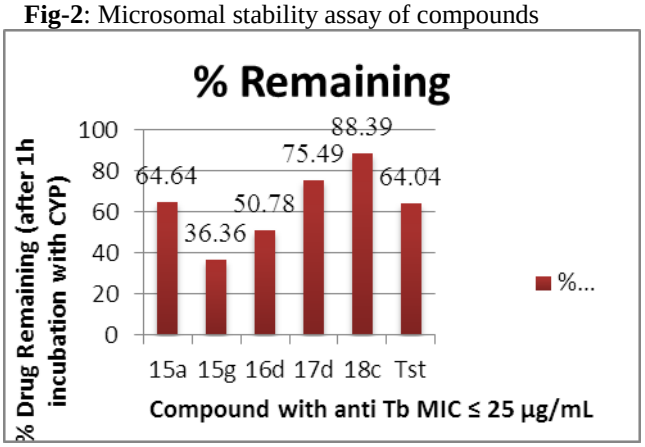


Table-IV: Microsomal stability of compounds with anti TB MIC ≤ 25 µg/mL

Compd	% Remaining [1]
15b	64.46
15g	36.36
16d	50.78
17d	75.49
18c	88.39
Testosterone(Ts)	64.04

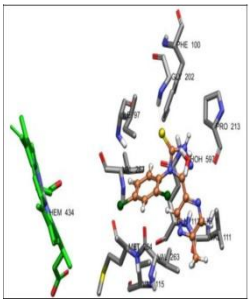
[1] Amount of drug remaining (%) after metabolisation by CYP enzymes after 1h.

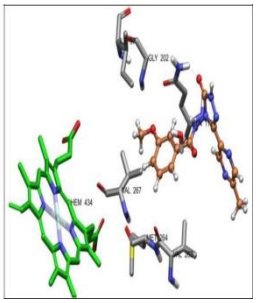
Fig-2: Microsomal stability assay of compounds

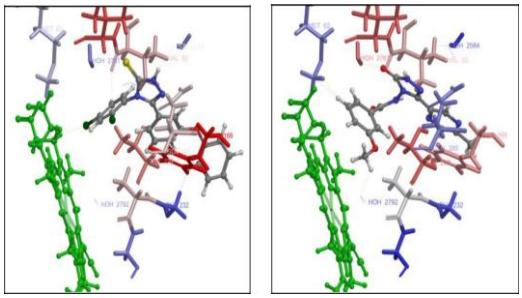


having anti Tb ≤ 25 µg/mL

C: CYP 125 with **17d**
D: CYP 125 with **18c**





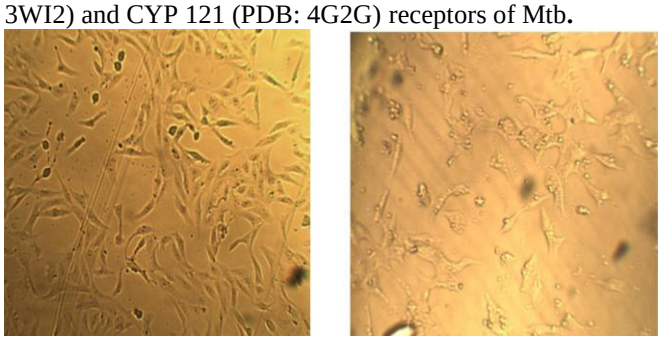


G: CYP 121 with 17d

H: CYP 121 with 18c

[a]Antitubercular activity against $H_{37}Rv$ strain, [b]Cytotoxicity evaluation by MTT assay method using Vero (African green monkey epithelial cells), [c]cytotoxicity evaluation by MTT assay method using HepG2 (hepatic carcinoma cells).

Fig-3: Docked poses of compounds with CYP 125 (PDB: 3WI2) and CYP 121 (PDB: 4G2G) receptors of Mtb**.**



A **B**

Fig-4: Effect of compound **15a** at 300 µg/mL on Vero cells: **A**. 80 % confluent Vero cells **B**. After 72h of incubation.

C **D**

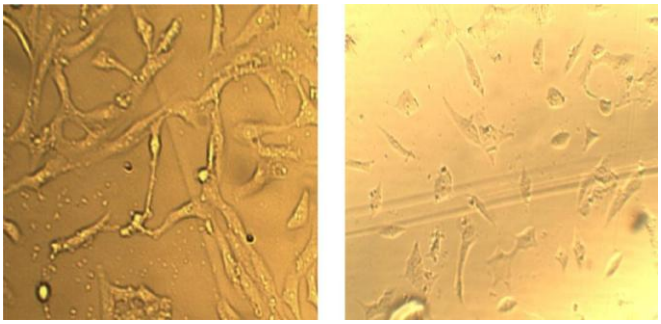


Fig-5: Effect of compound **15a** at 300 µg/mL on HepG2 cells: **C**. 90 % confluent HepG2 cells. **D**. After 24h Incubation.

IV. CONCLUSION

With a hope that development of new and effective and concurrent anti tubercular agents, was made to synthesize substituted 1,2,4-traizole derivatives of **13-18** with drug-like characters. We were great for synthesizing number triazole subsidiaries with calculable security profile, worthy metabolic Strength Furthermore hostile to tubercular action under 12. 5µg/ml. A standout amongst the exacerbates might have been Indeed going animated against MDR strain for mycobacterium tuberculosis toward 25µg/ml. Our endeavor to depict those relationship the middle of Different parameters in docking score Furthermore logP values need not yielded fancied majority of the data of the degree foreseen. We feel that there will be further degree will investigate 1,2,4-triazoles clinched alongside point of interest. This might additionally help should land at An All the more serious SAR.

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