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**Research Article**



# Design, Synthesis, Characterization and Biological Evaluation of Some Novel Benzothiazole Derivatives as Anti Tubercular Agents Targeting Glutamine Synthetase-I

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**ABSTRACT**
This study focuses on the design of benzithiazole ligands as prospective inhibition of Glutamine synthetase in the Mycobacterium tuberculosis. These derivatives were synthesized by two methods one by the condensation of 2 aminobenzothiazole derivatives, (beta keto ester), and aromatic aldehyde (1a-1d) and the other method by condensation of 2 amino benzothiazole with chloroacetyl chloride which is then reacted with ammonium thiocynate (2a), which form pyrimido and thiazolidine derivatives of benzothiazole. The synthesized compounds were characterized by IR, $^1$H NMR and GC MASS spectroscopy. These compounds evaluated for anti TB activities by Microplate Alamar Blue Assay (MABA) method. The most promising anti TB activity compound (1c) showed MIC at 6.25 g/ml that is equal to that of the standard drugs like Streptomycin and Ciprofloxacin.

**Keyword:** Benzothiazole; docking; streptomycin; antitubercular activity; MABA

## INTRODUCTION

Today, Tuberculosis is one of the leading causes of death worldwide. The World Health Organization (WHO) estimates that one-third of the world's population is currently infected with M.tuberculosis, and that 1.3 million deaths result from these infections each year. A number of once active anti-tuberculosis drugs have now became inactive due to the ever-increasing rise in drug resistant strains of tuberculosis. The multidrug resistant TB (MDR-TB) occurs only when drug-susceptible TB is improperly or incompletely treated [1]. According to WHO, MDR-TB is defined as a resistance to two of the most effective first line TB agents: Rifampicin and Isoniazid. Emergence of Multi-resistant (MDR) strains and high susceptibility of human immunodeficiency virus (HIV) infected persons to the disease has forced scientist to look for novel anti-tuberculosis agents [2].

*M. tuberculosis* in fact possesses four Glutamine Synthetase homologues, of which only one, the product of the *glnA1* gene is highly expressed and essential for the growth of the bacteria both *in vitro* and *in vivo*. It has a well-characterized role in bacterial nitrogen metabolism. *Mt*GS plays an important role in cell wall biosynthesis, specifically via the production of a poly-L-glutamate-glutamine component found exclusively in pathogenic mycobacteria [3]. The inhibition of Glutamine Synthetase secreted by M. tuberculosis is sufficient to halt the growth of the bacterium, suggesting that TB-GS might be a valid target for anti-tuberculosis drug-design.

Benzothiazole derivatives have been found to possess wide spectrum of biodynamic properties. Many of them have been reported for anti-inflammatory [5], antitumor [6], antiparkinsonism [7], antimicrobial [8] and anticonvulsant activities [9].

Therefore, it was considered meaningful to explore the synthesis of compound built upon benzothiazole skeleton incorporating pyrimido and thiazolidino moiety with hope of potentiating the activity of two such units in the same compound.

One of the methods describes the synthesis of pyrimido[2,1-*b*]benzothiazole derivatives from 2-amino benzothiazoles and *β*-halo esters [10,11]. Even though in some methods *β*-ketosters, *α*-halo acids and malonates have been used [12].

It is proposed to synthesis pyrimido[2,1-b] benzothiazole derivatives in solvent-free condition (Scheme 1). Several benzothiazole derivatives were synthesized and were screened for their in vitro antitubercular activity against H37Rv strain of Mycobacterium tuberculosis by Microplate Alamar Blue Assay method.

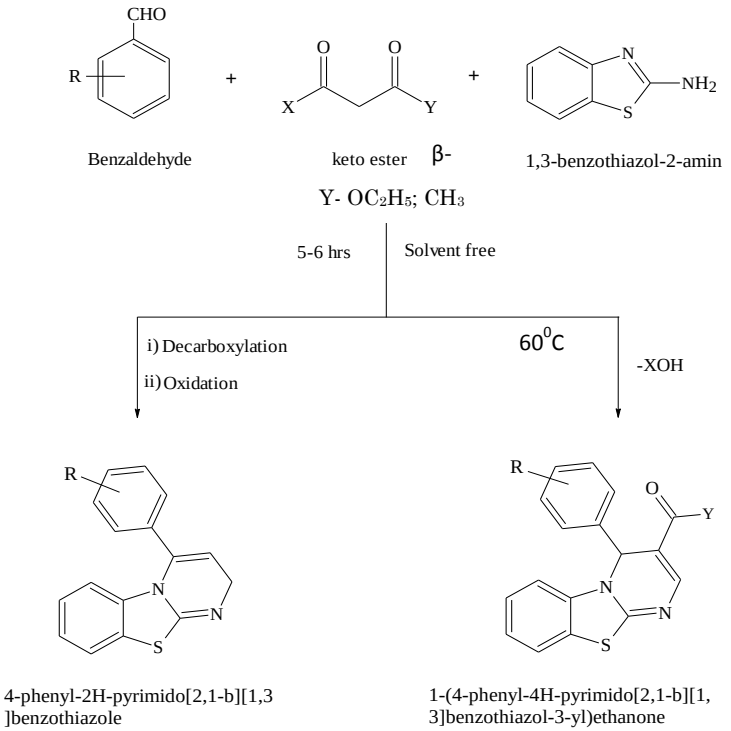
## MATERIALS AND METHODS

### Molecular modeling

Compounds were designed to obey the Lipinski's Rule of Five and then docked using AutoDock® 4.2.5.1. program [16]. The X-ray co-crystal structure of Glutamine Synthetase I (PDB ID: 3ZXR) [17] was used for docking. The binding model of the compounds with MtGS I were studied. The top scoring compounds were chosen for the study. In silico toxicity prediction was done using **OSIRIS**® Property Explorer.

### Synthetic procedure

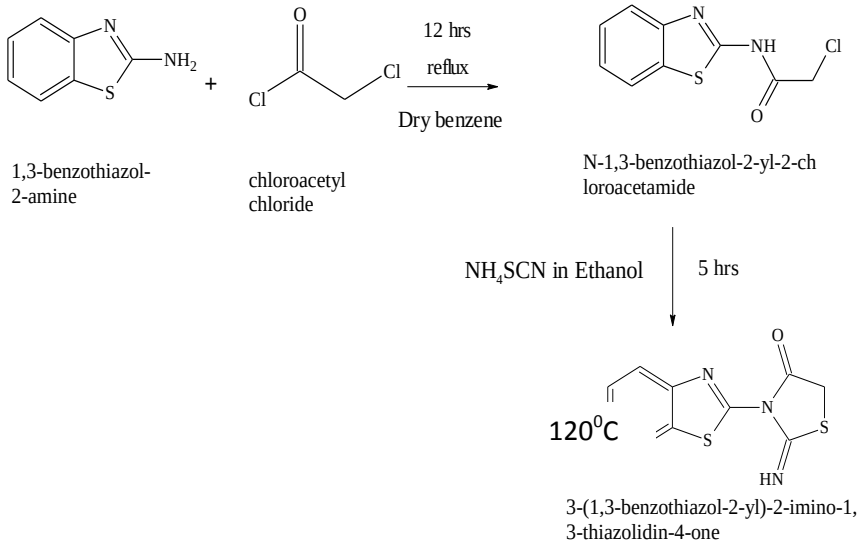
The general method for the synthesis of substituted benzothiazole derivatives are outlined below.




4-phenyl-2H-pyrimido[2,1-b][1,3]benzothiazole

1-(4-phenyl-4H-pyrimido[2,1-b][1,3]benzothiazol-3-yl)ethanone

**Scheme 1: synthesis of 4*H*-Pyrimido [2, 1-*b*] benzothiazole derivatives (1a-1d)**




**Scheme 2: Synthesis of Imino-3-(6-substitutedbenzo[d]thiazol-2-yl) thiazolidin-4-one**

**Characterization**

The melting point (MP) of the compounds was determined in open capillary tube and values are reported uncorrected. Thin layer chromatography was done to assess the course of reaction and the purity of the intermediates and the final compounds. Visualization of the compounds on chromatographic plates was done by exposure to iodine vapors.

The IR absorption spectra were recorded by FTIR model 84005 Shimadzu using KBr disk. 1H-NMR spectra were recorded on Bruker Advance 500 (300MHz) Spectrometer in CDCl3/DMSO-d6 as a solvent, the chemical shifts (δ) are expressed in ppm using TMS as internal standard. Mass spectra were measured using a high-resolution GC-MS (DFS) Thermo spectrometer with EI (70 EV).

**Antitubercular activity**

The synthesized molecules were screened for their activity to inhibit the growth of the Mycobacterium tuberculosis. The anti-mycobacterial activity of compounds is assessed against M. tuberculosis using Alamar Blue micro plate assay (MABA). Briefly, two hundred micro litter (200µl) of deionised sterile water added to all outer perimeter wells of sterile 96 wells plate. This is to reduce evaporation of medium in the test wells during incubation. Hundread micro litter (100µl) of middle brook 7H9 broth and serial dilution of compounds added directly on 96 wells plates. The final drug concentrations tested ranges from 100 to 0.2 micro gram/ml. Plates were covered and sealed. Then incubated at 370C for five days. After this, 1:1 mixture of freshly prepared Alamar blue reagent and 10% tween 80 (25µl) of was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The Minimum Inhibitory Concentration (MIC) is defined as lowest drug concentration, which prevents the colour change from blue to pink [20].

**RESULT AND DISCUSSION**

**Chemistry**

The compounds with top scoring function were identified by using AutoDock® 4.2.5.1.docking program. The molecules with good docking score (table 1) were selected ie, those compounds which have the least binding energy with the target. The binding models of the compounds with MtGS 1 and interaction view are displayed in the Figure 1 and Figure 2. **OSIRIS**® Property Explorer identified the toxicity of the chosen compounds. The results shown in the Table 2. The chosen aromatic and heterocyclic pyrimido and thiazolidino benzothiazole derivatives were synthesized according to the scheme 1 and 2.

**Table 1: The molecules with good docking score were mentioned as below**

| Code Name | Docking Score |
|---|---|
| **1a** | -7.51 |
| **1b** | -7.77 |
| **1c** | -8.45 |
| **1d** | -6..67 |
| **2a** | -6.41 |

**Table 2: Toxicity prediction of the synthesized compounds by Osiris®**

| Sample | Mutagenicity | Tumorogenicity | Irritant | Reproductive effect |
|---|---|---|---|---|
| **1a** | + | + | + | + |
| **1b** | + | + | + | + |
| **1c** | + | + | + | + |
| **1d** | + | + | + | + |
| **2a** | + | + | + | + |

[+] indicates absence of toxicity.
[-] indicates Presence of toxicity.

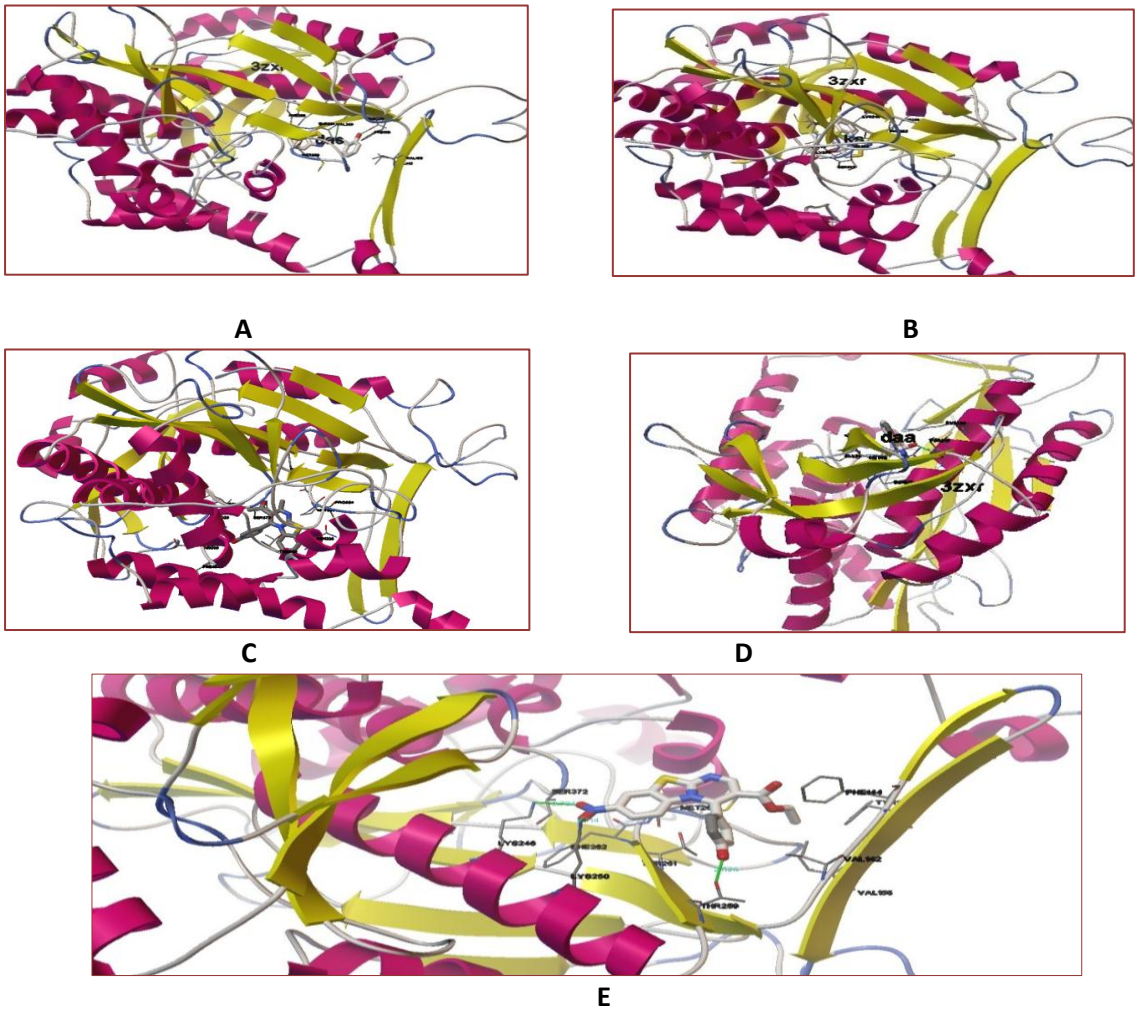



**Figure 1: Docking poses**

A) Compound **1a** with MtGS1. B) Compound **1b** with MtGS1.
C) Compound **1d** with MtGS 1. D) Compound **2a** with MtGS1.
E) Compound **1c** with MtGS 1

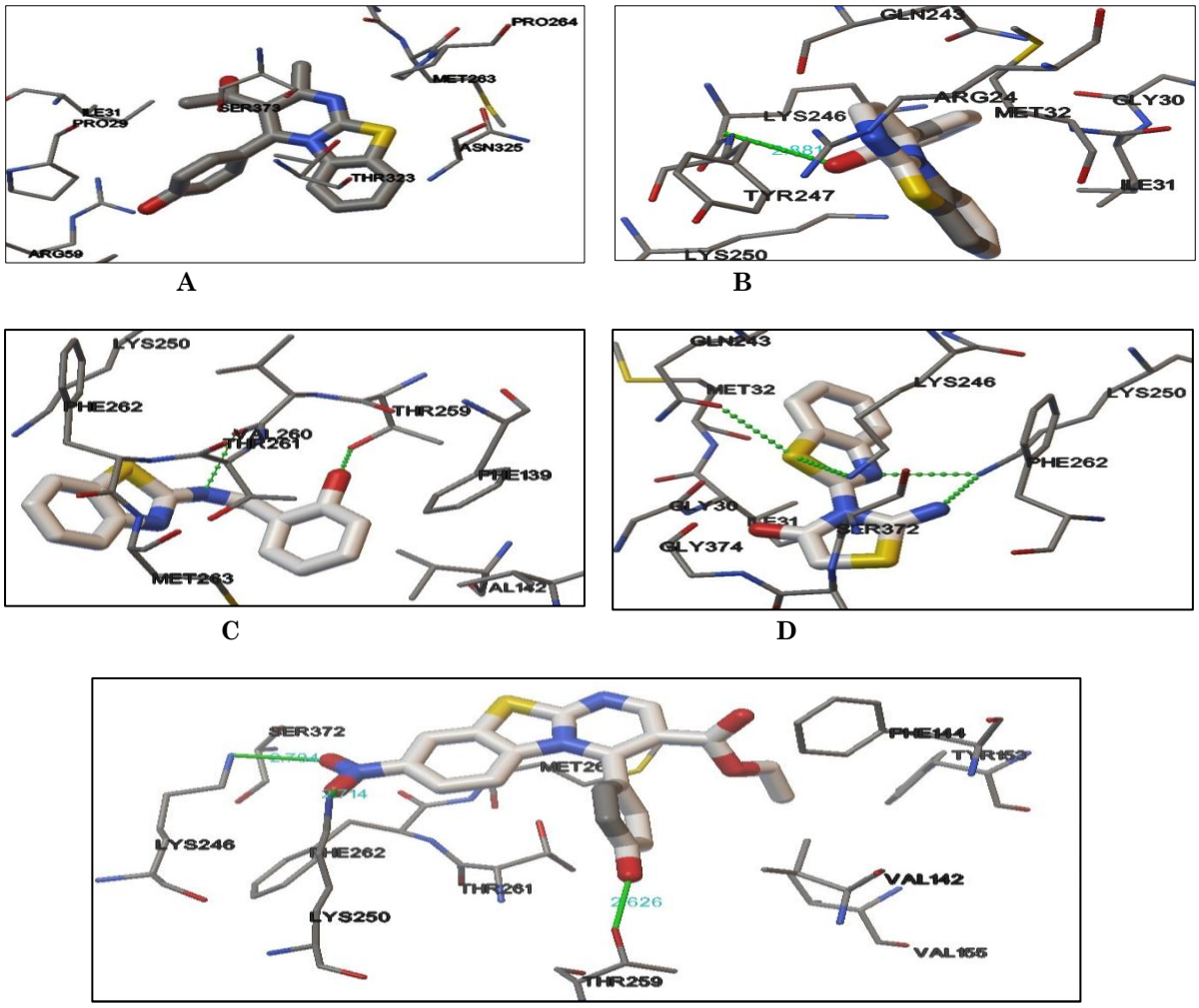



**Figure 2: Protein Ligand Interaction view**

A) Compound **1a** with MtGS 1 B) Compound **1b** with MtGS1
C) Compound **1d** with MtGS 1 D) Compound **2a** with MtGS 1
E) Compound **1c** with MtGS 1

**Experimental section**

**Scheme 1: General Procedure for the Synthesis of 4*H*-Pyrimido [2, 1-*b*] benzothiazole derivatives**

A mixture of 2-aminobenzothiazole (0.01mol) and aromatic aldehyde derivatives (0.01mol) and ß keto ester (0.01mol) were heated at 60°C in the solvent-free conditions for 5 - 6 hr. Completion of the reaction was confirmed by TLC (Petroleum ether: EtOAc 1:4). At the end of the reaction, the mixture was washed 3 times (3 × 20 ml) with water and diethyl ether.

**Scheme 2: Procedure for the Synthesis of Imino-3-(6-substitutedbenzo[d]thiazol-2-yl) thiazolidin-4-one**

A mixture of 2 amino benzothiazole (0.01mol) and chloroacetyl chloride (0.01mol) in dry benzene (50ml) in the presence of potassium carbonate (0.15mol) was refluxed for 12 hours. The mixture was stirred with water (100 mL) and filtered. The solid product was then washed with 5% Sodium bicarbonate (NaHCO3) solution and subsequently with water. The crude product was dried and crystallized from ethanol. Then this product (0.01mol) was added to ammonium thiocynate (0.03mol) in ethanol (30ml), and heated under reflux for 5 hours at 1200C, and then kept overnight. Then the sample was collected, washed with water and re-crystallized using ethanol.

The reaction products of all the reactions were purified initially by different workup processes to remove unreacted starting materials if any and then by recrystallization using suitable

solvents. Every step was optimized in to achieve quantitative yields.

The physical data of the synthesized are reported in the Table 3.

**Table 3: Physical data of the synthesized compounds**

| Compound | Molecular weight | Yield % | Melting point | Rf value |
|---|---|---|---|---|
| 1a | 336.4 | 67% | 198-200$^0$C | 0.68 |
| 1b | 280.3 | 59% | 115-117$^0$C | 0.85 |
| 1c | 400.4 | 74% | 198-200$^0$C | 0.68 |
| 1d | 254.3 | 69% | 118-120$^0$C | 0.86 |
| 2a | 249.3 | 70% | 198-200$^0$C | 0.63 |

**Characterization**

The FTIR spectra of the final products showed the absence of parent functional group and the presence of the new functional group ie. C=NH. For all the synthesized compounds the characteristic peak for C=NH stretching at 1450.34cm-1 was obtained. IR absorption of the synthesized compounds showed the presence of aliphatic CH stretching vibration at 2923.87cm-1. All the compounds showed the presence of NH stretching vibration between 3500-3390cm-1 and aromatic CH stretching vibration between 3080-3030cm-1.The absorption band for compound **1c** showed the strong band at 1512.08 cm-1 to indicate the presence of Nitro group. In 1HNMR spectra of certain derivatives, a signal was observed at □□7.2-8.4, which showed the presence of aromatic ring and bands around □□3.2 showed the presence of CH2.

**Synthesis of 1-[4-(4-hydroxyphenyl)-2-methyl-4*H*-pyrimido[2,1-*b*][1,3] benzothiazol-3-yl]ethanone (1a, C19H16N2O3S):** Yield: 67%; Melting point :198-2000C. IR (KBr, cm-1) :3440cm-1 (OH),2923 cm-1 (CH), 1735 cm-1 (C=O), 1488 cm-1 (C=N). 1H-NMR (DMSO- d6) δ ppm: 9.9(s, 1H, OH), 6.7-8 (m, 8H, Ar-H), 2.5(d, 2H, CH3), 1.1-1.3(m, 4H, CH3), MS (m/z): 337.17(M+); Anal. Calcd. For C19H16N2O3S (336.41): C67.84, H 4.79, N 8.33. Found: (337.17) C 67.43, H 5.36, N 8.28.

**Synthesis of 2-(2*H*-pyrimido[2,1-*b*][1,3]benzothiazol-4-yl)phenol(1b, C16H12N2OS):** Yield : 59% ; Melting Point : 115-1170C . IR (KBr, cm-1): 3433 cm-1(OH), 2923 cm-1 (CH), 2352 cm-1 (NH), 1643 cm-1 (C=C), 1450 cm-1 (C=N). 1H-NMR (DMSO- d6) δ ppm: 8.8(s, 1H, OH), 7.9(d, 1H, CH), 7.7 -8.0(d, 2H, CH), 7.1-7.5(m, 7H, Ar-H), 4.1(s, 2H, CH). MS (m/z): 279.29(M-1) Anal. Calcd. For C16H12N2OS (280.34): C 68.55, H 4.31, and N 9.99. Found: (279.29) C 68.80, H 3.97, N 10.03.

**Synthesis of Ethyl 4-(4-hydroxyphenyl)-8-nitro-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carboxylate(1c, C16H12N2OS):** Yield : 74%; Melting point : 198-2000C. IR (KBr, cm-1) : 3440 cm-1(OH), 2854 cm-1(CH), 2322 cm-1(NH), 1728 cm-1 (C=O), 1512 cm-1(-NO2), 1450 cm-1(C=N). 1H-NMR (DMSO- d6) δ ppm: 7.9-8.1(m, 4H, pyirimidine), 7.1-7.7(m, 8H, Ar-H), 6.7-6.9(m, 3H, CH), 6.5(S, 1H, OH), 2.2(d, 2H, CH3) MS (m/z): 401.36(M+) Anal. Calcd. For C19H18N3O5S (400.42): C 56.99, H 4.53, N 10.49. Found: (401.36) 56.85, H 4.77, N 10.47.

**Synthesis of 2-[(*Z*)-(1,3-benzothiazol-2-ylimino)methyl]phenol(1d, C14H10N2OS):** Yield : 69%; Melting Point : 118-1200C. IR (KBr, cm-1): 3409 cm-1(OH), 3062 cm-1 (Ar-CH), 2368 cm-1 (C=C),1450 cm-1 (C=N), 1H- NMR (DMSO-d6) δ ppm:12.1(s, 1H, OH), 6.2-7.9(m, 7H, Ar-H), 2.5-2.9(t, 1H, CH) MS(m/z):254.26(M). Anal. Calcd. For C14H10N2OS (254.31) : C 66.12, H 3.96, N 11.02. Found: (254.26) C 66.12, H 3.96, N 11.02.

**Synthesis of 3-(1,3-benzothiazol-2-yl)-2-imino-1,3-thiazolidin-4-one(2a, C10H7N3OS2):**Yield : 70%; Melting Point : 198-2000C. IR (KBr, cm-1): 3170 cm-1 (CH), 2067 cm-1 (NH), 1650 cm-1 (C=O),1407 cm-1 (C=N). 1HNMR (DMSO- d6) δ ppm: 7.0-7.5 (m, 5H, Ar-H), 7.6(t, 2H, CH). MS (m/z) : 250.12(M+). Anal.

Calcd. For C10H7N3OS2 (249.31): C 48.17, H 2.83, N 16.85. Found: (248.96) C 48.08, H 2.43, N 16.05.

**In vitro Antitubercular activity screening**

All the synthesized compounds showed anti-mycobacterial activity in varying degrees against the microorganism tested. The organism tested was susceptible to all the synthesized compounds and the minimum inhibitory concentration for the compounds varied between 12.5 and 6.25µg/ml. The data pertaining to these observations are presented in the Table.4. Inhibition was compared with the standard drugs like Pyrazinamide- 3.125 µg/ml, Ciprofloxacin-6.25µg/ml and streptomycin-6.25µg/ml. Compound **1c** was the most sensitive at 6.25µg/ml and that is similar to the standard drugs. The other compounds **1b, 1d and 2a** were sensitive at 12.5µg/ml, ie., they were less active than the standard drugs.

**Table 4: Biological Evaluation of the Synthesized Compounds by MABA method**

| Sample | 100 µg/ml | 50 µg/ml | 25 µg/ml | 12.5 µg/ml | 6.25 µg/ml | 3.12 µg/ml | 1.6 µg/ml | 0.8 µg/ml |
|---|---|---|---|---|---|---|---|---|
| 1a | **S** | **S** | **S** | R | R | R | R | R |
| 1b | **S** | **S** | **S** | **S** | R | R | R | R |
| 1c | **S** | **S** | **S** | **S** | **S** | R | R | R |
| 1d | **S** | **S** | **S** | **S** | R | R | R | R |
| 2a | **S** | **S** | **S** | **S** | R | R | R | R |
| Pyrazinamide | **S** | **S** | **S** | **S** | S | S | R | R |
| Ciprofloxacin | **S** | **S** | **S** | **S** | S | R | R | R |
| Streptomycin | **S** | **S** | **S** | **S** | S | R | R | R |

S - Sensitive; R-Resistant; Strain: M.tuberculosis (H37RV strain): ATCC No-27294

## CONCLUSION

A novel series of 4*H*-Pyrimido [2, 1-*b*] benzothiazole derivatives and Imino-3-(6-substitutedbenzo[d]thiazol-2-yl) thiazolidin-4-one and 3-(1,3-benzothiazol-2-yl)-2-imino-1,3-thiazolidin-4-one were synthesized, characterized and evaluated for the anti tubercular activity. These compounds exhibited modest inhibition. One compound ie., ethyl 4-(4-hydroxyphenyl)-8-nitro-3, 4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]benzothiazole-3-carboxylate (1c) with MIC value of 6.25 µg/ml was the most active in this series. Binding model of the synthesized compounds with Glutamine Synthetase was also studied. Biological data and molecular modeling outcomes revealed the 4*H*-Pyrimido [2, 1-*b*] benzothiazole moiety upon high inhibition of (Mycobacterium tuberculosis Glutamine Synthase-1) MtGSI enzyme activity which is essential for the Mycobacterium Tuberculosis. Further structural modifications of the synthesized compounds will aid in the development of potential molecule against the tuberculosis pathogen.

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## CONFLICT OF INTEREST STATEMENT

The authors have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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