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DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL THIADIAZOLE, IMIDAZOLE AND INDOLE DERIVATIVES AS ANTITUBERCULAR AGENTS AGAINST TARGET ENZYME GLUTA MINE *SYNTHETASE I*

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ABSTRACT

Tuberculosis is a chronic granulomatous disease which is a major health problem in developing countries. The Anti-tubercular drugs for the treatment of Tuberculosis are complicated by the existence of Multi Drug Resistance (MDR) tuberculosis, Extensively Drug Resistance (XDR) tuberculosis and Totally Drug Resistant (TDR) tuberculosis. In this research work, thiadiazole, imidazole and indole derivatives were designed and docked against target enzyme of *Mtb Glutamine synthetase* I (PDB ID: 3zxr) using Auto Dock 4® tools. The best binding score of the docked molecule were screened for drug likeness (Lipinski's rule) using Molinspiration® followed by toxicity by OSIRIS® property explorer. Anti-tubercular activity was evaluated against *Mycobacterium tuberculosis* ($H_{37}RV$) by microplate alamar blue assay (MABA). In the present study, the synthesized compounds (a,b,c,d,e,f and g) show promising activity between 6.25µg/ml to 1.6µg/ml against *Mycobacterium tuberculosis* similar to the standard *drugs* like pyraziamide and streptomycin. The toxicity studies like acute toxicity and cytotoxicity were performed.

KEYWORDS: Anti-tubercular agents, *Glutamine synthetase* I, Thiadiazole, Imidazole, Indole.

INTRODUCTION

Tuberculosis (TB) is the second leading cause of human death from infectious diseases worldwide. Current estimates suggest that one third of the world's population are infected resulting in some 2 million deaths per year. The Nineteenth World Health Organization (WHO) Tuberculosis Report indicates that TB is one of the world's deadliest communicable diseases. According to Global Tuberculosis Report 2017, by the World Health Organization, TB the chronic granulomatous disease is the leading cause of death worldwide in developing countries.[1] The Anti-tubercular drugs for the treatment of Tuberculosis are rendered ineffective by the development of Multi Drug Resistance (MDR) tuberculosis, Extensively Drug Resistance (XDR) tuberculosis and Totally Drug Resistant (TDR) tuberculosis. TB treatment is challenging and time-consuming. It requires appropriate treatment regimens for at least six months via directly observed therapy (DOT) and follow-up support. Treatment requires a minimum of six months in two separate phases. The duration of phase one is about two months and involves four drugs (Isoniazid, Rifampicin, Pyrazinamide And Ethambutol), followed by four months of phase two (using Isoniazid and Rifampicin).The present treatment has some limitations such as drug resistances, drug toxicity and intolerance, drug–drug interactions and poor patient adherence due to the long duration of the treatment. Therefore, there is an urgent need of new drug molecules with lesser side effects of treatment which are more effective.[2]

M. tuberculosis (Mt) in fact possesses four Glutamine Synthetase (GS) 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*. *Mt* GS plays an important role in cell wall biosynthesis and nitrogen metabolism, specifically via the production of a poly-L-glutamate-glutamine component found exclusively in pathogenic mycobacteria. New therapeutic strategies to combat *M. tuberculosis* are urgently needed. The enzyme glutamine synthetase (GS) was identified as a potential antitb target.[3]

Glutamine synthetase is an enzyme that plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine:

$$\text{Glutamate} + \text{ATP} + \text{NH}_3 \longrightarrow \text{Glutamine} + \text{ADP} + \text{phosphate}$$

The irreversible inhibitor of *M. tuberculosis* extracellular *Glutamine synthetase* blocks bacterial multiplication. The growth inhibition is correlated with a marked reduction in the virulence- associated cell wall component poly-glutamate/glutamine. Remarkably, the enzyme inhibitor has no effect against nonpathogenic mycobacteria, which do not export glutamine synthetase.[3]

Thiadiazole is a versatile moiety that exhibits a wide variety of biological and pharmacological properties such as Anti-tubercular, Analgesic, Anti-inflammatory, Anti-convulsant, Anti-oxidant, Anti-hypertensive, Anti-depressant, and Anti-fungal activities. Schiff bases of thiadiazole derivatives were designed, docked, synthesized and screened against glutamine synthetase1, the target.[4]

Imidazole nucleus has been reported for its wide applications in medicinal chemistry and also has been reported to have significant anti tubercular activity. Several imidazole derivatives were synthesized and screened for their in vitro anti tubercular activity against H37Rv strain of Mycobacterium tuberculosis by Microplate Alamar Blue Assay method[5]

An indole derivative are reported for their various biological and pharmacological activities and has prompted researchers to develop novel drug molecules containing indole as basic scaffold is indole derivatives were also designed synthesized characterized and screened.[6]

MATERIALS AND METHODS

Molecular modeling and Drug Design

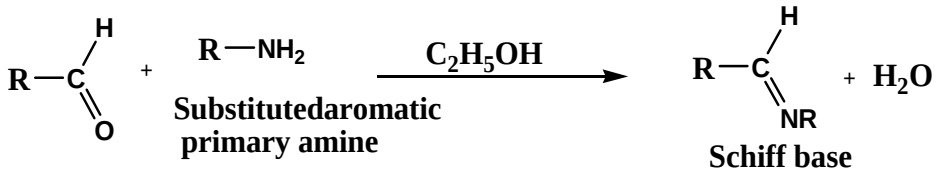
The compounds were designed to conform Lipinski's Rule (drug likeness) and then docked by using AutoDock® 4.2.5.1[7] program. Molecular docking is generally used to detect the protein-ligand orientation and interaction. The Target Enzyme is Glutamine Synthetase I (PDB ID: 3ZXR) was used for docking. .About 200 molecules were designed and docked against *Glutamine synthetase* (PDB ID: 3zxr), and visualized by Molegro Molecular Viewer® which helps in analyzing the energies and binding interactions. The top scoring compounds were chosen for further study.[8]

***In silico* – Drug likeness:** Lipinski's rule of five is to evaluate drug likeness.[9] Molinspiration® which is an online tool to calculate the molecular properties. The structures are drawn using chemsketch and docked by use of online tools using Auto dock tools.[10]

***In silico* –Toxicity prediction:** The designed molecules were also screened to predict their toxicity risk by using OSIRIS®-Property-Explorer on line tool.[11]

Experimental procedure

Scheme: I: Equi-molar mixture of the aldehyde and the amine in ethanol was refluxed for about 4-5 hours at 60 to 80°C temperature. The progress of the reaction was monitored by TLC. On completion of reaction, the mixture was poured onto crushed ice. The precipitate was filtered and re-crystallized from ethanol.

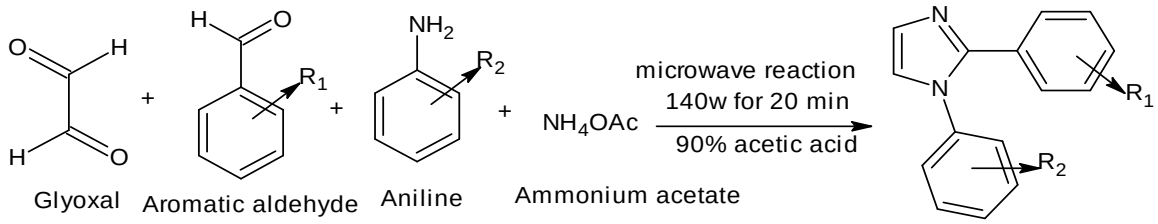


Substitutedaromatic primary amine

Substituted aromatic Aldehyde

Schiff base Compounds a,b,c

Scheme: II: A Mixture of 1 mol of Glyoxal, 1 mol of substituted aromatic aldehyde and 1 mol of primary amine and 0.1 mol of Ammonium acetate was taken in an Round bottom flask and subjected to reflux in a condenser for 7-8 hours. On completion of reaction as monitored by TLC at an interval of 30 minutes. The sticky mass was transferred in to an acetone: water (6:4) mixture. The reaction was monitored for completion every 30 mts by TLC. The precipitate that was obtained after completion of reaction was filtered, dried and purified by re crystallization.



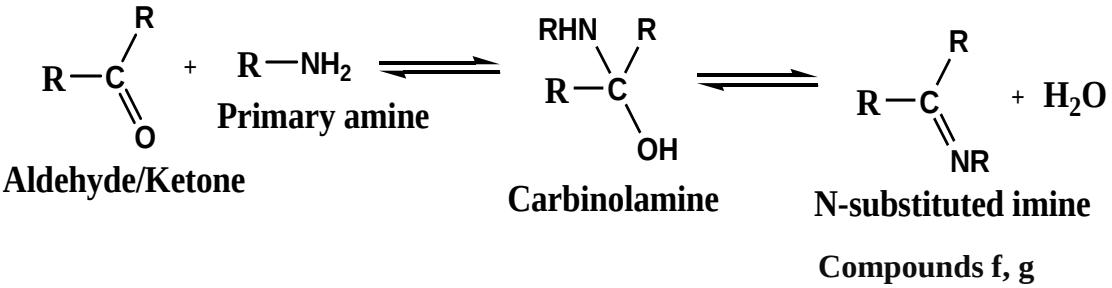
Glyoxal Aromatic aldehyde Aniline Ammonium acetate

Imidazole derivative

Compounds d, e

Scheme: III Equimolar quantities of ketone (0.01mol) and para-substituted amine (0.01mol) were added into 20ml of absolute ethanol and 5ml of glacial acetic acid was added to it. Reaction mixture was refluxed for 24hrs at 60ºC. Completion of reaction was confirmed by TLC. The product obtained was filtered, dried and re-crystallized.

Ketones used: Isatin, Chloro isatin; Amines Used: 4-fluoroaniline, 4-amino 3, 5-dichloro pyridine, 4-methoxy benzylamine, Anisidine, 2-amino benzimidazole.



Compounds f, g

RESULTS AND DISCUSSION

***In silico* study results:** The designed molecules were docked with target enzyme *Glutamine synthetase I*. Docking view Fig 1, Fig 2. Protein and ligand Interaction image Fig 3, Fig 4.

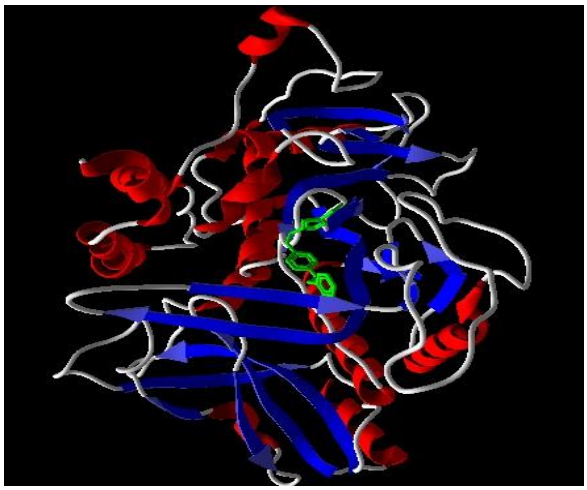


Fig 1: Compound a)

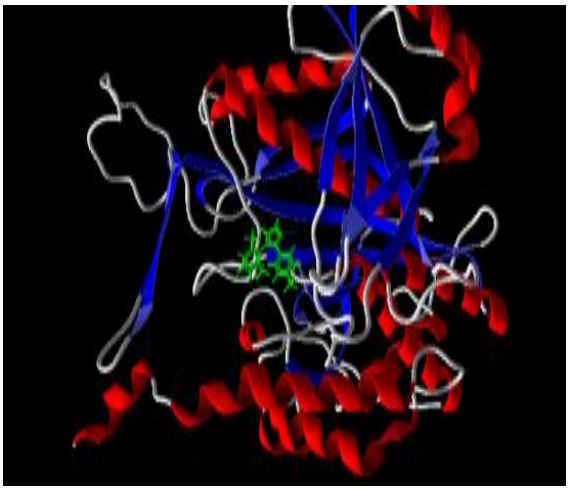


Fig 2: Compound e)

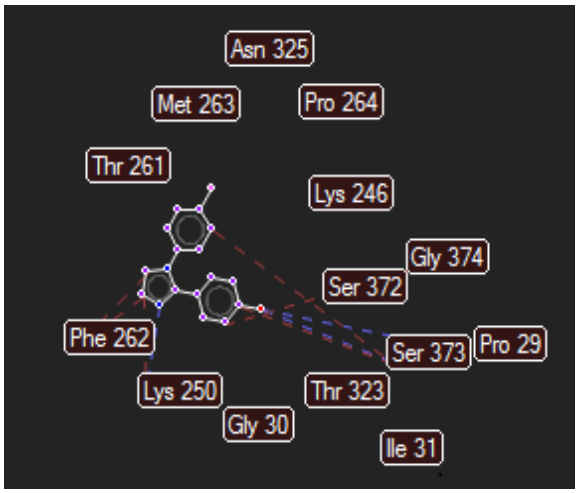


Fig 3: Compound d)

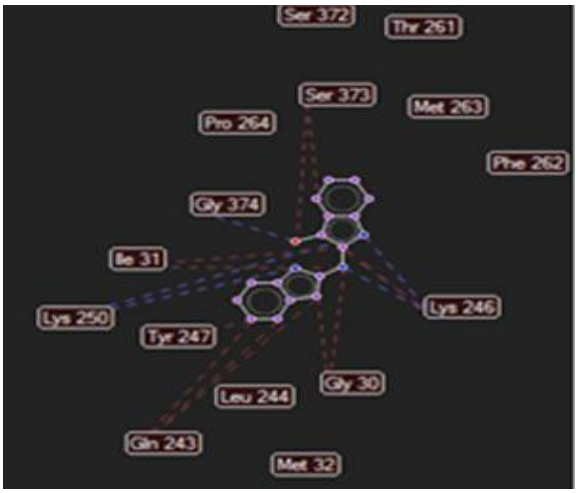
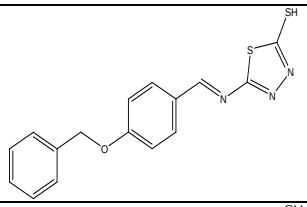
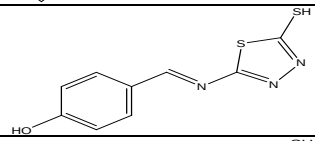
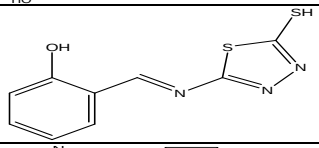
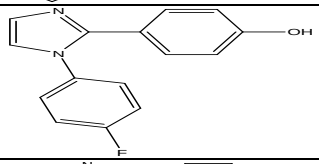
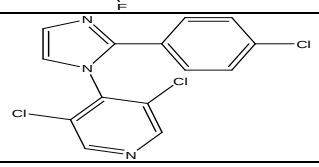
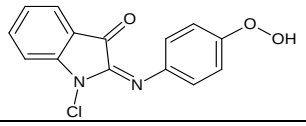
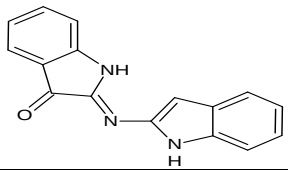


Fig 4: Compound g)

Table 1: Physical properties of the synthesized compounds.

Sample code	Compounds Structure	Mol. Formula	Mol. Wt	Docking Score in Kcal/mol	M.P	Color	Yield
a		$C_{16}H_{13}N_3OS_2$	327.43	-6.26	129-131°c	Pale Yellow	78%
b		$C_9H_6N_4O_2S_2$	266.29	-6.73	170-171°c	Yellowish Green	53%
c		$C_9H_7N_3OS_2$	237.30	-5.73	174-176°c	Pale Brown	48%
d		$C_{15}H_{11}FN_2O$	254.25	-5.85	110°c	Dark brown	74%
e		$C_{14}H_8Cl_3N_3$	324.59	-5.73	120°c	Light brown	86%
f		$C_{14}H_9ClN_2O_3$	286.72 g/mol	-5.90	210ºC	Brown	80%
g		$C_{32}H_{28}N_4O_4$	269.58 g/mol	-6.32	193ºC	Dark Red	70%

Spectral Studies

Compound a:: IR: 3112.17 cm^{-1}(Ar-C-H str), 2885.30 cm^{-1} (Al-CH_2-str), 1604.66 cm^{-1} (C=N str), 1257.50 cm^{-1} (C-O-C str), 694.32cm^{-1} (C-S-C str), NMR: δ (5.3 ppm, triplet, 2H), (7.1-7.5 ppm, multiplet, 6H), (8.6-8.7 ppm, multiplet, 4H),(9.9 ppm, singlet, 1H), MASS: 327.96(M^+).

Compound b:: IR: 3625.91 cm^{-1} (Alc O-H str), 3132.27 cm^{-1} (Ar C-Hstr), 1604.66 cm^{-1} (C=N str), 1288.35 cm^{-1} (C-O str), 709.75 cm^{-1} (C-S-C str), NMR: δ (6.9 ppm, singlet, 1H),(7.1-7.2 ppm, multiplet, 4H),(13.2 ppm, singlet, 1H), MASS: 237.95(M^+).

Compound c: IR: 3124.45 cm^{-1} (Ar C-H str), 1612.37 cm^{-1} (C=N str), 1481.22 cm^{-1} (C-NO_2 str), 686.61 cm^{-1} (C-S-C str), NMR: δ (5.5 ppm, doublet, 1H), (7.1-8.3 ppm, multiplet, 4H), (13.2 ppm, singlet, 1H), MASS: 267.03(M+1)

Compound d: Yield: 74%; Melting point:110^{o}c ;IR (KBr, cm^{-1}):1288.35cm^{-1} (C-F Str);3301.89cm^{-1}(OH Str); 1635.52cm^{-1}(C=N Str); 1157.20cm^{-1}(C-N Str). 1H NMR (DMSO-d6) δ ppm: 6.4-7.3(7H), 7.7-7.9(3H), 8.4-8.6(1H). MS (e/z) **: 255.07(M+).**

Compound e: Yield:86% ; Melting Point: 120^{o}c; IR(KBr,cm^{-1}):794.61cm^{-1}(C-Cl- Str); 1095.49cm^{-1}(C-N- Str); 1573.80cm^{-1}(C=N- Str); 3109.02cm^{-1}(C-H Str). 1H NMR (DMSO-d6) δ ppm: 6.6-6.8(1H), 7.8-8.2(1H). MS(e/z)**:** 324.81(M).

Compound f: IR (CM-1): 3078 [Ar- CH str],1257[C-O-C str],1573[Ar C=C str], 1697[C=N str],3433[OH str] H1NMR:; MASS (g/mol): Actual mass:286.72 g/mole, Expected mass: 287.01g/mole.

Compound g: IR (CM-1): 3078 [Ar- CH str],1257[C-O-C str],1573[Ar C=C str], 1697[C=N str],3433[OH str] H1NMR:7.7-7.9 (8H, s, Ar-H), 8.96(1H, s, OH); MASS (g/mol): Actual mass:269.58 g/mole, Expected mass:270.00 g/mole.

Biological Evaluation: The antitubercular activities of the synthesized compounds were determined by Microplate Alamar Blue Assay method (**MABA**) [12]. The Standard Strain used was *Mycobacterium tuberculosis* (Vaccine strain, H37 RV strain): ATCCNo- 27294. Anti-TB Result: The results of the MABA test to determine the activity of the synthesized compounds at different concentration is shown in Figure 5 and 6.

Note: S-Sensitive R-Resistant

Standard values for the Anti-Tb test which was performed. Pyrazinamide- 3.125µg/ml; Ciprofloxacin-3.125µg/ml; Streptomycin- 6.25µg/ml.

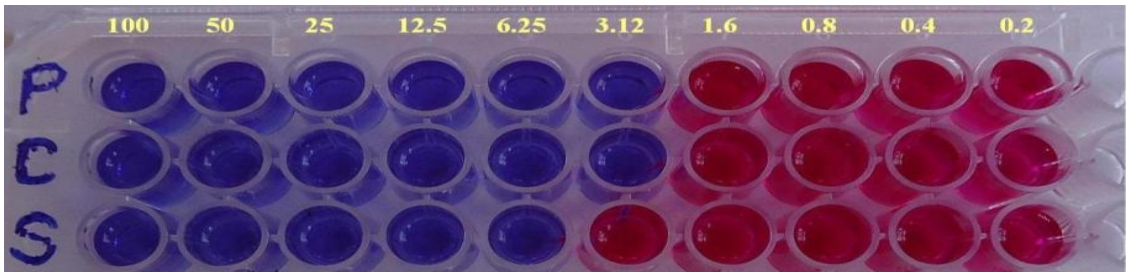


Fig 5: MABA Reports: Standard Drugs Photograph.

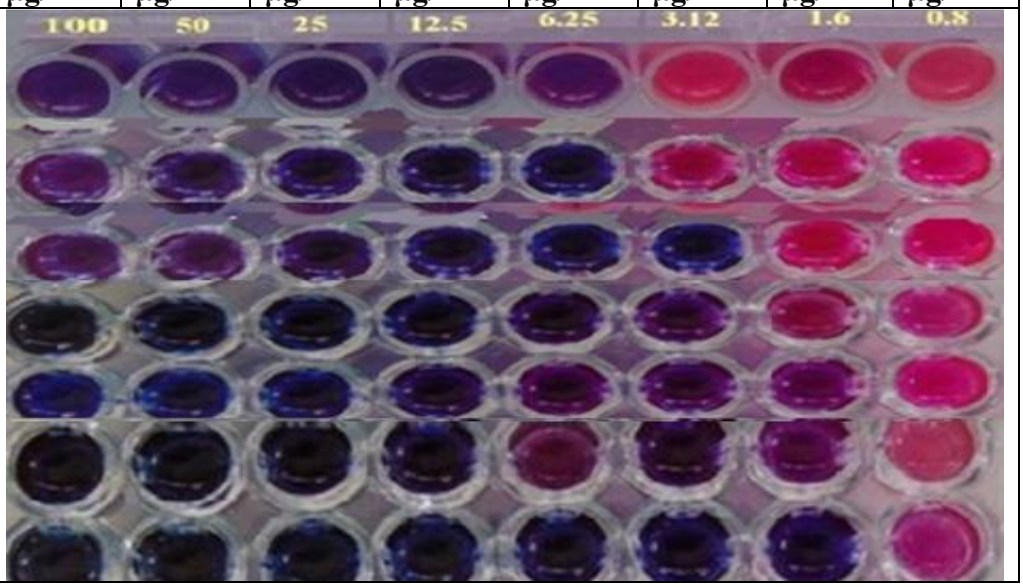
Sample Code	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml	0.8 µg/ml
a								
b								
c								
d								
e								
f								
g								

Fig 6: MABA Reports of Sample compounds.

Acute Oral Toxicity Study: The purpose of testing for acute toxicity is to evaluate the degree of toxicity in a quantitative and qualitative manner (Limit test) as per the OECD guidelines (423).[13] The synthesized compounds were chosen for acute oral toxicity study (Limit test) using albino mice as per (OECD guidelines (423)). After administration of molecules animals were observed for behavioral signs of toxicity like motor activity, tremor etc., and no significant toxic signs were observed during 14 days. The results of the acute toxicological studies revealed that the administration of these molecules by oral route upto 2000mg/kg/b.w did not produce any mortality and it was tolerated.

Cytotoxicity Study: Cyto toxicity study of the synthesized compounds was performed on VERO cell line[14,15] and the results of Inhibitory concentration were tabulated in Table 2.

Table 2: Cytotoxicity results.

Concentration (µg/ml)	a	b	C	d	e	f	g
500	98.06	53.54	69.74	66.39	56.34	96.66	98.05
250	72.52	31.23	57.38	47.67	49.12	97.65	98.02
125	48.85	22.06	39.96	24.45	40.80	77.63	96.42
64.5	26.06	9.65	20.83	0.23	36.81	60.66	90.70
31.25	15.13	4.08	10.03	13.02	21.97	38.54	67.45
IC_{50} from Prism	121.5	456.2	201.9	291.2	271.5	45.05	21.86

IC_{50} – Half maximal inhibitory concentration

The reported values of the compounds were compared with standard drugs. The IC_{50} for Rifampicin is 113 µg/ml on vero cell line. Therefore as compared to Rifampicin the synthesized compounds were found to be more cytotoxic.

CONCLUSION

The study concluded that the synthesized compounds effectively inhibit the chosen target *Glutamine synthetase* I which is an essential for the *Mycobacterium tuberculosis.*

The in vitro evaluation results of synthesized compounds were compared with standard drugs, and found that, the compounds e, f and g shows promising activity at 1.6 µg/ml , where as compounds c and d were active at 3.125 µg/ml. Compounds a and b showed activity at 6.25 µg/ml against *Mycobacterium tuberculosis.* Further *in vivo* evaluation of the synthesized compounds will aid in the future development of potential molecule against the pathogen. The toxicity studies like the acute toxicity and cyto toxicity studies were performed and reported.

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