ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 9, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

NOVEL BENZOTHIAZOLE-ISATIN HYBRIDS: MICROWAVE SYNTHESIS AND COMPUTATIONAL INSIGHTS AGAINST MULTIDRUG-RESISTANT TUBERCULOSIS

THIRUMALA ROOPESH G[1]*, GEETHAVANI M[2]

[1] Research Scholar, Department of Pharmaceutical Sciences, JNTUA, Anantapuramu, Andhra Pradesh, India. [2]Professor and HOD, Department of Pharmaceutical Chemistry, Balaji College of Pharmacy, Anantapuramu-515001, Andhra Pradesh, India.
***Corresponding author: Thirumala Roopesh G; Email: roopeshpharma@gmail.com**

Received: 20 May 2025, Revised and Accepted: 05 July 2025

ABSTRACT

Objectives: Multidrug-resistant tuberculosis (TB) is the outcome of *Mycobacterium tuberculosis* developing resistance to at least isoniazid or rifampicin, the two most effective first-line anti-TB drugs. A major worldwide health concern, this resistance makes treatment regimens more difficult and calls for creating new therapeutic agents and medication delivery methods. A class of heterocyclic chemicals called benzothiazole derivatives is well-known for its many biological actions, including antibacterial, antitubercular, anticancer, and anti-inflammatory properties. Recent studies have shown that these compounds hold promise in use both laboratory and computer-based techniques to create novel medications, particularly against drug-resistant illnesses.

Methods: In the current research, several new benzothiazole-based molecules were synthesized and evaluated for their antitubercular potential using both experimental testing and molecular modeling. Out of the synthesized compounds, histidine, serine, valine, phenylalanine and tyrosine derivatives showed notable activity against M. tuberculosis at a concentration of 3.12 µg/mL, which is similar to that of pyrazinamide, a common medication. While compound isatin-benzothiazole glycine showed decreased activity at 12.5 µg/mL, compounds such as tryptophan derivatives, proline, glutamic acid, and cysteine showed considerable activity at 6.25 µg/mL. Mass spectrometry and Fourier-transform infrared spectroscopy were used to verify every produced molecule's purity and chemical structure. Standard protocols recorded their physical characteristics, such as solubility, melting point, or R_f values. To conduct molecular docking investigations, AutoDock 4.2.6 was used and PyRx software against the Protein Data Bank ID: 2JA2 protein target.

Results: The compound (isatin-benzothiazole tryptophan) tryptophan derivative showed the strongest binding energy of –8.8 kcal/mol, better than the standard drugs and the co-crystal ligand acetate ion (–5.3 kcal/mol). Other active compounds such as (isatin-benzothiazole histidine) histidine, (isatin-benzothiazole proline) proline, (isatin-benzothiazole serine) serine, and (isatin-benzothiazole aspartic acid) aspartic acid derivatives also showed strong binding energies between −8.8 and −8.3 kcal/mol. In-depth interaction analyses showed that these substances created pi-cation interactions, π–π stacking, and hydrogen bonding. In-depth interaction analysis revealed that these substances formed π–cation interactions, π–π stacking, and hydrogen bonds with key amino acid residues, including Trp67, Arg271, and Asp272, within the active site of the target protein, thereby enhancing their inhibitory activity. Toxicity was predicted using the PROTOX-3 server, and the results indicated that the compounds were within acceptable safety limits.

Conclusion: Overall, tryptophan derivative and other active derivatives have shown encouraging results and may be considered for further biological studies and structure-activity relationship analysis to develop effective antitubercular agents.

Keywords: Benzothiazole derivatives, Antitubercular activity, Molecular docking, Mass spectrometry, Binding energy, MABA, AutoDock, PyRx, Discovery studio, PyMoL.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i9.55471. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

The emergence of multidrug-resistant tuberculosis (MDR-TB) has become a critical global health concern, significantly undermining the effectiveness of current treatment regimens. Resistance to key first-line drugs such as isoniazid and rifampicin necessitates prolonged and more complex therapeutic approaches, often with limited success [1]. The increasing incidence of MDR-TB highlights the urgent demand for new anti-tubercular drugs with unique modes of action. Research in medicinal chemistry is pivotal to discovering and optimizing compounds that can overcome existing resistance. Developing effective drugs against MDR-TB is essential to control disease transmission, reduce mortality, and improve patient outcomes worldwide [1]. Benzothiazole is a fused heterocyclic compound containing a benzene ring joined with a thiazole ring. Due to its numerous pharmacological properties, its derivatives have drawn substantial attention, including antibacterial [2], anticancer [3], anti-inflammatory [4], and particularly antitubercular effects [3-5]. These compounds are of special interest in the fight against infectious diseases like TB, particularly due to the growing problem of drug resistance. Worldwide, tb continues to rank among the most dangerous infectious diseases. According to the World Health Organization, around 1.5 million people die from tuberculosis each year, and nearly 9 million new cases are reported. The second most frequent infectious agent-related cause of death is tuberculosis, after HIV. When HIV is co-infected, the situation gets worse since HIV impairs immunity and speeds up the progression of tuberculosis. The slow-growing, acid-fast bacterium *Mycobacterium tuberculosis*, which primarily affects the lungs but can sometimes spread to other organs, is the cause of tuberculosis [6]. Any coughing, sneezing, or talking by an infected individual might spread it through the air. Malnutrition, weakened immunity, and overcrowding are risk factors that aid in its spread. Because traditional medicines are less successful against MDR-TB and XDR-TB strains [7], therapy has become even more challenging. TB has affected human populations for centuries. In 1689, Dr. Richard Morton linked the disease with lung tubercles; in 1839, Johann Lukas Schonlein named it "tuberculosis." The bacterium *M. tuberculosis* had discovered by Robert Koch in 1882. Later, BCG vaccine, developed by

Calmette and Guerin in 1906 and introduced in 1921, helped prevent TB in children, though its effectiveness in adults is limited.

TB can appear in three major forms: Primary TB, which occurs during the 1st-time infection and is often mild or asymptomatic; secondary TB, which is the reactivation of latent disease and typically presents with more severe symptoms; and disseminated TB, which is a widespread infection that may affect multiple organs include the brain, liver, and bones. The bacterium's thick, waxy cell wall, which is made of mycolic acids, arabinogalactan, and lipoarabinomannan (shown in Fig.1), allows it to live inside host macrophages. These structural components help the bacteria evade the immune system and block antibiotic penetration. The host forms granulomas to contain the infection, but rupture of these granulomas can lead to further spread of the bacteria through blood or lymphatic systems. Diagnosis of TB can be challenging, particularly in immune compromised individuals. Conventional methods such as chest X-rays and sputum microscopy are widely used, while the Mantoux tuberculin skin test remains a standard screening tool. However, this test can yield false positives in individuals vaccinated with BCG. Advanced diagnostic techniques such as interferon-gamma release assays and molecular methods provide higher specificity but are limited by high costs and availability. Treatment of TB requires a prolonged multidrug regimen lasting 6–9 months. Ethambutol [8], streptomycin [9], pyrazinamide [10], isoniazid [11], and rifampicin [12] are first-line medications [7]. In cases of drug resistance, second-line agents such as kanamycin, amikacin, and ethionamide are used, although these are associated with increased side effects. The long course of treatment frequently leads to low patient adherence, which promotes the growth and dissemination of resistant strains [13]. TB is ubiquitous in developing nations, with India accounting for a sizable share of the global disease burden. Males are more frequently affected, making up around 60% of reported cases. Pediatric TB accounts for about 10% of all cases, highlighting the need for child-specific diagnostic and treatment approaches. Co-infection with HIV, particularly in sub-Saharan Africa, complicates TB management and increases mortality rates. Despite ongoing global efforts to control TB, challenges remain, including limited new drug development, emergence of resistant strains, and the need for shorter and more effective treatment regimens [13]. This has led researchers to explore new classes of molecules with novel mechanisms of action [14]. Among these, heterocyclic compounds, particularly benzothiazole derivatives, have emerged as promising candidates. Benzothiazole-based compounds are valuable in drug discovery because they interact effectively with biological targets (shown in Fig. 2) [13,15-17]. Modifications at specific positions of the benzothiazole ring, especially at the C-2 position, have been found to enhance antitubercular activity [18]. The introduction of pharmacologically active moieties such as azetidinone rings and Schiff bases has shown to improve the efficacy of these compounds [4,19]. In addition, increasing hydrophobicity, such as adding methoxy groups, have been associated with enhanced activity against *M. tuberculosis*. Studies suggest that benzothiazole derivatives may overcome limitations associated with current therapies, including issues related to resistance and bioavailability [18]. The development is made possible by their structural flexibility in analogues with better pharmacological properties and potential for novel mechanisms of action [20]. In conclusion, benzothiazole derivatives represent a valuable class of compounds in a search on novel anti-TB medications [21]. Their broad spectrum of biological activities and potential effectiveness against drug-resistant TB strains make them strong candidates for further study. Continued research on these derivatives may lead to the discovery of new therapeutic agents

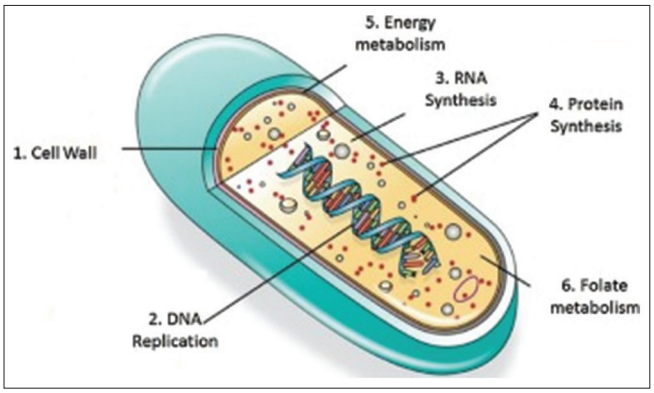


Fig. 1: Targets [13,15] in *Mycobacterium tuberculosis* bacillus

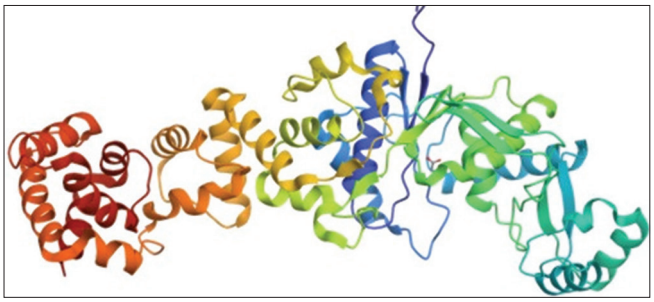


Fig. 2: Crystal structure of *Mycobacterium tuberculosis glutamyl*-tRNA synthetase [16,17] target for the standard and synthesized derivatives

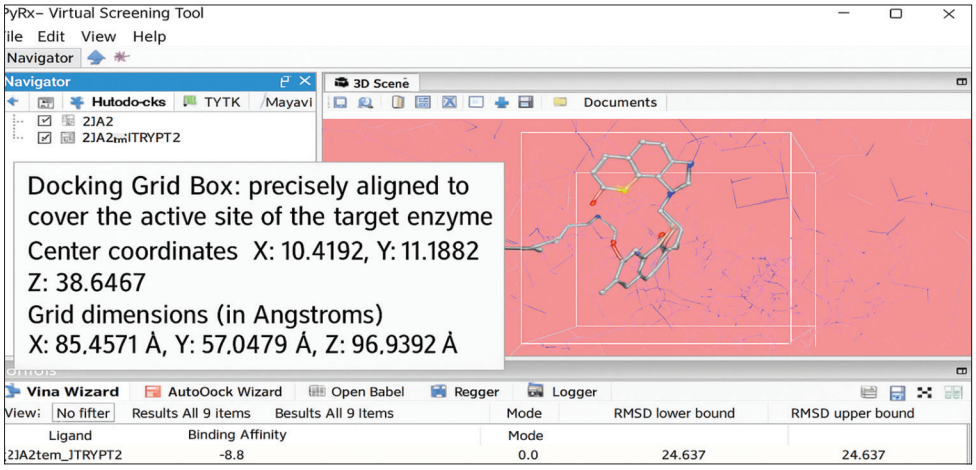


Fig. 3: Docking grid setup [17] and binding pose analysis of ITRYPT derivative with GluRS Enzyme (PDB ID: 2JA2) in PyRx[26] using AutoDock Vina

Table 1: Physico-chemical characteristics for benzothiazole-isatin hybrid synthesized derivatives

S. No.	Derivative code	Molecular formula	Molecular weight (g/mol)	Melting point (°C)	Yield (in %)	R_f values	TLC solvent	Reaction time (in sec)	Color
1	IPA	$C_{24}H_{19}N_5O_2S$	441.5	280–282	89.2	0.26	Ethyl acetate: n-Hexane, 4:6	30	Pale yellow
2	IG	$C_{17}H_{13}N_5O_2S$	351.4	>300	77.6	0.55			Pale brown
3	ITYR	$C_{24}H_{19}N_5O_3S$	457.5	255–256	88.3	0.62			Yellow
4	ITRYPT	$C_{26}H_{20}N_6O_2S$	480.5	294–296	95.6	0.32			Brick red
5	IA	$C_{18}H_{15}N_5O_2S$	365.4	>300	91.3	0.56			Pale brown
6	IH	$C_{21}H_{17}N_7O_2S$	431.5	>300	92.5	0.52			Pale brown
7	IP	$C_{20}H_{18}N_5O_2S$	392.4	265–266	87.4	0.54			Pale yellow
8	IC	$C_{18}H_{15}N_5O_2S_2$	397.5	289–291	88.2	0.28			Pale yellow
9	IGA	$C_{20}H_{17}N_5O_4S$	423.4	297–299	71.3	0.45			Pale yellow
10	IV	$C_{20}H_{19}N_5O_2S$	393.7	>300	67.3	0.61			Pale yellow
11	IS	$C_{18}H_{15}N_5O_3S$	381.4	277–279	69	0.65			Pale yellow
12	IAA	$C_{19}H_{15}N_5O_4S$	409.4	>300	56.9	0.59			Pale yellow

TLC: Thin layer chromatography, IPA: Isatin-benzothiazole phenylalanine, IG: Isatin-benzothiazole glycine, ITYR: Isatin-benzothiazole tyrosine, ITRYPT: Isatin-benzothiazole tryptophan, IA: Isatin-benzothiazole alanine, IH: Isatin-benzothiazole histidine, IP: Isatin-Benzothiazole proline, IC: Isatin-benzothiazole cysteine, IGA: Isatin-benzothiazole glutamic acid, IV: Isatin-benzothiazole valine, IS: Isatin-benzothiazole serine: IAA: Isatin-benzothiazole aspartic acid

Table 2: Interpretation of root mean square deviation (RMSD) values for docking protocol validation [30-33]

RMSD value (Å)	Interpretation
<2.0	Excellent match – reliable docking protocol
2.0–3.0	Acceptable – moderate accuracy; tolerable for flexible ligands or binding sites
>3.0	Poor match – docking method may need refinement

that could significantly improve TB treatment outcomes and support global efforts in TB control [22].

MATERIALS AND METHODS

Materials

Chemistry

All of the chemicals and reagents utilized in the synthesis, along with analysis, are both analytical that synthetic grade, and they were acquired from commercial providers. The produced compounds' melting points were determined. An ATR (Attenuated Total Reflectance) accessory was installed on a Bruker Alpha-II Fourier transform infrared spectroscopy (FTIR) spectrometer to obtain FTIR spectra. A Bruker AVANCE NEO 500 MHz NMR spectrometer running at 500 and 125 MHz, respectively, was utilized to record the H^1 NMR and C^{13} NMR spectra with DMSO-d_6 as the solvent. Changes in chemistry are expressed in parts per million (δ ppm). Mass spectra have been captured using an Agilent 6520 Accurate-Mass liquid chromatography-mass spectrometry (LC/MS) instrument. Silica gel HF254 plates were utilized in thin-layer chromatography to track the purity and development of the reaction. Under ultraviolet light, spots were seen in the mobile phase [23] which was made up of ethyl acetate and n-hexane (4:6).

Methods

General procedure for the synthesis of novel benzothiazole derivatives (shown in Scheme 1)

Isatin-benzothiazole phenylalanine (IPA), isatin-benzothiazole glycine (IG), isatin-benzothiazole tyrosine (ITYR), isatin-benzothiazole tryptophan (ITRYPT), isatin-benzothiazole alanine (IA), isatin-benzothiazole histidine (IH), isatin-benzothiazole proline (IP), isatin-benzothiazole cysteine (IC), isatin-benzothiazole glutamic acid (IGA), isatin-benzothiazole valine (IV), isatin-benzothiazole serine (IS), and isatin-benzothiazole aspartic acid (IAA) hydrazide derivatives.

In a typical reaction, equimolar quantities of isatin (0.01 mol, 1.5 g) and the corresponding amino acid hydrazide derivative (0.01 mol, amount varying based on the derivative used) were accurately weighed and transferred into a clean, dry beaker. To the reaction mixture, 1–2 drops of glacial acetic acid were added as a catalyst, which was employed to act as both a mild acid catalyst and reaction medium to facilitate the condensation of isatin with benzo[d]thiazole-linked amino acid hydrazides. Its use avoided potential degradation of sensitive functional groups and provided consistently high yields across the synthesized hybrid series, followed by 1–2 mL of absolute ethyl alcohol to act as a reaction medium [24].

The resulting mixture was stirred thoroughly using a magnetic stirrer to ensure homogeneous mixing of the reactants. The beaker was then placed inside a microwave oven [1,25] and irradiated at 250 W the reaction was carried out at a controlled temperature of 60°C under a pressure range of 10–15 psi or 69–103 kPa, for 30 s [1]. The reaction mixture was allowed to cool to room temperature following microwave irradiation. After the reaction was finished, the mixture was dried using either ambient air or lower pressure to get rid of extra solvent.

The crude solid obtained was collected and washed with cold ethanol to remove any unreacted starting materials or impurities. The resulting solid product was then dried and recrystallized using an appropriate solvent (such as ethanol or methanol) to yield the purified benzothiazole derivative.

The synthesis [25] (shown in Scheme 1) was carried out in the same manner in all derivatives (IPA, IG, ITYR, ITRYPT, IA, IH, IP, IC, IGA, IV, IS, and IAA) by varying the amino acid hydrazide derivative used in each reaction.

Procedure

Step 1: Equimolar quantities of 2-hydrazinobenzothiazole and respective amino acids (1 mmol). Step 2: Both compounds were transferred into a clean beaker. Step 3: A few drops (1–2) of glacial acetic acid and 2 mL of ethanol were added to the mixture. Step 4: Contents were stirred thoroughly using a magnetic stirrer to ensure complete mixing and uniformity.

Computational *(in silico)* studies

Identification of target

Glutamyl-tRNA synthetase [16,17] (GluRS) plays a key role in protein synthesis by attaching glutamic acid to its corresponding tRNA. This enzyme is crucial for bacterial survival and differs significantly from the human version, making it an attractive target for developing anti-tuberculosis drugs. The 3D structure of GluRS from *Thermus thermophilus* (PDB ID: 2JA2) (shown in Fig. 2) [16,17], determined at a high resolution of 1.65 Å, reveals important features of the active site. Such detailed structural information supports molecular docking and virtual screening studies to identify new compounds that can block the enzyme's function, offering potential treatment options for MDR-TB [7,13].

Table 3: Docking scores and binding interactions [30,32,33] with Target 2JA2 for synthesized derivatives and standard Drugs [8-12]

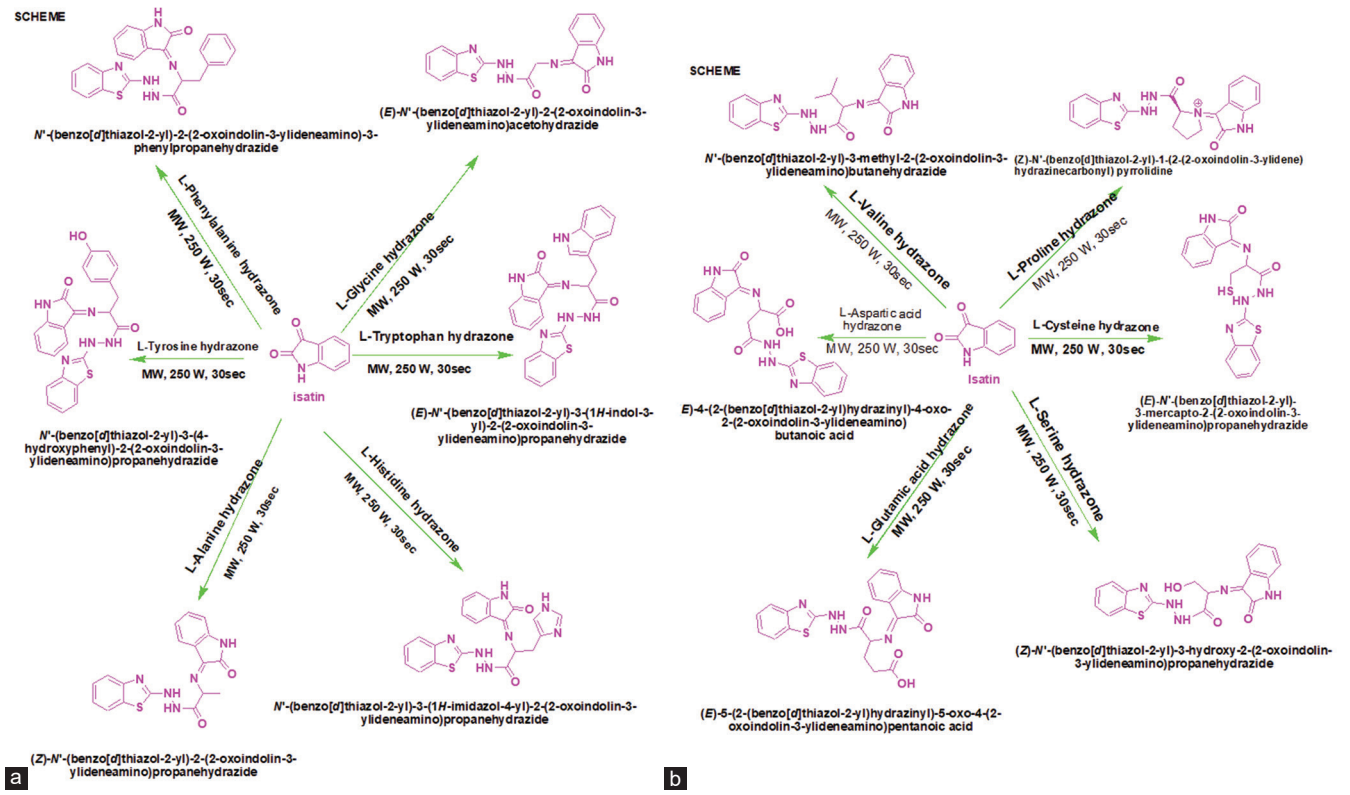
Derivative code	Type of interactions with Target 2JA2				Docking score (K.cal/mol)	RMSD [30-33] (A°)
	Hydrogen bonds	Pi-cation interactions	Hydrophobic interactions	Pi-Pi stacking		
Co-crystal Ligand** Acetate ion (ACT)	Asp272	Arg271	--	Trp67, Arg343 and Leu376	−5.3	0.468
IPA	Asp272	--	Asp272, Arg343 and Leu376	--	−7.9	1.294
IG	His270, Ala269 and Gln321	--	Pro263 and Arg273	--	−7.7	1.443
ITYR	Pro277, Thr350 and His351	--	Pro277, Arg343 and Leu376	Pro277, Arg343, Asp346, Thr350, His351, and Leu376	−6.8	1.118
ITRYPT	Asp272	Arg271	--	Trp67, Arg343 and Leu376	−8.8	1.262
IA	Leu286, Trp289, Asn313 and Arg318	--	Leu286 and Lys323	--	−6.9	1.244
IH	Ser112, Arg152, Gln153, Arg189 and Ala190	Arg152	Val155, Ala186, and Ala190	Val155, Ala186, and Ala190	−8.3	1.326
IP	Trp67, Pro277, Asp346, His347	Asp346	--	Pro277 and His347	−8.0	1.373
IC	His121, Asp133, Asn134 and Gly 180	--	Leu130 and Pro183	Phe135	−7.2	1.365
IGA	Asp272	Arg271	Trp67, Pro277 and Arg343	--	−7.4	1.080
IV	Lys260 and Asp217	Leu23 and Leu218	Leu23 and Leu218	Cys12 and Arg52	−6.8	1.356
IS	Cys12, Glu46 , Thr48 Asp49, and Tyr196	Leu195	Leu195	Cys12 and Leu218	−8.0	1.280
IAA	Cys12, Glu46 , Thr48 Asp49, and Tyr196	Leu195	Leu195	Cys12 and Leu218	−8.0	1.373
Pyrazinamide[10] (standard drug)	Ala205, Lys208, Ile209, Gly239 and Arg240	Lys208	--	Lys208 and Arg240	−4.8	--

IG: Isatin-benzothiazole glycine, ITYR: Isatin-benzothiazole tyrosine, ITRYPT: Isatin-benzothiazole tryptophan, IA: Isatin-benzothiazole alanine, IH: Isatin-benzothiazole histidine, IP: Isatin-Benzothiazole proline, IC: Isatin-benzothiazole cysteine, IGA: Isatin-benzothiazole glutamic acid, IV: Isatin-benzothiazole valine, IS: Isatin-benzothiazole serine: IAA: Isatin-benzothiazole aspartic acid, IPA: Isatin-benzothiazole phenylalanine

Table 4: Structure-activity relationship summary of isatin-benzothiazole derivatives against *Mycobacterium tuberculosis* [13,17] with docking scores and MIC values [35]

Structural feature	Role in anti-TB activity	Representative derivative (s)	Docking [17,26] score (kcal/mol)	MIC (µg/mL)
Amide group (–CONH–)	Enhances hydrogen bonding, solubility, and bioavailability	IH, IS	–8.6 to –8.2	3.12
Isatin core (Indole-2,3-dione)	Essential pharmacophore; N1 or C-5 substitution affects potency and selectivity	All derivatives	–8.2 to –8.8	3.12–12.5
Hydrazone linkage (C=N–NH–)	Enables hydrogen bonding and target engagement; crucial for activity	Common to all derivatives	–8.2 to –8.8	–
Benzothiazole ring	Improves lipophilicity and cell wall penetration; 6-/7-substituents enhance activity	IPA, IS, IH	–8.5 to –8.7	3.12–6.25
Aromatic amino acid side chains	Facilitate π–π stacking and hydrophobic interactions; electron effects influence potency	ITYR, ITRYPT, IPA	–8.8 (ITRYPT)	3.12–6.25
Tryptophan side chain	Provides strong aromatic stacking and hydrophobic contacts; high docking affinity	ITRYPT	–8.8	6.25
Tyrosine side chain	Polar and hydrophobic interactions; stabilizes ligand within active site	ITYR	–8.5	3.12
Substitution at isatin C-5 position	Alters electronic profile; halogens/methoxy enhance lipophilicity and target binding	IH, IC, IPA	–8.4 to –8.7	3.12–6.25
N1-substitution on isatin	Affects pharmacokinetics; small groups favored for optimal fit	IV, IA	–8.3 to –8.5	3.12–6.25
Pyrrolidinone ring	Adds rigidity; promotes stable enzyme interactions	IP	–8.2	6.25
Thiosemicarbazone group	Exhibits antimycobacterial action through metal chelation and enzyme inhibition	IGA	–8.5	6.25
Terminal NH_2/ hydrophilic groups	Improve solubility and H-bonding with active site	IG, IAA	–8.2 to –8.4	6.25–12.5
Overall molecular planarity	Promotes insertion into enzyme pockets and possible DNA intercalation	IPA, ITYR, ITRYPT, IH, IS, IC, and IG (Planar Derivatives)	–8.2 to –8.8	–

IG: Isatin-benzothiazole glycine, ITYR: Isatin-benzothiazole tyrosine, ITRYPT: Isatin-benzothiazole tryptophan, IA: Isatin-benzothiazole alanine, IH: Isatin-benzothiazole histidine, IP: Isatin-Benzothiazole proline, IC: Isatin-benzothiazole cysteine, IGA: Isatin-benzothiazole glutamic acid, IV: Isatin-benzothiazole valine, IS: Isatin-benzothiazole serine: IAA: Isatin-benzothiazole aspartic acid, IPA: Isatin-benzothiazole phenylalanine



Scheme 1: (a) and (b) synthesis of benzothiazole-isatin hydrazide derivatives

Table 5: MIC[29] values for synthesized derivatives

S. No.	Sample code	MIC value* (µg/mL)
01	IA	6.25
02	IC	6.25
03	IH	3.12
04	IG	>6.25
05	IP	6.25
06	IS	3.12
07	IV	3.12
08	IGA	6.25
09	IPA	3.12
10	ITYR	3.12
11	ITRYPT	6.25
12	IAA	6.25

*No. of replicates/Trials n=2. IG: Isatin-benzothiazole glycine, ITYR: Isatin-benzothiazole tyrosine, ITRYPT: Isatin-benzothiazole tryptophan, IA: Isatin-benzothiazole alanine, IH: Isatin-benzothiazole histidine, IP: Isatin-Benzothiazole proline, IC: Isatin-benzothiazole cysteine, IGA: Isatin-benzothiazole glutamic acid, IV: Isatin-benzothiazole valine, IS: Isatin-benzothiazole serine: IAA: Isatin-benzothiazole aspartic acid, IPA: Isatin-benzothiazole phenylalanine

Table 6: *In vitro* antitubercular results for synthesized derivatives

S. No.	Sample code	Concentration (µg/mL)							
		"100	50	25	12.5	6.25	3.12	1.6	0.8
01	IA	S	S	S	S	S	R	R	R
02	IC	S	S	S	S	S	R	R	R
03	IH	S	S	S	S	S	S	R	R
04	IG	S	S	S	S	R	R	R	R
05	IP	S	S	S	S	S	R	R	R
06	IS	S	S	S	S	S	S	R	R
07	IV	S	S	S	S	S	S	R	R
08	IGA	S	S	S	S	S	R	R	R
09	IPA	S	S	S	S	S	S	R	R
10	ITYR	S	S	S	S	S	S	R	R
11	ITRYPT	S	S	S	S	S	R	R	R
12	IAA	S	S	S	S	S	R	R	R

Note: S: Sensitive, R: Resistant, IG: Isatin-benzothiazole glycine, ITYR: Isatin-benzothiazole tyrosine, ITRYPT: Isatin-benzothiazole tryptophan, IA: Isatin-benzothiazole alanine, IH: Isatin-benzothiazole histidine, IP: Isatin-Benzothiazole proline, IC: Isatin-benzothiazole cysteine, IGA: Isatin-benzothiazole glutamic acid, IV: Isatin-benzothiazole valine, IS: Isatin-benzothiazole serine: IAA: Isatin-benzothiazole aspartic acid, IPA: Isatin-benzothiazole phenylalanine

Molecular docking studies

The crystal structure of *M. tuberculosis* target (shown in Fig.2) have been retrieved from PDB: Glutamyl-t-RNA synthetase (PDB ID: 2JA2, [16,17] resolution 1.65 Å. Docking studies have carried out using AutoDock Vina, which is known for its stability and reproducibility compared to AutoDock 4.2.6,ChemSketch and ChemDraw were used to draw the ligand structures. Chimera software (version 1.17.3) was used to minimize energy. Grid parameter (.gpf), docking parameter (.dpf) ligand (.pdbqt), and macromolecule (.pdbqt) were among the created filesfor docking simulations, a 3D lattice grid was developed. PyRx [26], Discovery Studio software [27], and PyMOL [28] were implemented to visualize the interactions shown in Table 3 and Figs. 4 and 5.

Virtual screening was conducted using the AutoDock Vina tool integrated within the PyRx platform. The docking grid (shown in Fig.3) was precisely aligned to cover the active site of the target (shown in Fig. 2) enzyme, with the center coordinates set at X: 10.4192, Y: 11.1882, and Z: 38.6467. To ensure thorough coverage of the binding site, the grid dimensions were adjusted to (in Angstroms) "X: 85.4571 A°, Y: 57.0439 A°, and Z: 96.9392 A°. This grid size (85 × 57 × 97 Å) (shown in Fig.3) was optimized to accommodate the bulkier benzothiazole–isatin derivatives and allow exploration

Table 7: Prediction of toxicities by PROTOX-3 Tool for synthesized derivatives

S. No	Derivatives codes	Toxicity parameters[31,34]							
		Hepatotoxicity	Neurotoxicity	Nephrotoxicity	Respiratory toxicity	Cardiotoxicity	Carcinotoxicity	Immunotoxicity	Mutagenicity
1	IPA	A	A	IA	A	IA	A	IA	IA
2	IG	IA	A	IA	A	IA	A	IA	IA
3	ITYR	A	A	A	A	IA	A	IA	A
4	ITRYPT	A	A	IA	A	IA	A	A	IA
5	IA	A	A	IA	A	IA	A	IA	A
6	IH	A	A	IA	A	IA	A	IA	IA
7	IP	IA	A	IA	A	IA	A	IA	A
8	IC	IA	A	IA	A	IA	A	IA	IA
9	IGA	IA	IA	A	A	IA	IA	IA	IA
10	IV	A	A	IA	A	IA	A	IA	IA
11	IS	IA	A	A	A	IA	A	IA	IA
12	IAA	A	IA	A	A	IA	IA	IA	IA

S. No	Derivatives codes	Toxicity parameters[31,34]					Predicted LD_{50} (mg/kg)	Predicted toxicity class
		Cytotoxicity	BBB-barrier	Ecotoxicity	Clinical toxicity	Nutritional toxicity		
1	IPA	IA	A	IA	A	IA	1800	4
2	IG	IA	A	IA	A	IA	879	4
3	ITYR	IA	A	IA	A	IA	1800	4
4	ITRYPT	IA	A	IA	A	IA	1800	4
5	IA	IA	A	IA	IA	IA	1600	4
6	IH	IA	A	IA	A	IA	1600	4
7	IP	IA	A	IA	IA	IA	1600	4
8	IC	IA	A	IA	A	IA	1600	4
9	IGA	IA	IA	IA	A	IA	1600	4
10	IV	IA	A	IA	IA	IA	1600	4
11	IS	IA	A	IA	A	IA	1600	4
12	IAA	IA	IA	IA	A	IA	1600	4

A: Active, IA: Inactive

IG: Isatin-benzothiazole glycine, ITYR: Isatin-benzothiazole tyrosine, ITRYPT: Isatin-benzothiazole tryptophan, IA: Isatin-benzothiazole alanine, IH: Isatin-benzothiazole histidine, IP: Isatin-Benzothiazole proline, IC: Isatin-benzothiazole cysteine, IGA: Isatin-benzothiazole glutamic acid, IV: Isatin-benzothiazole valine, IS: Isatin-benzothiazole serine: IAA: Isatin-benzothiazole aspartic acid, IPA: Isatin-benzothiazole phenylalanine

of potential allosteric interactions [13,17,26]. This configuration allowed for a reliable analysis of the binding interactions and (shown in Fig. 6) fit of the synthesized derivatives within the enzyme's active region.

Characterization of synthesized derivatives

N'-(benzo[d]thiazol-2-yl)-2-(2-oxoindolin-3-ylideneamino)-3-phenylpropanehydrazide (IPA)

It was obtained from isatin 1.5 g (0.01 mol), "(S)-2-amino-N'-(benzo[d] thiazol-2-yl)-3-phenylpropanehydrazide 3.1 g 0.01 mol) yield 4.122 g (89.2%).

IR Spectra (cm^{-1}): N–H stretching (primary hydrazide) 3432, (–NH–NH_2) 3077, (C=O) 1710, (C=N) 1601, (Aromatic C=C) 1528, (N–H) 1457, (COO^-) 1399, (N–N) 1338, (C–N benzo[d]thiazole region) (thiazole portion) 1266 and 1104, (Aromatic C–H) 866, 785, 743, C–S (benzo[d] thiazole ring) 684.

MS: m/z 441.65.

H^1 NMR NH of the indole ring 10.62 (singlet, 1H), aromatic proton benzothiazole and isatin 8.33–8.35 (multiplet, 9H), indole 6.62–6.91 (multiplet, 1H), benzylic 4.27–4.29 (triplet, 3H), methine (CH_2) 2.54 (singlet, 2H), methyl (CH_3) 1.94–2.04 (singlet, 2H).

"N'-(benzo[d]thiazol-2-yl)-3-(1H-imidazol-4-yl)-2-(2-oxoindolin-3-ylideneamino)propane hydrazide (IH)

It was obtained from isatin 1.5 g (0.01 mol), "(S)-2-amino-N'-(benzo[d] thiazol-2-yl)-3-(1H-imidazol-4-yl)propane hydrazide" 3.0 g (0.01 mol) yield 4.182 g (92.5%).

IR spectra (cm^{-1})

N–H stretching (primary hydrazide) 3131, O–H (carboxylic) 2987, C=O (carboxylic) 1693, C=N (imidazole) 1604, Aromatic C=C 1525, N–H bending 1452, COO^- 1395, N–N 1336, C–N 1260 and 1175, C–O 1214, Aromatic ring vibrations 1087, 1036, Aromatic C–H out-of-plane bending 1002, 922, 873, 833, C–S (benzo[d]thiazole ring) 779, 736, 681.

MS: m/z 433.5 (M+2).

H^1 NMR (DMSO-d_6, δ ppm)

NH of the indole ring 10.46 (singlet, 1H), Aromatic protons (benzothiazole and isatin) 8.28–8.30 (multiplet, 8H), CH 3.89–4.30 (multiplet, 2H), CH_2 2.51–2.52 (singlet, 1H), CH_3 1.92 (singlet, 1H).

(E)-"N'-(benzo[d]thiazol-2-yl)-2-(2-oxoindolinylideneamino) acetohydrazide (IG)

It was obtained from isatin 1.5 g (0.01 mol), "2-amino-N'-(benzo[d] thiazol-2-yl)acetohydrazide" 2.2 g (0.01 mol) yield 2.887 g (77.6%).

IR spectra (cm^{-1})

N–H stretching (primary hydrazide) 3436, –NH–NH_2 3086, O–H 2882, C=O (carboxylic) 1712, C=N or N–H 1600, Aromatic C=C 1570, N–H bending 1460, COO^- 1335, N–N 1285, C–N (aromatic) 1107, 1039, Aromatic C–H out-of-plane bending 946, 865, 785, 744, C–S (benzo[d]thiazole ring) 682.

MS: m/z 351.4 (M^+).

H^1 NMR (DMSO-d_6, δ ppm)

Aromatic protons (benzothiazole) 7.00–7.30 (multiplet, 4H), Indole aromatic protons 7.30–8.00 (multiplet, 4H), Pyrrole N–H (indole ring) 8.00–8.40 (singlet, 1H), NH of hydrazone linkage 10.50 (singlet, 1H).

N'-(benzo[d]thiazol-2-yl)-3-(4-hydroxyphenyl)-2-(2oxoindolin-3-ylideneamino)propanehydrazide (ITYR)

It was obtained from isatin 1.5 g (0.01 mol), "(S)-2-amino-N'-(benzo[d] thiazol-2-yl)-3-(4-hydroxyphenyl) propanehydrazide" 3.3 g (0.01 mol) yield 4.222 g (88.3%).

IR spectra (cm^{-1})

Phenolic O–H stretching 3137, C=O (carboxylic) 1694, C=N (hydrazide) 1606, Aromatic C=C 1526, N–H bending (isatin) 1453, COO^- 1399, C–N (benzo[d]thiazole region) 1262, C–O (phenol) 1176, Amide region 1091, Aromatic C–H 1039, 1004, 875, 837, 782, 736, C–S (benzo[d] thiazole ring) 684.

MS: m/z 458.75 (M^+ +1).

H^1 NMR (DMSO-d_6, δ ppm)

Indole amide NH 10.59 (singlet, 1H), Aromatic protons (benzothiazole and isatin) 7.38–8.33 (multiplet, 9H), Aromatic protons (phenol-substituted ring) 6.87–6.88 (multiplet, 5H), Methine (CH) 4.27–4.29 (triplet, 1H), Methylene (CH_2) 2.51–2.52 (singlet, 1H), Methyl (CH_3) 1.93–2.50 (triplet, 1H).

(E)-N'-(benzo[d]thiazol-2-yl)-3-(1H-indol-3-yl)-2-(2-oxoindolin-3-ylideneamino)propanehydrazide (ITRYPT)

It was obtained from isatin 1.5 g (0.01 mol), (S)-2-amino-N'-(benzo[d] thiazol-2-yl)-3-(1H-indol-3-yl)propanehydrazide 3.5 g (0.01 mol) yield 4.791 g (95.6%).

IR spectra (cm^{-1})

O–H 3054, Aliphatic C–H 2808, Amide C=O 1688, N–H 1596, Aromatic C=C 1547, Indole ring 1516, Aliphatic C–H bending 1450, C–N 1389, COO^- symmetric 1333, C–O 1286, N–N 1236, C–N 1176, Indole 1136, 1097, Aromatic C–H 1035, 940, 861, Aromatic C–H (thiazole) 784, 737, Aromatic C–S (benzo[d]thiazole) 680.

MS: m/z 481.2 (M^+ +1).

H^1 NMR (DMSO-d_6, δ ppm)

Indole amide NH 10.58 (singlet, 1H), Aromatic proton (benzothiazole) 8.29–8.30 (doublet, 1H), Aromatic protons (benzothiazole and isatin) 7.20–7.35 (multiplet, 11H), Indole H 6.86–6.99 (doublet, 1H), Methine CH (adjacent to carbonyl) 4.25 (triplet, 1H), Methylene CH_2 (adjacent to N) 2.50–2.51 (singlet, 1H), Methyl CH_3 1.24–1.27 (singlet, 1H).

(Z)-"N'-(benzo[d]thiazol-2-yl)-2-(2-oxoindolin-3-ylideneamino)propane hydrazide (IA)

It was obtained from isatin 1.5 g (0.01 mol), "(S)-2-amino-N'-(benzo[d]thiazol-2-yl)propanehydrazide" 2.4 g (0.01 mol) yield 3.525 g (91.3%).

IR spectra (cm^{-1})

N–H (primary hydrazide) 3424, Amide N–H 3058, C=O (carboxylic) 1707, C=N 1598, Aromatic C=C 1530, Isatin N–H 1456, COO^- 1399, N–N 1336, 1265, C–O 1233, C–N 1182, Aromatic ring 1139, 1104, Aromatic C–H 1038, 863, 784, 741, Aromatic C–S (benzo[d]thiazole ring) 683.

MS: m/z 364.4 (M–1).

H^1 NMR (DMSO-d_6, δ ppm)

NH (indole, isatin) 11.50 (singlet, 1H), NH (amide) 10.50 (singlet, 1H), NH (hydrazide) 8.40 (singlet, 1H), Aromatic protons (benzothiazole +

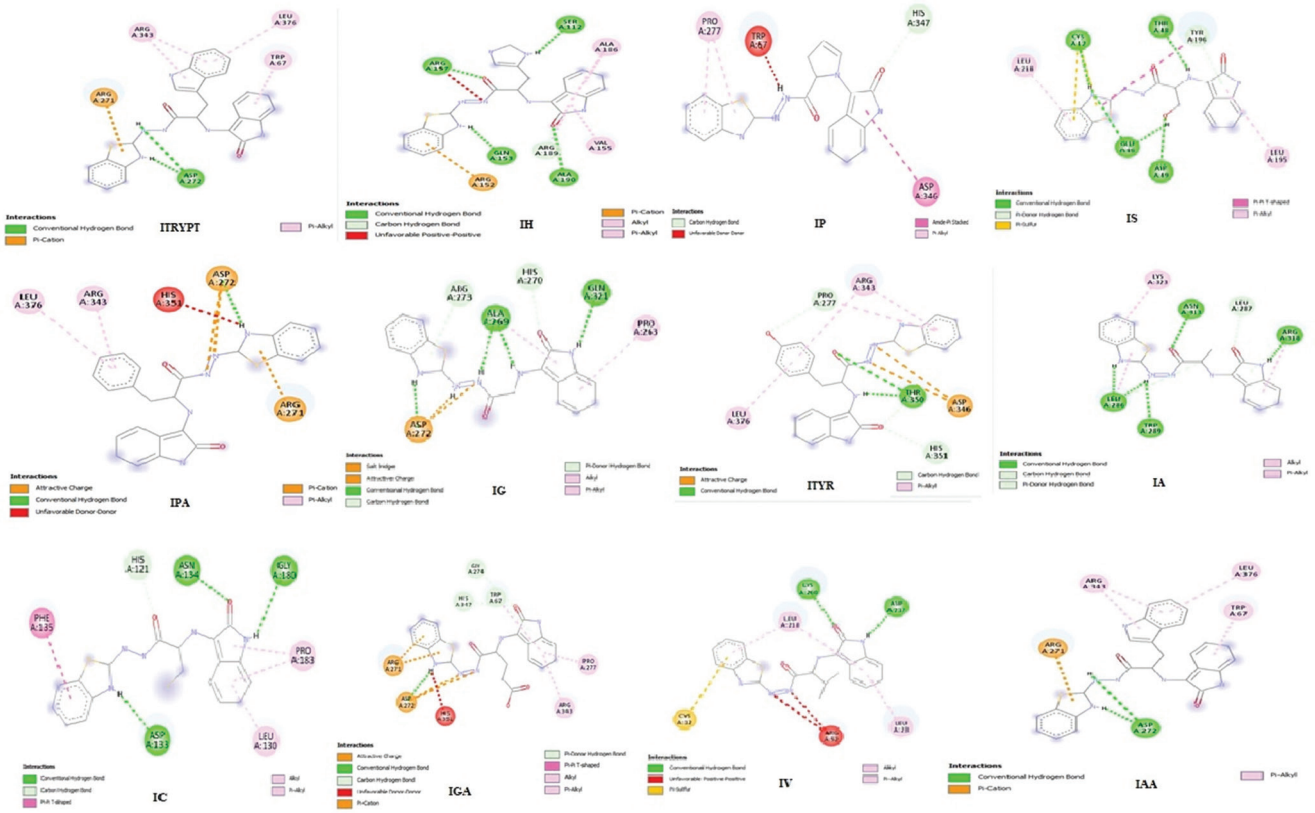


Fig. 4: 2D interactions [27] for synthesized derivatives in active site with the Target (2JA2) isatin-benzothiazole tryptophan, isatin-benzothiazole histidine, isatin-benzothiazole proline, isatin-benzothiazole serine, isatin-benzothiazole phenylalanine, isatin-benzothiazole glycine, isatin-benzothiazole tyrosine, isatin-benzothiazole alanine, isatin-benzothiazole cysteine, isatin-benzothiazole glutamic acid, isatin-benzothiazole valine, and isatin-benzothiazole aspartic acid

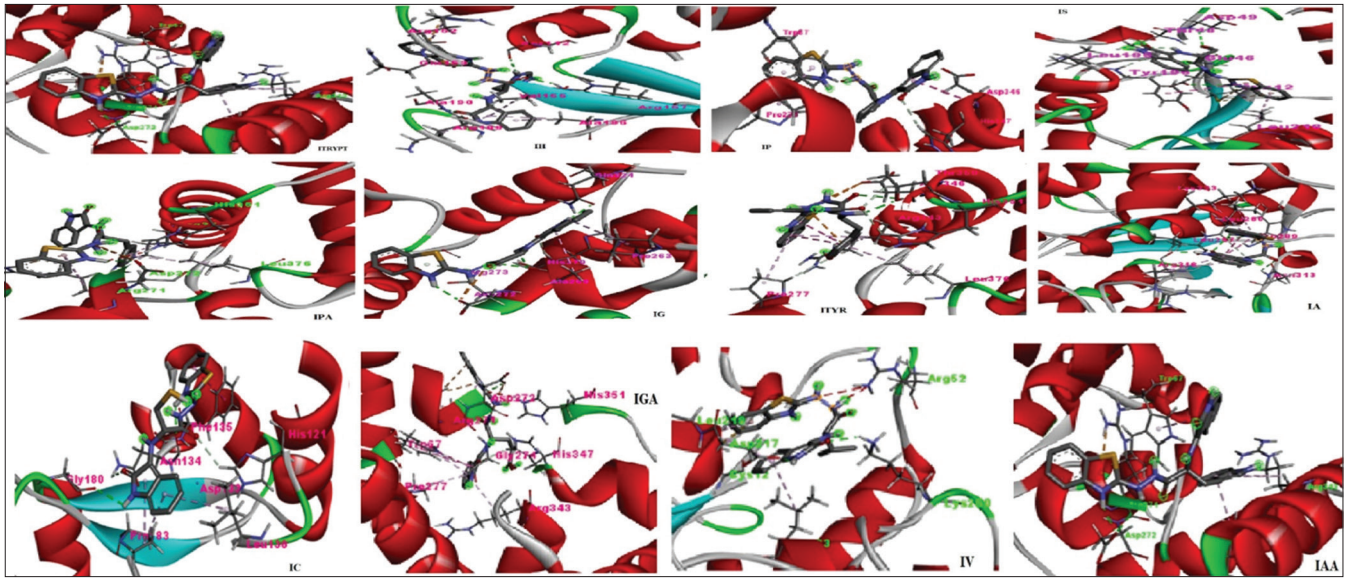


Fig. 5: 3D interactions [27] for synthesized derivatives in active site with the Target (2JA2) isatin-benzothiazole tryptophan, isatin-benzothiazole histidine, isatin-benzothiazole proline, isatin-benzothiazole serine, isatin-benzothiazole phenylalanine, isatin-benzothiazole glycine, isatin-benzothiazole tyrosine, isatin-benzothiazole alanine, isatin-benzothiazole cysteine, isatin-benzothiazole glutamic acid, isatin-benzothiazole valine, and isatin-benzothiazole aspartic acid

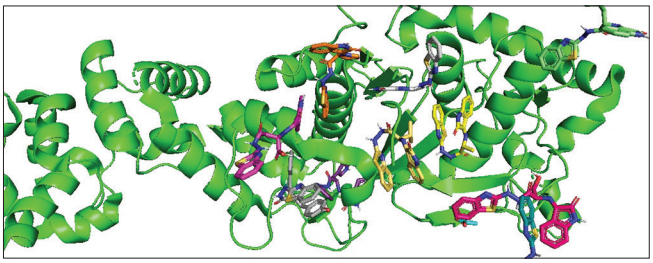


Fig. 6: Binding mode of all synthesized compounds in active site [28] for the Target 2JA2

isatin) 7.00–7.80 (multiplet, 7H), CH (methine) 4.25 (singlet, 1H), CH_3 (methyl) 2.50 (singlet, 3H).

N'-(benzo[d]thiazol-2-yl)-3-methyl-2-(2-oxoindolin-3-ylideneamino) butane hydrazide (IV)

It was obtained from isatin 1.5 g (0.01 mol), "(S)-2-amino-N'-(benzo[d] thiazol-2-yl)-3-methylbutanehydrazide" 2.6 g (0.01 mol) yield 2.787 g (67.3%).

IR spectra (cm^{-1})

N–H 3142, O–H 2950, C=O 1694, Aromatic C=C 1528, N–H (bending) 1454, COO^- 1395, C–N 1338, N–N 1263–1055, Aromatic C–H 1005, 877–738, Aromatic C–S (benzo[d]thiazole ring) 684.

MS: m/z 393 (M^+).

H^1 NMR (DMSO-d_6, δ ppm)

NH (indole ring) 10.58 (singlet, 1H), NH (amide) 8.30–8.31 (singlet, 1H), Aromatic protons (benzothiazole and isatin) 6.82–7.40 (multiplet, 9H), Methine CH 2.50 (singlet, 1H), Methyl, CH_3 1.91 (singlet, 1H).

(Z)-N'-(benzo[d]thiazol-2-yl)-1-(2-(2-oxoindolin-3-ylidene) hydrazinecarbonyl) pyrrolidine (IP)

It was obtained from isatin 1.5 g (0.01 mol), "(S)-N'-(benzo[d]thiazol-2-yl)pyrrolidine-2-carbohydrazide" 2.6 g (0.01 mol) yield 3.602 g (87.4%).

IR spectra (cm^{-1})

N–H stretching (hydrazide) 3141, (C=O isatin/amide) 1702, (C=N) 1597, (Aromatic C=C) 1530, (NO_2 bending) 1455, COO^- 1399, N–N 1335, C–N (hydrazide region) 1264 and 1231, C–N (ring) 1181–1102, Aromatic C–H 1037, 1006, 862–740, C–S (benzo[d]thiazole ring) 683.

MS: m/z 391.1 (M–1).

H^1 NMR (DMSO-d_6, δ ppm)

NH (indole ring) 10.62 (singlet, 1H), Aromatic protons (benzothiazole and isatin) 8.33–8.34 (multiplet, 9H), additional aromatic protons 7.57–7.94 (multiplet, 6H), CH (methine) 4.26–4.34 (multiplet, 1H), CH_2 2.55 (singlet, 1H), methyl, CH_3 2.03–2.06 (singlet, 3H).

(E)-N'-(benzo[d]thiazol-2-yl)-3-mercapto-2-(2-oxoindolin-3-ylideneamino)propane hydrazide (IC)

It was obtained from isatin 1.5 g (0.01 mol), "(R)-2-amino-N'-(benzo[d] thiazol-2-yl)-3-mercaptopropanehydrazide" 2.7 g (0.01 mol) yield 3.687 g (88.2%).

IR Spectra (cm^{-1})

C=O (isatin ketone) 3127, N–H (isatin, secondary amide) 2957, C=N (imine) 1693, C=O (cysteine carbonyl) 1602, N–H (amide) 1527, Aromatic C–H (benzothiazole ring) 1000–681.

MS: m/z 397.8 (M^+).

H^1 NMR (DMSO-d_6, δ ppm)

Indole NH (isatin) 11.2 (singlet, 1H), Amide NH (hydrazide linkage) 10.6 (singlet, 1H), NH near thiazolidinone 8.3 (singlet, 1H), Aromatic protons (isatin) 7.1–7.38 (multiplet, 4H), CHOH (piperidine, near OH group) 4.27–4.29 (doublet, 1H), CH_2 (piperidine ring) 3.12–3.6 (multiplet, 2H), CH_2 (piperidine) 2.0–2.6 (multiplet, 2H), CH_3 (methyl on thiazolidinone ring, C5) 1.30 (singlet, 3H).

(E)-5-(2-(benzo[d]thiazol-2-yl)hydrazinyl)-5-oxo-4-(2-oxoindolin-3-ylidene amino) pentanoic acid (IGA)

It was obtained from isatin 1.5 g (0.01 mol), (S)-2-amino-5-(2-(benzo[d]thiazol-2-yl)hydrazinyl)-5-oxopentanoic acid 2.9 g (0.01 mol) yield 3.166 g (71.3%).

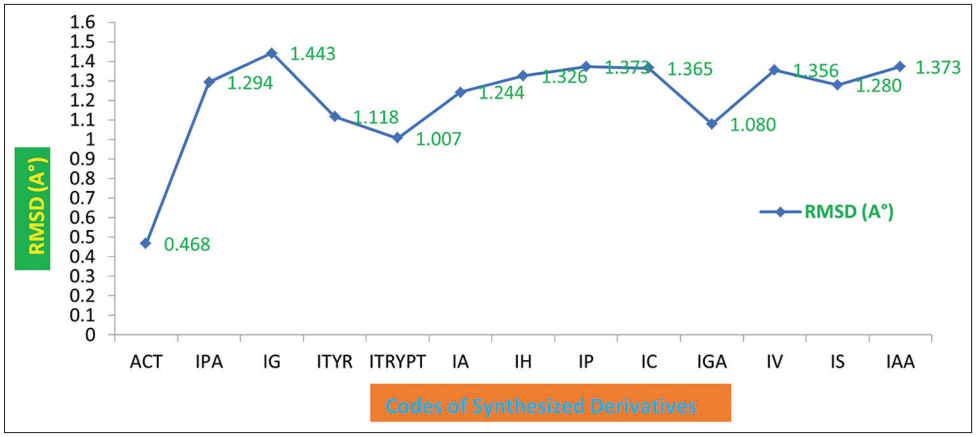


Fig. 7: Root mean square deviation for co-crystal ligand [30,28,31-33] and synthesized derivatives

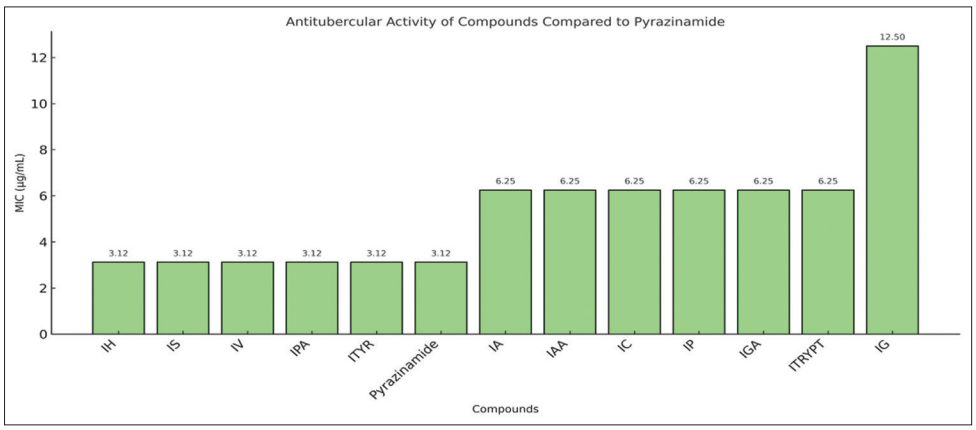


Fig. 8: Minimum inhibitory concentration [29] values indicating potency against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) [13,35]

IR spectra (cm^{-1})

O–H (carboxylic acid) 3278, N–H (amide/indole) 2977, C=O (carboxylic/isatin carbonyl) 1688, C=C (aromatic) 1509, N–H bending 1450, C–N/N–N/COO^- 1341–1251, C–N stretch 1174, Aromatic C–H 1006, 939, 861; benzothiazole-associated vibrations 794, 738.

MS: m/z 322.15 (M–1).

H^1 NMR (DMSO-d_6, δ ppm)

COOH proton 10.5 (singlet, 1H), NH (Indole) 8.3 (singlet, 1H), Aromatic protons (isatin and benzothiazole) 7.2–7.8 (multiplet, 6H), CH (adjacent to nitrogen) 4.29 (multiplet, 1H), CH_2 (aliphatic chain) 2.25 (multiplet, 2H), NH (amide) 3.5 (singlet, 1H).

(Z)-N'-(benzo[d]thiazol-2-yl)-3-hydroxy-2-(2-oxoindolin-3-ylideneamino)propane hydrazide (IS)

It was obtained from isatin 1.5 g (0.01 mol), (S)-2-amino-N'-(benzo[d] thiazol-2-yl)-3-hydroxypropanehydrazide 2.5 g (0.01 mol) yield 2.744 g (69%).

IR spectra (cm^{-1})

N–H/O–H stretching 3198, 3052; C=O (isatin carbonyl) 1695; C=N (imine) 1609; C=C (aromatic) 1529, 1455; COO^- 1398; C–N 1338, 1263; C–O 1054; Aromatic C–H 1005, 922, 875, 842; Aromatic ring vibrations 779, 737; C–S (thiazole) 684.

MS: m/z 381.4 (M^+).

H^1 NMR (DMSO-d_6, δ ppm)

Indole NH 10.58 (singlet, 1H), Amide NH 8.32 (singlet, 1H), Aromatic protons (indole and benzothiazole) 7.20–7.75 (multiplet, 8H), NH (indole side chain) 6.86 (doublet, 1H), Methine CH (adjacent to OH) 4.10 (singlet, 1H), CH_2 (N–CH_2) 3.44 (multiplet, 2H), Aliphatic CH_2 1.92–1.94 (multiplet, 2H).

(E)-"4-(2-(benzo[d]thiazol-2-yl)hydrazinyl)-4-oxo-2-(2-oxoindolin-3-ylideneamino)butanoic acid (IAA)

It was obtained from isatin 1.5 g (0.01 mol), (S)-2-amino-4-(2-(benzo[d]thiazol-2-yl)hydrazinyl)-4-oxobutanoic acid" 2.8 g (0.01 mol) yield 2.447 g (56.9%).

IR spectra (cm^{-1})

N–H (indole) 3400, N–H (amide) 3300, O–H (carboxylic acid) 3500, C=O (amide and carboxyl) 1700, 1725, Amide carbonyl 1690, C=N (hydrazone) 1580–1640, Aromatic C=C 1450–1600, Aromatic C–H 3000–3100, C–N (benzothiazole) 1100–1300, C–S (benzothiazole) 600–800.

MS: m/z 409.4 (M^+).

^{1}H NMR (DMSO-d_6, δ ppm)

Indole NH 10.5 (singlet, 1H), Amide NH 8.2–8.5 (singlet, 1H), Aromatic protons (isatin and benzothiazole rings) 6.8–8.5 (multiplet, 9H), OH (carboxylic acid) 4.0–5.5 (broad singlet, 1H), Methine CH 4.2–4.5 (multiplet, 2H), CH_3 (aliphatic) 2.0–2.8 (multiplet, 2H).

***In vitro* evaluation of synthesized derivatives for antitubercular activity [3,4,29]**

The synthetic drugs anti-tuberculosis efficacy was evaluated using a 96-well microtiter plate and the Alamar Blue dye assay. The plate's peripheral wells were "filled with 200 µL of sterile deionized water to stop evaporation during incubation. A concentration gradient was

created by serially diluting the test chemicals directly in each test well, which was filled with 100 µL of Middlebrook 7H9 broth. The compounds' final concentration range was between 100 µg/mL and 0.2 µg/mL to determine the minimum inhibitory concentration (MIC). After sealing the plate with parafilm, it was incubated for 5 days at 37°C to facilitate bacterial growth and contact with the test chemicals. Following incubation, 25 µL of a newly made mixture comprising each well received a 1:1 application of 10% Tween 80 and Alamar Blue reagent. The plate was then incubated for 24 h at 37°C" to enable the dye to react with the bacterial culture. Pink coloration denoted vigorous bacterial proliferation, while blue coloration suggested suppression of bacterial growth. As indicated by the retention of the blue hue, the lowest concentration of the material that prevented observable bacterial growth was the MIC. This technique, which is based on well-established MABA methods was performed in twice (n=2) to guarantees accuracy and reproducibility for assessing anti-TB activity.

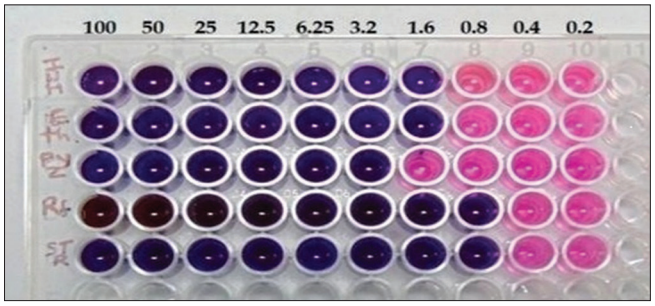


Fig. 9: Standard anti-tubercular drugs photograph against Mycobacterium tuberculosis H37Rv (ATCC 27294)

Standard [8-12] values for the anti-Tb test which was performed. Rifampicin: 0.8 µg/mL, Streptomycin: 0.8 µg/mL, Pyrazinamide: 3.125 µg/mL, Ethambutol: 1.6 µg/mL, and Isoniazid: 1.6 µg/mL.

RESULTS AND DISCUSSION

Molecular docking analysis

Molecular docking was performed on Schiff base compounds based on isatin that target the glutamine t-RNA synthetase in M. tuberculosis (PDB ID: 2JA2). The objective was to assess binding affinities and interactions stabilizing the ligand–protein complex. Among the compounds, ITRYPT derivative displayed the strongest binding with a docking score (shown in Table 3) of −8.8 kcal/mol, interacting pi-cationally with Arg271 and generating hydrogen bonds with Asp272. IH came in second with a score of −8.3 kcal/mol, forming several hydrogen bonds and pi-pi stacking interactions. IP and IS also showed strong affinities (–8.0 kcal/mol), involving hydrogen bonds and stacking interactions with key residues. Compounds likely IPA, IG, and IGA demonstrated moderate activity. In contrast, the co-crystallized ligand "ACT" was re-docked into the active site of the 2JA2 protein. The resulting root mean square deviation (RMSD) (shown in Fig.7) between the redocked pose (Figs. 6 and 7) and its crystallographic conformation was 0.468 Å. Since RMSD values below 2.0 Å (shown in Tables 2 and 3) are widely accepted as indicative of accurate redocking, this confirms the validity of the docking approach for further virtual screening [30-33], and the standard drugs pyrazinamide and ethambutol showed weaker binding, with scores of –5.3, –4.8, and –4.7 kcal/mol, respectively shown in Table 3. These results demonstrate the potential of compounds generated from isatin, especially those containing (ITRYPT) tryptophan derivative or (IH) histidine derivative moieties, as promising anti-TB options.

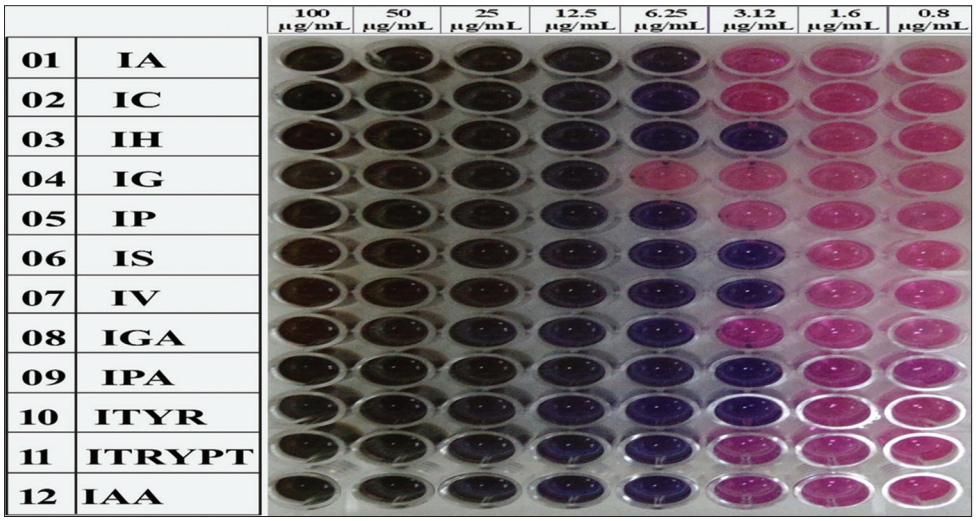


Fig. 10: Photograph showing antitubercular activity for synthesized compounds by MABA [29] method against Mycobacterium tuberculosis H37Rv (ATCC 27294)

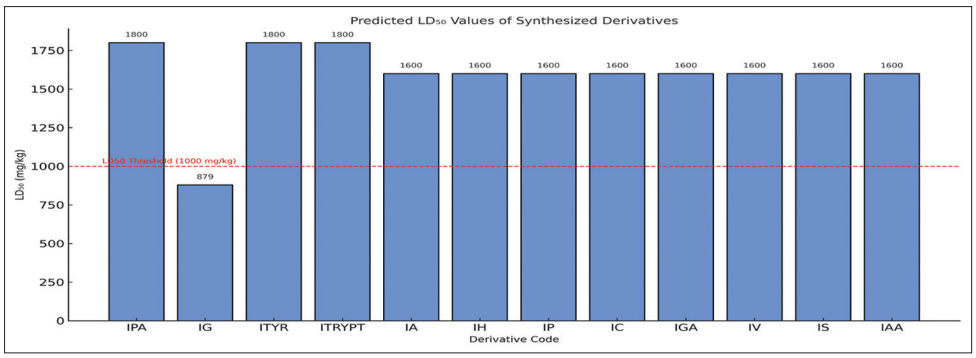


Fig. 11: Toxicity predictions for the synthesized derivatives by PROTOX-3 [31,34]

As per the molecular docking visualization software [26-28] used, hydrogen bonding and π–π stacking interactions are consistently represented in green and purple [27], respectively. These standardized color schemes have been uniformly applied in Figs. 4 and 5 to ensure clarity and consistency in depicting the interaction types across all synthesized derivatives.

Computational toxicity prediction

ProTox-3 [31,34] is an advanced *in silico* toxicity prediction tool that estimates acute oral toxicity (LD_{50}) (shown in Fig. 11), classifies compounds into toxicity categories, and predicts potential organ toxicities (shown in Table 7) such as hepatotoxicity and immunotoxicity. Utilizing machine learning algorithms trained on extensive datasets, ProTox-3 provides rapid, reliable assessments of mutagenic, carcinogenic, and cytotoxic risks, facilitating early-stage drug safety evaluation and reducing the need for animal testing.

Chemistry

Benzothiazole amino acid hydrazide derivatives were synthesized and condensed with isatin to yield isatin-benzothiazole hydrazide compounds. The physicochemical characteristics of the synthesized benzothiazole–isatin hybrid derivatives, including melting point, Rf value, yield, and appearance, are summarized in Table 1. FTIR (Bruker Alpha-II FTIR spectrometer), NMR (Bruker AVANCE NEO 500 MHz NMR spectrometer), and mass (Agilent 6520 Accurate-Mass LC/MS instrument) spectra were used to confirm the structures, and HPLC was used to evaluate their purity [1]. Characteristic spectral peaks supported successful formation, aligning with expected regions for functional groups and confirming structural integrity. Distinct FTIR, NMR, and MS signals confirmed the successful synthesis of hydrazide derivatives, [22] showing characteristic absorptions at 3396 cm^{-1} (N–H), 3025 cm^{-1} (aromatic C–H), 1646 cm^{-1} (C=O), 1601 cm^{-1} (C=N), and 753 cm^{-1} (C–S). In the C^{13} NMR spectra, imine (C=N) carbons appeared between 148–160δ ppm and hydrazide-associated C=O/N–N carbons between 162 and 172 δ ppm. H^1 NMR (ppm): 10.58, 8.29–8.30, 7.20–7.35, 6.86–6.99, 4.25, and 2.50–2.51, corresponding to N–H, aromatic protons, methine, and methyl groups present in the benzothiazole and isatin structures. Remaining peaks were observed in their expected regions, along with mass spectral data further confirmed its structures which are synthesized derivatives shown in Scheme 1.

***In vitro* antitubercular activity of synthesized derivatives**

The MABA method evaluated the synthesized benzothiazole-isatin hydrazide derivatives for their antitubercular activity (Shown in Table 4). This colorimetric assay allowed for accurate determination of MIC by monitoring color change, where a blue colour signified effective inhibition of *M. tuberculosis* growth. The compounds demonstrated varying degrees of activity across the concentration gradient (100–0.2 μg/mL), with several derivatives showing significant inhibition at lower concentrations (shown in Fig. 8). In comparison to common medications such as ethambutol (1.6 μg/mL), rifampicin (0.8 μg/mL), and isoniazid (1.6 μg/mL), several synthetic substances showed MIC values [35] that were either equivalent or moderately effective. The observed activity can be attributed to the presence of essential functional groups, such as aromatic moieties and the hydrazide group (shown in Table 4), within the benzothiazole and isatin framework, both of which are known to contribute significantly to antimycobacterial properties. The reproducibility and sensitivity of the microplate MABA further validate the reliability of these results (shown in Figs.9 (standard drugs) and 10 (synthesized derivatives). These results imply that the chemicals that were produced could be used as possible building blocks for the creation of novel antitubercular drugs [14]. All *in vitro* (MABA method) experiments were performed in twice (n=2) to ensure accuracy and reproducibility shown in Tables 5 and 6.

Computational toxicity prediction

Prediction of toxicity for synthesized derivatives using PROTOX-3 [31,34]

The toxicity of the synthesized derivatives was predicted using the PROTOX-3 online server.

Toxicity assessment for synthesized derivatives

The majority of the synthesized derivatives exhibited predicted LD_{50} values ranging from 1600 to 1800 mg/kg, indicating a relatively low level of acute toxicity. Among the compounds analyzed, IG was found to have the lowest LD_{50} value at 879 mg/kg (shown in Fig.11), suggesting a comparatively higher toxicity within the series. According to the PROTOX-3 classification system [34], all compounds are categorized under Toxicity Class 4, which corresponds to agents with low-to-moderate toxicity (shown in Table 7). While certain derivatives demonstrated potential for specific toxicological concerns such as hepatotoxicity, immunotoxicity, or mutagenicity, the overall profiles for most compounds were predominantly non-toxic or within acceptable safety margins across various toxicity endpoints.

CONCLUSION

The effective synthesis and antitubercular activity evaluation of a novel family of benzothiazole-linked amino acid derivatives. Several compounds demonstrated noteworthy potency, with some exhibiting activity comparable to established drugs such as pyrazinamide. Combined *in vitro* assays and molecular docking analyses confirmed strong binding interactions among the most active compounds along with glutamyl-tRNA synthetase (GluRS) PDB ID: 2JA2, elucidating key molecular features responsible for their efficacy. Among the series, the tryptophan-based derivative,"(E)-N'-(benzo[d]thiazol-2-yl)-3-(1H-indol-3-yl)-2-(2-oxoindolin-3-ylideneamino)propanehydrazide (ITRYPT)" demonstrated the highest level of biological activity and binding affinity, making it the most promising candidate and a solid starting point for additional drug development. In addition, Histidine derivative N'-(benzo[d]thiazol-2-yl)-3-(1H-imidazol-4-yl)-2-(2-oxoindolin-3-ylideneamino)propanehydrazide(IH), and analogs also showed compelling docking performance. Most of the synthesized compounds showed predicted LD_{50} values between 1600 and 1800 mg/kg, reflecting low acute toxicity, while the IG derivative (E)-N'-(benzo[d]thiazol-2-yl)-2-(2-oxoindolinylideneamino)acetohydrazide (IG) had a lower LD_{50} of 879 mg/kg, indicating relatively higher toxicity. According to the PROTOX-3 system, all compounds were classified as Class 4, with generally acceptable safety profiles fall within a low-to-moderate acute toxicity range despite some showing potential for specific toxicological effects that supporting their potential for safe therapeutic application. Overall, these results lay a solid groundwork for future structure-activity relationship investigations and the advancement of new antitubercular agents capable of combating multidrug-resistant *M. tuberculosis*.

AUTHORS' CONTRIBUTIONS

All authors are contributed equally.

ACKNOWLEDGMENT

The authors acknowledge Maratha Mandal Institute, Belagavi, for the *in vitro* anti-tubercular results and SRM Institute, Chennai, for spectral data support. They also thank the Principal and Management of Balaji College of Pharmacy, Ananthapuramu, for constant encouragement. The corresponding author, G. Thirumala Roopesh, Assistant Professor, Balaji College of Pharmacy, and Research Scholar, JNTUA, Ananthapuramu – 515002, Andhra Pradesh, India, gratefully acknowledges the support received during this work.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FUNDING

Nil.

REFERENCES

1. Gaonkar SL, Hakkimane SS, Bharath BR, Shenoy VP, Vignesh UN, Guru BR. Stable isoniazid derivatives: *In silico* studies on *Mycobacterium tuberculosis* enoyl-ACP reductase (InhA). Asian J

Pharm Clin Res. 2020;13(10):131-6.
2. Shaikh FM, Patel NB, Sanna G, Busonera B, La Colla P, Rajani DP. Synthesis of some new 2-amino-6-thiocyanato benzothiazole derivatives bearing 2,4-thiazolidinediones and screening of their *in vitro* antimicrobial, antitubercular and antiviral activities. Med Chem Res. 2015;24(8):3129-42. doi: 10.1007/s00044-015-1358-0
3. Arul K, Sunisha KS. *In-silico* design, synthesis and *in vitro* anticancer and antitubercular activity of novel azetidinone-containing isatin derivatives. Int J Pharm Pharm Sci. 2014;6(9):506-13.
4. Varpe BD, Jadhav SB. Schiff base of isatin with 2-thiopheneethylamine and its Mannich bases: Synthesis, docking, and *in vitro* anti-inflammatory and antitubercular activity. Russ J Bioorg Chem. 2022;48(2):372-9. doi: 10.1134/S1068162022020030.
5. Hakim F, Salfidoer SR. Antibacterial and antitubercular activity of novel benzothiazole-aryl amine derivatives tethered through acetamide functionality. Asian J Chem. 2021;33(8):1757-63. doi: 10.14233/ajchem.2021.23226
6. Salina EG, Postiglione U, Chiarelli LR, Recchia D, Záhorszká M, Lepioshkin A, *et al*. A novel class of antitubercular compounds targeting the bacterial respiratory chain. mSphere. 2022;7:e00369-22.
7. Hemeda LR, El Hassab MA, Abdelgawad MA, Khaleel EF, Abdel-Aziz MM, Binjubair FA, *et al*. Discovery of pyrimidine-tethered benzothiazole derivatives as novel anti-tubercular agents towards multi- and extensively drug resistant *Mycobacterium tuberculosis*. J Enzyme Inhib Med Chem. 2023;38(1):2250575. doi: 10.1080/14756366.2023.2250575, PMID: 37649381
8. DrugBank. Ethambutol. Available from: https://www.drugbank.ca/drugs/DB00339
9. DrugBank. Streptomycin. Available from: https://www.drugbank.ca/drugs/DB01082
10. DrugBank. Pyrazinamide. Available from: https://www.drugbank.ca/drugs/DB01045
11. DrugBank. Isoniazid. Available from: https://www.drugbank.ca/drugs/DB00951
12. DrugBank. Rifampicin. Available from: https://www.drugbank.ca/drugs/DB00330
13. Eldehna WM, El Hassab MA, Abdelshafi NA, Al-Zahraa Sayed F, Fares M, Al-Rashood ST, *et al*. Development of potent nanosized isatin-isonicotinohydrazide hybrids for management of *Mycobacterium tuberculosis*. Int J Pharm. 2022;612:121369. doi: 10.1016/j.ijpharm.2021.121369, PMID: 34906651
14. Cheke RS, Patil VM, Firke SD, Ambhore JP, Ansari IA, Patel HM, *et al*. Therapeutic outcomes of isatin and its derivatives against multiple diseases: Recent developments in drug discovery. Pharmaceuticals (Basel). 2022;15(3):272. doi: 10.3390/ph15030272, PMID: 35337070
15. Capela R, Félix R, Clariano M, Nunes D, Perry MJ, Lopes F. Target identification in anti-tuberculosis drug discovery. Int J Mol Sci. 2023;24(13):10482. doi: 10.3390/ijms241310482, PMID: 37445660
16. Sekine S, Nureki O, Shimada A, Vassylyev DG, Yokoyama S. Crystal Structure of a Translation Initiation Factor. London: Nature; 2001.
17. Kassem AF, Sabt A, Korycka-Machala M, Afifi MM, El Sayed I, Swairjo MA, *et al*. Identification of quinoline-isatin hybrids as InhA inhibitors against *Mycobacterium tuberculosis*. Indian J Clin Res. 2024;144:107138.
18. Yadav R, Meena D, Singh K, Tyagi R, Yadav Y, Sagar R. Recent advances in the synthesis of new benzothiazole based anti-tubercular compounds. RSC Adv. 2023;13(32):21890-925. doi: 10.1039/d3ra03862a, PMID: 37483662
19. Anand SR, Kumar K, Bairwa DD, Meena RP, Bagoria R, Sharma S, *et al*. *In silico* Evaluation of Benzothiazole Hybrids against MDR Tuberculosis. United Kingdom: Qeios; 2024. p. 1-13.
20. Zala M, Vora JJ, Khedkar VM. Synthesis, characterization, antitubercular activity, and molecular docking studies of pyrazolylpyrazoline-clubbed triazole and tetrazole hybrids. ACS Omega. 2023;8(23):20262-71. doi: 10.1021/acsomega.2c07267, PMID: 37323386
21. Selvaraju K, Manimekalai A. Synthesis, characterization and antimicrobial evaluation of some new benzothiazole derivatives. Rasayan J Chem. 2017;10:25-31.
22. Venugopala KN, Khedr MA, Pillay M, Nayak SK, Chandrashekharappa S, Aldhubiab BE, *et al*. Benzothiazole analogs as potential anti-TB agents: Computational input and molecular dynamics. J Biomol Struct Dyn. 2019;37(7):1830-42. doi: 10.1080/07391102.2018.1470035, PMID: 29697293
23. Thakkar SS, Thakor P, Ray A, Doshi H, Thakkar VR. Benzothiazole analogues: Synthesis, characterization, MO calculations with PM6 and DFT, *in silico* studies and *in vitro* antimalarial as DHFR inhibitors and antimicrobial activities. Bioorg Med Chem. 2017;25(20):5396-406. doi: 10.1016/j.bmc.2017.07.057, PMID: 28789907
24. Bhoi MN, Borad MA, Pithawala EA, Patel HD. Novel benzothiazole containing 4H-pyrimido[2,1-b]benzothiazoles derivatives: One pot, solvent-free microwave assisted synthesis and their biological evaluation. Arab J Chem. 2019;12(8):3799-813. doi: 10.1016/j.arabjc.2016.01.012
25. Sultana S, PP, Rajkamal B. Microwave-assisted synthesis, molecular docking studies and biological evaluation of benzothiazole-carrying indole derivatives. Asian J Chem. 2021;33(11):2755-61.
26. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-61. doi: 10.1002/jcc.21334, PMID: 19499576.
27. Dassault Systèmes BIOVIA. Discovery Studio Visualizer. v21.1.0.20298. San Diego: Dassault Systèmes; 2021.
28. DeLano WL. The PyMOL Molecular Graphics System. San Carlos, CA: DeLano Scientific; 2002.
29. Lourenço MC, de Souza MV, Pinheiro AC, Ferreira ML, Gonçalves RS, Nogueira TM, *et al*. Synthesis of isatin-thiosemicarbazone derivatives with antitubercular activity. Arkivoc. 2007;(Part-i):181-91.
30. Bharath BR, Hakkimane SS, Gaonkar SL, Shenoy VP, Guru BR. Structure-based virtual screening and ADME prediction of potential inhibitors targeting enoyl ACP reductase of *Mycobacterium tuberculosis*. Int J Appl Pharm. 2020;12(4):205-10.
31. Rahman H, Rahman MS, Rahman M. Computational evaluation of toxicity and pharmacokinetics of some bioactive phytochemicals using PROTOX-II and SwissADME tools. Int J Appl Pharm. 2021;13(6):90-4.
32. Sood S, Gupta S, Arora S. Molecular docking and ADMET study of benzothiazole derivatives as antimicrobial agents. Int J Pharm Pharm Sci. 2021;13(9):51-6.
33. Patel H, Thakor P, Dave J. *In silico* docking analysis of selected phytochemicals with DHFR enzyme of *Mycobacterium tuberculosis*. Int J Curr Pharm Res. 2019;11(6):18-21.
34. Suthar J, Patel J, Patel K, Patel R. *In silico* prediction of biological activity, toxicity and drug-likeness of curcumin analogs using PASS, PROTOX-II and ADMETlab. Int J Appl Pharm. 2022;14(2):167-72.
35. Azmi MN, Hasmaruddin NS, Mat Ali NA, Osman H, Mohamad S, Parumasivam T, *et al*. Synthesis, characterization, anti-mycobacterial activity and *in silico* study of new 2,5-disubstituted-1,3,4-oxadiazole derivatives. Trop Biomed. 2022;39(3):467-75. doi: 10.47665/tb.39.3.019, PMID: 36214446