Green and microwave-assisted synthesis of isoniazid-benzothiazole hybrids: In-silico and in-vitro evaluation against MDR-Tuberculosis

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Abstract

Tuberculosis (TB) continues to pose a significant global health challenge, exacerbated by the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of '*Mycobacterium tuberculosis'*. Isoniazid (INH), a key first-line anti-TB drug, is increasingly compromised by resistance and adverse effects, necessitating novel therapeutic strategies. This study explores the potential of eight benzothiazole derivatives 4-Nitrobenzoic Acid (4-NBA), 3-Nitrobenzoic Acid (3-NBA), 4-Aminobenzoic Acid (4-ABA), 5-Sulfosalicylic Acid (5-SSA), Salicylic Acid (SA), Cinnamic Acid (CA), Phthalic Acid (PA), and Gallic Acid (GA)—in combination with INH to improve anti-TB efficacy. Antimycobacterial activity was evaluated using the Alamar Blue assay, with INH and 4-NBA exhibiting the most potent effects at 1.6 µg/mL. Moderate activity was observed for 3-NBA and 4-ABA at 25 and 50 µg/mL, respectively. *Insilico* molecular docking via AutoDock Vina revealed that derivatives BI-6, BI-5, and BI-8 displayed strong binding affinities for Target 6R9W,2JA2 and 2BVC (-10.7 to -10.1 kcal/mol), (-8.5 to -8.2 kcal/mol) and (-8.9 to -8.6 kcal/mol), exceeding that of INH, and all eight compounds **(BI-1 to BI-8)** demonstrated favourable interactions with the target protein. These results highlight the potential of benzothiazole-INH combinations to enhance TB treatment efficacy, combat resistance mechanisms, and provide a promising basis for the development of next-generation anti-TB agents. Insilico toxicity analysis using ProTox-3 revealed that BTZ-INH derivatives (BI-1 to BI-8) generally exhibit moderate oral toxicity, with BI-5 and BI-8 emerging as the safest candidates, while others showed varying organ-specific toxicities, highlighting the need for further validation.

Keywords: Benzothiazole derivatives, Combination therapy, Molecular docking, MABA, Multidrug-resistant, Tuberculosis.

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Introduction:

Tuberculosis (TB) remains a major global health concern, with the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of '*Mycobacterium tuberculosis'* posing significant challenges to Incomplete[1] treatment plans, incorrect prescriptions, and the widespread use of inferior treatments are some of the causes of the growing resistance to first-line medications like isoniazid (INH) and rifampicin (RF)[2]. MDR-TB is subjected by resistance to at least INH and RIF, but XDR-TB shows increased resistance to second-line medications, resulting in fewer and less effective treatment alternatives [3]. Treatment complexity is further increased by *M. tuberculosis's* sluggish growth rate, latent cell formation capabilities, and persistence inside macrophages[4].Second-line antitubercular drugs, such as ethionamide[3], cycloserine, and fluoroquinolones, often have limited efficacy, increased toxicity, and higher costs, necessitating the search for novel therapeutic agents[5]. Because of their strong antibacterial qualities and capacity to block important enzymes involved in the formation of bacterial cell walls, benzothiazole derivatives have garnered attention with this context[6]. To improve bacterial penetration and activity, structural changes have been investigated, such as adding hydrophobic substituents [7].Notably, compounds such as 4-Nitrobenzoic Acid (4-NBA) and 3-Nitrobenzoic Acid (3-NBA) have demonstrated promising antitubercular potential and may be suitable for combination therapies[8].This work uses the Alamar Blue assay to assess the antitubercular activity of eight benzothiazole compounds in conjunction with INH. The objective is to assess their effectiveness against drug-resistant *M. tuberculosis* strains and to elucidate their mechanisms of action through molecular docking studies [9].Using AutoDock Vina, the docking study investigates the interactions between the active derivatives and crucial protein targets necessary for *M. tuberculosis* [10] survival and replication. The results shed light on benzothiazole derivatives' potential as viable options for creating cutting-edge treatment plans to combat MDR-TB and XDR-TB[11].

The well-known antibiotic isoniazid (INH) is used extensively to treat tuberculosis (TB), and it is essential in the fight against the illness[1], Antimicrobial, antitubercular, and antioxidant activity are only a few of the many pharmacological characteristics of benzothiazole, a molecule with a fused 1, 3-thiazole and benzene ring (Fig.1). In order to improve the effectiveness of existing available anti-TB medications, recent research has centred on generating new scaffolds and derivatives[3].The broad-spectrum biological activity

of benzothiazole derivatives, particularly their antibacterial and antimycobacterial qualities [12], have made them attractive candidates with this respect. When combined with conventional anti-TB medications, these compounds have shown promise for synergistic effects, providing a workable plan to combat drug resistance[13].Using eight distinct benzothiazole derivatives, including 4-Nitrobenzoic Acid (4-NBA), 3-Nitrobenzoic Acid (3-NBA), 4-Aminobenzoic Acid (4-ABA), 5-Sulfosalicylic Acid (5-SSA), Salicylic Acid (SA), Cinnamic Acid (CA), Phthalic Acid (PA), and Gallic Acid (GA), this study examines the antitubercular potential of isoniazid (INH) (Fig.1). Together with common anti-TB medications like Rifampicin, Ethambutol, Pyrazinamide, and Streptomycin, the 'Micro Alamar Blue assay' was used to evaluate the compounds' in vitro antitubercular activity at doses ranging from 100 µg/mL to 0.8 µg/mL. Additionally, the binding interactions of the most active compounds—4-NBA, CA, and GA—with the target protein 6R9W (Fig.3), were investigated using molecular docking studies using AutoDock Vina [14]. The study's findings are intended to aid in the creation of novel combination treatments by providing information on plausible mechanisms of action and opening the door for additional drug candidate optimization in the fight against tuberculosis, especially in light of the expanding problem of drug resistance[8].Continued research on these derivatives may lead to the discovery of new therapeutic agents that could significantly improve TB treatment outcomes and support global efforts in TB control [12].The development is made possible by their structural flexibility in analogues with better pharmacological properties and potential for novel mechanisms of action [21].To control TB, researchers have led to explore new classes of molecules with novel mechanisms of action [25]. 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. Modifications at specific positions of the benzothiazole ring, especially at the C-2 position, have been found to enhance antitubercular activity[26].The introduction of pharmacologically active moieties like azetidinone rings and Schiff bases has shown to improve the efficacy of these compounds[27]. Additionally, increasing hydrophobicity, such as adding methoxy groups have been associated with enhanced activity against Mycobacterium tuberculosis. Studies suggest that benzothiazole derivatives may overcome limitations associated with current therapies, including issues related to resistance and bioavailability [26].

Materials and Methods:

Materials

All 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 FTIR (ATR, Attenuated Total Reflectance) accessory was installed on a Bruker Alpha-II FTIR spectrometer to obtain FTIR spectra. A Bruker NMR spectrometer running at 400 MHz, respectively, was utilized to record both the 1H NMR and 13C NMR spectra with DMSO-d_6 as the solvent. Changes in chemistry are expressed in δ ppm. Mass spectra have been captured using an Agilent 6530 Q-TOF LC/MS instrument. 'Silica gel HF254 plates were utilized in TLC to track the purity and development of the reaction. Under UV light, spots were seen in the mobile phase[25] which was made up of n-hexane & ethyl acetate (5:5).

Methodology (Experimental Work):

Synthetic procedure:

INH derivatives were synthesized following a procedure adapted from established literature[14]. A mixture of 0.01 mol of INH and an equivalent amount of primary amine derivatives was combined in a beaker containing 3 mL of ethyl alcohol. The reaction mixture was stirred for three hours with a magnetic stirrer, and the mixture's progress was monitored using thin-layer chromatography (TLC) Stationary Phase: Pre-coated silica gel GF254, Mobile Phase: N-hexane: ethyl acetate at the ratio 5:5 %v/v.
After completing the reaction mixture and let it cool, the product was separated by filtering[25].

General procedure:

To prepare the target compounds for use, 0.01 mol of INH (Compound A) was reacted with 0.01 mol of carboxylic acids derivatives, including 4-Nitrobenzoic acid, 3-Nitrobenzoic acid, 4-Aminobenzoic acid, 5-Sulfosalicylic acid, Salicylic acid, Cinnamic acid, Phthalic acid, and Gallic acid (shown in Scheme-1). The reactants were placed in a 250 mL beaker, and a few drops of ethyl alcohol and glacial acetic acid were added. The mixture was then heated under reflux for 30 minutes[16].

Purification:

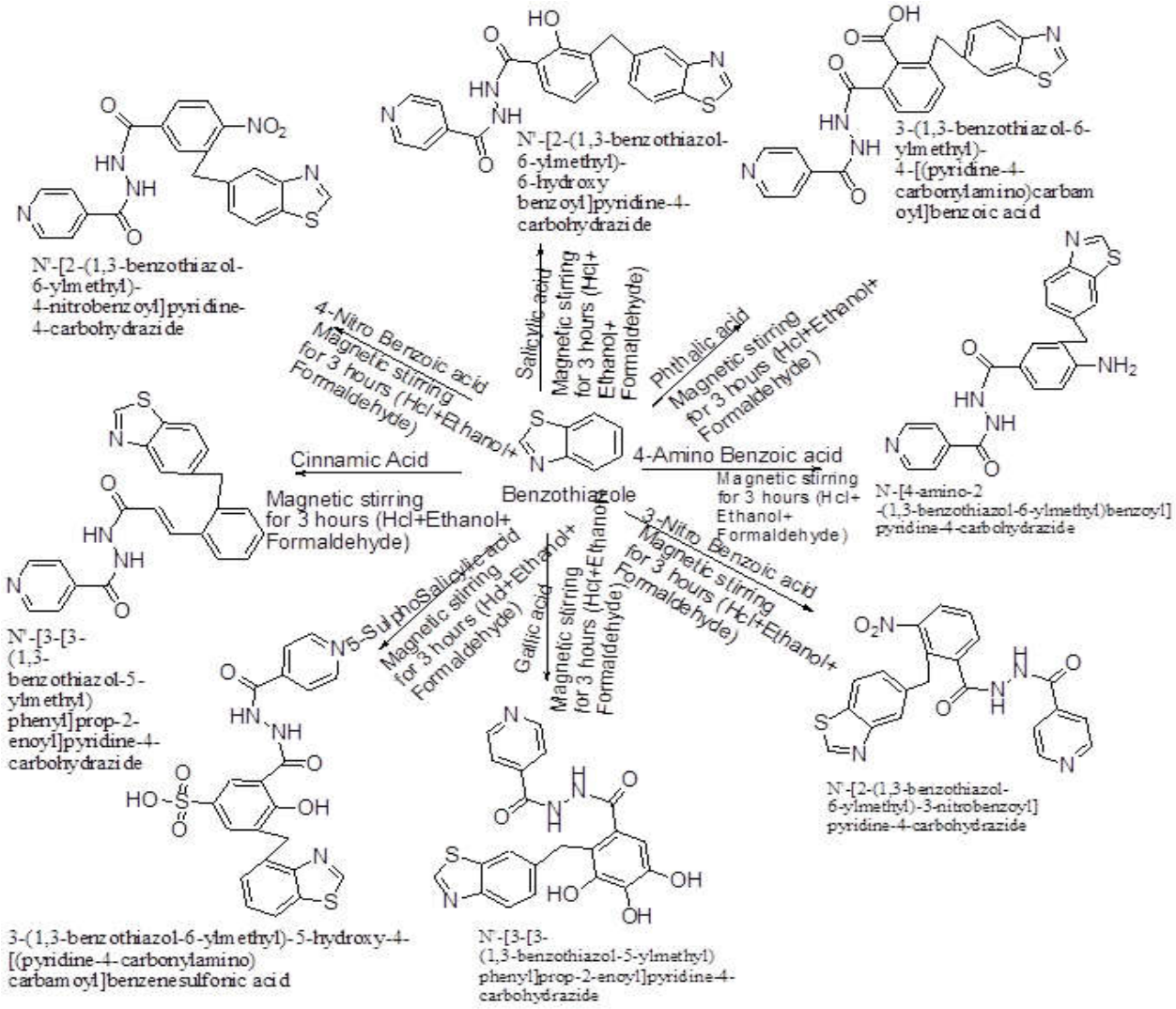
Following the reaction, the resultant crystals were filtered and treated with activated charcoal at 45°C. To achieve further purification, the crystals were recrystallized using 90% ethanol [16].

Purity assessment:

The purity of the final compounds was evaluated by determining their melting points and verifying their homogeneity through TLC analysis [16].

Spectral Characterization:

Eight compounds in all were produced and analyzed by Mass spectrometry [17], FTIR,1H NMR, and 13C NMR.



Scheme-1: Synthesis of Benzothiazole –Isoniazid derivatives

Computational (*Insilico)* Studies:

Identification of Target:

Glutamyl-tRNA synthetase [29] (GluRS) plays a vital 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) (Fig.3), 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 [16].

Molecular Docking (in silico) Studies

The crystal structures of three *Mycobacterium tuberculosis* targets have been retrieved from PDB: glutamyl-t-RNA synthetase (PDB ID: 2JA2, 6R9W and 2BVC[29], with respective resolutions 1.65, 1.75 and 2.10 Å(Shown in Fig.3). 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 files for docking simulations, a 3D lattice grid was developed. PyRx[28], Discovery Studio software[38] and PyMOL[39] were implemented to visualize the interactions.

Virtual screening was conducted using the AutoDock Vina tool integrated within the PyRx platform. The docking grid[28] was precisely aligned to cover the "active site of the target enzyme", amid the centre 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 configuration allowed for a reliable analysis of the binding interactions and fit of the synthesized derivatives within the enzyme's active region.

Invitro(Biological activity)

Anti-Tuberculosis (TB) Activity Assay Using Microplate Alamar Blue Dye Method [17-18]:

1. Preparation of the Plate:

To prevent medium evaporation during incubation, 200 µL of sterile deionized water is added to the outer perimeter wells of a sterile 96-well plate.

2. Addition of Broth and Test Compounds:

Each test well receives 100 µL of Middlebrook 7H9 broth. Serial dilutions of the test compounds are prepared directly in the wells to obtain the desired concentration range.

3. Concentration Range:

The final concentrations of the test compounds should range from 100 µg/mL to 0.2 µg/mL**,** ensuring an effective gradient for MIC determination. (Table.2, Fig. 4 and 5)

4. Incubation:

The plate is covered and sealed with parafilm before being incubated at 37°C for five days**.** This allows bacterial growth and interaction with the test compounds.

5. Addition of Alamar Blue Reagent:

Following incubation, 25 µL of a freshly prepared 1:1 mixture of Alamar Blue reagent and 10% Tween 80 is added to each well

6. Further Incubation:

The dye is permitted to interact with the bacterial culture by incubating the plate for an extra 24 hours.

7. Result Interpretation:
   - Blue colour**:** Indicates the absence of bacterial growth.
   - Pink colour: Suggests bacterial growth is present. (Fig.4 and 5)

8. Determination of MIC:

The lowest concentration of the test chemical that stops apparent bacterial growth—which is shown by the blue color change—is known as the minimum inhibitory concentration or MIC (Table.2).

This approach ensures repeatability and reliability in results by utilizing known methodologies for assessing anti-TB activity using the micro Alamar Blue assay.

Computational Toxicity Prediction

ProTox-3[40] is an advanced *insilico* toxicity prediction tool that estimates acute oral toxicity (LD_{50}), classifies compounds into toxicity categories, and predicts potential organ toxicities 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.

Results and Discussion:

Physico-chemical Properties for the Synthesized Derivatives

The physicochemical profiling of BTZ-INH derivatives (BI-1 to BI-8) revealed overall compliance with Lipinski's Rule of Five [31], indicating good drug-likeness. All compounds had acceptable molecular weights, hydrogen bond donors and acceptors, and logP values below 5. Most derivatives also satisfied Veber's criteria for rotatable bonds and polar surface area, suggesting favourable oral bioavailability [32]. Notably, BI-3, BI-5, and BI-6 showed the most optimal balance of parameters (Table-1).

SPECTRAL CHARACTERIZATION OF SYNTHESIZED DERIVATIVES

N'-[2-(1,3-benzothiazol—yl methyl)-4-nitrobenzoyl]pyridine-4-carbohydrazide (BI-1)

Yield: 90%; Color: Pale-yellow powder; mf: $C_{21}H_{15}N_5O_4S$; R_f:0.92; mp.: 240-242°C"; FT-IR (ATR, ν_{max} cm^{-1}): 1275.11(C-N Str.),1275.77(C-S Str.),1600.51(C=N Str.),1684.37(C=C Str.),1684.37(C=0 Str.),3061.08(O-H Str.),2836.24(C-NH Str.),3115.44(C-NH2Str.); ^{1}HNMR: 9.23 (s, 1H);CH(aromatic)7.35,7.92,8.11,9.01 (m, 4H);(CH_2,Methylene Bridge)3.34 (s, 1H); CH(Aromatic ,4-NBA) 8.09,8.18 (d, J=3.6 Hz, 2H) ;NH(Secondary Amide) 8.314,8.320 (s, 1H); CH(Pyridine) 7.96,9.06 (m, 4H),; ^{13}C NMR: CH(BTZ, Aromatic)120.7,122.3,125.8;CH_2-C 140.3,C=N-151,155.8,C-S-135.6;(CH_2,Methylene Bridge) 38.1;CH(4-NBA,Aromatic)118.37,128.9,140.6,C-N152.3;CONH(4-NBA) 165, CONH(Pyridine) 164.9;CH(Pyridine) 122.8,149.8; MS :Mass (200 eV), m/z (I_{rel}, %): m/z: 433.08 (100.0%), 434.09 (23.0%), 435.08 (5.0%), 435.09 (3.5%), 434.08 (2.6%), 436.08 (1.1%); HRMS ESI-MS (m/z): 434.56 [M+H] +(positive-ion mode), 431.24[M-H] −(negative-ion mode), $C_{21}H_{15}N_5O_4S$, Calculated, m/z:433.08.;Elemental analysis: Found, % C 58.19; H 3.49; N 16.16; O 14.77; S 7.40. $C_{21}H_{15}N_5O_4S$; Calculated, %: C 58.22; H 3.46; N 16.12; O 14.72; S 7.39.

N'-[2-(1,3-benzothiazol-6-ylmethyl)-3-nitrobenzoyl]pyridine-4-carbohydrazide (BI-2)
Yield: 93.2%; Color: Pale-yellow powder; mf: $C_{21}H_{15}N_5O_4S$; R_f: 0.65;mp.: 260-262°C; FT-IR(ATR, $\nu_{max}cm^{-1}$):1276.40(C-N Str.), 1107.42(C-S Str.),1537.05(C=N Str.),1685.34(C=C Str.),1602.05(C=0 Str.),2982.02(O-H Str.),3115.44(C-NH Str.).^{1}H NMR:C=N (BTZ) 9.23(s, 1H);CH (AROMATIC) 7.35,7.92,8.11,9.06 (m,5H);(CH_2,Methylene Bridge)3.81(s, 1H); CH(Aromatic,3-NBA)7.51,8.22,8.31 (d, J=2.8 Hz, J= 3.6 Hz, 2H);NH (Secondary Amide) 8.319,8.359 (s, 1H); CH(Pyridine)7.97,9.26 (m, 3H); ^{13}C NMR: CH (BTZ, Aromatic)120.7,122.3,125.8;CH_2-C 140.3,C=N-151,155.8,C-S-135.6;(CH_2,Methylene Bridge) 38.1;CH (4-NBA,Aromatic) 118.37,128.9,140.6,C-N 152.3; CONH(4-NBA) 165, CONH(Pyridine) 164.9;CH(Pyridine) 122.8,149.8;**MS:** Mass (200 eV), m/z (I_{rel}, %): m/z: 433.08 (100.0%), 434.08 (23.0%), 435.04 (5.0%), 435.04 (3.5%), 434.07 (2.6%), 436.08 (1.1%); HRMS ESI-MS (m/z): 434.92 [M+H] + (positive-ion mode), 430.44[M-H] − (negative-ion mode). $C_{21}H_{15}N_5O_4S$; Calculated, m/z: 433.08.; Elemental analysis: Found, % C 58.19; H 3.49; N 16.16; O 14.77; S 7.40. $C_{21}H_{15}N_5O_4S$; Calculated, %: C 58.19; H 3.47; N 16.15; O 14.76; S 7.38.

Supporting Information

Supporting information, which includes detailed characterization data (FTIR (ATR), 1H, 13C NMR and MS) for synthesized compounds (BI-3-BI-8)

Biological (Invitro) Activity Evaluation
The physicochemical data of the compounds were analyzed, and their in vitro antitubercular activity was evaluated using the MABA method [18], compared against standard drugs like isoniazid, rifampicin, streptomycin, ethambutol, and pyrazinamide [33-37](Fig.4). Most compounds exhibited significant activity at concentrations up to 50 µg/mL, with some showing potent effects at 1.6 µg/mL. Chemical substances BI-2, 4, 5, 6, and 8 showed activity at 100 µg/mL, whereas BI-3 and BI-7 showed equipotent action at 50 µg/mL. In the assay, blue color indicated active (sensitive) chemicals, while pink indicated inert ones. One molecule, BI-1, showed great potency [17] at 1.6 µg/mL [Fig. 5].

Antitubercular activity study

The *invitro* antitubercular activity of several newly synthesized compounds was evaluated using the Microplate Alamar Blue Assay (MABA) method, comparing them against standard drugs such as isoniazid, rifampicin, streptomycin, ethambutol, and pyrazinamide (DrugBank, 2023)[33-37]. Most compounds demonstrated significant activity at concentrations up to 50 µg/mL, with some exhibiting potent effects at 1.6 µg/mL [2]. Compound BI-1 specifically shown considerable potency at 1.6 µg/mL. The MABA methods colorimetric assay revealed pink for inactive compounds and blue for active (sensitive) ones.

Using the (MABA) Microplate Alamar Blue Assay [18], the synthetic Benzothiazole-INH derivatives (BI-1 to BI-8) were evaluated for their vitro antimycobacterial efficacy. The activity was compared with standard antitubercular drugs, including Isoniazid, Rifampicin, Ethambutol, Pyrazinamide, and Streptomycin [33-37]. Most derivatives displayed significant activity, with notable sensitivity observed at varying concentrations. Among the derivatives, BI-1 (4-NBA) exhibited the highest potency, remaining sensitive at concentrations as low as 1.6 µg/mL. BI-2 (3-NBA) showed moderate activity with sensitivity up to 25 µg/mL, while BI-3 (4-ABA) was sensitive up to 50 µg/mL. Compounds BI-4 (5-SSA), BI-5 (SA), BI-6 (CA), and BI-8 (GA) demonstrated limited sensitivity, with activity observed only at the

maximum concentration (100 µg/mL). BI-7 (PA) showed sensitivity at 50 µg/mL but became resistant at lower concentrations.

The produced Benzothiazole-INH compounds (BI-1 to BI-8) (shown in Scheme.1) show great promise as antitubercular drugs, meeting the urgent demand for novel therapies against drug-resistant strains of *Mycobacterium tuberculosis* (Mtb)[17]. BI-1 (4-NBA) was the most efficacious of these drugs, with a minimum inhibitory concentration (MIC) of 1.6 µg/mL, outperforming popular treatments such as isoniazid and rifampicin. This is in line with previous research findings and implies that the presence of nitro groups is crucial in boosting antibacterial action [Table 2]. With MIC values as high as 25 µg/mL and 50 µg/mL, respectively, BI-2 and BI-3 demonstrated moderate activity. However, other compounds, such as BI-4, BI-5, BI-6, and BI-8, needed greater doses to be effective, suggesting that they need to be further refined to increase their potency.

Computational (*Insilico)* Studies

Docking Studies

Molecular docking studies were conducted using AutoDock Vina to explore the binding interactions between the synthesized derivatives and three key protein targets of *Mycobacterium tuberculosis*: 2BVC, 6R9W, and 2JA2 (Fig.3)[29]. The significance of these targets to the pathogen's metabolic functions resulted to their selection.

The crystal structures of three *Mycobacterium tuberculosis* targets (Fig.2) were retrieved from the Protein Data Bank (PDB): glutamine synthetase (PDB ID: 2BVC, resolution 2.10 Å) [29], the InhA enzyme in complex with the AP-124 inhibitor (PDB ID: 6R9W, resolution 1.75 Å), and glutamyl-tRNA synthetase (PDB ID: 2JA2, resolution 1.65 Å [Figure 3]. Docking studies were carried out using AutoDock Vina, which is known for its stability and reproducibility compared to AutoDock vina [28],·ChemSketch and ChemDraw were used to draw the ligand structures, and Chimera software (version 1.17.3) was used to minimize energy [20], Grid parameter (.gpf) [28], docking parameter (.dpf) ligand (.pdbqt), and macromolecule (.pdbqt) were among the created files for docking simulations, a 3D lattice grid was developed and PyRx[28], and Discovery Studio software [38] were implemented to visualize the interactions.

Docking Studies on Antitubercular Compounds: Evaluation of Binding Affinities[28] and Interactions with *Mycobacterium tuberculosis* Targets[20,29]

Compounds over all BI-1, 5, and 6 exhibited notable docking results, with binding energies of -8.9, -8.3 and -10.7 kcal/mol, forming hydrogen bonds with seven amino acids. The results of the docking investigations showed that BI-1, BI-7, and BI-8 had high affinities for the 2BVC target, whereas BI-6, BI-5 and BI-8 had great binding affinities for the 6R9W target Similarly, the 2JA2 target was successfully attacked by BI-5, BI-7, and BI-4 shown in the Table 3.Theseinteractions imply that the compounds are very promising as lead candidates for antitubercular drug development.

Docking Analysis of Synthesized Compounds with Target Proteins

The binding affinities and interaction mechanisms of compounds BI-1, BI-5, BI-6, BI-7, BI-8, and BI-4 against three protein targets—6R9W (InhA enzyme), 2BVC (glutamine synthetase) [20], and 2JA2 (glutamyl-tRNA synthetase) [28]—were examined using molecular docking experiments [Table 3]. With a binding energy of -10.7 kcal/mol, BI-5 demonstrated the highest affinity for 6R9W, followed by BI-1 (-8.9 kcal/mol) and BI-6 (-8.3 kcal/mol), according to the estimated binding energies.BI-6 showed a strong hydrogen bond with important residues in the active site [2] of the 6R9W target (InhA enzyme). Compounds BI-5 and BI-8 exhibited

significant interactions with GLY14, ASP64, GLY96, and GLY204[2].The observed higher binding affinities of such compounds for the InhA enzyme were likely the result of these interactions[4].When it came to glutamyl-tRNA synthetase, or 2JA2, BI-5 showed hydrogen bonding with GLY22. BI-7 has interactions with THR196 and GLY22. BI-4 established hydrogen connections with THR48, CYS12, and SER14[2]. These results suggest that these substances can interact with glutamyl-tRNA synthetases active site in an efficient manner. The hydrogen bond formation and measured binding energies imply that these synthesized compounds may have inhibitory activity against the protein targets under study, which calls for additional research through in vitro and in vivo experiments.

The docking results indicated that the derivatives had favourable binding energies, better those of standard drugs.BI-1 demonstrated a binding energy of -8.9 kcal/mol for the target protein 2BVC, involving hydrogen bonding interactions involving significant amino acids such ASP145 and LYS265 [Figure 6].For 6R9W, BI-6 exhibited the highest binding affinity (-10.7 kcal/mol), forming hydrogen bonds with residues like GLY14 and ASP64 [Figure 7].For 2JA2, BI-5 showed strong binding (-8.3 kcal/mol), interacting with amino acids such as GLY22 and THR48 [Figure 8].Overall, compounds BI-6, BI-5, and BI-8 demonstrated superior binding affinities toward 6R9W, while BI-1, BI-7, and BI-8 showed higher affinities for 2BVC. Compounds BI-5, BI-7, and BI-4 exhibited strong interactions with 2JA2 shown in the [Table 3]. The molecular docking results support the theory that these derivatives possess significant antitubercular potential due to their strong protein-ligand interactions. According to the results, these substances—in particular, BI-1, BI-6, and BI-5—have encouraging potential as antitubercular agents and should be further optimized and subjected to preclinical research to assess their therapeutic efficiency against strains[17] of *Mycobacterium tuberculosis* (Mtb) that are resistant to drugs.

Molecular docking studies, conducted using AutoDock Vina, revealed favourable interactions between these derivatives and key protein targets involved in essential metabolic pathways of Mtb. These targets include Glutamine synthetase (2BVC), Enoyl-ACP Reductase InhA (6R9W), and Glutamyl-tRNA synthetase (2JA2). Notably [23] BI-6 displayed the strongest binding affinity (-10.7 kcal/mol) to InhA, an enzyme critical for fatty acid biosynthesis in Mtb, indicating its potential to disrupt this vital metabolic pathway.The survival and replication of the bacterium depend on the strong connections that BI-1 and BI-5 showed with 2BVC and 2JA2, respectively. The analysis of structure-activity relationships (SAR) indicates that electron-withdrawing groups, particularly nitro substituent's, greatly increase the antibacterial efficacy of these compounds.These findings align with prior SAR studies, which suggest that the addition of such functional groups and aromatic rings improves both drug effectiveness and binding capabilities [22]. Compared to existing antitubercular drugs (Table 2 and Table 3), the prepared derivatives demonstrated superior binding affinities, underscoring their potential to overcome the limitations of current treatments. This suggests a promising avenue for developing effective therapies against drug-resistant Mtb strains. Nonetheless, further investigation is required to validate the clinical potential of these derivatives, including optimization of their structures, pharmacokinetic profiling, and in vivo evaluations [24].The results of this investigation indicate BI-1, BI-7, BI-6, BI-5, BI-4 and BI-8 shown strong binding affinities towards crucial protein targets of Mycobacterium TB, according to the molecular docking analysis carried out with this investigation. These substances showed promising promise as powerful inhibitors by interacting strongly with important amino acid residues. According to these results, BI-6, BI-5, and BI-8 show promise as lead compounds for the creation of new anti-tubercular drugs.BI-5 and the insight obtained from this investigation provide a solid foundation for the structure-based design and

optimization of new therapeutic agents that target TB, as well as intriguing opportunities for the development of new antitubercular drugs.

Insilico Assessment for Synthesized derivatives by PROTOX-3 Tool [40]

In silico toxicity profiling of eight synthesized BTZ-INH derivatives (BI-1 to BI-8) was conducted using the ProTox-3 platform (Table 4 and Fig 9). All compounds were classified under toxicity class 4, indicating moderate oral toxicity with predicted LD_{50} values between 850 and 1500 mg/kg. Hepatotoxicity and respiratory risks were consistently predicted across the series, suggesting potential concerns related to liver and pulmonary effects. Carcinogenic potential was evident in most derivatives, excluding BI-4, BI-7, and BI-8, while mutagenicity appeared limited to BI-1, BI-2, and BI-6.

Compounds BI-3 and BI-6 demonstrated elevated neurotoxicity risks, whereas BI-5 and BI-8 showed comparatively safer profiles with minimal mutagenic and carcinogenic liabilities. Blood Brain Barrier (BBB) permeability was predicted in several derivatives, indicating possible CNS involvement. Except for BI-1 and BI-2, clinical toxicity was anticipated in the remaining compounds. These computational insights serve as a useful tool for identifying promising candidates for subsequent experimental toxicological validation (Table.4 and Fig.9).

Conclusion:

Molecular docking studies with this work revealed that the drugs BI-6, BI-5, and BI-8 had strong binding affinities towards key Mycobacterium TB protein targets. BTZ-INH derivatives demonstrated favourable drug-like properties, with BI-3, BI-5, and BI-6 exhibiting the most optimal profiles for oral bioavailability. Strong interactions between these substances and essential amino acid residues indicated that they might be able to successfully inhibit the targeted proteins.The results indicate that BI-6, BI-5, and BI-8 hold promise as lead compounds for the development of new anti-tubercular agents. The findings from this molecular docking investigation offer a solid basis for the structure-based design and further optimization of novel therapeutic agents for tuberculosis, particularly BI-5, BI-6, and BI-8, showed strong binding affinities toward key *M. tuberculosis* targets, with BI-6 achieving the highest score (−10.7 kcal/mol) against InhA, highlighting their potential as promising multi-target antitubercular agents. The in silico findings indicate that BTZ-INH derivatives possess moderate oral toxicity with distinct organ-specific risks, highlighting BI-5 and BI-8 as promising leads for further preclinical evaluation.

Declaration of Interest

"The authors declare no conflicts of interest for publishing this paper."

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