Design, Synthesis, Characterization, in-silico and in-vitro Evaluation of Novel 2-mercapto Benzimidazole Derivatives Tethered With Isoniazid for the Treatment of Tuberculosis

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Research Article

Keywords: 2-Mercapto benzimidazole, Isoniazid, Anti-tubercular activity, Molecular docking, Synthesis, Characterization, Mycobacterium tuberculosis

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DESIGN, SYNTHESIS, CHARACTERIZATION, *IN-SILICO* AND *IN-VITRO* EVALUATION OF NOVEL 2-MERCAPTO BENZIMIDAZOLE DERIVATIVES TETHERED WITH ISONIAZID FOR THE TREATMENT OF TUBERCULOSIS

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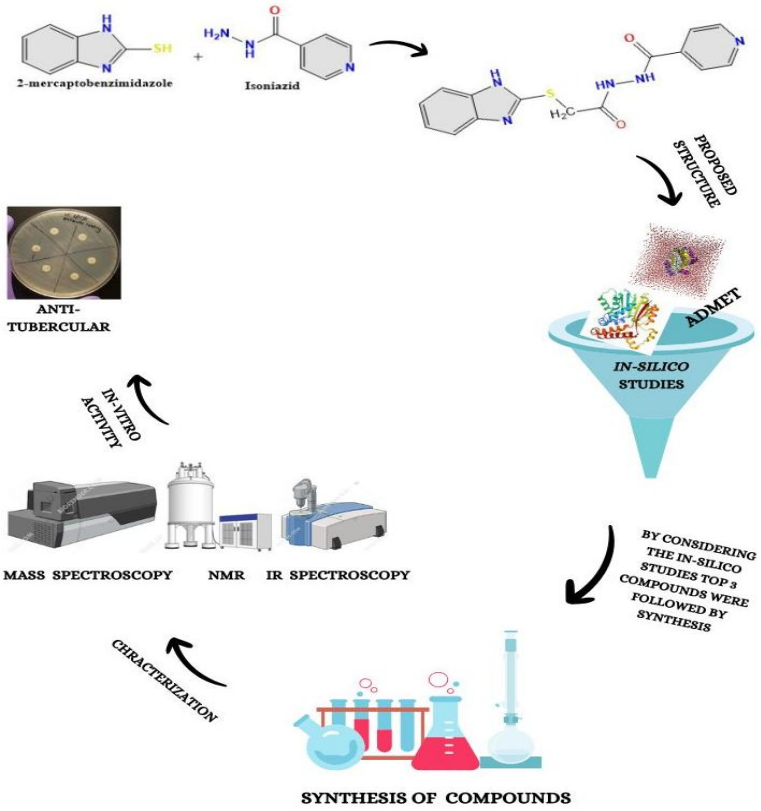
Graphical Abstract



Abstract

2-Mercapto benzimidazole derivatives are well-recognized for their diverse pharmacological activities, including anti-tubercular, antimicrobial, anti-ulcer, analgesic, and anticancer properties. Isoniazid, a first-line antitubercular agent, has been extensively used in the treatment of *Mycobacterium tuberculosis* infections. Based on these insights, the present study aimed to design and synthesize novel 2-mercapto benzimidazole derivatives tethered with isoniazid to explore their potential as anti-tubercular agents.An in-silico approach was employed to evaluate the binding efficiency of the designed molecules with key mycobacterial targets. The top-scoring compounds from molecular docking studies were selected for synthesis. Three derivatives were successfully synthesized via condensation and acetylation reactions. Structural confirmation of the synthesized compounds was carried out using FT-IR, ^1H-NMR, and mass spectrometry. The anti-tubercular activity of the synthesized derivatives was assessed using the agar well diffusion method against *Mycobacterium tuberculosis*. The results demonstrated that the synthesized compounds exhibited noteworthy anti-tubercular activity, comparable to or exceeding that of isoniazid, indicating their potential as promising lead molecules for further development.

Keywords: 2-Mercapto benzimidazole, Isoniazid, Anti-tubercular activity, Molecular docking, Synthesis, Characterization, *Mycobacterium tuberculosis*

Introduction

According to the *Global Tuberculosis Report 2021*, approximately 10 million individuals were diagnosed with tuberculosis (TB) in 2020, with an estimated 1.5 million fatalities, underscoring TB as one of the top ten causes of mortality worldwide and the leading cause of death from a single infectious agent (1). The COVID-19 pandemic further disrupted TB control efforts, resulting in reduced access to diagnosis and treatment, which is projected to cause an additional 1.4 million TB-related deaths between 2020 and 2025 (1).

Origin and Pathogenesis of Tuberculosis

Tuberculosis is caused by *Mycobacterium tuberculosis*, a slow-growing, aerobic, acid-fast bacillus primarily transmitted via airborne droplets. Upon inhalation, the bacilli reach the lungs, where they are phagocytosed by alveolar macrophages. If not effectively neutralized by the host immune system, the bacteria replicate and form granulomatous lesions, known as tubercles. These can disseminate via the bloodstream, affecting other organs such as the kidneys, lymph nodes, and bones (2). A majority of infected individuals harbor latent TB, which can reactivate under immunocompromised conditions such as HIV infection, malnutrition, or immunosuppressive therapy.

Etiology and Molecular Pathogenesis

Understanding the etiology and molecular pathogenesis of TB is critical for developing effective interventions. *M. tuberculosis* evades host immunity by inhibiting phagosome-lysosome fusion, altering cytokine signaling, and persisting within macrophages. Genomic studies have facilitated the identification of virulence factors and mutations linked to drug resistance, contributing to advancements in diagnostics and therapeutic strategies (3).

Clinical Manifestations

Clinical symptoms of TB depend on the site and stage of infection. Pulmonary TB, the most prevalent form, typically presents with:

- Chronic cough (lasting ≥3 weeks)
- Hemoptysis
- Chest pain
- Fatigue and malaise
- Anorexia and weight loss
- Night sweats and fever
- Dyspnea
- Lymphadenopathy (in extrapulmonary cases)

These symptoms often overlap with other respiratory illnesses, posing diagnostic challenges.

Diagnostic Methods

Accurate and timely diagnosis is pivotal for TB management. Traditional methods such as sputum smear microscopy and culture are limited by low sensitivity and long processing time. Molecular diagnostic tools like the GeneXpert MTB/RIF assay offer rapid and sensitive detection, including rifampicin resistance. Additional techniques such as loop-mediated isothermal amplification (LAMP), chest radiography, and computed tomography (CT) further enhance diagnostic accuracy (4).

Treatment Strategies

First-line anti-TB therapy for drug-susceptible TB includes a four-drug regimen administered over six months:

- **Isoniazid (INH):** Inhibits mycolic acid synthesis, essential for bacterial cell wall integrity.
- **Rifampin (RIF):** Inhibits DNA-dependent RNA polymerase, preventing bacterial transcription.
- **Ethambutol (EMB):** Disrupts arabinogalactan synthesis, impairing cell wall construction.
- **Pyrazinamide (PZA):** Effective against semi-dormant bacilli in acidic environments.

The intensive phase includes all four drugs for two months, followed by a continuation phase with INH and RIF for four months (5).

Adverse Effects

Anti-TB drugs are associated with several adverse effects that may compromise treatment adherence:

- **Hepatotoxicity** (most common with INH, RIF, PZA)
- **Peripheral neuropathy** (linked to INH)
- **Gastrointestinal discomfort**
- **Hypersensitivity reactions**

These side effects necessitate close clinical monitoring and appropriate management strategies (6).

Drug Resistance and Emerging Therapies

The emergence of drug-resistant TB, particularly multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains, poses a significant challenge. MDR-TB is resistant to at least INH and RIF, while XDR-TB also resists fluoroquinolones and second-line injectable drugs. These strains require prolonged treatment with more toxic and expensive second-line agents.

Recent studies have focused on identifying resistance-associated gene mutations (e.g., *katG*, *rpoB*, *inhA*) and developing novel therapies, including host-directed therapy (HDT), immunomodulators, and new drugs such as bedaquiline and delamanid. These advancements aim to shorten treatment duration and improve patient outcomes (7, 8).

2-Mercaptobenzimidazole: Structural and Pharmacological Significance

2-Mercaptobenzimidazole is a bicyclic heterocyclic compound comprising a fused benzene and imidazole ring system, in which a thiol (-SH) functional group is substituted at the second position of the imidazole moiety (Figure 1). This scaffold possesses significant pharmacophoric features that contribute to a wide range of biological activities. Derivatives of 2-mercaptobenzimidazole have demonstrated diverse therapeutic properties, including anti-tubercular, anti-ulcer, anticancer, antibacterial, antifungal, and anti-inflammatory effects (9–15).

Given its broad-spectrum pharmacological profile, 2-mercaptobenzimidazole represents a privileged structure in medicinal chemistry. Consequently, extensive research has been dedicated to synthesizing and evaluating its derivatives for potential therapeutic applications. The benzimidazole nucleus, in particular, plays a pivotal role in the design and development of novel bioactive compounds due to its structural versatility and biological relevance.

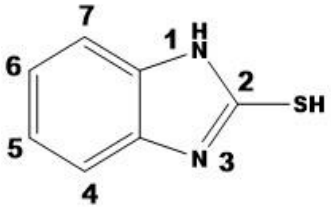


Figure 1. Chemical structure of 2-mercaptobenzimidazole.

Isoniazid: Pharmacology and Mechanism of Action

Isoniazid (INH), chemically known as pyridine-4-carbohydrazide, is a hydrazine-containing heterocyclic compound derived from nicotinic acid (Figure 2). It is widely recognized as a first-line antimycobacterial agent used in the treatment and prophylaxis of tuberculosis (TB). Although the precise mechanism of action is not completely understood, isoniazid is known to inhibit InhA, an NADH-dependent enoyl-ACP reductase involved in the biosynthesis of mycolic acids—essential components of the mycobacterial cell wall [16–17].

Resistance to isoniazid arises primarily due to reduced cell wall permeability or mutations in the *katG* and *inhA* genes, which encode catalase-peroxidase and enoyl reductase, respectively. Beyond its anti-TB activity, isoniazid has also demonstrated potential efficacy in cancer and bacterial infections, expanding its relevance in therapeutic research.\g

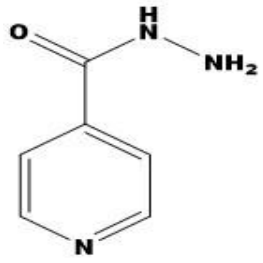


Figure 2. Chemical structure of isoniazid.

In Silico Studies in Drug Discovery

With the advent of computational technologies, in silico approaches have become integral to modern drug discovery, enabling efficient identification, design, and optimization of lead molecules. These techniques streamline the early stages of drug development, including virtual screening, molecular docking, pharmacokinetic profiling, and toxicity prediction.

In the present study, a structure-based drug design approach was employed to develop novel 2-mercaptobenzimidazole derivatives conjugated with isoniazid. Computational tools such as AutoDock Vina, PyRx, and PyMOL were utilized to predict binding affinities and interactions with target proteins. The most promising compounds, based on docking scores and pharmacokinetic properties, were selected for synthesis and further evaluation. These in silico methodologies significantly accelerate the drug development process while reducing experimental costs and enhancing hit-to-lead optimization. (18)

Materials and Methods

Molecular Docking

Molecular docking is a computational method used to predict the binding orientation and affinity between a small molecule (ligand) and a macromolecular target, typically a protein. It is widely employed in rational drug design to identify potential drug candidates based on their binding interactions with specific biological targets (19).

Protein structures were retrieved in .pdb format from the Protein Data Bank (PDB). Protein preparation was carried out using Discovery Studio 2020 by removing water molecules, heteroatoms, and adding polar hydrogens. Residue alignment and correction were verified using Swiss PDB Viewer.

Ligand structures were designed using ChemDraw and converted to three-dimensional conformations using Chem3D, then saved in .sdf format. Molecular docking was performed using AutoDock Vina through PyRx software, and docking results were visualized using PyMOL and Discovery Studio.

Molecular Dynamics Simulation

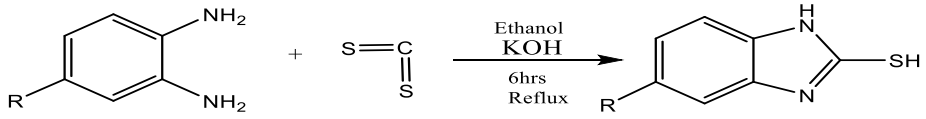
To assess the stability and dynamic behavior of the ligand–protein complexes, molecular dynamics (MD) simulations were conducted using the Desmond module of the Schrödinger suite. The docked complexes were solvated using an explicit water model and neutralized with counterions. MD simulations were performed for 100 nanoseconds under standard NPT ensemble conditions.

ADMET Analysis

Pharmacokinetic and toxicity profiles, including absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters, were predicted using **SwissADME** and **pkCSM** web servers. These in silico tools help assess the drug-likeness and safety of the designed compounds, facilitating the selection of optimal leads for synthesis and biological evaluation (20).

Synthetic Scheme

Step 1

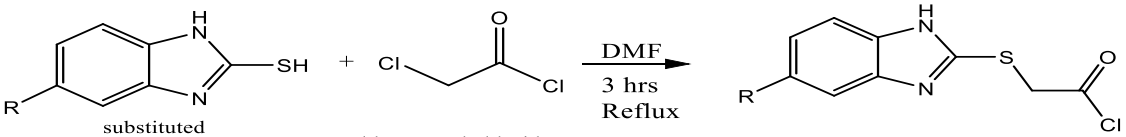


substitited o-phenylene diamine

carbon disulphide

Intermediate 1

Step 2

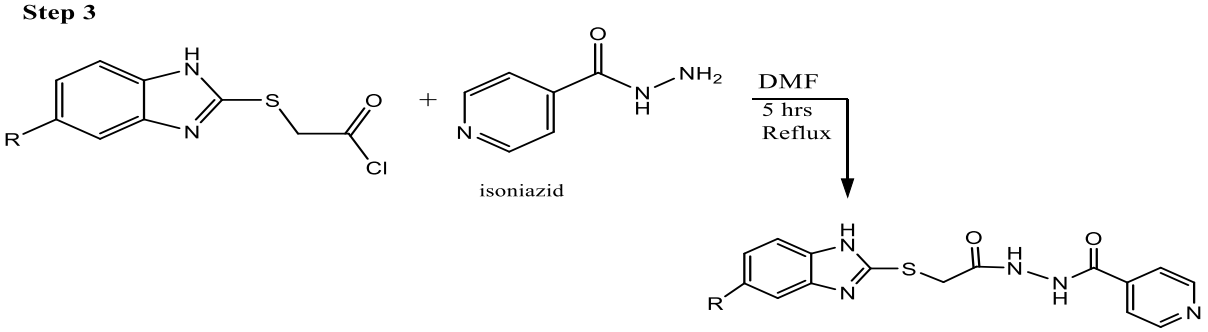


substituted 2-mercaptobenzimidazole

chloroacetyl chloride

Intermediate 2

Step 3



isoniazid

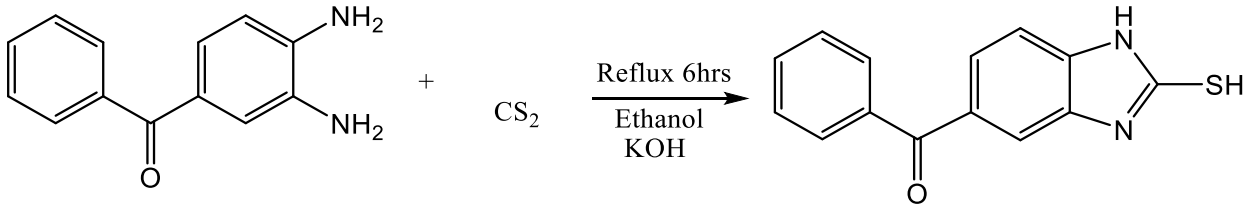
2-mercaptobenzimidazole derivative tethered with isoniazid

Chemical and reagents

All the chemicals and reagents utilized were AR and LR grade procured from Central drug house (CDH) Pvt. Ltd., HIMEDIA Laboratories Pvt. Ltd., RANKEM, Merck.

Procedure for synthesis of (2-mercapto-1H-benzo[d]imidazol-5-yl)(phenyl)methanone [BVSNRJ 3A]

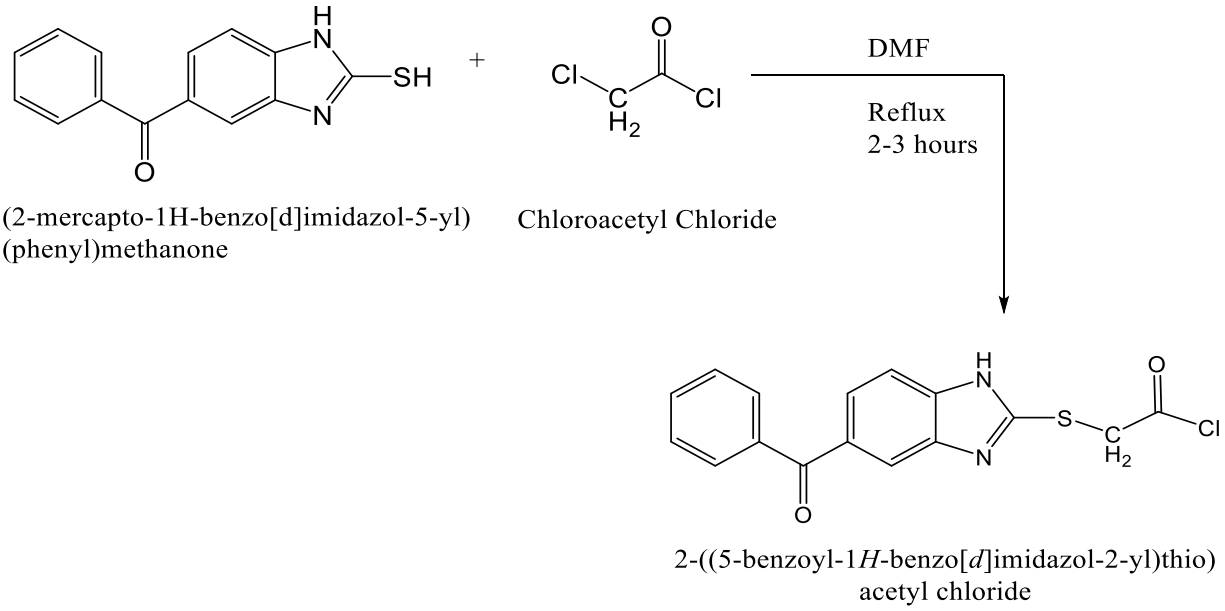
1.9 g; 0.03 mol of Potassium hydroxide was dissolved in a mixture of ethanol (30ml) and water (20 mL) water. Carbon disulfide (2.69 g; 0.03 mol) is then added with stirring, and the mixture was allowed to boil at 80 °C. 3,4-Diamino benzophenone (6.35 g; 0.03 mol) was dissolved in 20 mL ethanol at room temperature and added dropwise into the KOH/CS_2 mixture, which proceeded to reflux for 6 hours at 75-85 °C. After the reaction was completed (as evaluated by TLC), the ethanol was evaporated and the white residue was diluted in water and precipitated with 50% dilute acetic acid. The product was then recrystallized using a water-ethanol (1:1) mixture to get (2-mercapto-1H-benzo[d]imidazol-5-yl)(phenyl)methanone. (21)



3,4-Diamino benzophenone Carbon disulphide (2-mercapto-1*H*-benzo[*d*]imidazol-5-yl) (phenyl)methanone

Procedure for synthesis of 2-((5-benzoyl-1H-benzo[d]imidazol-2-yl) thio)acetyl chloride [BVSNRJ 3B]

2-mercapto-1H-benzo[d]imidazol-5-yl)(phenyl)methanone (2.54g;0.01mol) is dissolved in Dimethyl formamide and then chloroacetyl chloride (1.12 ml; 0.01 mol) is added into the mixture and allow to reflux on the water bath for 2-3 hours.The reaction mixture after confirming with the TLC was poured into crushed ice, the precipitate of compound 2-((5-benzoyl-1H-benzo[d]imidazol-2-yl)thio)acetyl chloride was collected by filtration and dried compound was recrystallized from glacial acetic acid. (22)



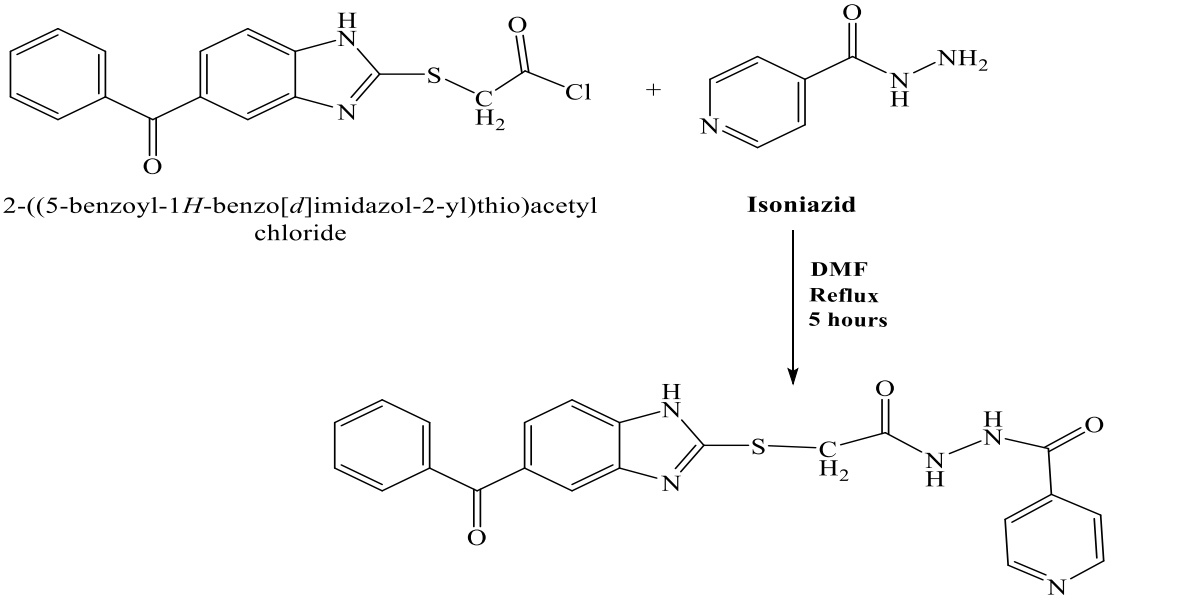
(2-mercapto-1H-benzo[d]imidazol-5-yl) (phenyl)methanone

Chloroacetyl Chloride

2-((5-benzoyl-1*H*-benzo[*d*]imidazol-2-yl)thio) acetyl chloride

Procedure for synthesis of N'-(2-((5-benzoyl-1H-benzo[d]imidazol-2-yl) thio) acetyl) isonicotinohydrazide [BVSNRJ 3C]

3.3g; 0.1mol of 2-((5-benzoyl-1H-benzo[d]imidazol-2-yl)thio)acetyl chloride and Isoniazid (1.37g; 0.1mol) are dissolved in DMF and allow to reflux for 5 hours, then pour the mixture into crushed ice, precipitate of compound N'-(2-((5-benzoyl-1H-benzo[d]imidazol-2-yl) thio) acetyl) isonicotinohydrazide was collected by filtration and dried compound was recrystallized by using ethanol.



2-((5-benzoyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride

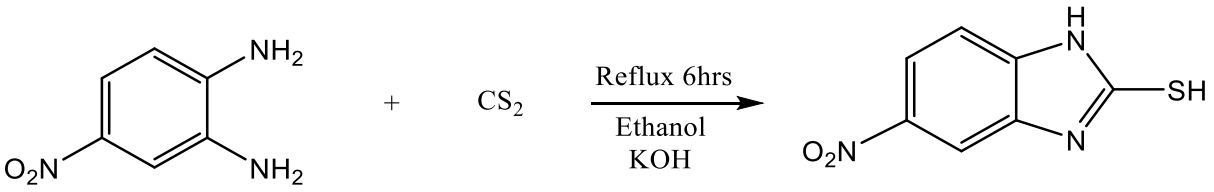
Isoniazid

N'-(2-((5-benzoyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl)isonicotinohydrazide

Chemical Formula: $C_{22}H_{17}N_5O_3S$

Procedure for synthesis of 5-nitro-1*H*-benzo[*d*]imidazole-2-thiol [BVSNRJ 8A]

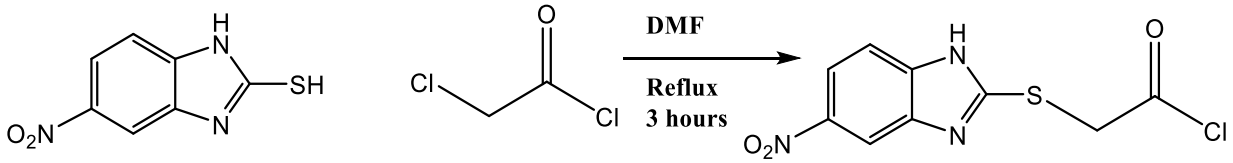
1.9 g; 0.03 mol of Potassium hydroxide was dissolved in mixture of ethanol (30ml) and water (20 mL) water. Carbon disulfide (2.69 g; 0.03 mol) is then added with stirring, and the mixture was allowed to boil at 80 °C. 3,4-Diamino nitrobenzene (4.60 g; 0.03 mol) was dissolved in 20 mL ethanol at room temperature and added drop wise into the KOH/CS_2 mixture, which proceeded to reflux for 6 hours at 75-85 °C. After the reaction was completed (as evaluated by TLC), the ethanol was evaported and the white residue was diluted in water and precipitated with 50% dilute acetic acid. The product was then recrystallized using a water-ethanol (1:1) mixture to get 5-nitro-1*H*-benzo[*d*]imidazole-2-thiol.



3,4-Diamino nitrobenzene | Carbon disulphide | 5-nitro-1*H*-benzo[*d*]imidazole-2-thiol
Molecular Weight: 195.20

Procedure for synthesis of 2-((5-nitro-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride [BVSNRJ 8B]

5-nitro-1*H*-benzo[*d*]imidazole-2-thiol (1.95g; 0.01mol) is dissolve in Dimethyl formamide and then chloroacetyl chloride (1.12 ml; 0.01 mol) is added into the mixture and allow to reflux on water bath for 3 hours. The reaction mixture was poured into crushed ice, the precipitate of compound 2-((5-nitro-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride was collected by filtration and dried compound was recrystallized from glacial acetic acid.

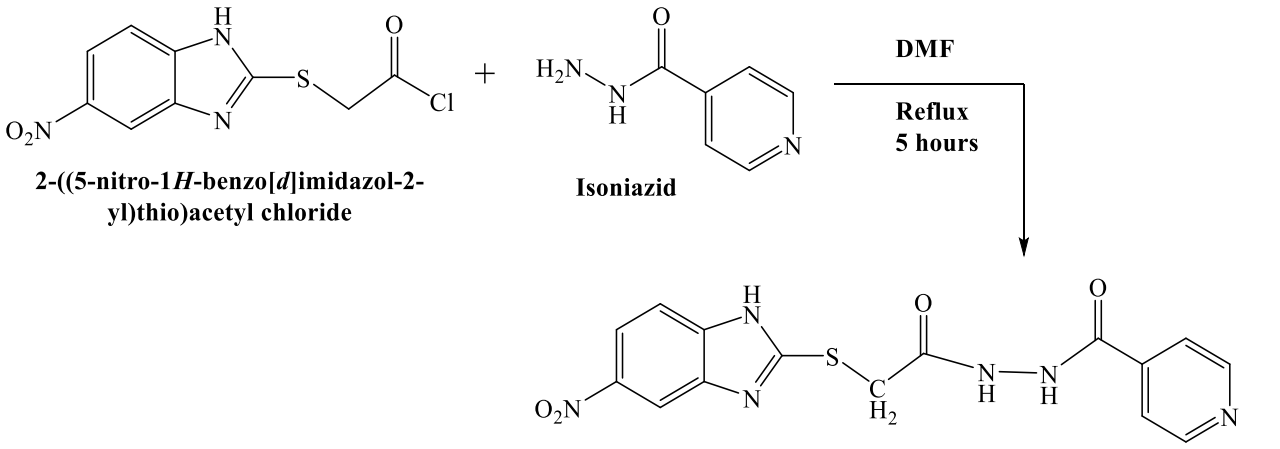


5-nitro-1*H*-benzo[*d*]imidazole-2-thiol | **Chloroacetyl Chloride** | **2-((5-nitro-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride**

Procedure for synthesis of N'-(2-((5-nitro-1H-benzo[d]imidazol-2-yl)thio)acetyl) isonicotinohydrazide [BVSNRJ 8C]

2.7g; 0.1mol of 2-((5-nitro-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride and Isoniazid 1.37g; 0.1 mol are dissolved in DMF and allow to reflux for 5 hours, then pour the mixture into crushed ice, precipitate of compound N'-(2-((5-nitro-1H-benzo[d]imidazol- 2yl)thio)acetyl)isonicotinohydrazide was collected by

filtration Dried compound was recrystallized by using ethanol.



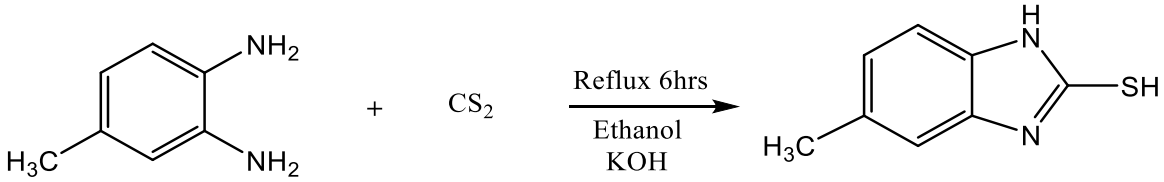
2-((5-nitro-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride

Isoniazid

N'-(2-((5-nitro-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl)isonicotinohydrazide

Procedure for synthesis of 5-methyl-1*H*-benzo[*d*]imidazole-2-thiol [BVSNRJ 9A]

1.9 g; 0.03 mol of Potassium hydroxide was dissolved in mixture of ethanol (30ml) and water (20 mL) water. Carbon disulfide (2.69 g; 0.03 mol) is then added with stirring, and the mixture was allowed to boil at 80 °C. 3,4-Diamino toluene (3.66 g; 0.03 mol) was dissolved in 20 mL ethanol at room temperature and added drop wise into the KOH/CS_2 mixture, which proceeded to reflux for 6 hours at 75-85 °C. After the reaction was completed (as evaluated by TLC), the ethanol was evaporated and the white residue was diluted in water and precipitated with 50% dilute acetic acid. The product was then recrystallized using a water-ethanol (1:1) mixture to get 5-methyl-1*H*-benzo[*d*]imidazole-2-thiol.



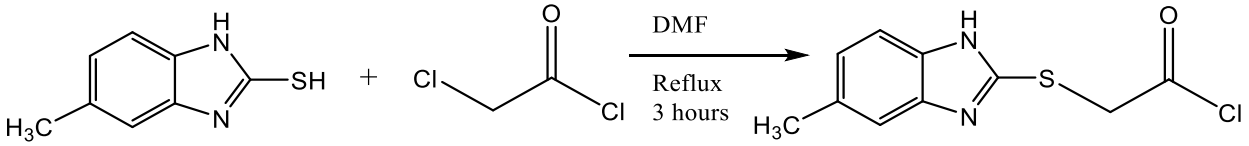
3,4-Diamino Toluene

Carbon disulphide

5-methyl-1*H*-benzo[*d*]imidazole-2-thiol
Chemical Formula: $C_8H_8N_2S$

Procedure for synthesis of 2-((5-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride [BVSNRJ 9B]

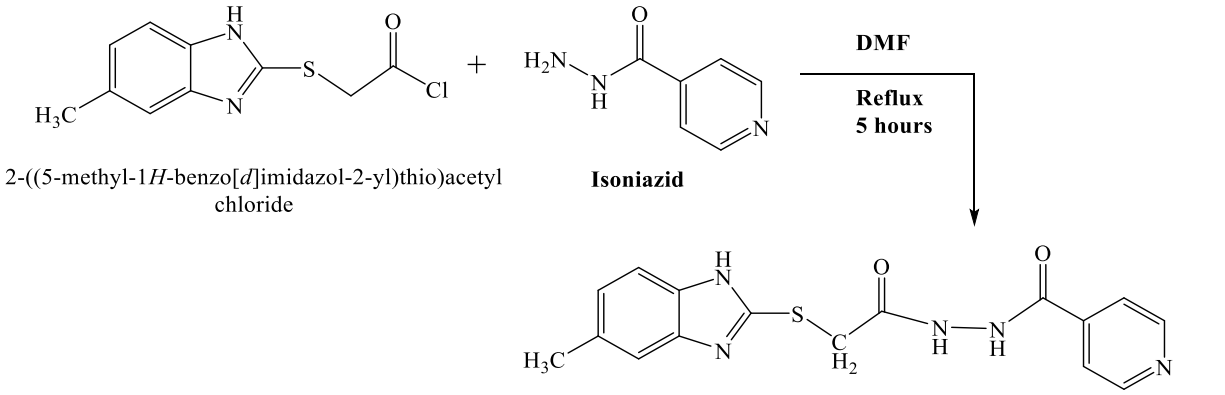
5-methyl-1*H*-benzo[*d*]imidazole-2-thiol (1.64g; 0.01mol) is dissolve in Dimethyl formamide and then chloroacetyl chloride (1.12 ml; 0.01 mol) is added into the mixture and allow to reflux on water bath for 3 hours.The reaction mixture was poured into crushed ice to get the precipitate of compound 2-((5-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride which was collected by filtration dried compound was recrystallized from glacial acetic acid.



5-methyl-1*H*-benzo[*d*]imidazole-2-thiol | **Chloroacetyl chloride** | **2-((5-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride**

Procedure for synthesis of N'-(2-((5-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl) isonicotinohydrazide [BVSNRJ 9C]

2.4g; 0.1mol of 2-((5-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride and Isoniazid 1.37g; 0.1mol are dissolved in DMF and allow to reflux for 5 hours, then pour the mixture into crushed ice, to get the precipitate of compound N'-(2-((5-methyl-1*H*-benzo[*d*]imidazole-2yl)thio)acetyl)isonicotinohydrazide dried compound was recrystallized by using ethanol.



2-((5-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride | **Isoniazid**

N'-(2-((5-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl)isonicotinohydrazide

In Vitro Anti-Tubercular Activity

Agar Well Diffusion Assay

The anti-tubercular activity of the synthesized 2-mercaptobenzimidazole–isoniazid derivatives was evaluated using the agar well diffusion method. Nutrient agar plates were prepared by pouring 20 mL of sterilized medium into sterile Petri dishes under aseptic conditions and allowed to solidify. A standardized inoculum (100 µL) of *Mycobacterium tuberculosis* H37Rv (ATCC 27294) was evenly spread across the surface using sterile cotton swabs.

Wells of 6 mm diameter were aseptically bored into the agar using a sterile cork borer. Isoniazid served as the positive control, while DMSO was used as the negative control. The plates were initially incubated at 4 °C for 4 hours to allow diffusion of the test compounds, followed by incubation at 37 °C for 48 hours. The diameter of the inhibition zones around each well was measured in millimeters, and the mean values were calculated from triplicate determinations to assess anti-TB activity [23].

Minimum Inhibitory Concentration (MIC) Assay

MIC values were determined according to the guidelines of the Clinical Laboratory Standards Institute (CLSI). Colonies of *M. tuberculosis* H37Rv exhibiting uniform morphology were collected from agar culture using a sterile loop and suspended in 10 mL of sterile Mueller–Hinton broth (MHB). The bacterial suspension was incubated at 37 °C until it reached the turbidity equivalent to 0.5 McFarland standard (absorbance 0.08–0.10 at 625 nm), representing approximately 1–2 × 10^8 CFU/mL.

Serial dilutions of the test compounds (25–100 µg/mL) were prepared, and 100 µL aliquots were added to each test well. The MIC was determined as the lowest concentration that inhibited visible bacterial growth, monitored spectrophotometrically at 625 nm.

RESULT AND DISCUSSION

Designing of molecules computationally

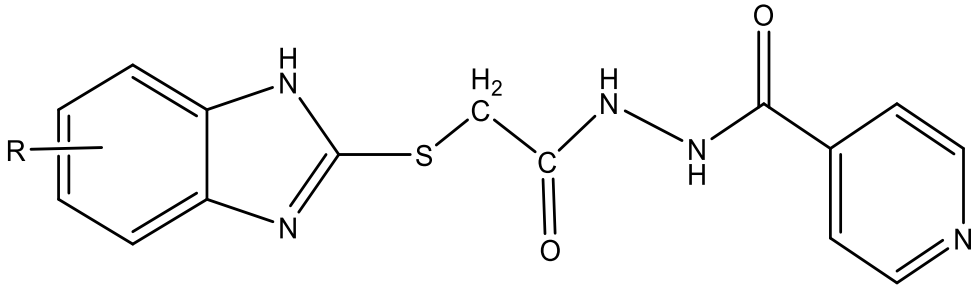


Figure 3. General structure of designed Compound

Table 1. Designed derivatives of 2-mercaptobenzimidazole derivatives

Proposed Compounds	Substitutions (R)
BVSNRJ 1C	5-H
BVSNRJ 2C	5-OCH_3
BVSNRJ 3C	5-C_6H_5CO
BVSNRJ 4C	4-Cl
BVSNRJ 5C	5,6-Dichloro
BVSNRJ 6C	5-COOH
BVSNRJ 7C	5-F
BVSNRJ 8C	5-NO_2
BVSNRJ 9C	5-CH_3

Molecular Docking:

Table 2. Molecular Docking results of Designed Compounds

Sr. No	Compound	Anti-Tubercular Activity 1KPI (InhA)

1	BVSNRJ 1C	-9.8
2	BVSNRJ 2C	-8.9
3	BVSNRJ 3C	**-11.5**
4	BVSNRJ 4C	-10.2
5	BVSNRJ 5C	-9.9
6	BVSNRJ 6C	-9.3
7	BVSNRJ 7C	-8.9
8	BVSNRJ 8C	**-10.5**
9	BVSNRJ 9C	**-10.4**
10	Isoniazid	-8.5

Molecular docking of the three synthesized compounds was performed against the InhA enzyme (PDB ID: 1KPI). Binding affinity scores ranged from –8.5 to –11.5 kcal/mol, indicating strong ligand–target interactions. All synthesized compounds exhibited better binding affinities compared to the standard drug isoniazid. Notably, compound BVSNRJ3C demonstrated the highest docking score of –11.5 kcal/mol, suggesting superior potential as an anti-tubercular agent.

Table 3. 2D interactions of compounds with 1KPI (InhA)

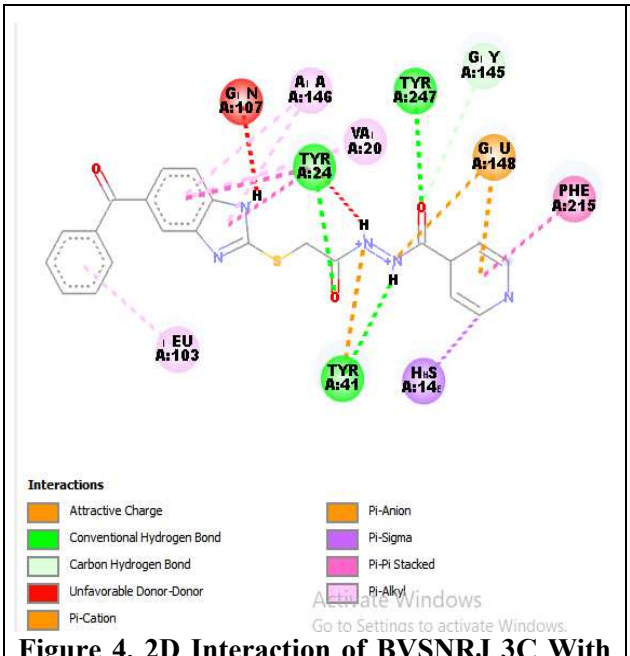


Figure 4. 2D Interaction of BVSNRJ 3C With 1KPI (InhA)

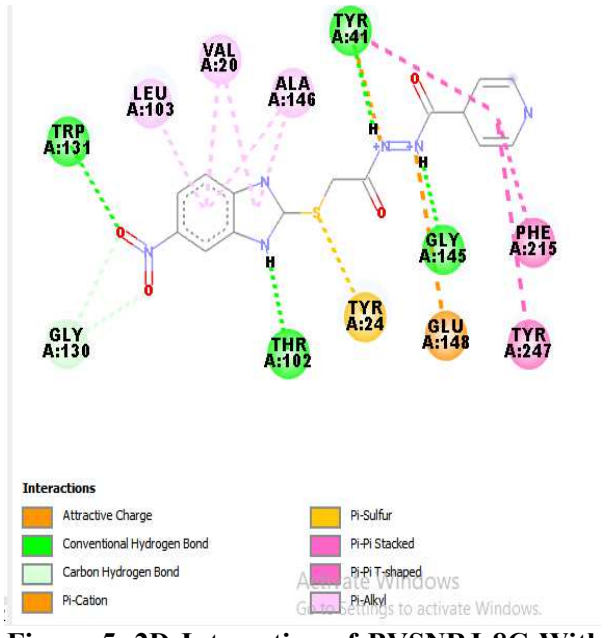


Figure 5. 2D Interaction of BVSNRJ 8C With 1KPI (InhA)

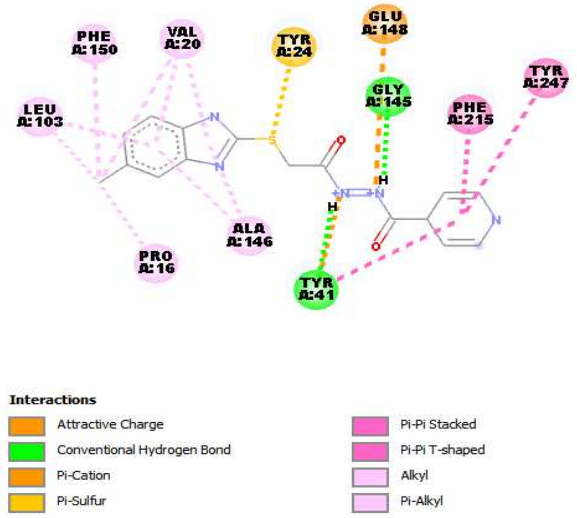


Figure 6. 2D Interaction of BVSNRJ 9C With 1KPI (InhA)

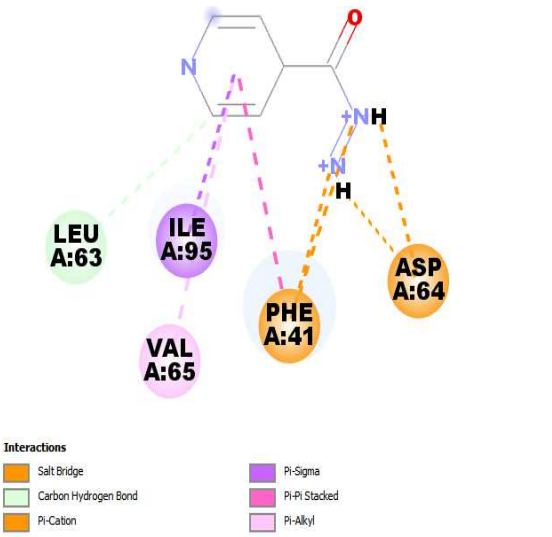


Figure 7. 2D Interaction of Isoniazid With 1KPI (InhA)

Table 4. Binding interactions of compounds with 1KPI (InhA)

Sr no.	Compounds	Type of Interaction	Interacting Amino acid
1	BVSNRJ 3C	Conventional H bond	TYR A:24, TYR A:41, TYR A:247
		Pi bonds	LEU A:103, VAL A:20, PHE A:215 ALA A:146
		Carbon-hydrogen bond	GLY A:145
2	BVSNRJ 8C	Conventional H bond	THR A:102, TRP A:131, TYR A:41, GLY A:145
		Pi bonds	LEU A:103, VAL A:20, ALA A:146, PHE A:215, TYR A:247
		Carbon-hydrogen bond	GLY A:130
3	BVSNRJ 9C	Conventional H bond	GLY A:145, TYR A:41
		Pi bonds	LEU A:103, VAL A:20, ALA A:146, PHE A:150, TYR A:24, PHE A:215, TYR A:247
4	Isoniazid	Pi bonds	LEU A: 63
		Carbon-hydrogen bond	ILE A:95, VAL A:65, PHE A:41, ASP A:64

ADMET Properties

Table 5. ADMET studies of designed compounds

Compounds	Molecular weight(g/mol)	Hydrogen bond acceptor	Hydrogen bond donor	Molecular refractivity	Log p

BVSNRJ3	431.47	5	3	115.81	1.61
BVSNRJ8	372.36	6	3	94.76	0.73
BVSNRJ9	341.39	4	3	90.90	1.40
Drug likeliness	<500	<10	<5	40-130	<5

Pharmacokinetic profiling was conducted as part of the computational studies to identify lead compounds with optimal safety, potency, and therapeutic potential. This in silico evaluation provides a preliminary assessment that supports further investigation through in vitro validation. Two predictive tools, SwissADME and pkCSM, were utilized to ensure a comprehensive analysis, as each offers unique and complementary data.

Predictions were based on the chemical structures, physicochemical properties, and adherence to Lipinski's Rule of Five, which evaluates drug-likeness. The results, summarized in Table 5, revealed that all synthesized compounds complied with Lipinski's criteria, indicating favorable pharmacokinetic behavior and a good safety profile. These computational findings support the advancement of these compounds for experimental validation in subsequent in vitro studies.

Table 6. Absorption and Distribution properties of synthesized compounds

Compounds	**Absorption**				**Distribution**		
	Water solubility	**Caco2 permeability**	**Intestinal absorption (human)**	**Skin Permeability (Log KP)**	**VDss (human) (Log L/Kg)**	**BBB permeability (Log BBB)**	**CNS permeability**
BVSNRJ 3C	-3.095	0.846	83.254	-2.735	0.441	-1.446	-3.198
BVSNRJ 8C	-3.055	0.019	72.864	-2.735	0.436	-1.359	-2.907
BVSNRJ 9C	-3.037	0.905	76.345	-2.735	**0.165**	-1.23	-2.927

Absorption parameters considered for the study were water solubility, Caco2 permeability, intestinal absorption and skin permeability. All the compounds have shown lesser water solubility indicating lesser absorption. A relative Caco2 permeability was obtained and a very good percentage intestinal human absorption was noticed.

Table 7. Metabolism Properties of synthesized compounds

Compounds	**CYP2D6 substrate**	**CYP3A4 substrate**	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**CYP3A4 inhibitor**
BVSNRJ 3C	No	No	No	No	Yes	No	No

BVSNRJ 8C	No	No	No	No	No	No	No
BVSNRJ 9C	No	No	No	No	No	No	No

Compounds are metabolised by various isoforms of cytochrome P450. 2D6 and 3A4 are the important P450s involved in drug metabolism. **Table 7** represents the predictions indicating the involvement of P450s in metabolism. A drug should be effectively metabolised from the body, once the expected therapeutic activity was obtained. Other Cytochrome P450s may be involved in metabolism were CYP1A2, CYP2C19 and CYP2C9.

Table 8. Excretion and Toxicity Properties of synthesized compounds

Compounds	**Excretion**		**Toxicity**				
	Total Clearance	**Renal OCT2 substrate**	**AMES toxicity**	**Max. tolerated dose (human)**	**hERG I inhibitor**	**Hepatotoxicity**	**Skin Sensitization**
BVSNRJ 3C	0.885	No	No	0.119	No	Yes	No
BVSNRJ 8C	0.643	Yes	Yes	0.091	No	Yes	No
BVSNRJ 9C	0.892	No	Yes	-0.125	No	Yes	No

Excretion parameters such as total clearance and Renal OCT2 substrate status were evaluated using pkCSM. Among the synthesized compounds, BVSNRJ8C demonstrated the highest clearance and was predicted to be a Renal OCT2 substrate, suggesting renal excretion as a primary route.

Toxicity predictions included maximum tolerated dose, hepatotoxicity, and skin sensitization. While all compounds showed no skin sensitization potential, they exhibited low maximum tolerated doses and a high likelihood of hepatotoxicity, similar to the standard drug Isoniazid. These findings suggest that hepatotoxicity may be within tolerable limits but warrants further in vitro validation.

Molecular Dynamics Simulation

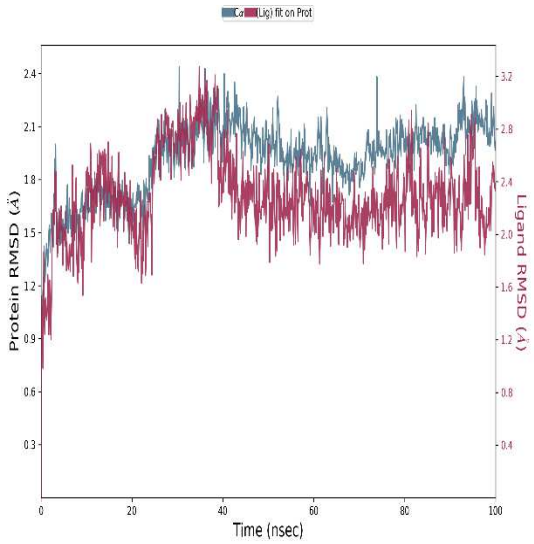


Figure 8. ***Protein-ligand RMSD plot***

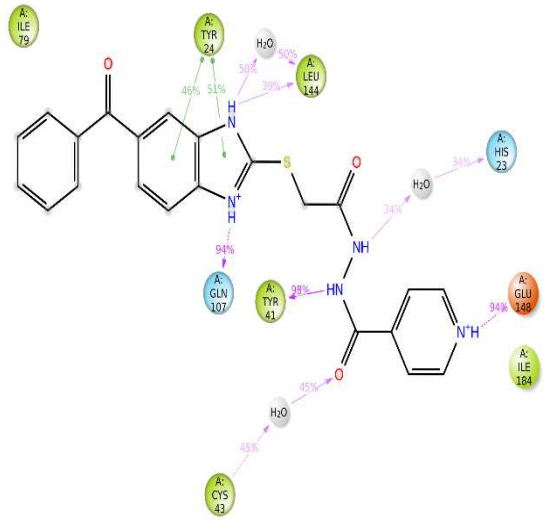


Figure 9. Ligand (BVSNRJ 3C) RMSF plot

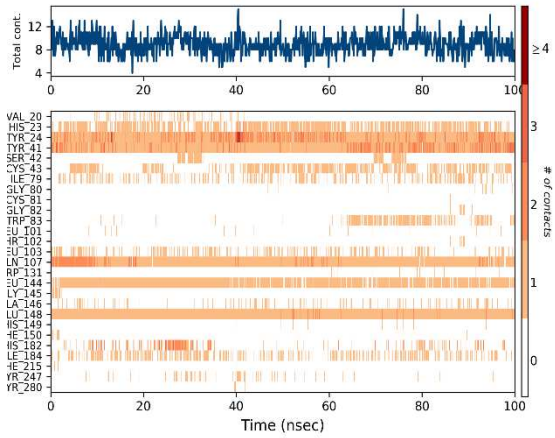


Figure 10. Protein-ligand contact timeline plot

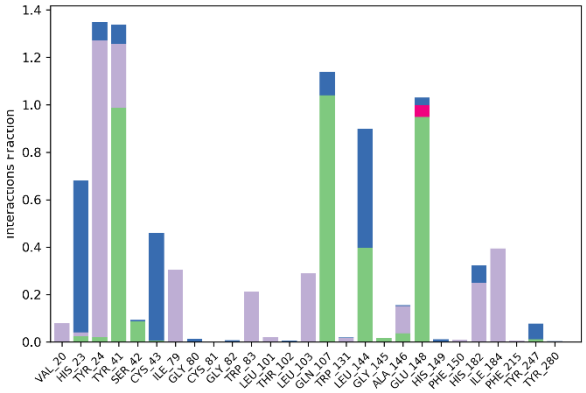


Figure 11. Histogram of protein-ligand complex

A 100 ns molecular dynamics simulation of compound **BVSNRJ3C** with **InhA (PDB ID: 1KPI)** was conducted using the Desmond module. The protein and ligand RMSD values remained stable throughout the simulation, indicating a well-maintained binding conformation. Key residues such as **Tyr24, Gln107, Glu148, and Tyr158** showed persistent interactions, including hydrogen bonding, hydrophobic contacts, water bridges, and π-π stacking.

Interaction timeline and fraction plots confirmed the stability and frequency of these contacts, highlighting the compound's strong binding affinity. These findings support BVSNRJ3C as a promising anti-tubercular lead for further in vitro validation.

Physical Data of synthesized compounds

Table 9. Physical data of synthesized compounds

Compound	IUPAC Name	Percentage yield (%)	Melting Point (°C)	R_f Value	Color	Mol. wt.
BVSNRJ 3A	(2-mercapto-1H-benzo[d]imidazol-5-yl)(phenyl)methanone	69.71	319-320	0.66	Light Brown	254.31
BVSNRJ 8A	5-nitro-1*H*-benzo[*d*]imidazole-2-thiol	83.79	263-265	0.42	Light orange	195.20
BVSNRJ 9A	5-methyl-1*H*-benzo[*d*]imidazole-2-thiol	86.88	271-273	0.45	Light brown	164.23
BVSNRJ 3B	2-((5-benzoyl-1H-benzo[d]imidazol-2-yl)thio)acetyl chloride	63.56	301	0.66	Brown	330.79
BVSNRJ 8B	2-((5-nitro-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride	64.28	258-259	0.62	Orange	271.68
BVSNRJ 9B	2-((5-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl chloride	66.48	293-295	0.71	Brown	240.71
BVSNRJ 3C	N'-(2-((5-benzoyl-1H-benzo[d]imidazol-2-yl) thio) acetyl) isonicotinohydrazide	61.06	196-199	0.55	Dark brown	431.47
BVSNRJ 8C	N'-(2-((5-nitro-1H-benzo[d]imidazol-2-yl)thio)acetyl) isonicotinohydrazide	70.07	178-179	0.76	Dark orange	372.36
BVSNRJ 9C	N'-(2-((5-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl) isonicotinohydrazide	61.69	165-168	0.60	Dark brown	341.39

Table 10. Interpretation of compound BVSNRJ 3C

IR Interpretation	BVSNRJ 3A	S-H Stretching (2571), S-H Bending (640), C=N (1607), AR C-H stretching (3046)
	BVSNRJ 3B	C=O stretching (1786), C-Cl Stretching (736), CH_2 stretching (2850), AR C-H Stretching (3096)
	BVSNRJ 3C	Amide C=O Stretching (1627), Amide N-H Stretching (3432), CH_2(2849), AR C-H Stretching (3045)

NMR	BVSNRJ 3C	Aromatic Proton (11) 7-8ppm, NH Proton (3) 12-13ppm, CH_2 Proton (2) 4ppm
MASS	BVSNRJ 3C	M.W= 431.47, M/z= M+1=432, Base peak= 361

Table 11. Interpretation of compound BVSNRJ 8C

IR Interpretation	BVSNRJ 8A	S-H Stretching (2519), S-H bending (696), C=N (1676), AR C-H stretching (3066)
	BVSNRJ 8B	C=O stretching (1732), C-Cl Stretching (762), CH_2 stretching (2940), AR C-H Stretching (3002)
	BVSNRJ 8C	Amide C=O Stretching (1625), Amide N-H Stretching (3125), CH_2 Stretching (2850), AR C-H Stretching (3097)
NMR	BVSNRJ 8C	Aromatic Proton (7) – 7-9ppm, NH Proton (3) 13-14ppm, CH_2 Proton (2)- 2-3ppm
MASS	BVSNRJ 8C	M. W= 372.36, m/z= M+1=373, Base Peak= 351.14

Table 12. Interpretation of compound BVSNRJ 9C

IR Interpretation	BVSNRJ 9A	S-H Stretching (2519), S-H bending (640), C=N (1660), AR C-H stretching (3081)
	BVSNRJ 9B	C=O stretching (1726), C-Cl Stretching (736), CH_2 stretching (2921), AR C-H Stretching (3096)
	BVSNRJ 9C	Amide C=O Stretching (1664), Amide N-H Stretching (3196), CH_2 Stretching (2954)
NMR	BVSNRJ 9C	Aromatic Proton (7) – 7-9ppm, NH Proton (3) 12-13ppm, CH_2 Proton (2)- 4ppm, CH_3 Proton (3)- 2-3ppm
MASS	BVSNRJ 9C	M.W= 341.39, $[M+1]^+$ = 342.09, Base Peak= 325.04

In-vitro Anti-tubercular Activity

The anti-tubercular potential of the synthesized compounds was evaluated against *Mycobacterium tuberculosis* H37Rv using the agar well diffusion method. The zone of inhibition (mm) was measured to assess the efficacy of the compounds. As shown in Table 13, compound BVSNRJ9C exhibited the highest activity (12 mm), followed by BVSNRJ3C (10 mm) and BVSNRJ8C (8 mm), compared to the standard drug Isoniazid (13 mm).

Table 13. Zone of Inhibition of Synthesized Compounds Against *M. tuberculosis*

Sl. No.	Compound Name	Zone of Inhibition (mm)
1	BVSNRJ3C	10

Sl. No.	Compound Name	Zone of Inhibition (mm)
2	BVSNRJ8C	8
3	BVSNRJ9C	12
4	Isoniazid	13

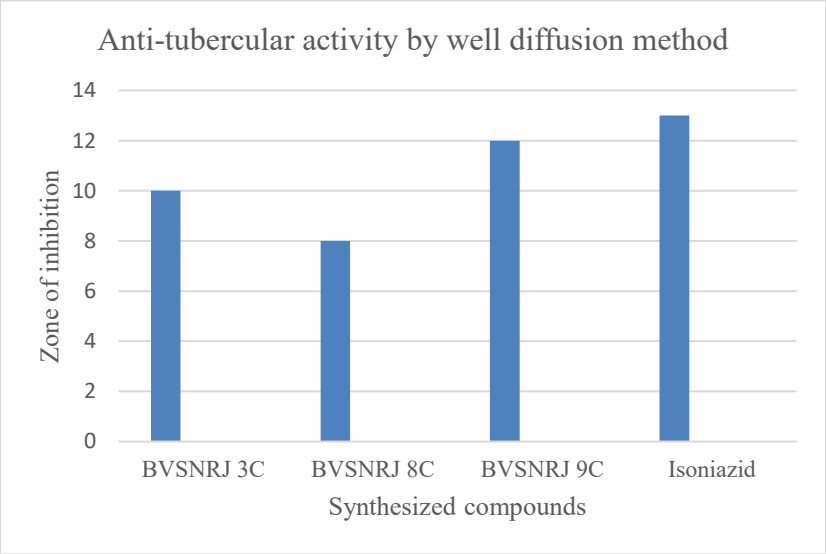


Figure 12. Anti-tubercular activity of synthesized compounds

Minimum Inhibitory Concentration (MIC) Analysis

The MIC values of the synthesized compounds were evaluated against *Mycobacterium tuberculosis* H37Rv at four different concentrations (2.5–10.0 µg/100 µL), and the percentage inhibition was calculated. As shown in **Table 13**, all compounds exhibited dose-dependent inhibitory effects. **BVSNRJ9C** showed the highest activity among the test compounds, achieving **78.18%** inhibition at 10 µg/100 µL, which is comparable to **Isoniazid** (**85.45%**).

Table 13. MIC Results (% Inhibition) of Synthesized Compounds

Compound	**2.5 µg/100 µL**	**5.0 µg/100 µL**	**7.5 µg/100 µL**	**10.0 µg/100 µL**
BVSNRJ3C	12.73	23.64	52.73	71.82
BVSNRJ8C	36.36	19.09	35.45	51.82
BVSNRJ9C	21.82	52.73	66.36	78.18
Isoniazid	61.81	65.45	80.91	85.45

Conclusion

The present study aimed to develop potent anti-tubercular agents by synthesizing novel 2-mercaptobenzimidazole derivatives tethered with isoniazid. Based on in-silico molecular docking results, three top-ranking compounds—**BVSNRJ 3C, BVSNRJ 8C, and BVSNRJ 9C**—were successfully synthesized and structurally characterized. The computational ADMET predictions suggested favorable pharmacokinetic and safety profiles, though these require further validation through in vitro and in vivo studies.

In vitro anti-tubercular activity assessed by the well diffusion method against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) demonstrated that all three synthesized compounds exhibited notable inhibitory effects, which may be attributed to the synergistic presence of the 2-mercaptobenzimidazole core and the isoniazid pharmacophore. Among them, **BVSNRJ 9C** showed the highest efficacy.

These findings provide a promising foundation for further optimization and in vivo evaluation of this compound class as potential anti-tubercular agents.

Conflict of Interest

The authors declare no conflict of interest.

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Author Contribution Declaration:

Nayana Ravindra Jawale contributed to the conceptualization, methodology design, data curation, software analysis, and manuscript writing. *B V Suma* supervised the research work, provided critical revisions, and guided the final review and editing of the manuscript. Both authors have read and approved the final manuscript.

Consent to Publish Declaration:

We have included the following statement in the manuscript as the study does not involve any identifiable individual data:
"Consent to Publish declaration: Not applicable."

Ethics and Consent to Participate Declarations:

As this study does not involve human participants, animal subjects, or any personal data requiring ethical approval, we have added the following declaration in the manuscript:
"Ethics and Consent to Participate declarations: Not applicable."

Declaration

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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