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ORIGINAL ARTICLE

Design, Synthesis, Molecular Docking and Antitubercular Evaluation of 2-Substituted 1-Methyl-1h-Benzimidazoles bearing an N-(2-(4-Hydroxy-3-Carboxyphenyl)Ethyl) Moiety

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ABSTRACT
In the present study, a series of 2-substituted 1-methyl-1H-benzimidazoles bearing an N-(2-(4-hydroxy-3-carboxyphenyl) ethyl) moiety was designed and synthesized to explore their potential as antitubercular agents. The synthesized compounds (P1–P6) were structurally confirmed through IR, ^1H NMR, and mass spectrometry. Their antimycobacterial activity was assessed using the Microplate Alamar Blue Assay (MABA), while molecular docking studies were performed with AutoDock Vina 1.5.6 against the 2Q1Y receptor. Among the series, compound P4 exhibited the most favorable docking score (–8.9), outperforming standard antitubercular drugs including isoniazid and para-aminosalicylic acid. Moreover, P4, P5, and P6 displayed strong binding affinity consistent with their in vitro activity, emphasizing the structural relevance of substitutions at the benzimidazole core. Collectively, these findings suggest that the synthesized benzimidazole derivatives, particularly P4, hold promise as lead candidates for the development of novel antitubercular therapeutics.
Keywords: *Benzimidazole derivatives; Anti-tubercular activity; Molecular docking; Microplate Alamar Blue Assay; 2Q1Y receptor*

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INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is a major infectious disease transmitted via respiratory droplets [1,2]. Once termed the "white plague," TB was identified by Robert Koch in 1882, a discovery that earned him the Nobel Prize [3–5]. Despite medical progress, TB has resurged, particularly in immunocompromised individuals such as AIDS patients [6–11].

Globally, TB remains a leading cause of mortality, with over 3 million deaths each year. Nearly one-third of the world's population carries latent infection, with ~8.8 million new cases and ~7,000 deaths daily [12–15]. Drug resistance further intensifies the crisis, with over 0.5 million multidrug-resistant TB (MDR-TB) cases reported among the 9 million new infections in 2012 alone [16]. The disease burden is highest in Asia and Africa (>80% prevalence) compared to <10% in the U.S. [17]. India, China, Indonesia, South Africa, and Nigeria account for the majority of global cases, with India alone reporting ~0.23 million MDR-TB cases annually [18–20].

As shown in Figure 1, TB incidence is disproportionately concentrated in regions such as South-East Asia and sub-Saharan Africa, with countries like India, China, South Africa, and Nigeria representing significant hotspots of disease transmission.

Table 1: Global Burden of Tuberculosis

Region	Estimated Incidence (per 100,000)	TB-Related Deaths	MDR-TB Cases (%)	HIV-TB Coinfection (%)
Africa	219	230,000	3.1%	30%
Southeast Asia	226	435,000	2.5%	10%
Western Pacific	96	150,000	1.8%	5%
Americas	28	25,000	1.2%	10%
Global Average	133	1.3 million	3.0%	8%

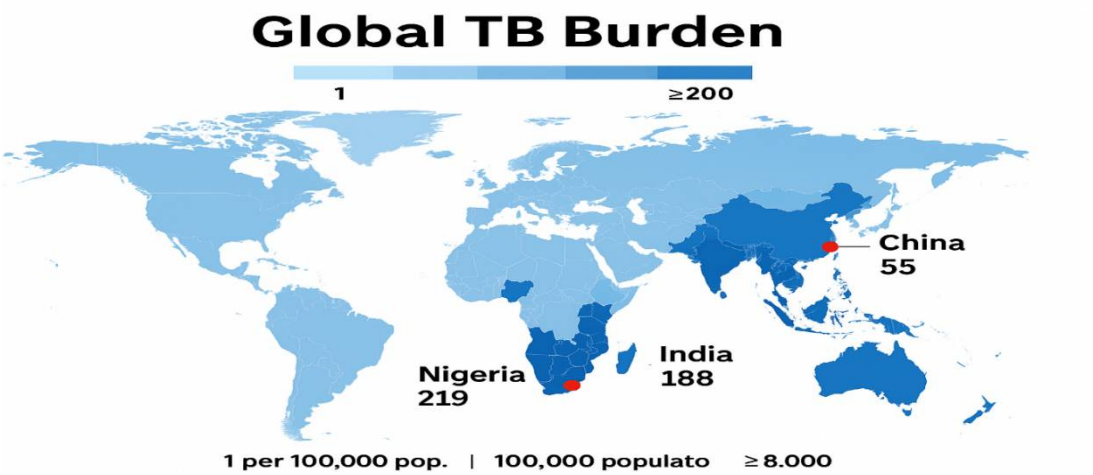


Figure 1: Global Burden of Tuberculosis

Figure 1 shows Global TB incidence rates by country (darker shades indicate higher incidence), highlighting high-burden nations such as India, China, South Africa, and Nigeria.

The urgent need for new chemotherapeutics has directed attention to heterocyclic scaffolds, with benzimidazole emerging as a key framework due to its broad pharmacological spectrum [25]. First synthesized by Debus in 1858 [26] via condensation of ***o***-phenylenediamine with mono-/dibasic acids [27], benzimidazole exhibits diverse activities including antitubercular, anticancer, antifungal, antihypertensive, antimicrobial, antiviral, and anti-inflammatory effects [28–39] (Figure 2). Its NH group further imparts unique acidic–basic properties [40].

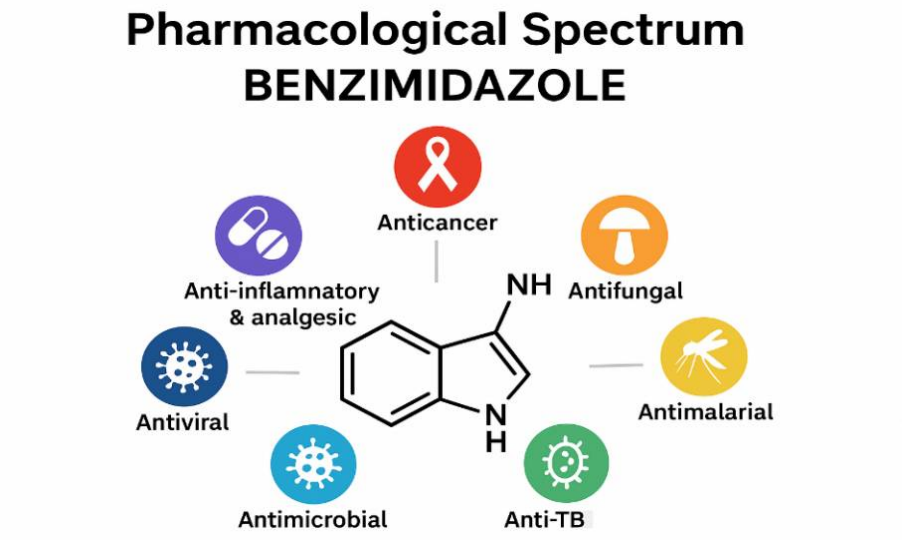


Figure 2: Pharmacological spectrum of benzimidazole derivatives

The infographic illustrates the broad range of biological activities associated with benzimidazole scaffolds, including antitubercular, anticancer, antifungal, antimicrobial, antimalarial, antiviral, antiparasitic, and anti-inflammatory/analgesic effects [41].

Structurally, benzimidazole (IUPAC: 1H-benzimidazole; synonyms: 1H-1,3-benzimidazole, 1H-benzo[d]imidazole) exhibits isosterism with indole and purine [42, 43] (Figure 3). This structural mimicry explains its significant role in biology, highlighted by the discovery of 5,6-dimethyl-1-(D-ribofuranosyl) benzimidazole as a key fragment of Vitamin B12 [82,83]. Furthermore, tautomerization at the N1 position renders the C5 and C6 carbons chemically equivalent, influencing substitution patterns and bioactivity [44].

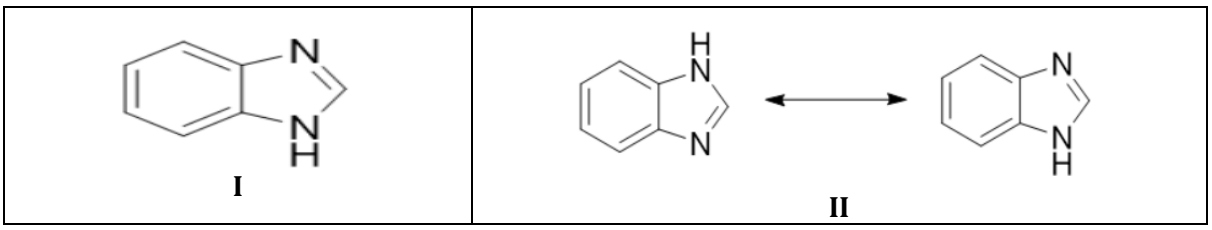
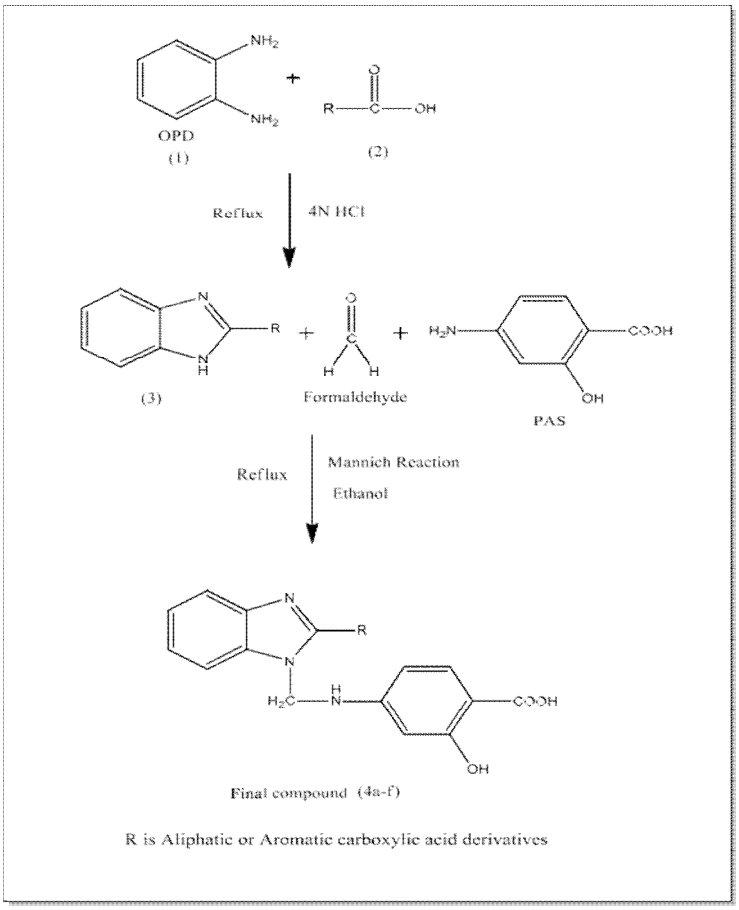


Figure 3: Structural representation.

Given these features, benzimidazole derivatives stand as promising scaffolds in anti-TB drug discovery, offering opportunities to combat drug resistance and improve therapeutic outcomes.

MATERIAL AND METHODS

All chemicals and solvents were of analytical grade (Sigma Aldrich, SD Fine Chemicals, Thomas Baker). Reactions were monitored by TLC on silica gel 60 F254 plates (Merck). Melting points were determined in Thiel's tube. IR spectra were recorded on a Shimadzu FTIR-1000 (KBr, 4000–400 cm^{-1}), ^1H NMR on a Bruker 400 MHz (DMSO-d6, TMS), and mass spectra by LC–MS.



Scheme-I: Synthetic route for preparation of benzimidazole derivatives

Table 2: Various Substitutions on Final compounds

Comp. Code	R/Ar
P1	-H
P2	$-CH_3$
P3	$-2-NH_2C_6H_5$
P4	$-2,4-NO_2C_6H_5$
P5	$-C_2H_4Cl$
P6	$-4-NO_2C_6H_5$

General procedure for the synthesis of Intermediates

o-Phenylenediamine and substituted carboxylic acids (0.01 mol) were refluxed in 4 N HCl at 100 °C (6–16 h). After TLC monitoring, the mixture was cooled, neutralized, and the precipitate recrystallized from ethanol to yield pure benzimidazole derivatives. [45]

General procedure for the synthesis of Final compounds (P1-P6)

Benzimidazole derivatives, para-aminosalicylic acid, and formaldehyde (0.02 mol each) were dissolved in ethanol (70 mL) and subjected to reflux (4 h). After solvent removal and trituration with petroleum ether (40–60 °C), PAS-conjugated benzimidazole derivatives were obtained in good yield.

Analytical data of synthesize benzimidazole derivatives

Table 3: Analytical data of synthesize benzimidazole derivatives

Compound code	Chemical Name	M.P (°C)	% Yield	IR (cm^{-1})	^1H NMR (δ, ppm)	Mass (M/Z)
P1	4-(((1H-benzo[d]imidazol-1-yl)methyl)amino)-2-hydroxybenzoic acid	145–150	85.25	N-H: 3502 OH: 3336 COOH: 3378 C=N: 1619 C=C: 1419	CH_2: 5.17 (2H, s) Ar-H: 6.37–7.96	401.15
P2	2-hydroxy-4-(((2-methyl-1H-benzo[d]imidazol-1-yl)methyl)amino)benzoic acid	139–140	80.90	N-H: 3595 OH: 3498 COOH: 1718 C=N: 1612 C-N: 1254	–	–
P3	4-(((2-(2-aminophenyl)-1H-benzo[d]imidazol-1-yl)methyl)amino)-2-hydroxybenzoic acid	273–276	65.80	N-H: 3352 OH: 3479 COOH: 3294 C=N: 1709 NH_2: 1277	CH_2: 5.36 (2H, s) Ar-H: 6.30–7.91	583.23
P4	4-(((2-(2,4-dinitrophenyl)-1H-benzo[d]imidazol-1-yl)methyl)amino)-2-hydroxybenzoic acid	215–220	78.20	N-H: 3471 OH: 3633 COOH: 3386 C=N/C=C: 1643 NO_2: 1516, 1342	–	–
P5	4-(((2-(2-chloroethyl)-1H-benzo[d]imidazol-1-yl)methyl)amino)-2-hydroxybenzoic acid	287–290	83.70	N-H: 3305 OH: 3629 COOH: 3379 C=N: 1631 CH_2Cl: 1234	–	–
P6	2-hydroxy-4-(((2-(4-nitrophenyl)-1H-benzo[d]imidazol-1-yl)methyl)amino)benzoic acid	215–219	62.50	N-H: 3236 OH: 3471 COOH: 3143 C=N: 1519 NO_2: 1560	CH_2: 5.43 (2H, s) Ar-H: 6.30–8.27	404.11

Molecular docking

Molecular docking was carried out using AutoDock Vina v1.5.6 [46], which enables rigid and flexible ligand docking within defined protein binding sites. The receptor structure was obtained from the Protein Data Bank or modeled through homology approaches. The primary goal was to predict optimal ligand–receptor binding conformations with minimum interaction energy, evaluated by scoring functions based on steric and electrostatic interactions [47].

Ligand structures of the synthesized benzimidazole derivatives were drawn in ChemDraw, converted into 3D models, and energy-minimized. Conformers were generated, and the lowest-energy conformer for

each compound was selected. Final structures were refined in PyMOL and saved in .pdb format for docking studies [47].

Preparation of ligands

Ligand structures were designed and optimized in ChemDraw Ultra 12.0, saved in. mol format, and converted to .pdb using PyMOL. Prior to docking in AutoDock Vina v1.5.6, water molecules and native ligands were removed from the target enzyme. Hydrogen atoms, torsions, and rotatable bonds were assigned, and the final ligand files were saved in .pdbqt format.

Preparation of protein

The crystal structure of FtsZ from *Mycobacterium tuberculosis* complexed with GTP-γ-S (PDB ID: 2Q1Y) was retrieved from the RCSB Protein Data Bank [48]. The structure was processed in Discovery Studio**,** where water molecules and the native ligand were removed, hydrogen atoms were added, and the binding site was defined before saving the receptor in .pdb format.

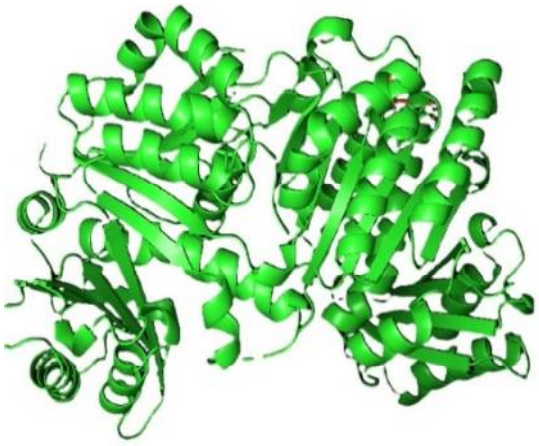


Figure 4: Crystal structure of the cell division protein FtsZ from Mycobacterium TB in association with GTP-gamma-S (PDB ID: 2Q1Y)

Anti- Myobacterial activity

Antitubercular Activity Evaluation

The antitubercular activity of synthesized compounds was evaluated by the Microplate Alamar Blue Assay (MABA) against *Mycobacterium tuberculosis* H37Rv [49–51]. Test compounds were dissolved, serially diluted in Middlebrook 7H9 broth (with OADC and Tween 80), and incubated with bacterial suspension in 96-well plates at 37 °C for 7 days. After addition of Alamar Blue and 24 h incubation, fluorescence was measured, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration inhibiting ≥90% of growth. Rifampicin and para-aminosalicylic acid were used as positive controls.

RESULT AND DISCUSSION

Chemistry

2-substituted benzimidazoles were synthesized by refluxing ***o***-phenylenediamine with substituted carboxylic acids in 4N HCl at 70–80 °C, monitored by TLC. Final derivatives were obtained by coupling these intermediates with para-amino salicylic acid using K_2CO_3 in ethanol, followed by recrystallization. All compounds were characterized by IR, NMR, and mass spectrometry.

Molecular docking study

All synthesized derivatives (P1–P6) were evaluated for anti-mycobacterial activity. Docking results (Table 4) showed compound P4 with the highest binding affinity (–8.9) against the FtsZ receptor (PDB ID: 2Q1Y). Receptor–ligand interactions of P4, PAS, and isoniazid are illustrated in 3D (Figures 5, 7, 9) and 2D (Figures 6, 7, 10) representations.

Table 4: Molecular docking results of benzimidazole derivatives showing Interaction for their anti-tubercular activity

Sr. No.	Log P	Docking Score Kcal/mol	Interaction
P1	1.78	-7.5	ASP A:84, GLU B:136 & GLY B:19 (CHB), GLY B:104 (Amide-Pi stacked), GLU A:87 (Pi-Anion)
P2	2.45	-6.7	ARG B:139 (CHB), ALA B:144 (Pi-Sigma), GLY B:103 (Amide Pi-stacked), PRO B:132 (Pi-Alkyl)
P3	3.03	-7.5	ASN B:22 (CHB), ARG B:126 & GLU A:36 (Pi- Cation/ Pi-Anion), ARG B:181 (Pi-Alkyl), VAL A:10 (Pi-Sigma)
P4	4.4	**-8.9**	ARG B:304, SER B:260 & THR B:306 (CHB), ALA B:262 (Pi-Alkyl)
P5	3.36	-6.9	ARG B:304 & VAL B:305 (CHB), GLN B: 192 (Halogen), SER B:227 (Carbon-hydrogen bond), ALA B:262& ILE B:225 (Pi-Alkyl)
P6	4.6	-7.4	ILE A:11 & GLU A:36 (CHB), ASP A:53 & GLU B:29 (Pi-Anion), VAL A:10 (Pi-Alkyl & Carbon-hydrogen bond)
PAS	**1.012**	**-4.7**	ASP B:43 (pi-anion), ALA B:70 (pi-alkyl), GLY B:104, GLY B:19, GLY B:107, THR B:106 & ASP A:81 (CHB), GLY B:105 & GLY B:17 (C-H Bond)
Isoniazid	**-0.64**	**-5.5**	ALA B:70 (pi-alkyl), ASP B:43 (pi-anion), GLY B:18 (Van der waals), GLY B:107 & THR B:106 (CHB)

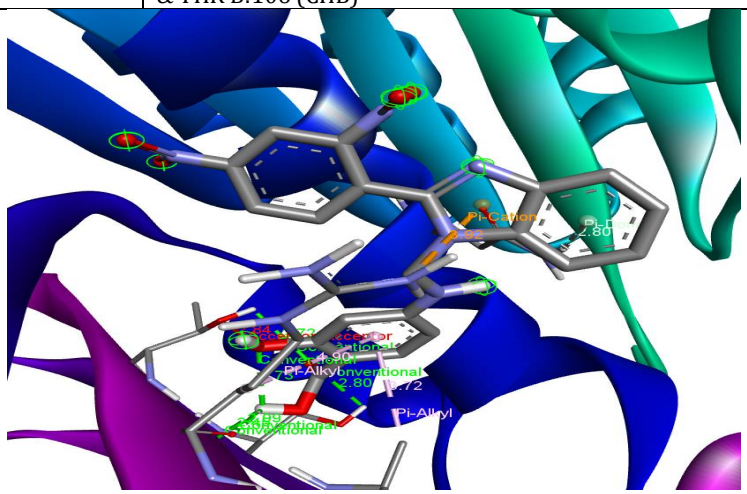


Figure 5: 3D receptor–ligand interaction of compound P4 with the FtsZ protein (PDB ID: 2Q1Y)

3D view of compound P4 bound to FtsZ protein (PDB ID: 2Q1Y). Key hydrogen bonds (dashed lines) and hydrophobic interactions are shown, illustrating the ligand's binding mode.

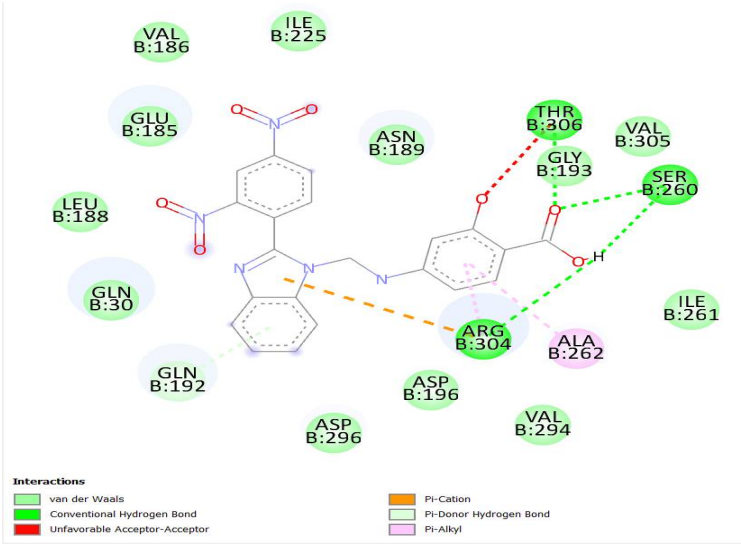


Figure 6: 2D receptor–ligand interaction of compound P4 with the FtsZ protein (PDB ID: 2Q1Y)

2D map showing key interactions of compound P4 with FtsZ protein (PDB ID: 2Q1Y), including hydrogen bonds and hydrophobic contacts.

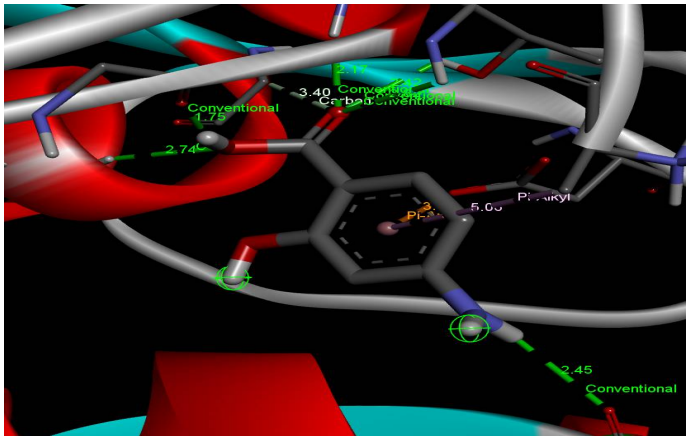


Figure 7: 3D receptor–ligand interaction of para-aminosalicylic acid (PAS) with the FtsZ protein (PDB ID: 2Q1Y)

3D view of PAS bound to FtsZ protein (PDB ID: 2Q1Y), showing key hydrogen bonds and hydrophobic interactions in the active site.

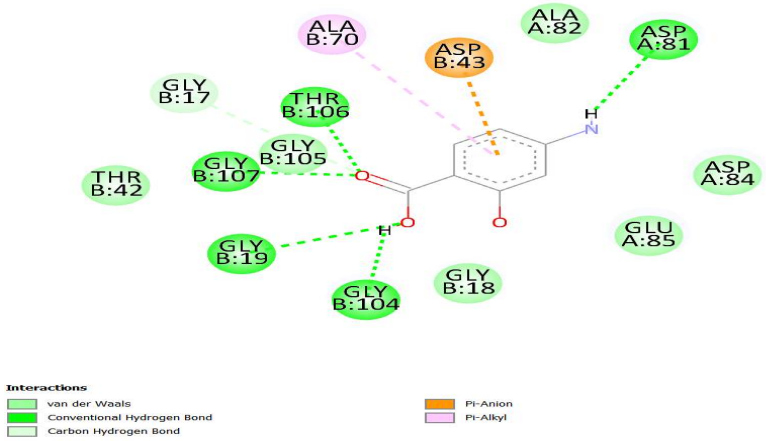


Figure 8: 2D receptor–ligand interaction of para-aminosalicylic acid (PAS) with the FtsZ protein (PDB ID: 2Q1Y)

Two-dimensional representation of para-aminosalicylic acid (PAS) interactions with FtsZ protein (PDB ID: 2Q1Y), highlighting hydrogen bonds, hydrophobic contacts, and key ligand–residue interactions in the binding site.

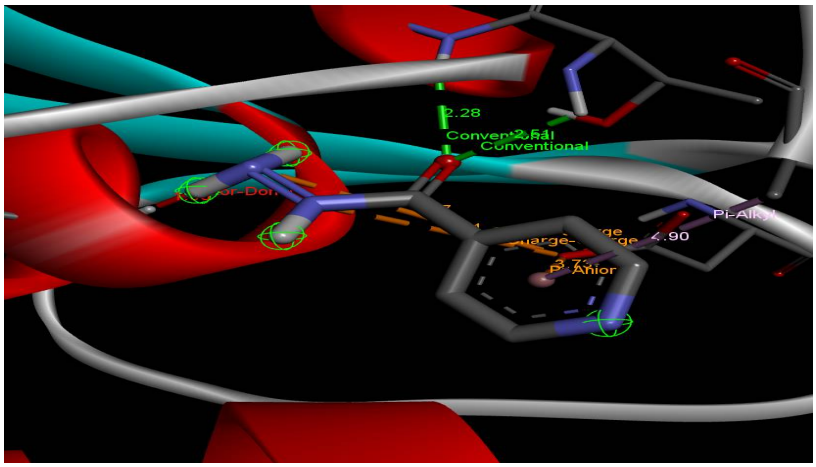


Figure 9: 3D receptor–ligand interaction of isoniazid with the FtsZ protein (PDB ID: 2Q1Y)

Three-dimensional representation of isoniazid bound to FtsZ protein (PDB ID: 2Q1Y). Hydrogen bonds are shown as dashed lines, and hydrophobic interactions are indicated, illustrating the binding mode of isoniazid within the active site.

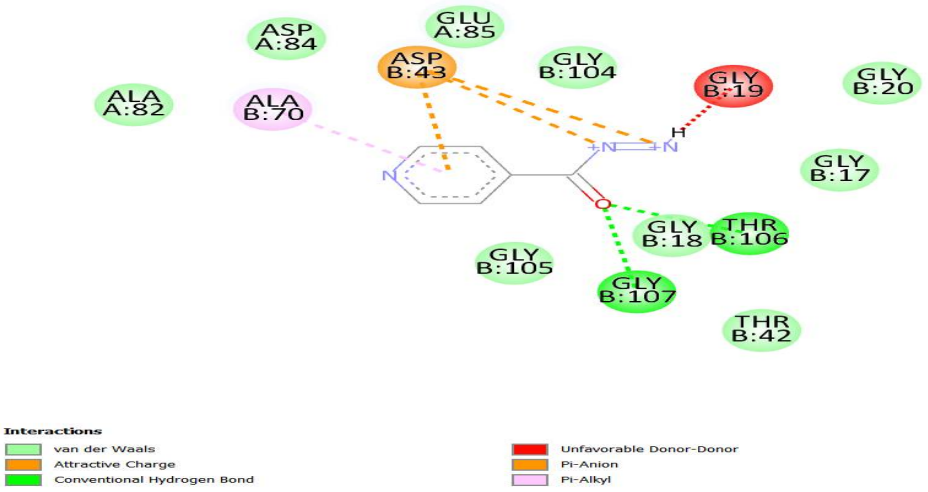


Figure 10: 2D receptor–ligand interaction of isoniazid with the FtsZ protein (PDB ID: 2Q1Y)

Two-dimensional representation of isoniazid interactions with FtsZ protein (PDB ID: 2Q1Y), showing hydrogen bonds, hydrophobic contacts, and key ligand–residue interactions in the binding site.

Pharmacological evaluation

The Microplate Alamar Blue Assay (MABA) was conducted at Andhra University, Visakhapatnam. Due to poor solubility, compounds were dissolved in DMSO for solution preparation. MIC values were determined and compared with standard drugs (rifampicin and PAS), using DMSO as a positive control.

- Each drug's test solution was added to a 96-well microplate containing Alamar Blue Reagent.
- Fluorescence was quantified with a microplate reader to determine the minimum inhibitory concentration (MIC).

The anti-tubercular activity of the synthesized derivatives was assessed using MABA (Figure 11), and the MIC values are summarized in Table 5. A comparative activity profile with PAS and Rifampicin is shown in Figure 12.

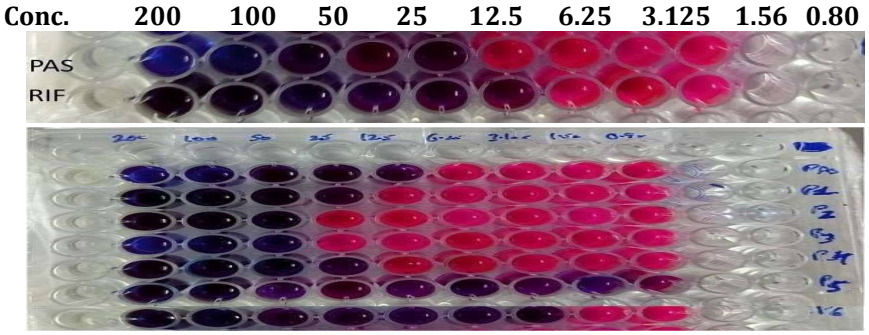


Figure 11: Microplate Alamar Blue Assay (Anti-tubercular activity results)

Table 5: Microplate Alamar Blue Assay

Sr. No.	Comp. Code	MIC (µg/ml) $H_{37}R_V$ Strain
1.	P1	25
2.	P2	50
3.	P3	50
4.	P4	25
5.	**P5**	**1.56**
6.	**P6**	**3.125**
7.	PAS	12.5
8.	Rifampicin	6.25

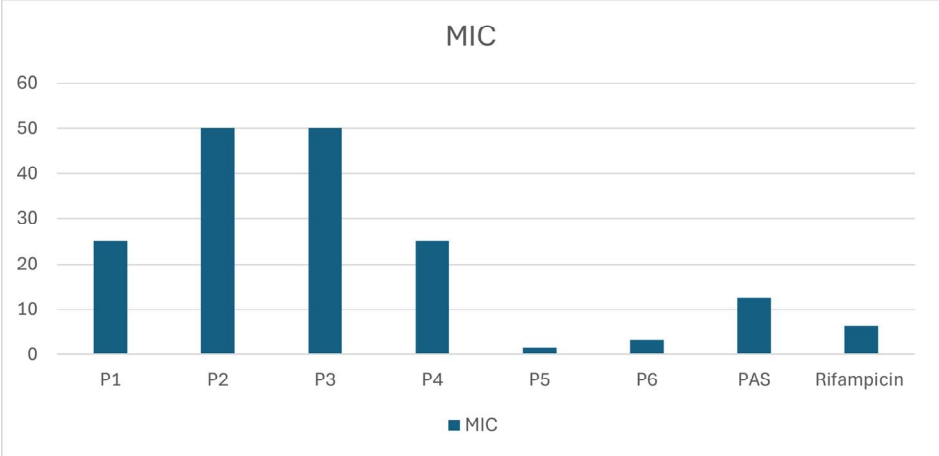


Figure 12: Graphical representation showing activity of derivatives (MIC values) with respect to PAS and Rifampicin

CONCLUSION

Tuberculosis remains a major global health challenge, demanding the discovery of new and effective therapeutics. In this study, benzimidazole derivatives were synthesized and evaluated, demonstrating significant anti-mycobacterial activity against the H37Rv strain. Molecular docking revealed strong affinity toward the FtsZ protein (PDB: 2Q1Y), with derivative P4 exhibiting superior binding compared to standard drugs such as PAS and isoniazid. Importantly, all derivatives displayed higher docking scores than conventional therapies, suggesting their potential as next-generation anti-TB agents. The Microplate Alamar Blue Assay further confirmed their bioactivity, establishing these compounds as promising scaffolds for rational drug design. Collectively, these findings not only highlight benzimidazole derivatives as valuable leads but also underscore their potential contribution to combating the escalating global TB burden.

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Conflict Of Interest

The authors declare that they have no conflict of interest.

Ethical Approvals

This study does not involve experiments on animal of human subjects.

Data Availability Statement

All data which are generated and analyzed are included within this research article.

Author Contribution Statement

P. N. S. performed the synthesis, characterization, biological evaluation, computational studies, data analysis, and manuscript preparation. M. R. K. supervised the research, critically reviewed the manuscript, and approved the final version.

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