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Original Article

Design, Synthesis and Evaluation of Substituted Benzimidazoles as Potent Polyketide Synthase 13 Inhibitors for Tuberculosis

Gokula Krishnu, Ganesh Paleti, Bharath Kumar Chagaleti, Ahmed Mueen, Shanthakumar Balakrishnan*

Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM Institute of Science and Technology (SRMIST), Kattankulathur, Chengalpattu, Tamil Nadu, INDIA.

ABSTRACT

Background: Tuberculosis remains a major global health concern, affecting millions of lives annually due to its high morbidity and mortality rates. The urgent need for effective treatments has driven research into novel therapeutic strategies. This study focuses on the development of anti-tubercular compounds targeting polyketide synthase 13, a crucial enzyme in the production of mycolic acids, which are essential for the integrity and drug resistance of the *Mycobacterium tuberculosis* cell wall. **Materials and Methods:** Substituted benzimidazole derivatives were designed through Structure-Activity Relationship investigations and molecular docking studies. The compounds were synthesized and tested for their antimicrobial activity using the Microplate Alamar Blue Assay. This assay was employed to determine the Minimum Inhibitory Concentration against *Mycobacterium tuberculosis* H37RV. Additionally, the well plate method was used to evaluate the effectiveness of these compounds against *Klebsiella pneumoniae* and *Staphylococcus aureus*. **Results:** Among the synthesized benzimidazole derivatives, compounds Ga2 and Ga3 demonstrated the highest potency against tuberculosis. These compounds exhibited superior binding affinity to polyketide synthase 13, exhibiting favourable amino acid interactions in diverse, effectively inhibiting mycobacterial growth, in turn justified by the *in silico* analysis The findings highlight the effectiveness of the designed derivatives in disrupting mycolic acid synthesis, thereby enhancing drug permeability and efficacy against *Mycobacterium tuberculosis*. **Conclusion:** This study demonstrates the potential of computational chemistry and structure-based drug design in identifying and optimizing novel anti-tubercular agents. The promising results of benzimidazole derivatives, particularly Ga2 and Ga3, against tuberculosis emphasize their potential as effective therapeutic candidates targeting specific enzymes critical to the bacterium's survival and pathogenicity.

Keywords: Tuberculosis, Polyketide synthase 13, Benzimidazole, *in silico* analysis, Microplate alamar blue assay.

Correspondence:
Dr. Shanthakumar Balakrishnan
Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRM Institute of Science and Technology (SRMIST), Kattankulathur, Chengalpattu-603203, Tamil Nadu, INDIA.
Email: shanthab@srmist.edu.in

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INTRODUCTION

Despite significant advancements in treatment, Tuberculosis (TB), a global epidemic caused by the pathogen *Mycobacterium tuberculosis* (M. tb), remains a major public health concern.[1] TB is one of the top ten deadliest diseases that prevails globally, responsible for 10% of deaths every year.[2] World Health Organization declares TB a global pandemic, with the highest incidence in 13 African and 6 Asian countries, half of new cases.[3] Despite a decrease in deaths to 1.4M however,10.5M new TB cases arise in 2022 and it remains a major threat, exacerbated by MDR-TB with high HIV prevalence.[4]

The complexity of TB treatment is compounded by the distinctive structural and chemical characteristics of M. *tb*, which contribute to the bacteria's capacity to enhance defiance to antibiotics.[5] Many antibiotics exhibit phenotypic drug resistance, influenced not by genetic alterations but by the metabolic state of the bacteria. Different vulnerable forms of drug-resistant tuberculosis exist namely: MDR-TB, extensively (XDR-TB), (XXDR-TB) and (TDR-TB). These categories represent significant public health threats, capable of causing widespread outbreaks and high mortality rates.[6-9]

The mycobacterial cell wall consisting of the mycolic acid layer, plays a critical role in the pathogen's design and resistance to drugs. Mycolic acids are key components of the M. *tb* cell envelope, crucial for the bacteria's survival and resistance mechanisms.[10] 1st-line anti-TB medications such as Isoniazid (INH) and ethionamide target the Fatty Acid Synthase II complex

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(FAS2), disrupting mycolic acid synthesis and compromising the bacterial cell wall. Polyketide synthase 13 (Pks 13) is a vital enzyme in mycolic acid production, that highlights its potential as a viable target for emerging TB treatments.[11,12]

The emergence of resistant forms of tuberculosis like multi-drug resistant and extensively drug-resistant has increased the demand for potent antitubercular agents. However, on exploring the recently reported vital targets, Pks 13 has emerged as a significant target due to its essential role in mycolic acid biosynthesis. Several potent Pks 13 inhibitors have also been identified over the past decade, but none have advanced to clinical trials. Thus, effective methods for discovering Pks 13 inhibitors are crucial, involving systematic Structure-Activity Relationship (SAR) studies to optimize these compounds for potential therapeutic use. Imidazole heterocycles, known for their biological and pharmacological activities, have also shown promise in TB treatment. Compounds like 4-nitroimidazoles, pretomanid and delamanid are in advanced clinical trials. High-Throughput Screening (HTS) and computational chemistry support their rapid development.[13,14]

Benzimidazole is a privileged structure in heterocyclic chemistry and a notable pharmacophore with diverse biological activities, including anti-parasitic, antimicrobial, antiviral, antifungal, anticonvulsant, antihypertensive, antihistaminic, analgesic, anti-inflammatory, anticancer and anticoagulant characteristics. Benzimidazoles, particularly the 2,5- and 2,6-disubstituted derivatives, have demonstrated considerable antimycobacterial activity against *Mycobacterium tuberculosis*.[15] The 2,5-disubstituted benzimidazole derivatives have exhibited high activity against M. tuberculosis. Although benzimidazoles' exact functional dynamics were not fully understood, their structural similarity to purines is hypothesized to contribute to the antimycobacterial activity.[16-18]

The study involves the design, synthesis and evaluation of 2-(1,3-diphenyl-1H-pyrazol-4-yl)-1H-benzo[d]imidazole derivatives using CADD to accelerate drug development. The compound designing process was guided by computational chemistry and high-throughput screening. To combat Multidrug-Resistant (MDR-TB) and Extensively Drug-Resistant tuberculosis (XDR-TB), this study aims to identify promising benzimidazole derivatives through various *in silico* methods and then it was synthesized and subjected to *in vitro* studies. These compounds target key enzymes such as Pks13, which are involved in the biosynthesis of mycolic acids. By focusing on this particular enzyme, the synthesized derivatives could provide effective therapeutic options against drug-resistant TB strains, addressing a critical global health issue.[19]

MATERIALS AND METHODS

Experimental Section

Materials

4-Hydroxy acetophenone, 4-Nitro aceophenone, 2,4-Dichloro acetophenone, Phenyl Hydrazine, Acetic acid, Methanol, Dimethyl formamide, Phosphorous oxychloride and Ammonium chloride. All the reactants and solvents were sourced from Southern India Scientific Corporation Limited, Chennai.

Methods

Using Chem3D Pro 12.0, the compounds were sketched and virtually docked them into the selected protein (PDB 5V3X). AutoDock 4.2 was utilized to predict the binding in docking. The UCSF Chimera, was involved in assigning charges and defining a grid around the active site. The compounds were ranked based on their predicted binding energy and interactions with the active site as determined by docking results. Visualization with Biovia Discovery Studio helped to analyze the docked complexes. The most promising candidates for the additional research were determined by evaluating drug-likeness and bioactivity with Molinspiration and ADMET properties with pkCSM software.[20-22] finally, the compounds were synthesised and the end of the reaction was confirmed via TLC.

Computational Studies

Molecular Docking

A combination of literature review and SAR studies involved in designing various benzimidazole derivatives by subsequently reacting with Phenyl hydrazine along with various substitiuted acetophenones. *In silico* evaluation led to the selection of potent compounds from the library using computer-aided binding prediction to justify the interaction strength to the target protein, alongside ADMET predictions and bioactivity assessments. The target protein, validated from the Protein Data Bank (PDB), had its structure evaluated with the Ramachandran plot. The Protein preparation involves selecting the relevant chain, removing bound molecules, defining the active site and setting up docking grids with energy minimization using the OPLS2005 force field. Ligands were minimized with MMFF94 and the docking results were analyzed using Biovia Discovery Visualizer.[23,24]

In silico Analysis

The molinspiration cheminformatics toolkit was used to measure the molecular properties of the selected compounds. SMILES codes were generated for the sketched compounds and uploaded to the server for analysis. Lipinski rule incorporates four key physicochemical parameters (H-bond acceptors≤10, MW<500 Da, H-bond donors≤5, log P≤5) that have been linked to the oral bioavailability of drugs reaching phase II clinical trials in approximately 90% of cases. ADMET properties, including

intestinal permeability and solubility, were analyzed with pkCSM to assess pharmacokinetic profiles and drug-like nature.[25]

Experimental work

Synthesis of the Compounds

The synthesis of the compounds involved three steps. First, phenyl hydrazine reacted with substituted acetophenones in an acidic solution to form yellow hydrazone crystals. Step two utilized Vilsmeier-Haack formylation, transforming the hydrazone into an orange solid using dimethylformamide and phosphorus oxychloride and were allowed to stirr for 5 hr. Finally, the obtained pyrazole aldehyde was condensed with o-phenylene diamine in acidic ethanol under stirring. The desired final product, benzimidazole derivatives, was precipitated as pale-yellow solids after pouring the reaction mixture into frozen water and purified with ethanol.

Characterization

The synthesized compounds were justified by their distinct melting points and Rf values. The end of the reaction was checked by Thin-Layer Chromatography (TLC) with a mixture of n-hexane and ethyl acetate as a solvent in the ratio 2:1. Further the compounds were scrutinised by Fourier-Transform Infrared spectroscopy (FTIR), elemental analysis, Nuclear Magnetic Resonance Spectroscopy (NMR) and mass spectrometry.

In vitro Antitubercular Activity

The synthesized compounds were checked for antitubercular activity by the MABA method using the standard procedure with standard drugs like isoniazid, ethambutol rifampicin and streptomycin.

Antimicrobial activity

The Kirby-Bauer test, a commonly used agar diffusion method, is used to determine antibiotic sensitivity in micro-organisms. This test involves placing antibiotic-impregnated discs (usually 1000 µg) on an agar plate containing bacteria. Following incubation, the antibiotic's efficiency in suppressing the bacterial growth is evidenced by clear zones around the discs that are measured in millimetres and antimicrobial activity was determined by using standard drugs that are indicated in the methodology.

RESULTS

Docking Analysis

Docking simulations were implemented to determine the interaction and affinity between ligands and the target protein (PDB 5v3x). Ligands with the highest predicted interaction and affinities were selected for synthesis and subsequent evaluation. Table 1 summarizes the docking results, while Figure 1 (2D) and Figure 2 (3D) illustrates the key binding affinity between the selected protein and ligand.

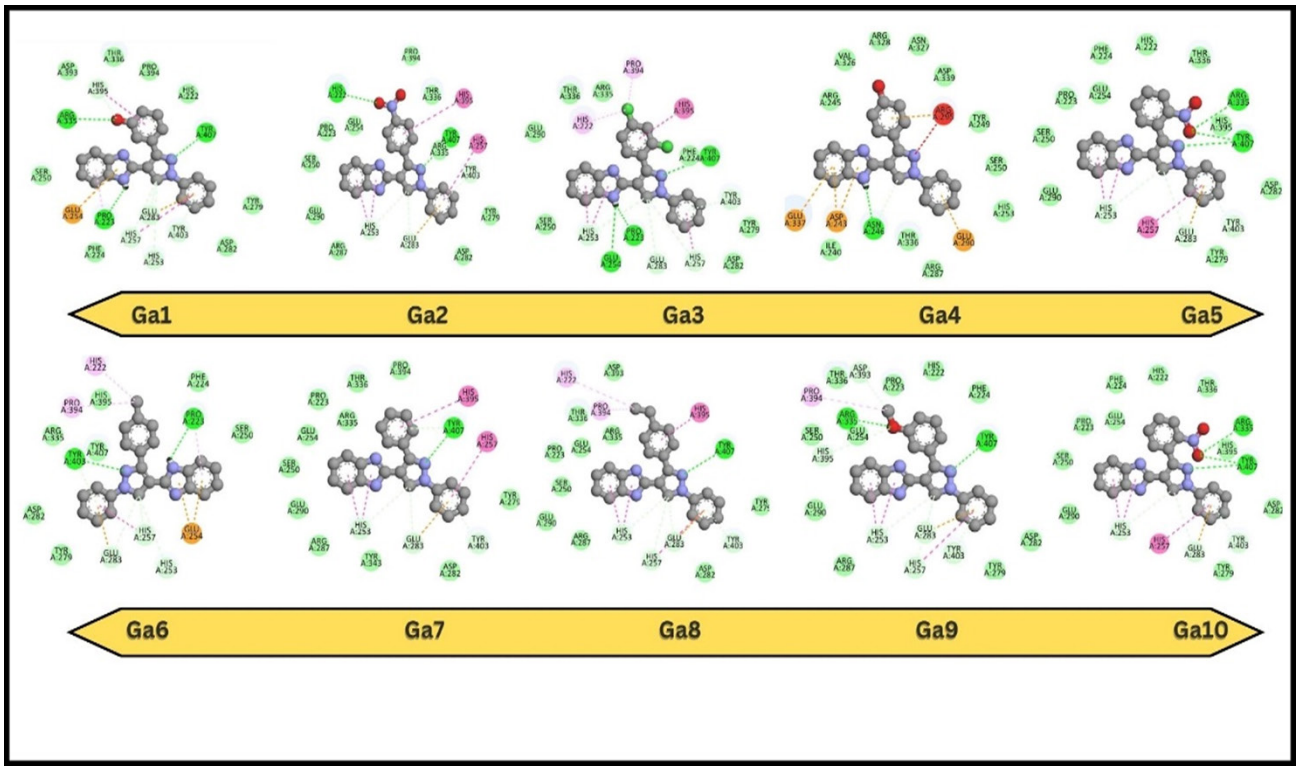

Figure 1: 2D Interactions of Docking.

Table 1: Molecular Docking Results.

Compound code	Binding energy (K.Cal)	Amino acids	Type of interactions
Ga1	-9.1	HIS A: 395 HIS A:257 GLU A:283 HIS A:253 TYR A:403	Vanderwaal's Interaction
		ARG A:335 PRO A:223 TYR A:407	Conventional H-bond
		GLU A:254	π-cation
Ga2	-10.6	HIS A:222 HIS A:253 GLU A:283 TYR A:403 ARG A:335	Vanderwaal's Interaction
		HIS A:222	Conventional H-bond
		HIS A:395 HIS A:257	π-alkyl
Ga3	-10.7	PRO A:394	Alkyl
		GLU A:254 PRO A:223	Conventional H-bond
		HIS A:257 TYR A:403 PHE A:224 GLU A:283 HIS A:253	Vanderwaal's Interaction
		HIS A:395 HIS A:222	π-alkyl
Ga4	-8.4	ASN A:246	Conventional H-bond
		GLU A:290 ARG A:295 GLU A:337 ASP A:243	π-cation
Ga5	-8.5	ARG A:335 TYR A:407	Conventional H-bond
		TYR A:403 GLU A:283 HIS A:253	Vanderwaal's Interaction
		HIS A:257	π-alkyl

Compound code	Binding energy (K.Cal)	Amino acids	Type of interactions
Ga6	-7.4	HIS A:222 PRO A:394	π-alkyl
		GLU A:283 HIS A:257 HIS A:253	Vanderwaal's Interaction
		GLU A:254	π-cation
		PRO A:223 TYR A:403	Conventional H-bond
Ga7	-7.3	HIS A:253 TYR A:403 GLU A:283	Vanderwaal's Interaction
		TYR A:407	Conventional H-bond
		HIS A:395 HIS A:257	π-alky
Ga8	-7.1	HIS A:222 PRO A:394	Alkyl
		HIS A:253 HIS A:257 GLU A:283 TYR A:403	Vanderwaal's Interaction
		TYR A:407	Conventional H-bond
		HIS A:395	Pi-alkyl
Ga9	-7.1	PRO A:394	Alkyl
		HIS A:395 HIS A:253 HIS A:257 TYR A:403 ASP A:393 GLU A:283	Vanderwaal's Interaction
		TYR A:407 ARG A:335	Conventional H-bond
Ga10	-6.8	HIS A:257	π-alkyl
		GLU A:283 TYR A:403 HIS A:253	Vanderwaal's Interaction
		TYR A:407 ARG A:335	Conventional H-bond

Pharmacokinetic Properties

An online tool, the PKCSM toxicity server, is used for predicting pharmacokinetic parameters. The designed compounds were pooled for *in silico* analysis and the best compounds were selected and analysed for the various pharmacokinetics parameters and it was summarized in Table 2.

Molecular Properties of Synthesized Compounds

The molecular properties and potential bioactivity of the selected compounds were calculated using Molinspiration. Key properties assessed includes the log P value for bioavailability, TPSA for absorption, volume, MW, total no. of atoms, Flexible bond, H-bond acceptors and H-bond donors. The results were presented in Table 3.

Table 2: **Pharmacokinetic and Toxicity Prediction.**

Compound code	Absorption		Distribution		Metabolism		Excretion		Toxicity	
Model	Water Solubility	Intestinal absorption	VDss (Human)	Fraction Unbounded	CYP2 D6	CYP 3A4	Total clearance	Renal OCT2 Substrate	Max total clearance	Oral Rat Acute T
Ga1	-2.89	82.47	0.15	0.29	No	Yes	0.68	yes	0.40	2.45
Ga2	-2.89	82.86	0.07	0.31	No	Yes	0.52	yes	0.41	2.47
Ga3	-2.89	82.46	0.14	0.29	No	Yes	0.59	yes	0.40	2.45
Isoniazid	-1.6	92.60	-0.35	0.72	No	No	0.72	No	1.16	2.30

Table 3: **Drug-Likeness Properties of Synthesized Compounds.**

Compound Code	log p	TPSA	N atoms	Mol.wt	Nrotb	Volume	Hydrogen Bond acceptor	Hydrogen Bond Acceptor
Ga1	4.37	66.74	27	352.40	3	312.32	4	2
Ga2	4.83	92.33	23	381.39	4	327.63	5	1
Ga3	6.15	46.51	28	405.29	3	331.37	3	1

Table 4: **Bioactivity of the Synthesized Compounds.**

Compound Code	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
Ga1	0.15	-0.01	0.38	0.11	-0.23	0.08
Ga2	-0.03	-0.10	0.16	-0.12	-0.34	-0.08
Ga3	0.14	-0.04	0.30	-0.03	-0.23	-0.03

Table 5: **Evaluation of Anti-Tubercular Activity in Synthesized Compounds.**

CompoundCode	Conc (µg/mL)							
	100	50	25	12.5	6.25	3.12	1.6	0.8
Ga1	S	S	R	R	R	R	R	R
Ga2	S	S	S	R	R	R	R	R
Ga3	S	S	S	R	R	R	R	R

Table 6: **Antimicrobial activity of the Synthesised compounds**

Compound code	Zone inhibition (mm)						
	Control	Standard				Sample	
		Chloramphenicol		Kanamycin		*S. aureus*	*K. pneumonia*
	Chloroform	*S. aureus*	*K. pneumonia*	*S. aureus*	*K. pneumonia*		
Ga1	10	21	27	13	30	18	19
Ga2	10	27	25	18	28	10	12
Ga3	10	21	27	19	31	11	15

Note: *S. aureus*-Staphylococcus, *K. pneumonia*-Klebsiella.

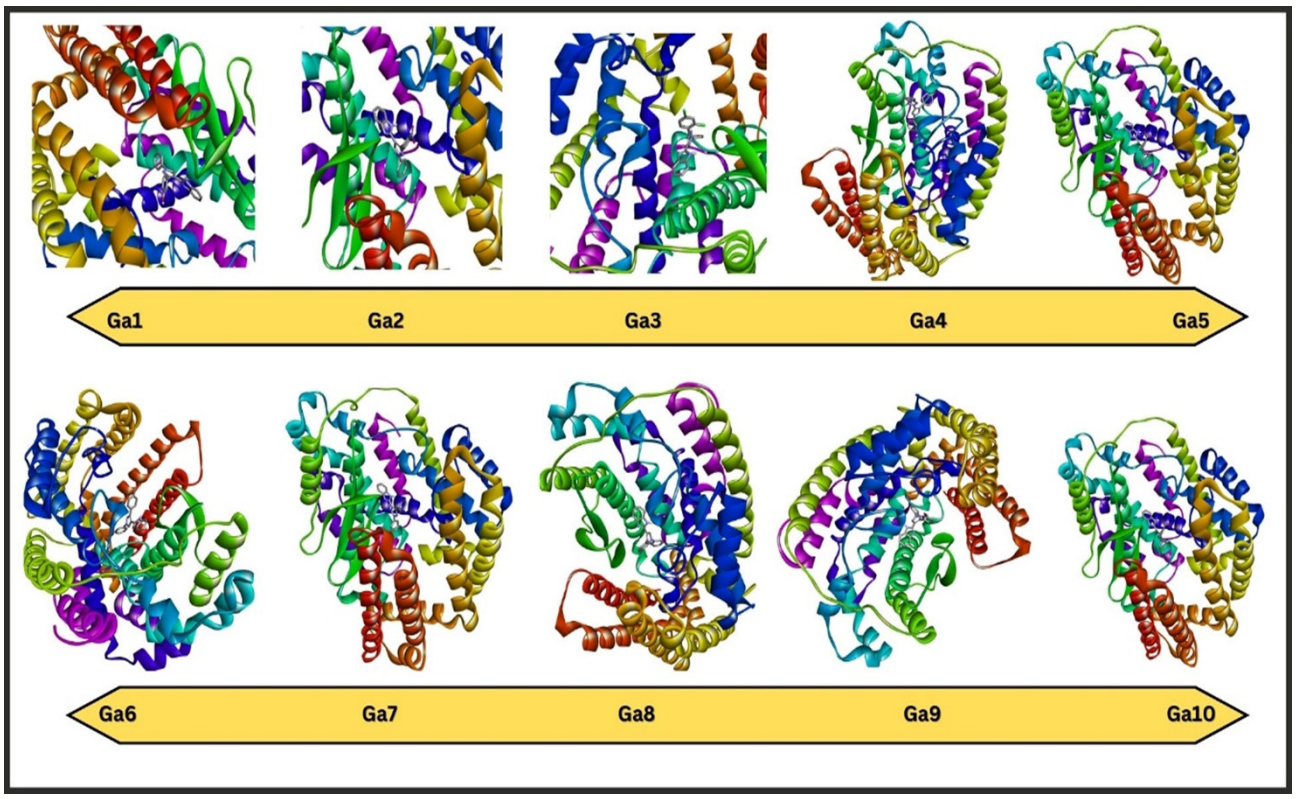

Figure 2: 3D Interaction of Docking.

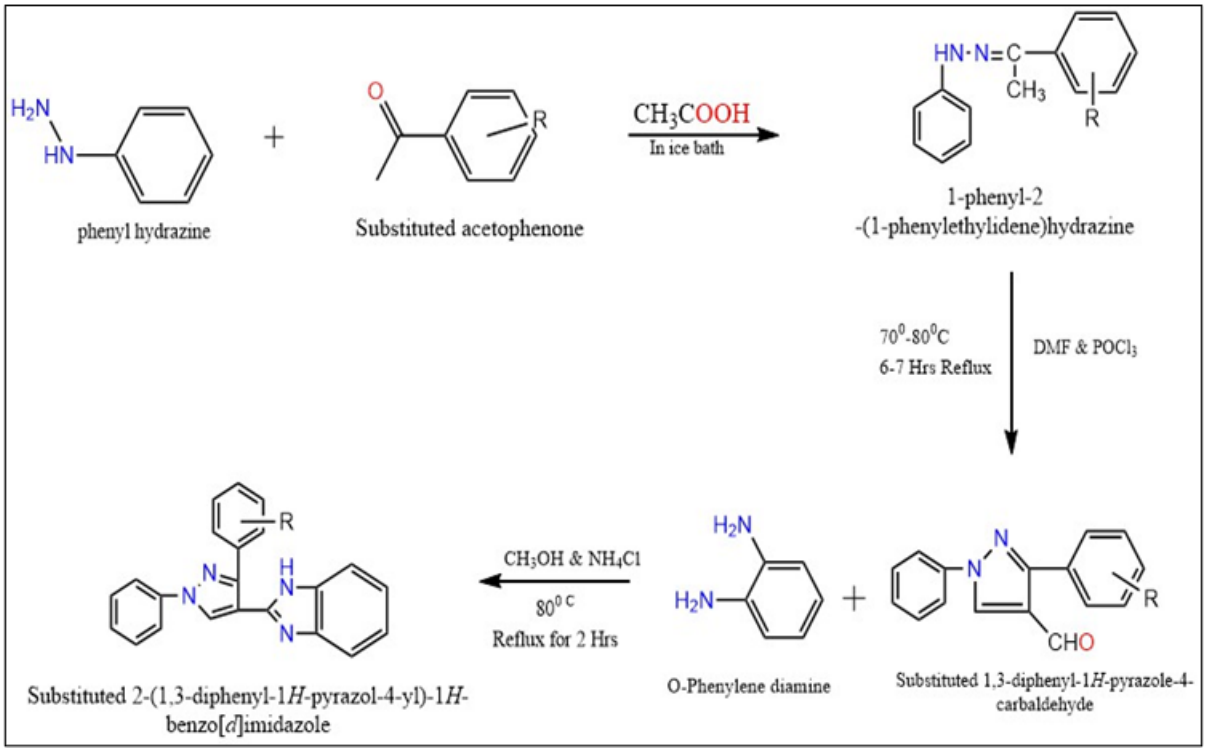

Figure 3: Synthesis Scheme of Substituted Benzimidazole Derivatives.

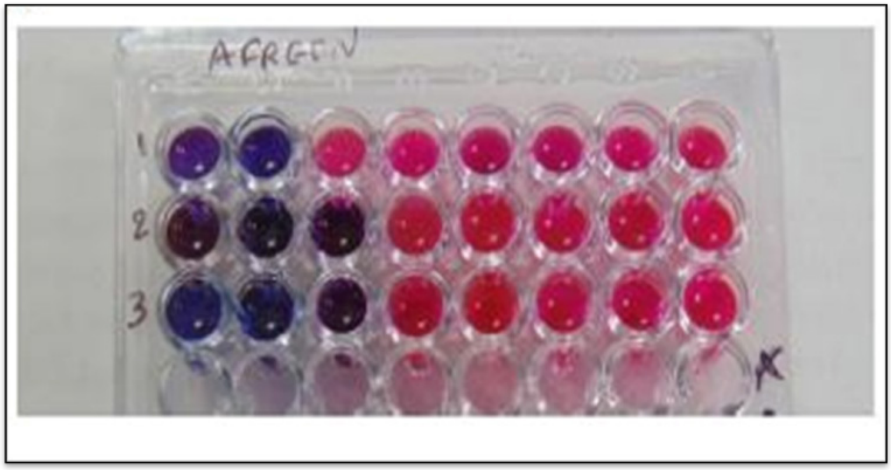

Figure 4: Anti-Tubercular Activity of Synthesized Compounds.

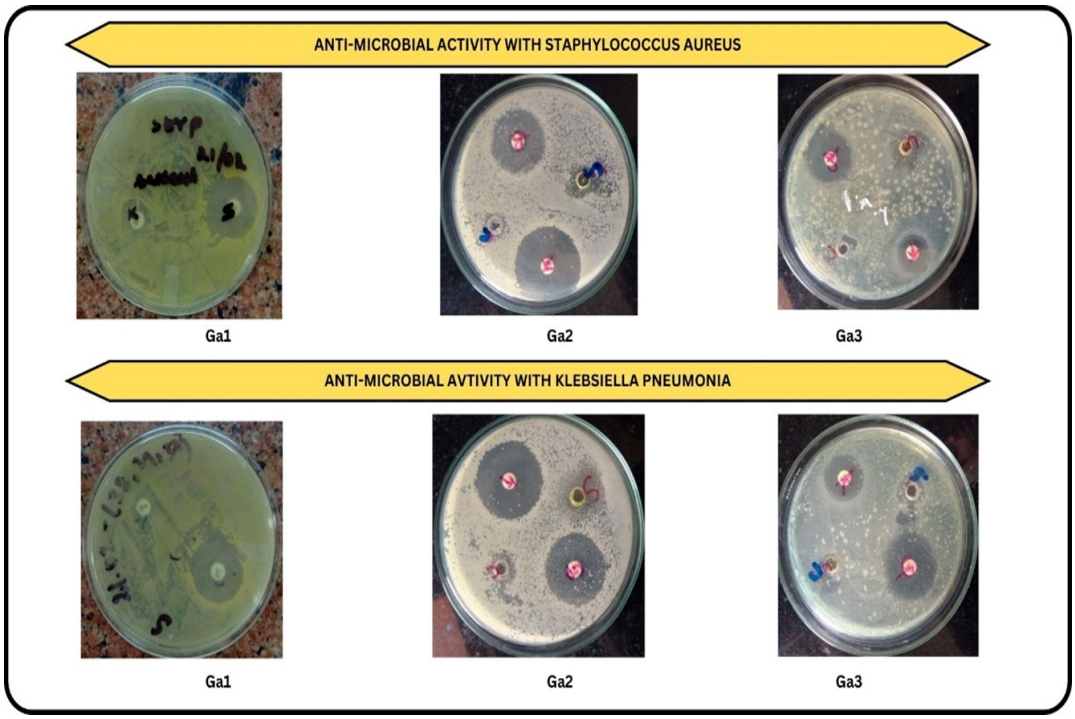

Figure 5: Antimicrobial Activity of Synthesized Compounds against *Staphylococcus aureus, Klebsiella pneumoniae*.

Bioactivity

The molinspiration online tool that were used for accessing the bioactivity of the selected compounds against various receptor types, including ion channels, nuclear ligand receptors, proteases, tyrosine kinase-linked receptors, enzyme inhibitors and G-protein coupled receptors. The results of this assessment were tabulated in Table 4.

Antitubercular Activity by Microplate Alamar Blue Assay test

The MABA test is one of the *in vitro* techniques used to assess the anti-TB activity of the synthesized compounds at concentration between 0.8-100 µg/mL and is presented in Table 5.

Antimicrobial Activity

The agar diffusion method, also known as the disc diffusion antibiotic sensitivity test or Kirby-Bauer test, is used to assess bacterial sensitivity to antibiotics. In this method, antibiotic-impregnated discs are placed on an agar plate inoculated with the bacterial sample (1000 µg) and the plate is then incubated. If an antibiotic inhibits bacterial growth or causes bacterial cell death, a clear area known as the zone of inhibition will appear around the disc, indicating the absence of visible bacterial growth. The findings from this test are compiled and presented in Table 6.

CHARACTERIZATION

3-(4-(1H-benzo[d]imidazol-2-yl)-1-phenyl-1H-pyrazol-3-yl) phenol (Ga1)

Yield 72%, Melting point: 115°C; IR (N-H): 3407, (O-H): 3349, (Benzene C=C): 1442, (Aromatic C-H): 3072; ^{1}H NMR(400 MHz, DMSO):9.22(s, 1H, -OH), 6.82(S, 1H, -CH), 7.27(S, 1H, -CH), 7.40(S, 1H, -CH), 7.14(S, 1H, -CH), 7.28 (D, 2H, -CH), 7.70(D, 2H, -CH), 12.56(S, 1H, -NH), 8.44(S, 1H, -CH), 7.68(D, 2H, -CH), 7.42(D, 2H, -CH), 7.52(S, 1H, -CH). MS (ESI) m/z: 352 [M]+1; Anal. Found C, 74.98%, H 4.58%, N 15.90%, O 4.54% calculated values: C 74.92%, H 4.51% N15.88%, O, 4.44% Molecular formula: $C_{22}H_{16}N_4O$, Molecular weight: 352.40, Nature: Colour of the compound is Pale yellow amorphous.

2-(3-(4-nitrophenyl)-1phenyl-1H-pyrazol-4-yl)-1H-benzo[d]imidazole (Ga2)

Yield 76%, Melting point: 119°C; IR (Aromatic C=C): 3336.25, (N-H): 3432.67, (C-NO_2): 1596, (C-N):1072; ^{1}H NMR(400 MHz, DMSO): 7.50(S, 1H, -CH), 7.36(S,1 H, -CH), 8.03(S, 1H, -CH), 7.70(D, 2H, -CH), 7.28(D, 2H, -CH), 12.56(S, 1H, -NH), 8.44(S, 1H, -CH), 7.68(D, 2H, -CH), 7.42(D, 2H, -CH), 7.52(S, 1H, -CH), MS (ESI) m/z: 381 [M]+1; Anal. Found C, 69.28%, H, 3.96%, N 18.36%, O, 8.39% Calculated values: 69.21%, H, 3.91%, N 18.31%, O, 8.22% Molecular formula: $C_{22}H_{15}N_5O_2$, Molecular weight: 381.40, Nature: Colour of the compound is orange amorphous.

2-(3-(2, 4-dichlorophenyl)-1-phenyl-1H-pyrazol-4yl)-1H-benzo[d]imidazole (Ga3)

Yield 80%, Melting point: 117°C; IR (Aromatic C-H): 3397, (C-Cl): 778, (Aromatic C=C): 1400;^{1}H NMR(400 MHz, DMSO): 8.26 (D, 2H, -CH), 7.97(D, 2H, -CH), 7.70(D, 2H, -CH), 7.28(D, 2H, -CH), 12.56(S, 1H, -NH), 8.44 (S, 1H, NH), 7.68 (D, 2H, -CH), 7.42 S(D, 2H, -CH), 7.52(S, 1H, -CH), MS (ESI) m/z: 405 [M]+1; Anal. Found C, 65.20%, H 3.48%, Cl, 17.49%, N, 13.82% Calculated values: C 65.11%, H 3.42%, Cl, 17.39%, N, 13.79% Molecular formula: $C_{22}H_{14}Cl_2N_4$, Molecular weight: 405.28, Nature: Colour of the compound is yellow amorphous.

DISCUSSION

The compounds were designed in accordance with the literature survey and SAR analysis and subjected to docking among them; two compounds namely Ga2 and Ga3 exhibited the strongest binding to the target protein at -10.6 and -10.7 Kcal. The protein and the ligand were interacted well in diverse, exhibiting strong conventional hydrogen bonding and Pi-alkyl interactions to the core.

Absorption, Distribution, Metabolism, Excretion and Toxicity of the Drugs showed promising characteristics for oral administration. Despite being slightly soluble in water, their high intestinal absorption (82.46%-82.86%) suggests efficient entry into the bloodstream. Additionally, the low volume of distribution (VDss: 0.07-0.15 L/kg) indicates the drug stays mainly in the blood with minimal tissue accumulation. The compounds interact with key metabolic enzymes at varying degrees, potentially impacting their elimination. While the total clearance falls within a moderate range (0.52-0.68 L/min), one case exhibits a prolonged elimination rate (0.41 L/min). Notably, the acute oral toxicity is very high (2.45-2.47 mg/kg), highlighting the potential for severe side effects.[26,27] several compounds possess desirable pharmacokinetic properties, making them strong candidates for further investigation.

The molecular properties of the synthesized compounds showed partition coefficient values ranging from 4.37 to 6.15, indicating moderate lipophilicity and mild hydrophilic in nature that alters the pharmacokinetic and bioavailability. The TPSA values ranges between 46.51 and 92.33 Å^2, suggesting that the compounds vary from moderately to highly polar responsible for bioavailability. The molar mass of the compounds ranges from 352.40 to 405.29 Da. Which is >500 indicating that the compounds are rapidly soluble and cleared by showing better absorption, distribution and overall bioavailability. Molecular volume determines the transport characteristics of the compounds such as intestinal absorption and BBB transport. The value ranges from 331.37 which is slightly deviated from the ideal range but it is within the desirable limits of 100-500. Additionally, the no. of H-bond donors, H-bond acceptors and flexible bonds fall within the acceptable range for orally administered drugs, in accordance to the Lipinski Rule of Five. Generally, the rotatable bonds alters the flexibility of the molecule that greatly affects the interactions being more flexible ultimately leading to reduced bioavailability. It also indicates that the compounds may have difficulty in crossing biological membranes and may be quickly metabolised. However the numbers of rotatable bonds were within the limits indicating good bioavailability. The H bond donors and acceptors that strongly affect the solubility, permeability and oral bioavailability leading to reduced efficacy, despite they were found to be in the acceptable range, hence the compounds shows good bioavailability.

The bioactivity of the compounds has the potential to interact with various cellular components, including G-Protein Coupled Receptors (GPCRs), nuclear receptors, enzymes and ion channels, both as inhibitors and proteases. The observed range of values for GPCR ligands (-0.03 to 0.15) suggests weak interactions, with GPCRs potentially acting as weak antagonists or weak agonists. Similarly, ion channels and nuclear receptors may experience weak modulation or interaction. Kinase inhibition shows a moderate range (0.16 to 0.38), indicating potentially a more pronounced effect. The compounds were also found to have weak inhibitory effects on proteases (-0.23 to -0.34) and minimal influence on general enzyme activity (-0.03 to 0.08).

The synthesis of substituted benzimidazole derivatives was carried out in three steps by conventional technique and the synthetic scheme is depicted in Figure 3. The compounds synthesised were found to have moderate yields ranging from 72-80% and showed distinct melting point in the range of 112-117. The color and nature of the compounds were yellow to orange and amorphous in nature. The Infrared and NMR spectral values showed characteristics absorption spectral values and coupling constant values respectively. The mass spectral values showed the characteristics molecular ion peak for all the compounds.

The compounds was examined using the MABA method[28] with concentrations ranging from 0.8 to 100 µg/mL. The activity image were depicted in Figure 4. Compounds Ga2 and Ga3 were found to be active at 25 µg/mL, while Ga1, Ga2 and Ga3 demonstrated activity at conc between 50 and 100 µg/mL.

The antimicrobial activity[29,30] of the compound Ga1 showed moderate activity exhibiting the zone of inhibition around 18 mm against *S. aureus* and 19 mm against *Klebsiella pneumonia*. With reference to the standard drugs. Compounds Ga2 and Ga3 were less active against *S. aureus* and *Klebsiella pneumoniae*. The results of this antimicrobial study were shown in Figure 5.

CONCLUSION

In this study, a series of benzimidazole derivatives were strategically designed based on computational predictions and subsequently synthesized using conventional synthetic methods. The synthesized compounds were rigorously characterized and optimized through techniques such as melting point determination, Thin-Layer Chromatography (TLC), Infrared (IR) spectroscopy, mass spectrometry, Nuclear Magnetic Resonance (NMR) spectroscopy and elemental analysis, ensuring their structural integrity and purity. Biological evaluation revealed that all synthesized compounds exhibited notable antimicrobial activity. Specifically, compounds Ga2 and Ga3 demonstrated strong inhibitory effects against the *Mycobacterium tuberculosis* H37Rv strain, while compound Ga1 displayed moderate activity. These findings suggest that compounds Ga2 and Ga3 hold significant potential as lead compounds in anti-tubercular drug development.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DMF: Dimethylformamide; **HIV:** Human Immunodeficiency Virus KB; **Testing:** Kirby-Bauer Antibiotic Testing; **MABA:** Microplate Alamar Blue Assay; **ADMET:** Absorption, Distribution, Metabolism, Excretion, Toxicity; **M.tb:** *Mycobacterium tuberculosis;* **MDR-TB:** Multidrug-resistant Tuberculosis; **Pks 13:** Polyketide Synthase 13; **SAR:** Structure-Activity Relationship; **CADD:** Computer-aided Drug Design; **TDR-TB:** Total Drug-resistant Tuberculosis; **TPSA:** Topological Polar Surface Area; **TLC:** Thin Layer Chromatography; **XDR-TB:** Extensively Drug-resistant Tuberculosis; **TB:** Tuberculosis; **MW:** Molecular weight; **H-BOND:** Hydrogen bond; **Conc:** Concentration.

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