Int. j. adv. multidisc. res. stud. 2025; 5(3):1534-1541

Received: 01-05-2025
Accepted: 11-06-2025

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

DprE1 Enzyme a Potential Target for Treating Tuberculosis: A Drug Repurposing Approach

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DOI: https://doi.org/10.62225/2583049X.2025.5.3.4488
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Abstract

Tuberculosis (TB), a serious infectious disease caused by *Mycobacterium tuberculosis*, which is challenging to treat due to its complex cell wall which is essential for bacterial survival and its resistance towards anti-tb drugs. The enzyme DprE1, crucial for mycolic acid synthesis in their cell wall, is a promising drug target. This study used an in-silico drug repurposing approach, screening 7,647 FDA-approved drugs and phytochemicals against DprE1. The DprE1 structure was refined via I-TASSER and YASARA, and validated with ProSA and PDBsum. Molecular docking with AutoDock Vina identified 11 compounds with strong binding affinities (≤ –18.8 kcal/mol), outperforming the reference inhibitor BTZ (–10.05 kcal/mol). Amphotericin B showed the highest binding affinity (–21.34 kcal/mol) and stable interactions in 100.1 ns MD simulations. Other promising candidates included Nystatin, Rifapentine, and Eltrombopag. In-vitro and in-vivo analyses further supported their potential highlighting the value of drug repurposing in accelerating TB drug discovery targeting DprE1.

Keywords: *Mycobacterium Tuberculosis* (Mtb), Multi Drug Resistance (MDR), DprE1 (Decaprenyl-phosphoryl-β-D-ribose-2′ epimerase), Tuberculosis (TB), Molecular Docking, Molecular Dynamics (MD), Arabinogalactan (AG), Lipoarabinomannan (LAM)

1. Introduction

Tuberculosis (TB) remains one of the deadliest infectious diseases worldwide, primarily caused by *Mycobacterium tuberculosis* (Mtb), a specialized human pathogen. TB primarily affects the lungs which is termed as pulmonary TB, but can also affect other organs such as the spine, lymph nodes, kidneys, and brain, termed extrapulmonary TB. It is classified into latent (non-contagious) and active (contagious) TB based on clinical presentation [2, 3]. Latent infections may persist asymptomatically for years and often reactivate under conditions such as poverty, malnutrition, or co-infection with HIV/AIDS. Although transdermal and gastrointestinal routes of transmission have also been observed, inhaling airborne droplets remains the primary route for TB infection [4].

Despite the availability of effective anti-TB regimens for decades, TB continues to pose a major global health threat. First-line treatment regimens include isoniazid (INH), rifampicin (RIF), ethambutol, and pyrazinamide. While these therapies have

historically been effective, the emergence of drug-resistant strains, including MDR-TB and extensively drug-resistant TB (XDR-TB), is severely challenging the global TB control efforts [1]. According to the World Health Organization (WHO) 2024 report, TB was reported in 193 out of 215 countries and regions, with over 10 million new cases and more than 1.6 million deaths annually. The emergence of MDR-TB, defined by resistance to at least isoniazid and rifampicin, accounts for a significant portion of these cases and presents an urgent public health challenge. Rifampicin-resistant or MDR-TB accounted for approximately 558,000 cases globally in 2017, with no substantial decline reported through 2022 [11].

The development of drug resistance in Mtb occurs because of multiple reasons, originating from its unique physiology, including slow metabolic rate, strong efflux mechanisms, an impermeable lipid-rich cell wall, and poor drug penetration [5, 7]. Additional factors such as patient non-compliance, inadequate treatment regimens, and comorbidities like diabetes further contribute to resistance. These challenges highlight the pressing need for the discovery of new drugs targeting novel mechanisms essential for Mtb survival [3, 12].

Mtb's cell wall is an attractive target for drug development due to its critical role in pathogen survival and resistance. The complex, waxy, and lipid-rich nature of the cell wall not only provides resistance to host immune responses and antibiotics but also plays a key role in virulence [13]. Its unique components, such as mycolic acids and arabinan, are synthesized by specific enzymes that have been identified as promising drug targets. In recent years, whole-cell screening has led to the identification of several candidate drug families targeting essential cell wall synthesis enzymes like MmpL3 and decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1) [14, 15].

DprE1, in particular, has emerged as a vital therapeutic target in TB drug discovery. It is a flavoenzyme involved in the biosynthesis of arabinogalactan (AG) and lipoarabinomannan (LAM), essential components of the Mtb cell wall. The enzyme catalyzes a two-step epimerization reaction: Converting decaprenyl-phosphoribose (DPR) to decaprenyl-phospho-2-keto-D-arabinose (DPX) and subsequently to decaprenyl-phospho-D-arabinose (DPA) with the assistance of DprE2. DPA is the sole precursor for AG and LAM, and the absence of alternative biosynthetic pathways underscores the indispensable nature of DprE1 for Mtb viability [16].

Structurally, DprE1 comprises two domains; a substrate-binding domain (residues 197–412) and a cofactor-binding domain (residues 7–196 and 413–461). It is localized on the cytoplasmic membrane, rendering it surface-accessible and favorable for inhibitor binding. The active site contains critical nucleophilic residues that are essential for its enzymatic activity and serve as potential anchoring points for drug design. Importantly, the highly conserved nature of DprE1 among Mtb strains enhances its appeal as a broad-spectrum drug target [14, 16].

Given its essential role and accessibility, DprE1 has been regarded as a "magic drug" target in TB therapy. Several candidate molecules targeting DprE1 have demonstrated promising results *in-vitro* and *in-vivo*, validating the approach of disrupting cell wall biosynthesis to combat TB. However, the rapid emergence of resistance even against novel agents requires continuous efforts to identify and validate novel inhibitors [17].

To address these challenges, the present study focuses on evaluating FDA-approved drugs for their potential to inhibit DprE1. Drug repurposing offers a time- and cost-efficient strategy for developing new TB therapies, particularly in light of the high expense and lengthy timelines associated with conventional drug development. Molecular docking, a widely used computational technique, was employed to predict the binding affinity and interaction patterns of selected compounds with the DprE1 enzyme. This approach helps prioritize candidates with strong binding potential and guides further experimental validation.

2. Methods

2.1 Software Configuration

Virtual screening and molecular dynamics (MD) simulations were conducted using Discovery Studio Visualizer (DSV), AutoDock Vina, and Schrodinger's Desmond v12. All computational work was carried out on a workstation running Ubuntu 22.10, equipped with an Intel® Core™ i7-14700 processor and an RTX™ 4060 GPU.

2.2 Protein Validation

The crystal structure of DprE1 (PDB ID: 4P8K) was retrieved from the RCSB Protein Data Bank. Structural refinement was performed using the I-TASSER server. The refined model was energy minimized using the YASARA server. Validation was carried out using ProSA (for Z-score) and PDBsum (Ramachandran plot) to assess the structural quality.

2.3 Ligand Preparation

A total of 7,647 compounds comprising FDA-approved drugs, phytochemicals, and small molecules were downloaded from DrugBank in .sdf format. The ligands were converted to 3D structures using RDKit and optimized with the MMFF94 force field. Structures were then converted to .pdbqt format using OpenBabel for compatibility with AutoDock Vina.

2.4 Docking Studies

Using DSV, the protein structure was pre-processed by removing water molecules, heteroatoms, and co-crystallized ligands. The binding pocket was defined based on known active site residues. AutoDock Tools 1.5.6 was used to add polar hydrogens, assign Kollman charges, and generate the .pdbqt format of the protein. AutoDock Vina was employed for docking using a grid box centered on the active site with dimensions of 21 × -11 × 0.3 Å and 1 Å spacing. Batch docking was performed for all ligands to evaluate their binding affinities.

2.5 Molecular Dynamic Simulations

Top-ranked protein-ligand complexes were subjected to MD simulations using Desmond. Simulations were conducted for 100.1 ns to assess complex stability and interactions. System setup included solvation, ionization, and NPT ensemble settings.

3. Results

3.1 Protein Structure Validation and Energy Minimization

The DprE1 protein (Figure 1) consists of 480 amino acids and functions as a homodimer associated with FAD. Energy minimization reduced the total energy from -187,321.3

kJ/mol to -27,241.1 kJ/mol, indicating a more stable conformation. Post-minimization validation revealed 74.9% of residues in the most favoured regions of the Ramachandran plot (PDBsum) as shown in Figure 2. ProSA analysis returned a Z-score of -9.68, confirming good model quality as shown in Table 1 and Figure 2.

Table 1: Protein structure validation and energy minimization results

Itasser ID	C-score	start energy	end energy	PDBsum				ProSA Z-score
				MA	GA	AA	DA	
S804542	-0.42	-187321.3	-27241.1	74.90%	3.20%	20.40%	1.50%	-9.68%

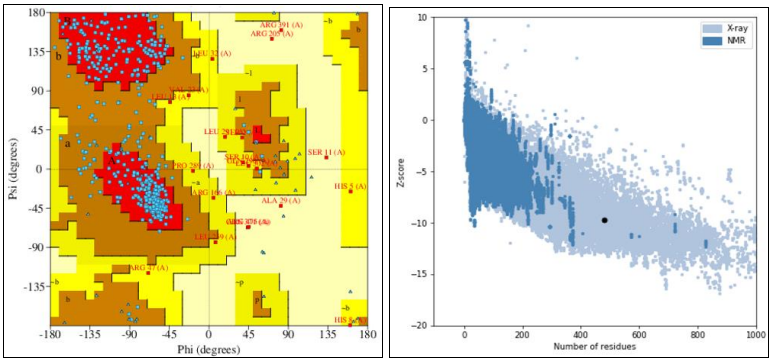
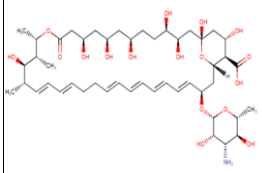
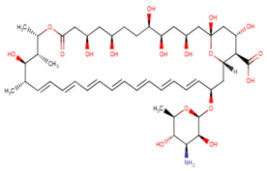
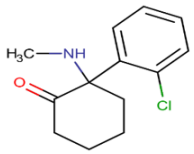
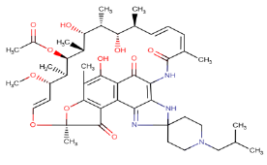
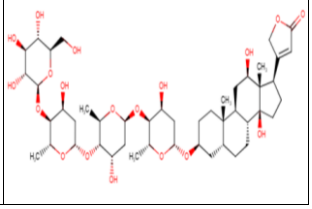
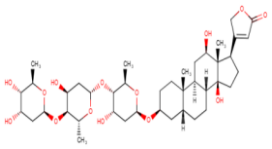
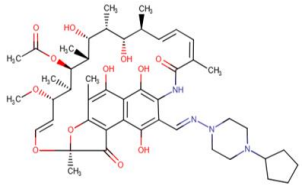
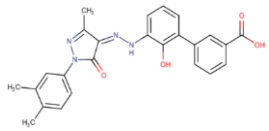
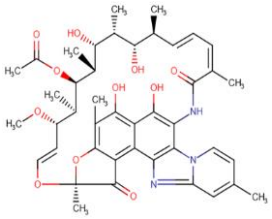
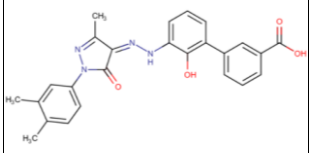
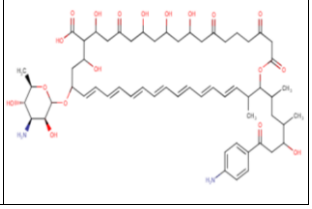


Fig 2: (A) Ramachandran plot of target protein Dpre-1 obtained from PDBsum server. **(B)** ProSA graph of the target protein generated by ProSA server

Table 2: Molecular Docking Results of top 11 molecules

S. No.	Drugbank ID	Name	Binding affinity Score kcal/mol	Structure
1	DB00646	Nystatin	-21.340	
2	DB00681	Amphotericin B	-21.062	
3	DB01221	Ketamine	-20.570	
4	DB00615	Rifabutin	-20.318	
5	DB01078	Deslanoside	-19.495	

6	DB00390	Digoxin	-19.288	
7	DB01201	Rifapentine	-19.124	
8	DB06210	Eltrombopag	-19.022	
9	DB01220	Rifaximin	-18.877	
10	DB12513	Omaveloxolone	-18.886	
11	DB01152	Candicidin	-18.836	

3.2 Ligand Processing

All 7,647 ligands were successfully converted to 3D format and optimized. Structures were processed and converted into .pdbqt file format for virtual screening.

3.3 Docking Outcomes

Eleven top molecules with high binding affinities (≤ -18.8 kcal/mol) were shortlisted (Table 2). Nystatin showed the strongest binding (-21.34 kcal/mol), followed by Amphotericin B (-21.06 kcal/mol), and Ketamine (-20.57 kcal/mol) (Table 2). All selected compounds showed better binding than the reference compound BTZ (-10.05 kcal/mol).

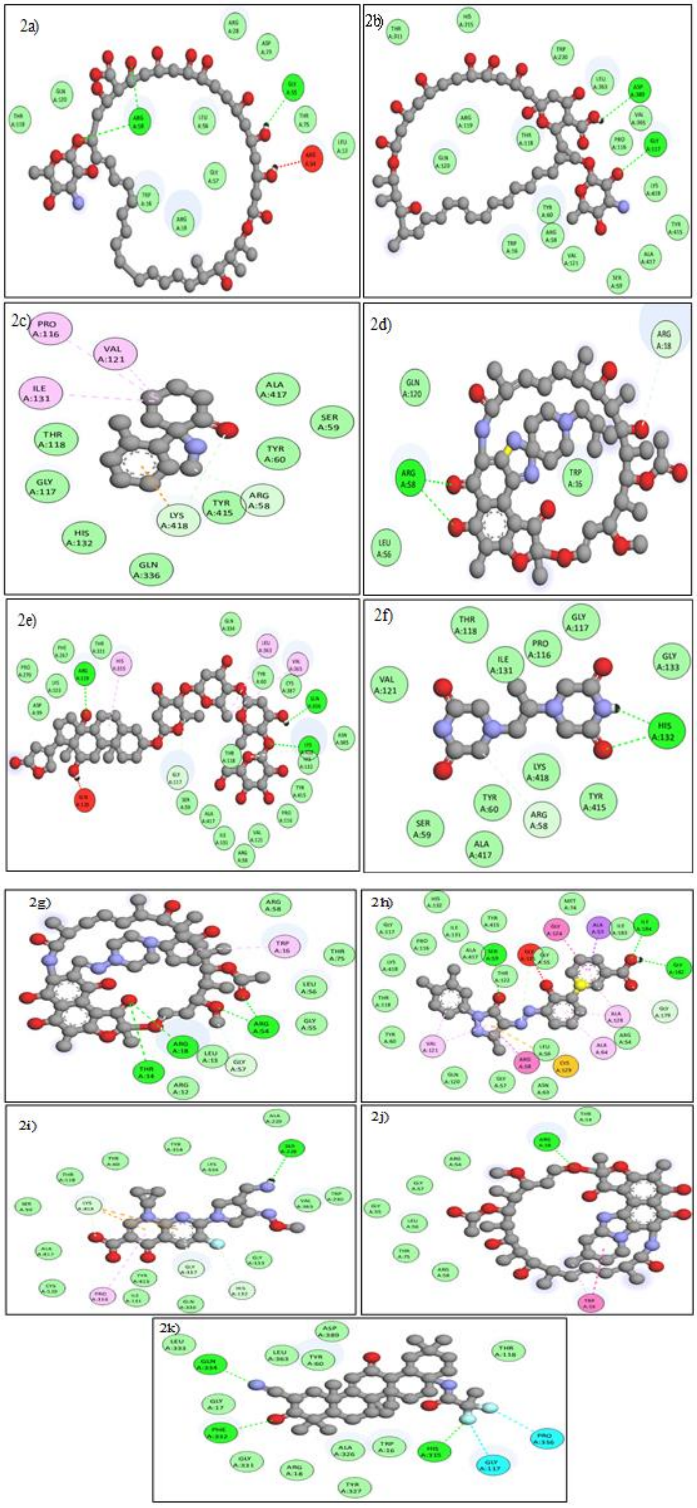


Figure 2: Molecular interactions of Docking Study. Molecular interaction studies of receptor Dpre-1 with top 11 molecules (Table 2). Here -Hydrogen bonds, -Carbon-hydrogen bond, -van der waals interactions, -pi-sulfur, -halogen, -Amide-pi stacked, -pi-alkyl, -pi-sigma, -unfavorable bump.

3.4 MD Simulation Analysis

MD simulations of DprE1 with 11 ligands for 100.1ns revealed that amphotericin B demonstrated the most stable and consistent binding. As indicated by low ligand RMSD values (<2.0 Å) and reduced RMSF in binding site regions as shown in Figure 7b. It maintained strong, persistent interaction with minimal structural perturbation. In contrast, ligands such as rifabutin (figure 7d), nystatin (figure 7a), rifapentine (Figure 7g), and eltrombopag (Figure 7h) showed higher RMSD and elevated RMSF values, suggesting weaker or transient binding. Overall, the results highlighted that amphotericin B showed a favorable dynamic behaviour, supporting its prioritization for further drug development studies targeting DprE1 (Figure 7).

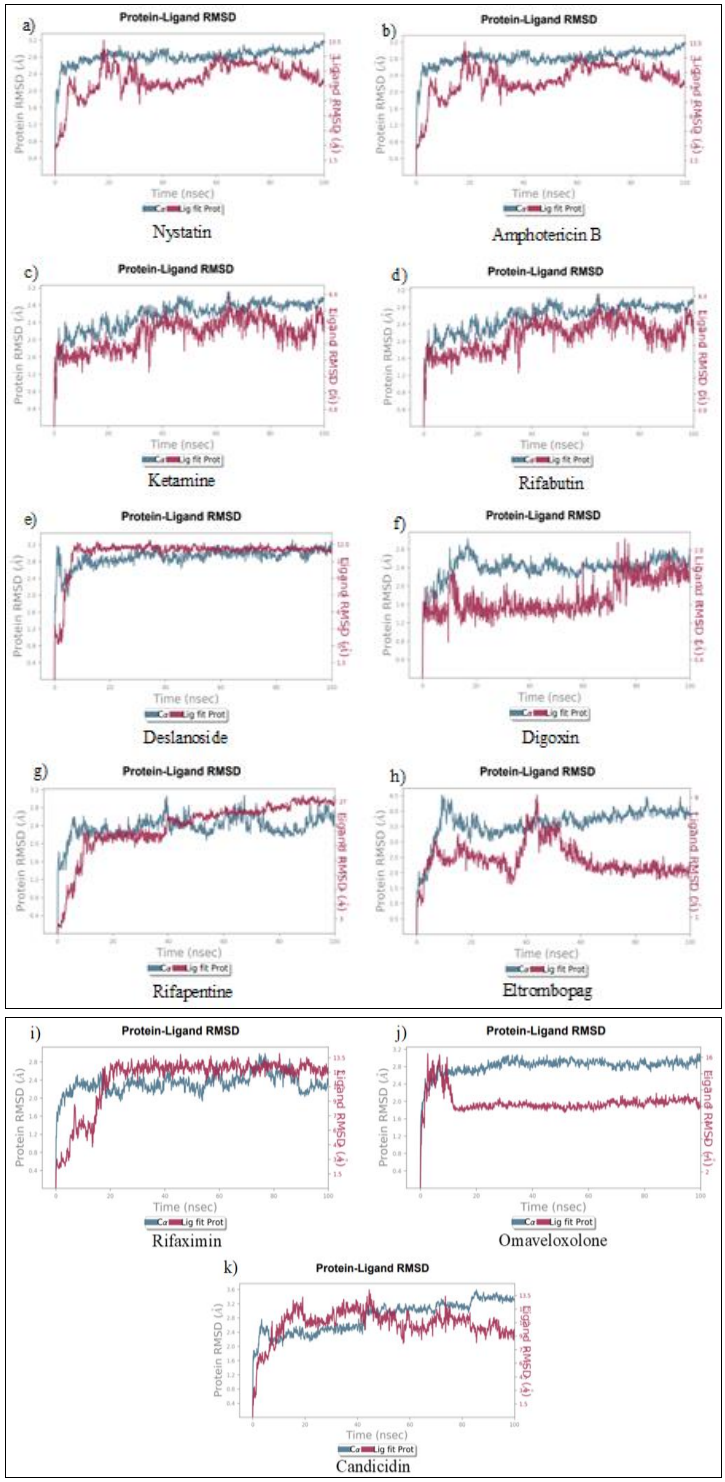


Fig 3: Molecular-Dynamics interaction studies. Root Mean Square Deviation (RMSD) plot for 11 complexes with the target protein Dpre-1 at 100.1 ns of MD simulation. The receptor is represented on the plot by blue colour and ligand is represented in red colour

4. Discussion

In the current landscape of drug development, *in-silico* drug discovery plays a pivotal role by offering a cost-effective and time-efficient alternative to traditional experimental methods. Screening thousands of compounds for potential therapeutic use through conventional means is often prohibitively expensive and time-consuming. One promising strategy is drug repurposing, which involves identifying new therapeutic applications for existing drugs. Computational methods significantly streamline this process by enabling virtual screening and molecular interaction studies with high precision and speed [17].

This study employed a structure-based drug design approach to explore the interaction dynamics between the *Mycobacterium tuberculosis* target protein DprE1 and various FDA-approved drugs. Molecular docking and dynamics simulations were used to understand the binding affinities and stabilities of these compounds at the atomic level. Given DprE1's vital role in mycobacterial cell wall biosynthesis, understanding its binding behavior is essential for the rational design of new anti-TB agents.

Previous studies, especially those involving the inhibitor BTZ (Benzothiazinone), have highlighted the potential of DprE1 as a drug target [20]. MD simulations in earlier work showed that the DprE1-BTZ complex reached equilibrium

around 3 ns during a 50 ns simulation, indicating stable binding. In the present study, MD simulations were extended to 100.1 ns to evaluate the long-term stability of newly identified drug candidates. The majority of these compounds exhibited stable RMSD values between 1.5 Å and 3.0 Å, with many maintaining binding fluctuations within 1.8 Å to 2.3 Å, reflecting improved and more sustained stability over a longer time frame than observed with BTZ.

Docking results further validated these findings. While BTZ showed a binding energy of -11.49 kcal/mol, all selected drugs from the screened library of 7647 compounds demonstrated significantly lower binding energies, ranging from -18.836 to -21.340 kcal/mol. This suggests stronger binding affinities and enhanced interaction potential compared to BTZ, underscoring their promise as effective DprE1 inhibitors.

Hydrogen bonding is a key factor in ligand-protein interaction, often correlating with binding specificity and stability. BTZ was previously shown to form only three hydrogen bonds with DprE1, primarily involving residues such as Lys134, Gln336, and Cys387. In contrast, several of the top compounds in this study—Nystatin, Eltrombopag, Candicidin, Rifapentine, and Amphotericin B—formed multiple hydrogen bonds with the important DprE1 residues, further supporting their strong binding profiles.

In addition to known binding residues, several new amino acids were identified as contributors to ligand interaction. While Gly55, Gly177, Gly182, Asp389, Gln336, Gln334, Tyr60, Thr14, and Lys418 were common binding residues shared between BTZ and the new compounds, previously unreported residues such as Pro316, Phe332, His132, Arg58, Ser59, and Gly288 were also involved in the interaction networks of the top candidates. The identification of these novel contact points suggests potential additional anchoring sites within the DprE1 active pocket, which may be exploited for developing next-generation anti-TB therapeutics with improved specificity and efficacy.

Among all tested compounds, Amphotericin B emerged as a particularly promising candidate. It not only demonstrated the lowest binding energy but also maintained excellent RMSD stability throughout the simulation period. These characteristics strongly suggest that Amphotericin B could serve as a potent DprE1 inhibitor, meriting further experimental validation through in-vitro and in-vivo studies.

5. Conclusions

This study highlights the potential of drug repurposing through in-silico methods to identify new treatments for tuberculosis, especially in the face of rising drug resistance. By targeting the essential enzyme DprE1, which plays a key role in the cell wall synthesis of *Mycobacterium tuberculosis*, we aimed to discover effective inhibitors from a library of already-approved FDA drugs.

Through molecular docking and molecular dynamics (MD) simulations, we identified several compounds with stronger binding affinities and more stable interactions with DprE1 than the previously studied reference drug, BTZ. In particular, Amphotericin B showed the strongest binding energy and maintained stable behavior throughout a 100.1 ns simulation, suggesting it could be a highly effective inhibitor of DprE1. Other compounds such as Nystatin, Rifapentine, and Eltrombopag also demonstrated promising results.

Additionally, our study identified several new amino acid residues within the DprE1 active site that were involved in binding with the selected compounds. These findings open new avenues for the design of more targeted and potent anti-TB drugs.

In summary, this *in-silico* approach has not only identified promising drug candidates for TB treatment but also expanded our understanding of key interactions within the DprE1 enzyme. These findings warrant further experimental validation and could contribute to the development of new, more effective therapies against tuberculosis.

6. Limitations

Although molecular docking and dynamics simulations offer valuable predictions, they are based on theoretical models and do not fully capture the complexity of biological systems. Consequently, the calculated binding energies may not directly correlate with actual inhibitory potency, suggesting the requirement of biochemical validation.

7. Recommendation

To build upon these findings, *in-vitro* assays such as enzyme inhibition and minimum inhibitory concentration (MIC) tests should be conducted to validate the efficacy of shortlisted compounds. *In-vivo* studies in suitable TB models are also recommended to assess pharmacokinetics, toxicity, and therapeutic potential. Further exploration of the newly identified DprE1-binding residues could assist in designing novel inhibitors with enhanced specificity. Finally, integrating ADME/Tox analysis and exploring combination therapies with existing TB drugs may improve clinical relevance and therapeutic outcomes.

8. Acknowledgement

I would like to express my sincere gratitude to Dr. Virupaksha A. Bastikar for conceiving the research idea and providing invaluable supervisory guidance throughout the project. I am also thankful to Mr. Abhijit Bhatkal for his essential contributions in guiding the research, analyzing the data, and providing valuable support in the preparation of the manuscript. My appreciation extends to Mr. Deepanshu Garg and Mr. Ayanjeet Chowdhury for their technical expertise in conducting Molecular Dynamics simulations and supporting the data analysis. I would also take this moment to thank Dr. Anant D. Kulkarni for his critical support based on his years of experience in Computational Simulations through this period & also take this opportunity to acknowledge Centre of Excellence (CoE) in Computational Science and Simulations (C2S2), Somaiya Vidyavihar University, for providing me with technical & systemic support. I would also like to thank Dr. Ramesh Paranjape and Dr. Laxmi Deshpande for their valuable suggestions and consistent support throughout the project. Finally, we acknowledge the collaborative efforts of all contributors and thank the individuals and institutions who indirectly supported this study.

9. Financial support and sponsorship

Nil.

10. Conflicts of interest

There are no conflicts of interest.

11. References

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