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Repurposing FDA-Approved Drugs as Potential Inhibitors of MbtB for Tuberculosis Therapy †

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Abstract

Tuberculosis (TB) remains a major global health concern, underscoring the urgent need for innovative therapeutic approaches. In this study, we aimed to identify potential inhibitors of phenyloxazoline synthase MbtB, an essential enzyme involved in the iron acquisition pathway of *Mycobacterium tuberculosis* and a promising target for drug development. To this end, a curated library of FDA-approved drugs from the ZINC database was systematically screened to uncover compounds with potential inhibitory activity against MbtB.Multiple conformations of the substrate's transition state structure were utilized as query models. Conformational ensembles for both the query molecules and compounds within the FDA-approved drug library were generated using Balloon (v1.8.2). Virtual screening was then conducted using ShaEP (v1.4.0), which evaluates shape and electrostatic potential similarity, resulting in the identification of several promising candidate inhibitors.Molecular dynamics simulation was performed to understand molecular-level interaction between the top 5 hits and the target protein. Top hits will be procured, testing their anti-TB activity in ion-rich and ion-deprived media, as reported in earlier publications.

Keywords: MbtB inhibitor; tuberculosis; FDA-approved drugs; molecular dynamics,

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1. Introduction

The deadly organism Mycobacterium tuberculosis (Mtb) is the cause of tuberculosis (TB), an infectious disease that has plagued humans for generations. This bacterium has now spread to become a global epidemic that is causing devastation all across the planet [1]. According to a 2021 study published by the World Health Organization (WHO), TB had a devastating effect on the mortality and morbidity rates worldwide in 2021, killing 1.6 million people (including 187,000 HIV-positive individuals) and affecting over 10.6 million people [2]. According to the most recent estimations from the WHO, one in four people has an active TB infection. Mtb is a serious bacterial infection that mainly affects the lungs, however it can spread to other body areas as well. There are difficulties in treating TB infection because of its capacity to switch between respiring and non-respiring situations without losing vitality due to different enzymatic processes [3]. As a worldwide health concern, it necessitates better living circumstances, easier access to medical care, and greater awareness and prevention initiatives to stop its spread.

The scientific community is now primarily focused on drug research and development due to the rise of infections worldwide. The discovery of p-amino salicylic acid in 1943, streptomycin in 1944, isoniazid, and pyrazinamide in 1952 marked the beginning of the development of antitubercular drugs. Rifampicin (1963), ethambutol (1961). The absence of global funding, resistive old drug targets, unviable new drug targets, and the failure of clinical trials of innovative pharmaceuticals were the main causes of the forty-year research halt that followed. A few clinically approved medications were introduced in an attempt to lessen the horrors connected to infectious tuberculosis: Bedaquiline (2012), Delamanid (2014), and Pretomanid (2019). [4] To date, these medications remain the sole option for treating drug-resistant tuberculosis. Due to the rise in many resistance cases, especially DR-TB, MDR-TB, XDR-TB, and TDR-TB cases, TB has recently turned into an epidemic [5,6]. These incidences of medication resistance appear to be possibly curable with current treatments. Furthermore, the current situation in combating tuberculosis has been worse due to the co-occurrence of illnesses both before and during the COVID era [7–9]. These elements make it necessary to look for new antitubercular drugs that target new targets through new mechanisms of action. This may pave the way for addressing drug resistance.

2. Materials and Methods

2.1. System Specifications and Software Employed

A workstation running Ubuntu 22.04 LTS (64-bit) with an Intel® Core™ i5-12400 CPU operating at 2.30 GHz, 16 GB of RAM, and an 8 GB Nvidia GeForce RTX 3050 GPU was used to execute molecular simulations. The ZINC database was used to download the FDA-approved drug library (fda.smi). AlphaFold and the Protein Data Bank provided the MbtA protein structure. AutoDock-GPU on Google Colab was used for molecular docking and virtual screening, while internal Python scripts were used to generate ligand conformers and docking summaries. AutoDock 4.2.6 was used to build protein–ligand complexes, and GROMACS 2022.4 was used to perform molecular dynamics simulations. LigPlot+ and PyMOL were used for visualization.

2.2. Ligand Preparation

Using Open Babel v2.4.0, the FDA-approved ligand set (fda.smi) from the ZINC database was transformed into separate.pdb files for virtual screening. The ligands were produced with the proper bond ordering and hydrogens, energy-minimized, and loaded into AutoDock-GPU on Google Colab. For docking investigations, the best conformations were stored as.pdbqt files.

2.3. Protein Preparation

The AlphaFold database provided the structure of Mycobacterium tuberculosis H37Rv's Phenyloxazoline Synthetase (MbtB). The active site was identified with the use of PDB-BLAST, which revealed sequence similarities with DhbE from Bacillus subtilis and MbtB from Mycobacterium smegmatis. Water molecules were eliminated, polar hydrogens were added, non-polar hydrogens were merged, AD4 atom types were assigned, and Gasteiger charges were added as part of the protein preparation process in AutoDock 4.2.6 (MGLTools 1.5.6). The protein with the reduced structure was preserved. pdbqt for docking.

2.4. Identification of Binding Site and Receptor Grid Generation

The active site was identified by aligning MbtB with the ligand-bound allowing comparison of conserved residues. A grid box was generated around the co-crystallized ligand's centroid, with receptor atom parameters set to a 1.00 Å van der Waals radius and 0.25 partial charge. The protein.gpf input file was created, and AutoGrid produced the protein.glg output, providing grid coordinates used for virtual screening (Table 1).

Table 1. Specifics of the grid parameter data utilized.

AutoDock 4.2.6							
Protein	Center grid box dimensions (Å)			Spacing (Å)	Coordinates for the center of the grid box		
	X-axis	Y-axis	Z-axis	0.375	X-axis	Y-axis	Z-axis
MbtB	50	50	50		−2.239	19.515	32.895

2.5. Docking-Based Virtual Screening Studies

By re-docking the co-crystallized ligand into the MbtB active site and computing RMSD, docking validation was carried out. The FDA-approved compounds (fda.smi) were then virtually screened using the same grid parameters in AutoDock-GPU (Google Colab). 200 runs for dependable sampling, 27,000 generations, 2,500,000 energy evaluations, and a population size of 150 were among the docking parameters. LigPlot+ v2.2 and PyMOL were used to view docked complexes.

2.6. Molecular Dynamics Studies

Molecular dynamics simulations were performed in GROMACS 2022.4 (GPU, single precision) using the CHARMM force field. The MbtB structure was prepared by removing water, adding hydrogens, and energy minimizing (steepest descent, 5 ns). The system was solvated with TIP3P water in a cubic box (10 Å edge), neutralized with counterions, and simulated under NVT and NPT ensembles at 300 K and 1 atm, with PME for electrostatics (12 Å cutoff). Simulations ran for 300 ns, saving data every 10 ps. Outputs (md300.xtc, md300.tpr, md300.edr) were used to calculate RMSD, RMSF, Rg, and SASA, with a stable frame selected for detailed H-bond interaction analysis.

2.7. Principal Component Analysis (PCA)

MD trajectories were subjected to Principal Component Analysis (PCA), also referred to as Essential Dynamics (ED), in order to detect dominating protein movements. By breaking down conformational changes, this technique lowers dimensionality and gives priority to large-scale motions, which frequently have the most biological significance.

3. Results

3.1. Molecular Docking Studies on MbtA

3.1.1. Validation of Docking Procedure

With a binding energy of −6.41 kcal/mol, a Ki of 20.06 μM, and an RMSD of 1.52 Å, redocking of MbtB demonstrated satisfactory stability for a small globular protein. The overlay of the co-crystallized and docked ligand conformations is displayed in Figure 1.

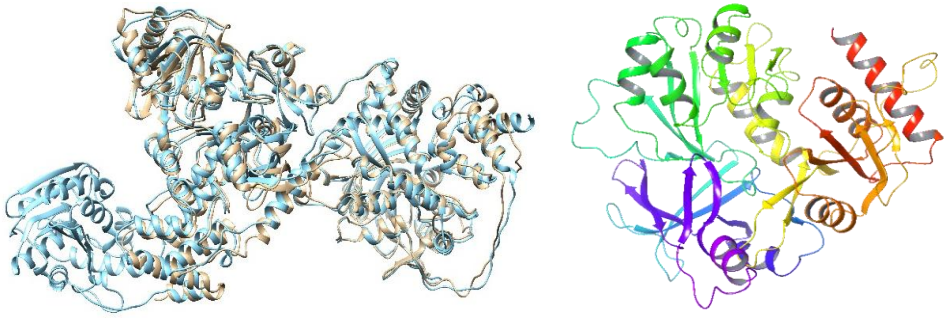

Figure 1. The superimposed overlay conformation of the docked internal ligand (Golden) concerning its crystallized conformation (Cyan) obtained from the co-crystallized complex structure.

3.1.2. Virtual Screening of FDA-Reported Library Through Molecular Docking

Using structure-based drug design, FDA-approved drugs were virtually screened against MbtB to identify safe candidates for TB and AMR therapy. Top hits were selected based on binding energies, docking scores, ligand efficiency, and key interactions, with the ten best-performing molecules detailed in Table 2.

Table 2. Comprehensive tabular overview of the top ten hits resulting from docking-based virtual screening of FDA library against MbtA receptor.

Drug Code	ZINC Code	Drug Name	Lowest_Binding_Energy
a_1338	ZINC000000113404	Teldrin	−15.42
a_60	ZINC000256433952		−14.56

Interaction analysis of the top-scoring compounds obtained through virtual screening.

Molecular docking results are interpreted using descriptors such as binding energy, electrostatic energy, hydrogen bonding, van der Waals energy, and solvation energy. Lower binding energies indicate stronger, more favorable interactions. Electrostatic and van der Waals energies guide ligand orientation and fit, while hydrogen bonds stabilize the complex and enhance specificity. Solvation energies account for physiological context and hydrophobic/hydrophilic balance. Together, these parameters provide a comprehensive view of ligand–receptor compatibility.

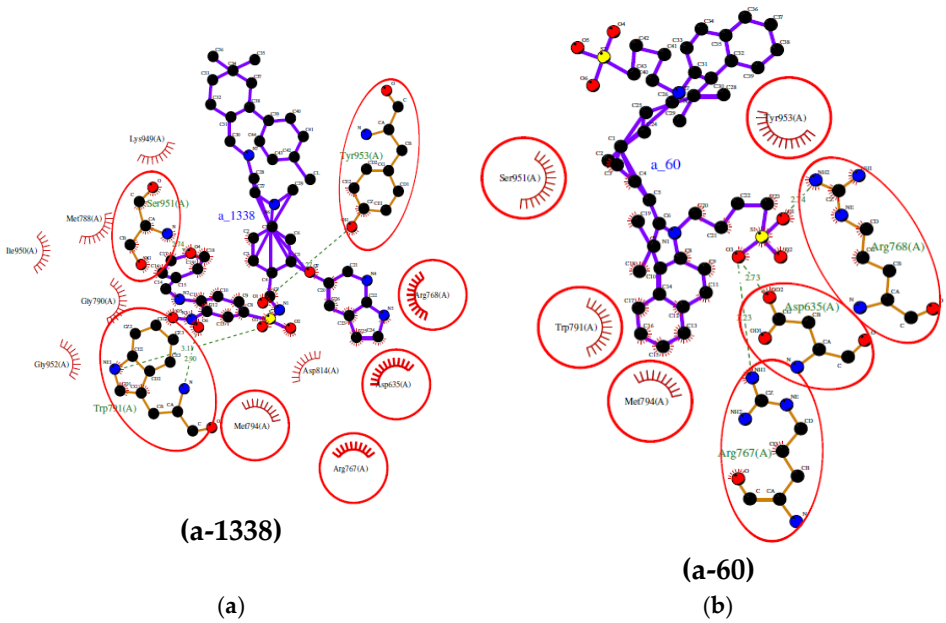

Figure 2. The docked confirmation of (**a**) a_1338 (**b**) a_60, in the active site of MbtB highlighting various interactions.

4. Principal Component Analysis (PCA)

PCA was used to analyze the primary motions of MbtB-ligand complexes during MD simulations. The first two principal components revealed that complexes a_1338-MbtB, a_66-MbtB,, while a_1338-MbtB and a_66-MbtB showed broader distributions, reflecting conformational flexibility. Overall, PCA indicated that ligand binding stabilizes the MbtB active site and restricts essential enzymatic motions, consistent with RMSD, RMSF, Rg, docking scores, and protein-ligand interaction analyses.

5. Discussion

MbtB, essential for mycobactin biosynthesis in *M. tuberculosis*, was targeted for virtual screening of FDA-approved drugs. Ten top hits showed strong binding (−15.42 to −14.56 kcal/mol), with seven selected for molecular dynamics. Simulations (300 ns) confirmed stable protein–ligand complexes, supported by RMSD, RMSF, Rg, SASA, H-bond, and PCA analyses. Key residues (e.g., Thr462, Gly330, Asp436) mediated stable interactions. These findings suggest seven promising candidates for repurposing as MbtB inhibitors against TB.

6. Conclusions

The rise of drug-resistant Mycobacterium tuberculosis highlights the need for novel therapeutic targets. Our study focused on the dual enzyme system MbtA–MbtB, essential for siderophore biosynthesis and linked to efflux pump activity. Using drug repurposing, FDA-approved drugs were virtually screened against MbtB, leading to the identification of seven promising inhibitors with established ADMET profiles. These findings demonstrate the potential of repurposing existing drugs to disrupt mycobactin biosynthesis and offer a practical strategy against TB and co-infections.

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