RESEARCH ARTICLE
10.5152/TrendsPharm.2024.23009

Virtual Screening of Potent Inhibitors for *Mycobacterium tuberculosis* Arabinogalactan Synthesis

ABSTRACT

Background: Tuberculosis remains the most prevalent infectious disease, requiring the identification of new targets and drugs due to emerging drug resistance for *Mycobacterium tuberculosis*. Virtual screening has the potential to be highly valuable for identifying new drug candidates by searching through compound databases. The present study involved screening a library of around 3.5 million selected compounds from the ZINC20 database and a reference inhibitor library from literature to identify strong inhibitors against the Decaprenylphosphoryl-β-D-ribose 2′-epimerase (DprE1) enzyme of *M. tuberculosis* through virtual methods.

Methods: A two-step selection of compounds was carried out from the ZINC20 database using stringent reference physicochemical ranks based on the reference inhibitor library. The resulting library comprised 2700 compounds from the ZINC database along with 21 reference inhibitors from the literature exhibiting MICs of 10 µM or lower. Docking simulations were performed for a total of 2721 compounds based on the AutoDock Vina tool using python-based computational drug discovery software, PyRx against the crystal structure of *M. tuberculosis* DprE1 enzyme (Protein Data Bank, PDB: 4P8K).

Results: After docking analysis of the selected final library (2721 in total), we determined 21 molecules as top-ranked compounds. The top-ranked compounds from the ZINC20 library had a ΔG in the range of -11 and -11.9 compared to -7.7 and -10.9 of the selected 21 reference inhibitors with MICs of 10 µM or lower. Among the whole library, ZINC000009429240 and Ref3 emerged as the highest ranked compounds, exhibiting a ΔG value of -11.9 and -10.9, respectively. ZINC000009429240 and Ref3 were determined to show H-bond interactions with 228S and 418K versus 132H, 228S and 60Y, respectively.

Conclusion: Here, we virtually identified a largely distinct set of compounds as effective inhibitors for the DprE1 enzyme of *M. tuberculosis*. The data gathered in this study may have the potential to be developed into effective drug molecules.

Key Words: Bioinformatics, chemistry, medical microbiology.

INTRODUCTION

Tuberculosis (TB) is among the top 10 leading causes of death on a global scale, with more than 1.5 million deaths and a 10 million annual new diagnosis according to the World Health Organization.[1] Due to the highly contagious nature of TB and its airborne transmission, drug resistant strains become difficult to control. The global efforts for preventing TB includes early detection, vaccination, efficient treatment and surveillance.[2] Currently, TB treatment consists of four old drugs including pyrazinamide (PYR), isoniazid (INH), ethambutol (EMB) and rifampicin (RFP) which are applied in combination. The incorrect use of the antibiotics resulted in the development of drug-resistant strains of *M. tuberculosis*.[3] The current worldwide strategy to combat drug resistant *M. tuberculosis* strains has been directed towards the development of novel TB drugs, especially those with novel targets.[4] TB drugs target and inhibit numerous essential pathways involved in cell wall formation (e.g., InhA, KasA, and Emb proteins), ATP synthase, nucleic acid synthesis (via DNA gyrase and topoisomerase IV inhibition), protein synthesis (targeting ribosomal proteins), fatty acid biosynthesis and many others.[5]

Over the past decade, new drugs have been approved for tuberculosis treatment. Some of these drugs target well-known important enzymes like InhA (enoyl-ACP reductase) while others effectively target different mechanisms to inhibit *M. tuberculosis*. Among

Nurbanu Yaşar[1]
Ahmet Serhat Ayvaz[2]
Gülay Dilek[3]

[1]*Department of Medical Microbiology, University of Mersin, Faculty of Medicine, Mersin, Türkiye*
[2]*Department of Pharmaceutical Microbiology, Zonguldak Bülent Ecevit University, Faculty of Pharmacy, Zonguldak, Türkiye*
[3]*Department of Pharmaceutical Basic Sciences, Zonguldak Bülent Ecevit University, Faculty of Pharmacy, Zonguldak, Türkiye*

Corresponding author:
Gülay Dilek
Address: Department of Pharmaceutical Basic Sciences, Zonguldak Bülent Ecevit University, Faculty of Pharmacy, Zonguldak, Türkiye
Email address: gulay.dilek@beun.edu.tr

Received: May 29, 2024
Revision Requested: June 6, 2024
Last Revision Received: June 9, 2024
Accepted: June 10, 2024
Publication Date: June 14, 2024

Cite this article as: Yaşar N, Ayvaz AS, Dilek G. Virtual screening of potent inhibitors for *Mycobacterium tuberculosis* arabinogalactan synthesis. *Trends Pharm.* 2024, 1, 9, doi: 10.5152/TrendsPharm.2024.23009

these targets, Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1) plays an important role in the biosynthesis of cell wall arabinogalactan. DprE1 is the target of two recently approved antituberculosis drugs, including delamanid (DLM) and pretomanid (PMD). These drugs are being used in the management of extensively drug-resistant TB (XDR TB).[6]

Drug screening aimed at inhibiting the mycolic acid synthesis through the targeting DprE1 has demonstrated significant promise in the development of novel drugs for tuberculosis.[7-9] In recent years, computational methods have made it possible for screening of millions of potential drug candidates for the treatment of tuberculosis.[10] In this study, we aimed to evaluate a large-scale of compounds via *in-silico* screening against DprE1 for the discovery of potential drug candidates for TB treatment.

MATERIAL AND METHODS

Construction of Compound Library And Preparation

The compounds used for the virtual screening were collected from the ZINC20 database (https://zinc20.docking.org).[11] The physicochemical properties of the DprE1 potent inhibitor library (included ~1519 compounds) from literature[12] were utilized for selecting compounds from ZINC20. The average values comprising standard deviations (SD) of molecular weight (MW), cLogP, H-bond donors, H-bond acceptors, topological polar surface area (TPSA), flexibility, rotatable bonds, druglikeness, and also toxicity properties were calculated from the potent DprE1 inhibitor library. In addition to physicochemical features, the compounds known to have MIC values lower than 10 μM were also screened for determining a cut-off Gibbs free energy (ΔG, kcal/mol) value.

Selected compounds were chosen from the ZINC20 database based on their purchasability, 3D structures, reactivities, and reference values in the range of 2.5-4.57 for cLogP and 300-500 for MW as the first step of selection. In the second step, these collected compounds underwent evaluation using physicochemical descriptors other than cLogP and MW through Datawarrior v06.01 software.[13] The reference range values used for selection were 70-120 for TPSA, 0.10-0.25 for flexibility, less than 10 for H-bond acceptors, less than 5 for H-bond donors, greater than 0 for druglikeness, and a range of 2-8 for rotatable bonds; in addition to avoiding high mutagenicity. Thus, the selection ensured compatibility to Lipinski's RO5 rule[14] even with some more strict parameters for a more accurate prediction for targeting *M.tuberculosis.*

Virtual Screening

Multiple docking simulations for the compounds selected were performed using the AutoDock Vina tool[15] in python-based computational drug discovery software, PyRx[16] against the crystal structure of *M. tuberculosis* DprE1 enzyme (Protein Data Bank, PDB: 4P8K).[17] The crystal structure used contained a complex with a potent inhibitor, and the active cavity was determined to include the space where the inhibitor interacted. The dimensions of this cavity were as follows: center (x, y, z) = (16, -20, 1.5), and dimensions (x, y, z) = (11, 16, 14). The crystal structure of M. tuberculosis DprE1 enzyme was imported into the software and underwent protein preparation steps including removing unwanted molecules such as water, adding polar hydrogen and charge; then converting the prepared protein to .pdbqt format using PyRx automatically. When preparing the ligands, all selected compounds from ZINC20 and the reference compounds from the literature[12] with minimum inhibitory concentrations lower than 10 μM were imported into PyRx. The integrated OpenBabel module was then used for multiple ligand minimization and conversion to .pdpqt format. Docking simulations for each ligand were run in 8 repetitions to calculate ΔG. After the docking simulations, only docked poses with lower total binding energy compared to those of reference compounds, with a root mean square deviation (RMSD) value ≤ 2 Å, and with successful binding in the selected cavity space were accepted as top-ranked compounds. The inhibition constant (Ki) was calculated from the binding energy (ΔG) using the formula: Ki = exp(ΔG/RT) (R is the universal gas constant [1.985 × 10–3 kcal mol–1 K–1] and T is the temperature [298.15 K]). The compounds were visually evaluated using PyMOL Molecular Graphics System.[18] 2D interaction profiles were obtained using Maestro v.11.8.012 of the Schrodinger 2018.4 (Schrodinger, LLC, New York, NY, 2018) Linux Ubuntu (2020) version.

Mutation Screening for the Interacting Amino Acids

DprE1 of *M. tuberculosis* has the potential for amino acid mutations. A mutation screening was conducted specifically for amino acid positions that interacted with reference inhibitors and top ranked compounds. Gene sequences related to DprE1 enzyme were gathered from Genbank (www.ncbi.nlm.nih.gov/genbank/), aligned, converted to amino acid sequences, and compared to the reference DprE1 sequence (NP_218307.1) of *M. tuberculosis* via BioEdit v7.7.1. It was assessed if there were any records of mutations at the positions where the compounds interacted.

RESULTS

In preparing the compound library in the ZINC20 database, we applied five basic selections based on molecular weight, cLogP, 3D structure, in-stock availability, and reactivity properties. The initial selection resulted in the collection and download of approximately 3.5 million compounds (accessed on March 15, 2024) in .sdf format. Subsequently importing the library into Datawarrior software led to a second selection process based on reference value ranges for H-bond donors, H-bond acceptors, topological polar surface area, flexibility, rotatable bonds, druglikeness criteria as well as toxicity properties. This rigorous process yielded a total of 2700 compounds with similar physicochemical descriptors clustering as those found in the potent DprE1 inhibitors' library.[12] Virtual screening involved 2700 compounds from the ZINC20 database and 21 reference inhibitors with a MIC of 10 μM or lower selected from the inhibitor library. The inhibitors with the highest MICs reported a ΔG in the range of -7.7 and -10.9. Ref3 showed the strongest interaction among the selected 21 inhibitors in the reference inhibitor library, with a ΔG of -10.9. A total of 21 compounds from the ZINC20 library were identified as top-ranked ones, exhibiting lower binding energies than those of Ref3. The top-ranked compounds from the ZINC20 library had a ΔG in the range of -11 and -11.9. Within these, ZINC000009429240 was found to be the top-ranked compound with a ΔG of -11.9. From the 2D interaction maps of the top-ranked compounds of the reference and the ZINC20 library, H-bond interactions with 228S and 418K versus 132H, 228S and 60Y; hydrophobic interactions with 116P, 131I, 316P, 365V and 415Y versus 116P, 316P and 365V were determined for ZINC000009429240 and Ref3, respectively (Figure 1). Amino acids positions of 228S, 116P, 316P and 365V were found to be common

positions that interact with both ZINC000009429240 and Ref3. The names, structures as SMILE format, binding energies, and Ki values of reference compounds (with MICs lower than 10 μM) as well as the top-ranked 21 compounds of the library from the ZINC20 database are given in Table 1.

Table 1. Energies and Inhibition Constant (Ki) Values of Top-Ranked Compounds from Reference[12] and ZINC20 Libraries

Compound name	SMILES	Binding energy (ΔG, kcal/mol)	Ki (μM)
Ref01	c1cc(ccc1OC2CCN(CC2)c3nc(=O)c4cc(cc(c4s3)N(=O)=O)C(F)(F)F)F	-9,7	0,076
Ref02	c1cc(ccc1C(=O)OC2CCN(CC2)c3nc(=O)c4cc(cc(c4s3)N(=O)=O)C(F)(F)F)F	-10,7	0,014
Ref03	**c1c(cc(c2c1c(=O)nc(s2)N3CCN(CC3)NC4CCCCC4)N(=O)=O)C(F)(F)F**	**-10,9**	**0,010**
Ref04	c1c(cc(c2c1c(=O)nc(s2)N3CCN(CC3)NCC4CCCC4)N(=O)=O)C(F)(F)F	-10,4	0,023
Ref05	c1ccc(cc1)c2nnc(n2C3CCCCC3)SCc4cc(cc(c4)N(=O)=O)N(=O)=O	-9,9	0,054
Ref06	CCCCCCn1c(nnc1SCc2cc(cc(c2)N(=O)=O)N(=O)=O)c3ccccc3	-9	0,249
Ref07	CCCCCCn1c(nnc1SCc2ccccc2)c3cc(cc(c3)N(=O)=O)N(=O)=O	-8,3	0,812
Ref08	c1c(cc(c2c1c(=O)nc(s2)N3CCC4(CC3)SCCS4)N(=O)=O)C(F)(F)F	-8	1,347
Ref09	COc1cc2c(cc1OC)CN(CC2)c3nc(=O)c4cc(cc(c4s3)N(=O)=O)C(F)(F)F	-9,5	0,107
Ref10	COc1ccc(cc1)Cn2c(nnc2SCc3cc(cc(c3)N(=O)=O)N(=O)=O)c4ccccc4	-9,7	0,076
Ref11	FC1=CC=C(OC2CCN(CC2)C(=O)C2CCN(CC2)C2=CC(=NC=N2)N2CCOCC2)C(F)=C1	-9,3	0,150
Ref12	c1ccc(cc1)c2nnc(n2c3ccc(c(c3)Cl)Cl)SCc4cc(cc(c4)N(=O)=O)N(=O)=O	-10,6	0,017
Ref13	c1ccc(cc1)Cn2c(nnc2SCc3ccc(cc3)Br)c4cc(cc(c4)N(=O)=O)C(F)(F)F	-9,9	0,054
Ref14	c1c(cc(c2c1c(=O)nc(s2)N3CCOCC3)N(=O)=O)C(F)(F)F	-9,2	0,177
Ref15	O=C(N1CC(C2=C(F)C=C(F)C=N2)=O)C(C3=CC=C(C#N)C=C3)(C)NC1=O	-9	0,249
Ref16	O=C1N(C(C(N1)(C2=CC=C(C#N)C=C2)CC)=O)CC(C3=CC=C(C=C3F)F)=O	-9,4	0,127
Ref17	O=C(N1CC(C2=CC=CC(C)=C2)=O)NC(C3=CC=C(S(=O)(N)=O)C=C3)(C)C1=O	-9,7	0,076
Ref18	O=C(N1CC(C2=CC=CC(F)=C2)=O)NC(C3=CC=C(S(=O)(N)=O)C=C3)(C)C1=O	-8,6	0,489
Ref19	O=C1C2=CC(C(F)(F)F)=CC(N3C(C)=CC=C3C)=C2SC(N4CCN(CC5CCCCC5)CC4)=N1	-8,7	0,413
Ref20	O=C(NC1=C(C(NC(OCC)=O)=O)C=CS1)C2=CC=C(C(N3CCCCC3)=O)C=C2	-10,2	0,033
Ref21	c1c(cc(c2c1c(=S)nc(s2)N3CCN(CC3)CC4CCCCC4)N(=O)=O)C(F)(F)F	-8,8	0,349
ZINC000009429240	**Cn1c(=O)c2c(-c3ccccc3)n3c(c2n(C)c1=O)[C@H](c1coc2ccccc2c1=O)OCC3**	**-11,9**	**0,002**
ZINC000021787978	Cc1cccc(-c2c3c(=O)n(C)c(=O)n(C)c3c3n2CCO[C@H]3c2coc3ccccc3c2=O)c1	-11,8	0,002
ZINC000021787983	Cc1cccc(-c2c3c(=O)n(C)c(=O)n(C)c3c3n2CCO[C@@H]3c2coc3ccccc3c2=O)c1	-11,7	0,003
ZINC000252672778	CCCCN1C(=O)C(=c2sc3n(c2=O)[C@@H](c2ccccc2)C(C(=O)OC)=C(C)N=3)c2ccccc21	-11,6	0,003
ZINC000000632944	O=C1/C(=c2\sc3nc4ccccc4n3c2=O)c2ccccc2N1Cc1ccccc1	-11,5	0,004
ZINC000013775581	O=C1/C(=c2/sc3nc4ccccc4n3c2=O)c2ccccc2N1Cc1ccccc1	-11,5	0,004
ZINC000101343925	O=C(Nc1ccccc1)[C@@H]1[C@@H]2C(=O)N(c3ccc4c(c3)OCO4)C(=O)[C@@H]2[C@H]2c3ccccc3C=NN21	-11,5	0,004
ZINC000252517668	O=C(Nc1ccccc1)[C@@H]1[C@@H]2C(=O)N(c3ccc4c(c3)OCO4)C(=O)[C@H]2[C@@H]2c3ccccc3C=NN12	-11,5	0,004
ZINC000252669789	O=C1C(=c2sc3nc4ccccc4n3c2=O)c2ccccc2N1Cc1ccccc1	-11,5	0,004
ZINC000002788016	Cc1nn(C)cc1/C=c1\sc2n(c1=O)[C@H]1c3ccccc3O[C@@](C)(N=2)[C@@H]1C(=O)Nc1ccccc1	-11,2	0,006
ZINC000008455064	Cc1ccccc1NC(=O)[C@@H]1[C@H]2c3ccccc3O[C@]1(C)N=c1s/c(=C/c3cnn(C)c3C)c(=O)n12	-11,2	0,006
ZINC000101455637	CC(=O)N1N=C(c2c(O)[nH]c(=O)n(-c3cccc(Cl)c3)c2=O)C[C@@H]1c1c[nH]c2ccccc12	-11,2	0,006
ZINC000101765650	CCC(=O)N1N=C(c2c(O)[nH]c(=O)n(-c3cccc(Cl)c3)c2=O)C[C@@H]1c1c[nH]c2ccccc12	-11,2	0,006
ZINC000016033791	Cc1cc(C)cc(N2C(=O)[C@@H]3[C@@H](Cc4ccc(O)cc4)N[C@]4(C(=O)Nc5ccc(F)cc54)[C@@H]3C2=O)c1	-11,1	0,007
ZINC000019960761	COc1ccc2[nH]c3c(c2c1)CCN1C(=O)c2cnc(N4CCCCC4)nc2C[C@H]31	-11,1	0,007
ZINC000020760244	CC(C)[C@H]1N[C@@]2(c3ccccc3-n3c2nc2ccccc2c3=O)[C@H]2C(=O)N(c3ccccc3)C(=O)[C@H]21	-11,1	0,007
ZINC000101765646	CCC(=O)N1N=C(c2c(O)[nH]c(=O)n(-c3cccc(Cl)c3)c2=O)C[C@H]1c1c[nH]c2ccccc12	-11,1	0,007
ZINC000000988062	Cn1c(=O)c2c(nc3n(-c4ccccc4)nc(C(=O)c4ccc5ccccc5c4)n23)n(C)c1=O	-11	0,008
ZINC000013575113	Cn1c(=O)c2c(-c3ccccc3)n3c(c2n(C)c1=O)[C@@H](c1coc2ccccc2c1=O)OCC3	-11	0,008
ZINC000035538889	CN1C(=O)[C@@H](c2c(-c3ccc4ccccc4c3)[nH]c3[nH]c(=O)[nH]c(=O)c23)c2ccccc21	-11	0,008
ZINC000252517669	O=C(Nc1ccccc1)[C@@H]1[C@@H]2C(=O)N(c3ccc4c(c3)OCO4)C(=O)[C@H]2[C@H]2c3ccccc3C=NN12	-11	0,008

The compounds with lowest ΔG from reference and ZINC libraries were indicated as bold.

The amino acid library containing 100 sequences of DprE1 mostly from diverse strains did not reveal any mutation profiles in amino acids interacting with compounds ZINC000009429240 and Ref3. The prepared mutation library of DprE1 is included in the Supplementary Figure 1.

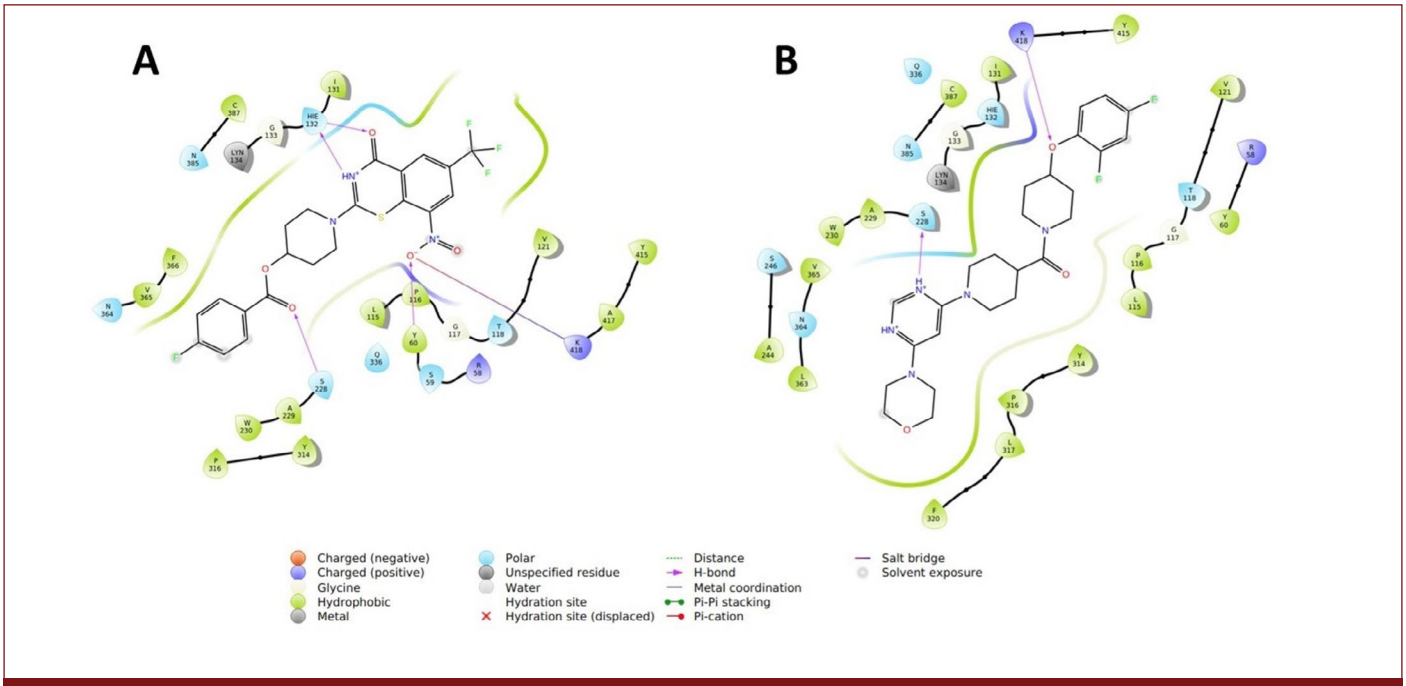


Figure 1. 2D Interaction Profiles of Top-Ranked Compounds of ZINC000009429240 and Ref3.

DISCUSSION

In the current study, virtual screening was performed using molecular docking with the ZINC20 database[11] to search for potent inhibitors against the DprE1 enzyme of *M.tuberculosis* and compared with DprE1 inhibitors reported in the literature.[12] The analysis identified 21 compounds with a binding energy range of -11 to -11.9 as the top-ranked molecules. Both ZINC000009429240 and Ref3 demonstrated a strong interaction with the enzyme cavity, exhibiting a ΔG of -11.9 and -10.9, respectively. These interactions involved H-bonding with amino acid positions of 228S and 418K versus 132H, 228S and 60Y, and hydrophobic interactions with 116P, 131I, 316P, 365V and 415Y versus 116P, 316P and 365V for ZINC000009429240 and Ref3, respectively. Additionally, it was observed that the amino acids at positions of 228S, 116P, 316P and 365V played an important role as they interacted with both ZINC000009429240 and Ref3. When compared with the prepared mutation library of DprE1, it turned out that these interacting amino acids were not affected by mutations in the enzyme.

In recent years, various proteins of *M.tuberculosis* have been targeted for screening of novel and potent inhibitors through various compound databases. The target proteins involved in various studies were DprE1,[19] adenylating enzymes of AspS and KatG,[20] protein kinase B (PknB),[21] glutamine synthetase (GS) in the nitrogen metabolism and cell wall synthesis,[22] β-ketoacyl-acyl carrier protein KasA,[23] malonyl Co-A acyl carrier protein transacylase (FabD),[24] 7,8-diaminopelargonic acid synthase (BioA),[25] and many others. Among these target proteins, DprE1 is particularly important due to its essential role in the synthesis of arabinogalactan and lipoarabinomannan, which are crucial components in mycobacterial cell wall biogenesis.[26]

Zgang et al.,[26] identified potential inhibitors from a virtual screening of 135,755 selected molecules in the ChemDiv dataset, which is also linked to the ZINC database. ZINC09833455 and ZINC32996629 were determined as hit compounds to inhibit DprE1. Due to their availability, certain compounds labeled as "for sale", but not "in stock", and the compounds outside the specified range of physicochemical descriptors such as TPSA and/or logP were excluded from this study. Kumar et al.,[19] conducted a virtual screening of bioactive molecules from the ChEMBL database against DprE1. They identified 4 compounds (ChEMBL2441313, ChEMBL2338605, ChEMBL441373, ChEMBL1607606) as potential inhibitors. However, these were excluded in this study because their molecular weights fell outside the specified range. Anand et al.,[27] once again utilized the ZINC database for virtual screening against a modeled DprE1 enzyme, screening a total of 19,365 molecules and identifying the top 10 hit molecules (ZINC06142089, ZINC18116975, ZINC00856140, ZINC01800489, ZINC06142069, ZINC06142038, ZINC03953443, ZINC01886310, ZINC01750499, ZINC11582332) with docking scores ranging from -5.8 to -8 kcal/mol. These compounds were not included in our library, possibly due to their mutagenicity characteristics. However, the docking scores for these compounds are lower than those of our hit molecules (-11 and -11.9) in the ZINC20 database. According to ZINC20, PubChem (https://pubchem.ncbi.nlm.nih.gov) and Pubmed (https://pubmed.ncbi.nlm.nih.gov) databases (accessed on March 30, 2024), none of the top-ranked compounds of ZINC20 have been reported as potential inhibitors of DprE1.

It's important to note that there is no consensus approach for

virtual screening and thus docking scores can vary for the same compounds and targets. Additionally, although the DprE1 enzyme has been an important target in virtual screening studies, none of them contain a selective library based on physicochemical properties of DprE1 inhibitors known from *in-vitro* data in the literature. Despite high binding affinity data obtained from various virtual screening studies, enabling these hit molecules to penetrate the highly complex cell wall of *M. tuberculosis* may require more stringent physicochemical features which could enhance their efficiency when tested in-vitro. Virtual screening is becoming increasingly important to discover new compounds that will inhibit essential molecules of *M. tuberculosis*. Data obtained from virtual screening has the potential to develope drug-like compounds into potent drug molecules.

CONCLUSION

Overall, we suggested a mostly diverse group of compounds as potent inhibitors of the DprE1 enzyme of *M. tuberculosis*. It would be highly interesting to evaluate the effectiveness of these hit compounds *in-vitro* to validate our approach further. The data gathered in this study may have potential to be developed into effective drug molecules.

Ethics Committee Approval: Ethical approval was not necessary due to the totally in-silico design of the study.

Informed Consent: Informed consent was not necessary due to the totally in-silico design of the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – N.Y., S.A., G.D.; Design – N.Y., S.A., G.D.; Data Collection and/or Processing – N.Y., S.A., G.D.; Analysis and/or Interpretation – N.Y., S.A., G.D.; Writing – N.Y., S.A., G.D.

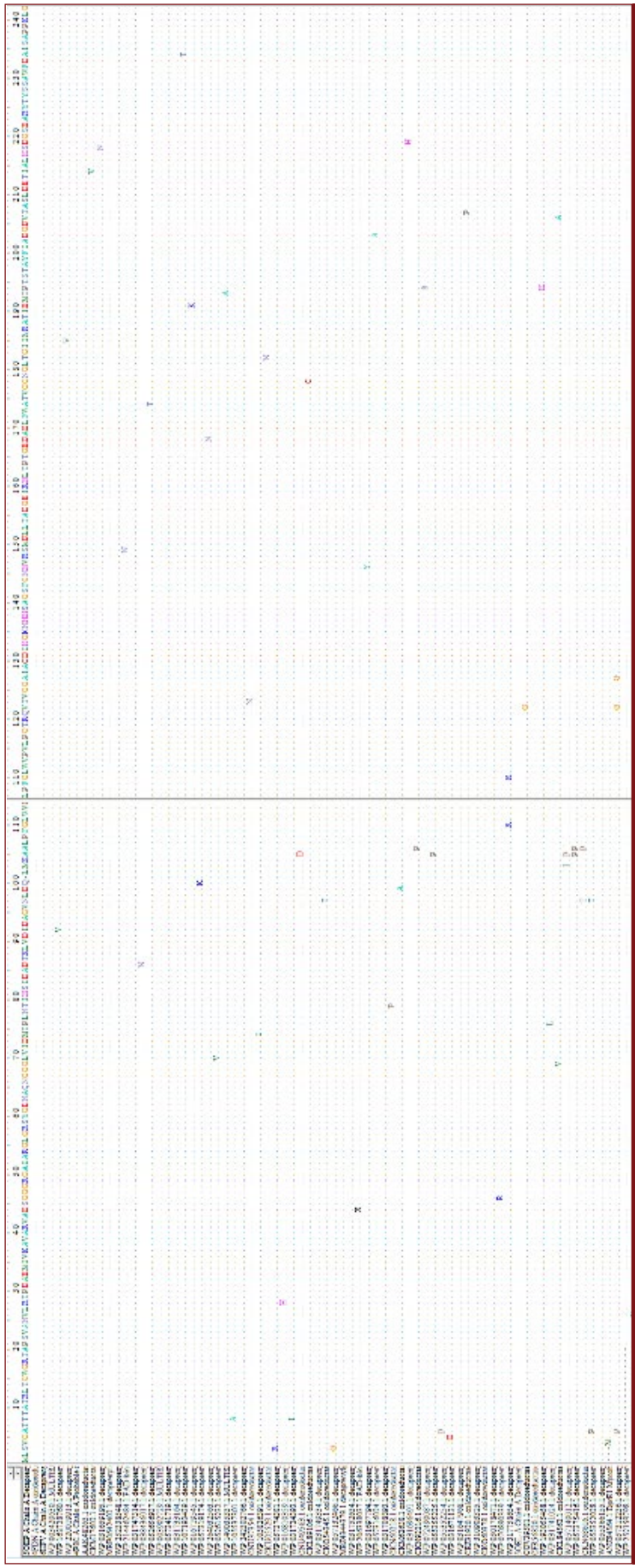
Declaration of Interests: The authors have no conflict of interest to declare.

Funding: The authors declared that this study has received no financial support.

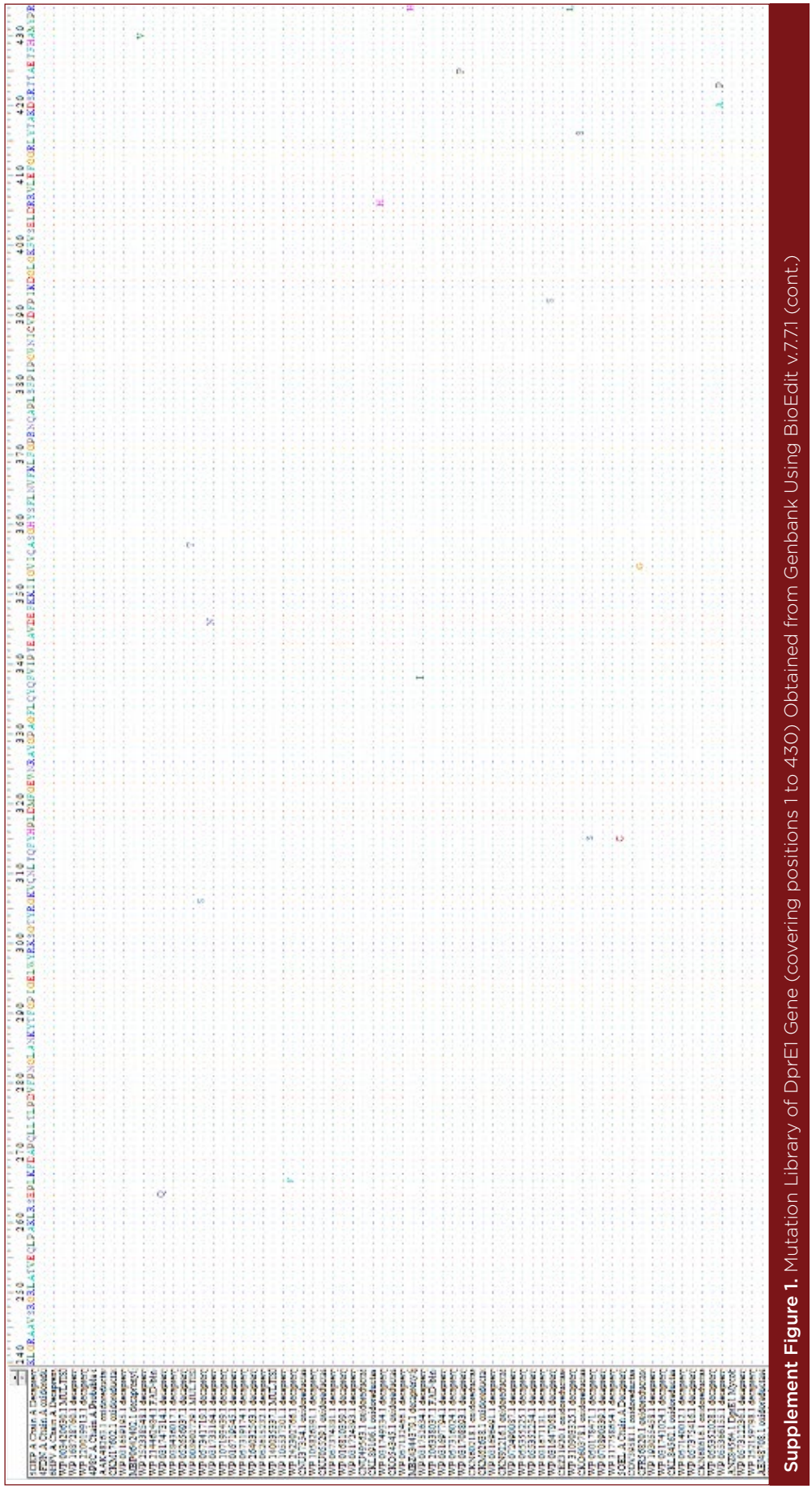
References

1. Salina EG. Mycobacterium tuberculosis infection: Control and treatment. *Microorganisms.* 2023 Apr 18;11(4):1057. **[Crossref]**
2. Chakaya J, Khan M, Ntoumi F, et al. Global Tuberculosis Report 2020 - Reflections on the Global tuberculosis report 2020 - Reflections on the global TB burden, treatment and prevention efforts. *Int J Infect Dis.* 2021;113 Suppl 1(Suppl 1):7-12. **[Crossref]**
3. Zumla A, Nahid P, Cole ST. Advances in the development of new tuberculosis drugs and treatment regimens *Nat Rev Drug Discov.* 2013 May;12(5):388-404. **[Crossref]**
4. Singh V, Chibale K. Strategies to combat multi-drug resistance in tuberculosis. *Acc Chem Res.* 2021 May 18;54(10):2361-2376. **[Crossref]**
5. Oh S, Trifonov L, Yadav VD, et al. Tuberculosis drug discovery: a decade of hit assessment for defined targets. *Front Cell Infect Microbiol.* 2021;11:611304. **[Crossref]**
6. Abrahams KA, Batt SM, Gurcha SS, et al. Dpre2 is a molecular target of the anti-tubercular nitroimidazole compounds pretomanid and delamanid. *Nat Commun.* 2023;14(1):3828. **[Crossref]**
7. MakarovV, NeresJ, Hartkoorn RC, et al. The 8-pyrrole-benzothiazinones are noncovalent inhibitors of Dpre1 from mycobacterium tuberculosis. *Antimicrob Agents Chemother.* 2015;59(8):4446-52. **[Crossref]**
8. Tiwari R, Miller PA, Chiarelli LR, et al. Design, syntheses, and anti-TB activity of 1,3-benzothiazinone azide and click chemistry products inspired. by BTZ043. *ACS Med Chem Lett.* 2016;7(3):266-270. **[Crossref]**
9. Chikhale R, Menghani S, Babu R, et al. Development of selective Dpre1 inhibitors: Design, synthesis, crystal structure and antitubercular activity of benzothiazolylpyrimidine-5-carboxamides. *Eur J Med Chem.* 2015;96:30-46. **[Crossref]**
10. Naidu A, Nayak SS, Lulu S S, et al. Advances in computational frameworks in the fight against TB: The way forward. *Front Pharmacol.* 2023;14:1152915. **[Crossref]**
11. Irwin JJ, Tang KG, Young J, et al. ZINC20-A free ultralarge-scale chemical database for ligand discovery. *J Chem Inf Model.* 2020;60(12):6065-6073. **[Crossref]**
12. Amado PSM, Woodley C, Cristiano MLS, et al. Recent advances of Dpre1 inhibitors against mycobacterium tuberculosis: Computational analysis of physicochemical and ADMET properties. *ACS Omega.* 2022;7(45):40659-40681. **[Crossref]**
13. Sander T, Freyss J, von Korff M, Rufener C. Datawarrior: An open-source program for chemistry aware data visualization and analysis *J Chem Inf Model.* 2015;55(2):460-473. **[Crossref]**
14. Benet LZ, Hosey CM, Ursu O, et al. BDDCS, The rule of 5 and drugability. *Adv Drug Deliv Rev.* 2016 Jun 1;101:89-98. **[Crossref]**
15. Trott O, Olson AJ. Autodock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.* 2010;31(2):455-461. **[Crossref]**
16. Dallakyan S, Olson AJ. Small-molecule library screening-by docking with pyrx. *Methods Mol Biol.* 2015;1263:243-50. **[Crossref]**
17. Neres J, Hartkoorn RC, Chiarelli LR, et al. 2-carboxyquinoxalines kill mycobacterium tuberculosis through noncovalent inhibition of Dpre1. *ACS Chem Biol.* 2015;10(3):705-14. **[Crossref]**
18. Schrödinger L, DeLano W. PyMOL. 2020. Retrieved from http://www.pymol.org/pymol
19. Niranjan K, Srivastava R, Prakash A, et al. Virtual screening and free energy estimation for identifying mycobacterium tuberculosis flavoenzyme Dpre1 inhibitors. *J Mol Graph Model.* 2021;102:107770. **[Crossref]**
20. Collins AP, Abdelfattah W. Structure-based virtual screening of essential mycobacterium tuberculosis enzymes AspS and KatG for potential inhibitors. *Bioinformation.* 2021;17(1):101-108. **[Crossref]**
21. Thongdee P, Hanwarinroj C, Pakamwong B, et al. Virtual screening to identify novel potential inhibitors for glutamine synthetase of mycobacterium tuberculosis. PknB with Antibacterial Activity. *J Chem Inf Model.* 2022;62(24):6508-6518. **[Crossref]**
22. Kumari M, Subbarao N. Virtual screening to identify novel potential inhibitors for glutamine synthetase of mycobacterium tuberculosis. *J Biomol Struct Dyn.* 2020;38(17):5062-5080. **[Crossref]**
23. Andrianov AM, Furs KV, Gonchar AV, et al. Virtual screening and identification of promising therapeutic compounds against drug-eesistant mycobacterium tuberculosis B-ketoacyl-acyl carrier protein synthase I (KasA). *J Biomol Struct Dyn.* 2023:1-13. **[Crossref]**

24. Sundararajan S, Karunakaran K, Muniyan R. Structure based virtual screening and discovery of novel inhibitors against FabD protein of mycobacterium tuberculosis. *J Biomol Struct Dyn.* 2023:1-12. **[Crossref]**
25. Singh S, Khare G, Bahal RK, et al. Identification of mycobacterium tuberculosis BioA inhibitors by using structure-based virtual screening. *Drug Des Devel Ther.* 2018;12:1065-1079. **[Crossref]**
26. Zhang G, Guo S, Cui H, et al. Virtual screening of small molecular inhibitors against DprE1. *Molecules.* 2018;23(3):524. **[Crossref]**
27. Anand R. Identification of potential antituberculosis drugs through docking and virtual screening. *Interdiscip Sci.* 2018;10(2):419-429. **[Crossref]**



Supplement Figure 1. Mutation Library of DprE1 Gene (Covering Positions 1 to 430) Obtained from Genbank Using BioEdit v.7.2.5



Supplement Figure 1. Mutation Library of DprE1 Gene (covering positions 1 to 430) Obtained from Genbank Using BioEdit v.7.7.1 (cont.)