Int. J. Pharm. Investigation, 2023; 13(1):129-138.
www.jpionline.org

Original Article

Computational Studies to Identify Potential Inhibitors Targeting the DprE1 Protein in *Mycobacterium tuberculosis*

Bashir A Sheikh[1] Basharat A Bhat[1], Masood A Rizvi[2], Zahoor Ahmad[3] Abdullah Almilaibary[4], Mustfa Alkhanani[5], Manzoor A Mir[1,*]

[1]Department of Bioresources, School of Biological Sciences, University of Kashmir, Srinagar, INDIA.
[2]Department of Chemistry, School of Physical and Mathematical Sciences, University of Kashmir, Srinagar, INDIA.
[3]Clinical Microbiology PK-PD/ Laboratory, Indian Institute of Integrative Medicine (IIIM), Srinagar, INDIA.
[4]Department of Family and Community Medicine, Faculty of Medicine, Albaha University, Albaha, SAUDI ARABIA.
[5]Department of Biology, College of Science, Hafr Al Batin University Hafr Albatin, SAUDI ARABIA.

ABSTRACT

Background: DprE1, which is a flavoenzyme, is very important for cell wall biosynthesis in *Mycobacterium tuberculosis (Mtb)* and for the pathogenesis, virulence, lethality, and stress resistance of the host. Drug-resistant tuberculosis is a challenging global human health issue, necessitating the development of novel, more effective treatment regimens without adverse effects. DprE1 represents a potential therapeutic target. It was explored as a drug target utilizing benzothiazoles (BTZ), which are enormously potential anti-bacterial agents and are currently being explored as anti-mycobacterial entities. **Materials and Methods:** We used virtual screening of bioactive molecules from PubChem and ZINC databases targeting DprE1, having bioactive thousands of molecules known for anti-microbial activity. In the present study, we selected 100 compounds as the most promising candidates to act as potential DprE1 inhibitors to control this emerging condition of tuberculosis infection. To identify the six top-ranked compounds, molecular docking was used to calculate the binding affinities (ranging from -8.3 to 10.0 kcal/mol) between various compounds (C1-C6) and the DprE1 protein. **Results:** Based on the results of an ADMET analysis, these six chemicals are safer potential drug candidates, as neither AMES toxicity nor carcinogenicity is present when toxicological properties are considered. Out of 6 compounds, the top-ranked compound exhibiting the best binding affinity against the drug target DprE1 (Pdb-id;4FEH) receptor was further subjected to molecular dynamic simulation for 100 nanoseconds to check the stability and trajectories by root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) graphs and interacting coordinates using Desmond Schrodinger Software. **Conclusion:** Our *in-silico* investigation identified potent inhibitors for the DprE1 protein of *Mtb,* and these compounds can be considered and recommended as the lead molecules in the treatment of tuberculosis.

Keywords: MD simulation, DprE1, *In silico* Screening, *Mycobacterium tuberculosis*, ADMET, Bioavailability, Cytotoxicity.

Correspondence
Dr. Manzoor Ahmad Mir
Head, Department of Bioresources, School of Biological Sciences, University of Kashmir, Srinagar-190006, Jammu and Kashmir, INDIA.
Email id: drmanzoor@kashmiruniversity.ac.in
ORCID ID 0000-0003-3297-1402

Received: 17-08-2022;
Revised: 24-09-2022;
Accepted: 14-10-2022

INTRODUCTION

The causative organism of tuberculosis (TB) infection, *Mycobacterium tuberculosis (Mtb)*, has been a risk to the health of humans for millennia, and it represents one of the world's deadliest infections.[1] According to the WHO, TB was one of the leading infectious causes of death worldwide in 2021, killing more than 1.5 million individuals across the globe.[1-3] Escalating transmission of AIDS-TB co-infections has been exacerbated due to the evolution of resistant forms of tuberculosis, including extensively drug-resistant TB (XDR-TB), multidrug-resistant TB (MDR-TB), and totally drug-resistant TB (TDR-TB) strains.[4-9] *Mycobacterium tuberculosis (Mtb)* has developed immense strategies to evade the host immune response leading to its establishment in the host organism.[10,11] The current treatment strategy for tuberculosis infection uses a combination of isoniazid, rifampicin, ethambutol, and pyrazinamide regimens, which takes 6–9 months to achieve a high cure rate for infected individuals.[12-14] Due to the prolonged treatment period, the currently available conventional treatment for tuberculosis has low compliance, resulting in the development of drug-resistant, multidrug-resistant, and highly drug-resistant strains of *Mtb.*[12] As a result, there is a pressing need to improve treatment outcomes by exploring novel chemical entities with significant anti-tuberculosis activity. To address the drug-resistant forms of

DOI: 10.5530/223097131750

TB, such as XDR-TB/MDR-TB/TDR-TB, it is critical to identify various potential and vulnerable drug targets and explore the most effective drug target inhibitors in *Mtb*.

Multiple proteins paying their role in the metabolism and survival of *Mtb* were investigated as possible therapeutic targets, and drug development is subsequently progressing. Mycobacteria have already developed intricate biosynthetic pathways that are well-organized and sustain their distinctive, thick cell walls, which help them retain cellular integrity, withstand stress and dormancy, and avoid detection by the immune system of the host.[10,11,15-18] Moreover, most of the drug targets are proteins or enzymes that display a significant role during the various metabolic processes in *Mtb,* which prominently encompasses the biosynthesis of fatty acids, the process of translation, biosynthesis of the cell wall, the process of translation, as well as other molecular mechanisms essential for the survival of *Mtb.*[15] Decaprenylphosphoryl-β-D-ribose 2'-epimerase (DprE1) is a flavoenzyme playing a role in *the* biosynthesis of *Mtb* cell wall.[19] DprE1 facilitates the epimerization of decaprenyl-phospho-arabinose (DPA) to an intermediate decaprenyl-phopsho-2-keto-D-arabinose (DPX) which is further reduced to DPA by decaprenyl phosphoryl-β-D-ribose-2-epimerase (DprE2).[15,20] Therefore, the proteins DprE1 and DprE2 are essential for the functioning and growth of *Mtb* cells.[20,21]

Consequently, the catalytic activity of DprE1 represents the possible drug targets in developing tuberculosis treatment regimens. Recently, benzothiazinone (BTZ) derivatives demonstrated increased potency for DprE1 inhibition and more significant potential when evaluated against various strains of MDR and XDR mycobacteria.[20,22] In drug screening, various chemical scaffolds with various structural differences are evaluated as DprE1 inhibitors. Depending on their interactions with the catalytic domains of DprE1, these inhibitors are classified as covalent or non-covalent.[22-24] Previous studies on the advancement of DprE1 inhibitors proposed that high throughput screening, molecular modeling, docking, functional genomics, and proteomics are all significant in identifying novel chemical scaffolds as potential TB chemotherapy molecules.[20,25] Using the PubChem and ZINC databases, we used insilico-based virtual screening to find potential chemical compounds that can serve as DprE1 inhibitors. In the present study, a library of 100 molecules with a structural resemblance to bedaquiline (FDA approved anti-TB drug) was generated to identify novel and promising agents against the DprE1, a vulnerable drug target in *Mtb.*[26] Bedaquiline and bis-coumarins are both heterocyclic compounds containing number of substituted aromatic rings in their structures. Derivatives of both chemical agents have been found to be quite effective against drug susceptible as well as drug resistant strains of *Mycobacterium tuberculosis.*[27,28]

Using this information, we examined a compound library (https://www.otavachemicals.com) containing more than 100 different compounds. The molecular docking and binding affinity estimation procedures identified six hit-molecules (C1-C6). The purpose of the molecular docking was to analyze the binding interactions between these compounds and the active site of the DprE1 enzyme. The top-scored hit-molecule was used in MD simulations for 100 ns of ligand binding state with the DprE1 protein complex. M D simulation was carried out on the DprE1 complex, and the top hit ligand to determine the molecular dynamics behaviors, including interaction and structural stability

We also assessed the compound's carcinogenicity, toxicological and biological activities, and ADME properties using *in silico* predictive tools. The current study found that six chemical entities, in general, and 3,3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one, in particular, may be investigated as potential lead molecules for the development of promising DprE1 inhibitors in tuberculosis therapy.

MATERIALS AND METHODS

Pre-processing

Virtual screening of prepared library was analyzed on Linux Operating System (Ubuntu 20.04) with high configuration with 40 cores, 1 TB SSD, and 32 GB RAM inbuilt workstation. It also had an all-in-built Java environment and internet connectivity for fast processes.[29]

Library Preparation

We used the anti-microbial compounds from publicly available chemical compound databases such as PubChem and ZINC databases. We also carried out the literature review regarding library preparation to find out the best ligands (bedaquiline related) against *Mtb* pathogen, especially against target receptors. After sorting compounds based on the repetitive entries, 100 compounds were found unique and used for ligand preparation.[30] The structure of most of the compounds was downloaded from PubChem and ZINC databases, and the structures of other compounds were drawn using the chem-sketch Marvin visualization (https://chemaxon.com/products/marvin). All the compounds for this study were selected based on their respective mechanism toward the targeted receptors so that we can predict the best outcome for the particular disease pathway. The complete library was downloaded, converted into SDF format, and then analyzed using the standard python script. For the virtual screening process, all the libraries were subjected to the pdbqt format of receptor protein.[30] Furthermore, all the compounds were subjected to pharmacological screening, such as ADME and toxicity, followed by the Lipinski rule of five.

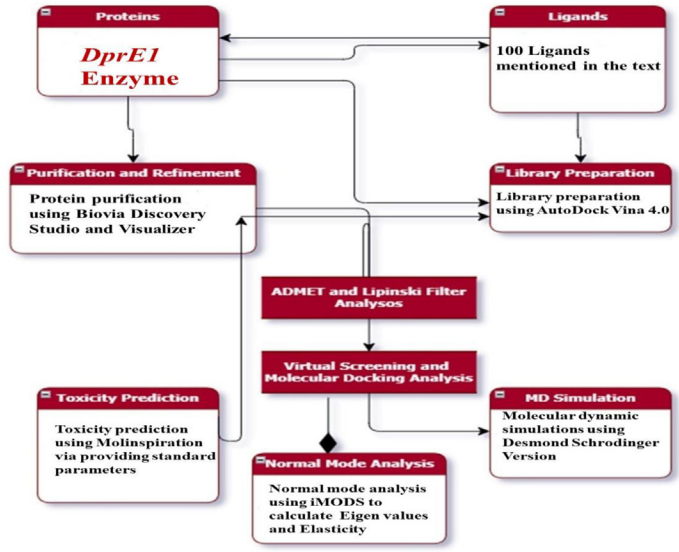

Figure 1: Workflow of the study.

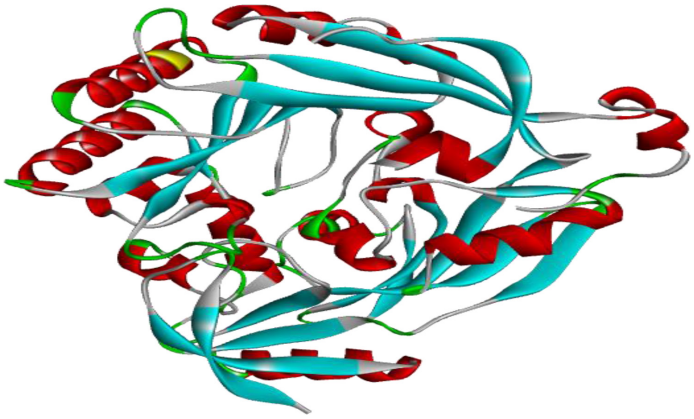

Figure 2: Crystal structure of DprE1 protein of *Mycobacterium tuberculosis*.

Retrieval and Refinement of receptors

The selected receptor, i.e., DprE1 (PDB ID: 4FEH), in PDB format was obtained from a protein data bank (https://www.rcsb.org) (Figure 1, 2). The two domains that make up the structure of DprE1 are the FAD-binding domain, composed of α/β folds, and the substrate-binding domain, which has extended conformation and antiparallel β- -sheets. Initially, receptor preparations were subjected to Biovia Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer). This step was followed by removing all the unwanted water molecules and chains, then adding polar hydrogen bonds with optimized salt concentrations because compounds should interact indigenously with the proteins and interrupt the pathways and enrichment of proteins.[31] Regarding grid preparation for receptors, 10 ^{0}A grid boxes were prepared before subjecting to molecular docking. The center grid parameters were also set to 43.00, 28.23, and 54.89 for x, y, and z coordinates.

Molecular Docking Studies

All the pdbqt files and contig files (which stored all the information regarding virtual screening) were kept in a directory for the virtual screening process.[32,33] The target receptor (prepared) was subjected to docking in contrast to the already prepared compound library using Autodock vina 4.0 (https://vina.scripps.edu/), and a Perl script was employed for multiple ligands molecular docking. In each docking experiment, 10 separate runs were carried out, and every pose was selected based on docking score and binding energies.[32] The virtual screening results were analyzed using Biovia Discovery studio and PyMol Schrodinger (https://pymol.org/2/).

Lipinski and ADMET Screening

All the ligands were subjected to ADMET and Lipinski analysis Swiss-ADME (http://www.swissadme.ch/) and pre-ADMET web server (https://preadmet.qsarhub.com/) to analyze the toxicity properties of compounds. Initially, all compounds were investigated for Lipinski analysis from a molecular docking perspective.[34,35] In terms of Lipinski's rule, any two properties violated by ligands were not considered for molecular docking and further study. The toxicity was predicted using the pre-ADMET web server in the case of PAINS (Pan Assay interference compounds) (https://www.molinspiration.com/), also known as frequent hitters that release the potent outcome in assays regardless of the target receptor. The most frequent PAINS are easily distinguished by how they were constructed.[34,35]

Boiled-egg analysis

The BOILED EGG model was utilized to predict the blood-brain barrier permeability and the gastrointestinal absorption of selected substances. Upon the analysis of the BOILED-Egg plot, compounds falling in the yellow area of the plot were considered to exhibit enhanced blood-brain barrier permeability, while compounds falling in the white zone of the plot were expected to have enhanced properties for gastrointestinal absorption. The Swiss-ADME web server was employed for the BOILED-Egg plot analysis.[36,37]

Molecular dynamic simulation

The best hit compound from selected compounds against the primary target receptor was subjected to molecular dynamics simulation to analyze the fluctuation and simulation graph between ligand and protein residues in nanoseconds. In the experiment, Desmond Schrodinger (https://www.schrodinger.com/products/desmond) was performed to investigate the molecule's stability and determine the involvement between homologous and heterologous structures.[38] For dynamic studies employing the OPLS force field with the typical parameters, Desmond Schrodinger V20.2 was utilized. Using Desmond's internal servers, the topologies and simulation files were prepared.[39]

Simulation run at 100 nanoseconds

The hit compounds were put through RMSD and RMSF calculations with protein and non-protein portions to find a better trajectory scale. With the OPLS force field and Newton's equation, the square deviation and fluctuation rate were shown by the RMSD and RMSF values. The concentration of the salts was kept at 0.15 M sodium and chloride ions expectedly to mimic the physiological conditions.[40,41] Desmond was performed in an NPT ensemble for corresponding time intervals of 70ns to 100ns at temperature 300k and pressure of 1.70 bar.[42]

Normal mode analysis

It is a computer-based simulation approach used to analyze the physical movements of atoms or molecules. It is possible to identify several key hydrogen bond interactions by M D simulations.[43] Improvements in protein docking or virtual screening are made possible by MD simulations.[44] To perform the dynamic simulations, the iMODS server (http://imods.chaconlab.org/) was used in the current study. The iMODS server facilitates the exploration of normal mode analysis and generates accessible information on pathways involving macromolecules or homologous structures.[45]

RESULTS

Ligands

The structure of most of the ligands was retrieved from PubChem and ZINC databases and other related sources, including published research studies. While in the unavailability of compounds' structures, the structures (ligands) were drawn using Chem-Draw and chem sketch. However, after analysis, six out of 10 hit compounds followed the typical structural resemblance with known standard anti-TB drugs and were carried forward for further studies. The (3D) structure of these six compounds or ligands (C1-C6) exhibits all the required parameters for insilico studies, showing the best docking score is provided in (Figure 3).

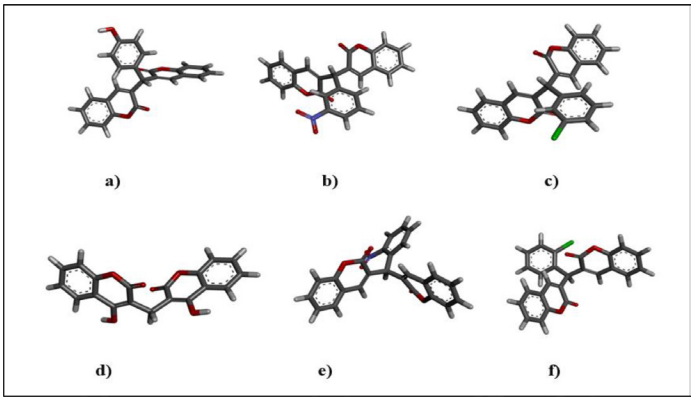

Figure 3: 3D- structure of all 6 hit compounds. a) 3,3'-((4-Hydroxyphenyl) methylene) bis (2H-chromen-2-one) (C1) b) 3,3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) (C2) c) 3,3'-((3-chlorophenyl) methylene) bis (2H-chromen-2-one (C3) d) 3,3'-methylenebis (4-hydroxy-2H-chromen-2-one) (C4) e) 3.3'((2-nitrophenyl) methylene) bis (2H-chromen-2-one) (C5) f) 3,3'((2-chlorophenyl) methylene) bis (2H-chromen-2-one) (C6).

Molecular Docking Analysis

One hundred (100) compounds were screened against the DprE1 receptor protein to analyze the binding affinities (Supplementary file). Consequently, all the compounds, including the top hit compound, were docked with the DprE1 protein to calculate the binding affinity of native ligands. The threshold value for all ligands was kept at -8.0 KJ/Mol to screen the best hit compounds for RMSD and stability against the protein of interest. All the compounds were equal to or greater than -8.0 KJ/Mol binding affinity from the prepared library. The best six compounds were identified from the library based on the threshold value and docking score obtained using Auto dock Vina.

Screening of Hit Compounds

All six selected top score compounds were assessed in accordance with the Lipinski rule of five. PAINS, gastrointestinal retention property along with null PAINS alert in the mechanism. All six selected compounds showed the best activity against the initial screening of hit compounds, as shown in (Table 1).

Bioactivity-based screening of hit compounds

All hit compounds were subjected to bioactivity screening using the Molinspiration web server. This server used input as a SMILES ID of all the six-hit compounds to determine the receptor-specific activity in ion channel modulators, GPCR ligands, kinase inhibitors, protease inhibitors, and nuclear receptor ligands, followed by enzyme activity inhibitors. We found that the compound 3.3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) possess an efficiently high degree of selectivity for the enzyme inhibitor activity due to the enzymatic mechanism of protein DprE1 (Table 2).

Toxicity based analysis

Toxicity is the final output of the hit compound screening process using the pre-ADMET web server, which was utilized to test compounds' toxicity screening and prediction (Table 3). Now 3.3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) (C2) compound was selected for toxicity prediction and observed the acceptable toxicity profiles and was selected as the final hit compound for molecular dynamic simulation using Desmond Schrodinger suite.[46]

Hit compound visualization

Biovia Discovery Studio Visualizer 2021 was used to visualize the docked poses of the hit compound, i.e., 3.3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) (C2) against DprE1 protein. Both 3-Dimensional and 2-Dimensional images were produced with the target receptor by using Biovia 2021. The docked

Table 1: Analysis of binding energies with ADMET properties of selected compounds.

ID	LogS	Lipinski's violation	Pains	BBB penetration	GI absorption	Permeability glycoprotein substrate
3,3'-((4-Hydroxyphenyl) methylene) bis (2H-chromen-2-one)	-10.22	1	0	No	Low	Yes
3,3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one)	-14.49	0	0	No	High	Yes
3,3'-((3-chlorophenyl) methylene) bis (2H-chromen-2-one	-2.07	1	0	No	High	No
3,3'-methylenebis(4-hydroxy-2H-chromen-2-one)	-1.09	2	0	Yes	Low	Yes
3,3'((2-nitrophenyl) methylene) bis (2H-chromen-2-one)	-9.01	0	0	Yes	High	No
3,3'((2-chlorophenyl) methylene) bis (2H-chromen-2-one)	-11.09	1	0	No	Low	N o

Table 2: Bioactivity analysis of the initially screened hit compounds.

ID	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
3.3'-((4-Hydroxyphenyl) methylene) bis (2H-chromen-2-one)	0.61	-0.34	-0.3	-0.51	0.29	0.55
3.3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one)	0.73	-0.45	-0.1	-0.15	0.02	0.74
3.3'-((3-chlorophenyl) methylene) bis (2H-chromen-2-one	0.47	0.32	-0.28	0.02	0.17	0.14
3.3'-methylenebis(4-hydroxy-2H-chromen-2-one)	0.22	0.43	0.76	0.13	0.19	0.55
3.3'((2-nitrophenyl) methylene) bis (2H-chromen-2-one)	0.03	-0.33	0.01	-0.37	-0.07	0.11
3.3'((2-chlorophenyl) methylene) bis (2H-chromen-2-one)	0.18	-0.52	-0.38	-0.7	0.46	-0.2

Table 3: Toxicity analysis of the final one-hit compounds.

ID	3.3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one)
algae_at	0.00461681
Carcino Mouse	Negative
Carcino Rat	Negative
daphnia at	0.00461681
hERG inhibition	Ambiguous
medaka at	5.87E-05
minnow at	0.000367322
TA100 10RLI	Negative
TA100 NA	Negative

positions of the hit compounds were superimposed on the docked position of the co-crystallized native ligand of the receptor. As observed, the amino acids, i.e., ILE 131, LYS 418, and GLN 336 of the receptor, displayed the interaction with the ligand residues involved in regulating the pathways, including ant-igenicity and the topology through cross-covariance transformations of the protein residues. The specific amino acid sequence of DprE1 was calculated to be 0.435 based on the bond length and ionizability, and it was determined to be non-antigenic.

The current study also believes that specific ligand selection criteria such as 3.3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) (C2) towards specific amino acids will result in accurate epitope prediction pursued by transmembrane topologies (Figure 4a-f).

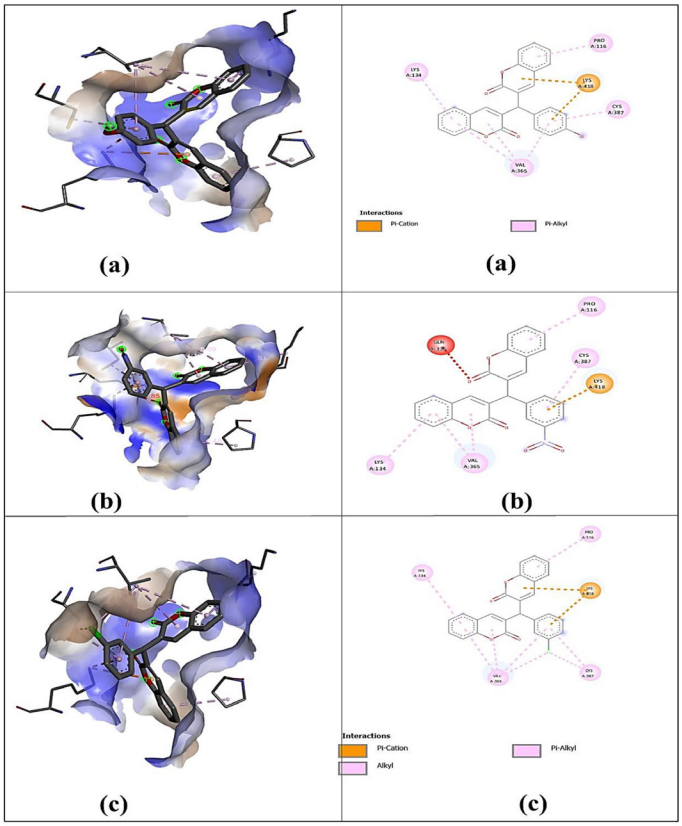

Figure 4a: 3D interactions of ligands with respective amino-acids of DprE1. (a). 3.3'-((4-Hydroxyphenyl) methylene) bis (2H-chromen-2-one) (C1). (b). 3.3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) (C2). (c). 3.3'-((3-chlorophenyl) methylene) bis (2H-chromen-2-one with (C3).

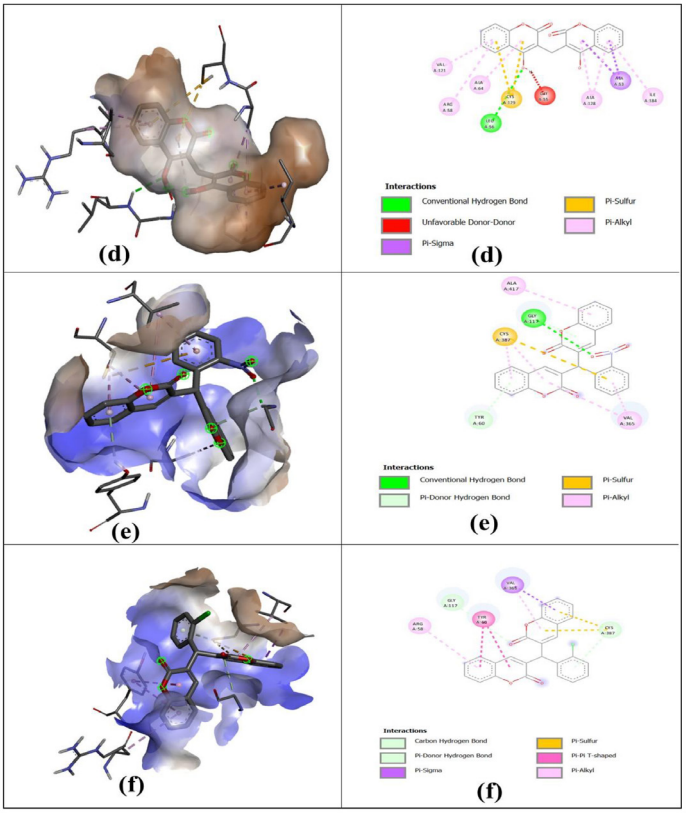

Figure 4b: 3D and 2D interactions of ligands with the respective amino-acids of DprE1. (d). 3.3′-methylenebis (4-hydroxy-2H-chromen-2-one) (C4). (e). 3.3′((2-nitrophenyl) methylene) bis (2H-chromen-2-one) (C5). (f). 3.3′((2-chlorophenyl) methylene) bis (2H-chromen-2-one) (C6).

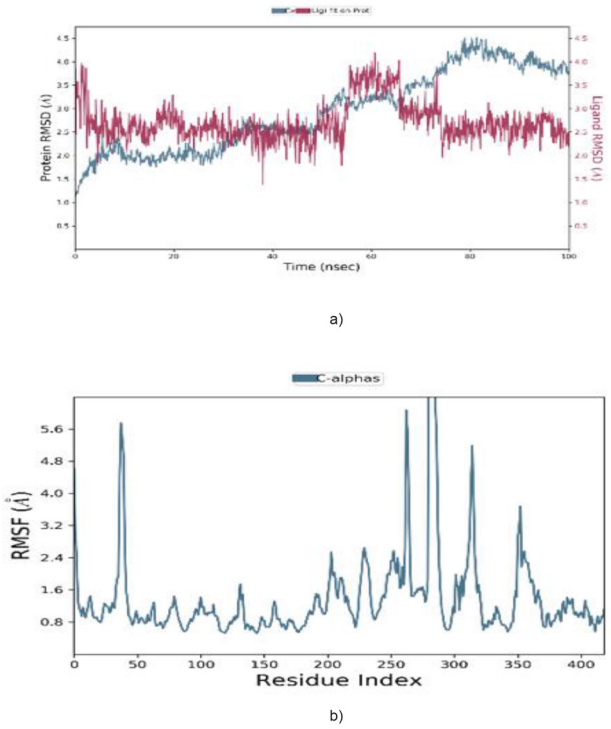

Figure 5a, 5b: Desmond Schrodinger is used to analyse the protein-ligand (C2) complex through molecular dynamics simulation (i) Root Mean Square Deviation is depicted in Image A (ii) The Root Mean Square Fluctuation is depicted in Image B (iii) Image C shows a protein-ligand interaction and histogram.

Molecular dynamic simulation

3.3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) (C2) was subjected to molecular dynamics simulation using Desmond Schrodinger v20.2 for 100 nanoseconds against DprE1 protein. The complex was considered for docking pose in binding site regions of receptors and led to computing the molecules with time, followed by Newton's equation for dynamic modulators. The OPLS force field was applied for 100 nanoseconds for a constant period concerning complex compounds.

The start of the 100 nanoseconds simulation runs revealed stable trajectories for the root mean square fluctuations and root mean square deviations. The RMSD values for the standard docked compound were displayed on the Y-axis, while the ligand RMSD was displayed on the X-axis on the right. The fluctuation in ligand RMSF was observed during the first 30ns of the standard trajectory. The system was standardized, and no changes in density, volume, or kinetic energies were detected[48] (Figure 5a, 5b, and 5c).

Normal mode analysis

Through the molecular docking analysis, it was observed that 3.3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) were exhibiting the highest binding affinities with the target protein.

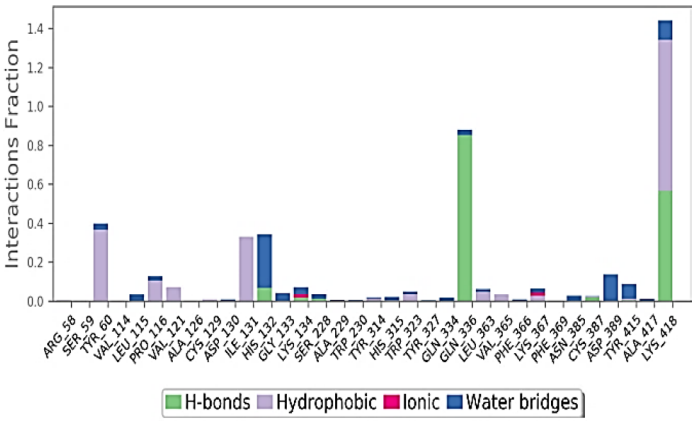

Figure 5c: Graphical representation depicts the interaction between protein and ligand.

Hence these were selected for the MD simulation. In the current study, the molecular dynamic simulation was done on the iMODS server, and the results were given the ID 0301132717957. This server analyzed internal coordinates based on the structural interaction between protein-protein types. iMODS was used to analyze stability, B factor, molecular mobility, variance, and eigenvalue calculation (Figure 6a -e).

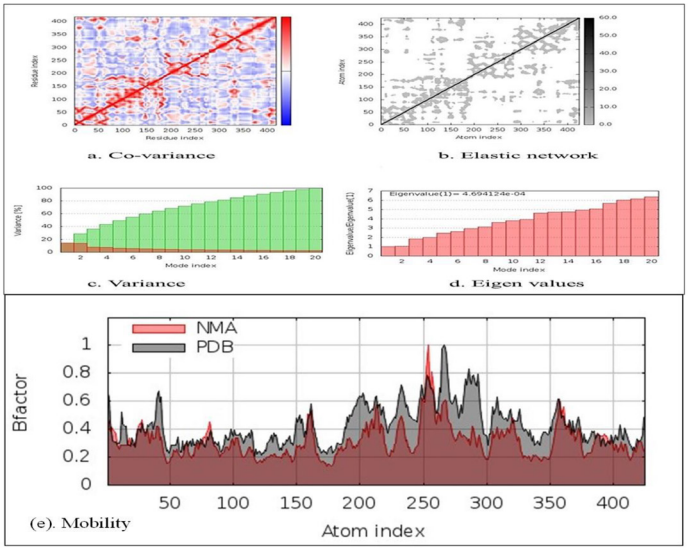

Figure 6: a) Covariance map (correlated (red), uncorrelated (white) or anti-correlated (blue) motions of coupled residues). b) Elastic network (Each dot denotes one spring within the respective atoms pair. The dots are colored based on the stiffness where the dark grey dots indicate the stiffer springs and vice versa). c) Variance (individual (red) and cumulative (green) variances). d) Eigenvalues (The eigenvalue associated with each normal mode represents the motion stiffness. Its value is directly related to the energy required to deform the structure. The lower the eigenvalue, the easier the deformation) e) B-factor or Mobility (The main-chain deformability measures the capability of a given molecule to deform at each of its residues).

DISCUSSION

Different high-throughput screening (HTS) strategies are utilized to screen drug-like small molecular chemical libraries in search of novel and active scaffolds (hit compounds) for lead generation.[48,49] Over the last century, two screening technologies have dominated the early-stage drug development process: target-based approaches and phenotypic screening. The anti-TB drug discovery field can explore the target-to-drug and drug-to-target tools.[51] The former strategy employs traditional techniques such as cutting-edge computer screens and biochemical assays. Still, it has yet to provide any therapeutic candidates for clinical trials, with a high attrition rate due to a lack of whole-cell activity.[51] The latter approach ensures whole-cell activity by testing chemical libraries against bacilli or model organisms; however, target identification is the rate-limiting step in this approach.[52] Numerous scientific advances have resulted in combining elements of both methodologies for the innovation of novel drug discovery tools that speed up screening new hits and leads with known targets and whole-cell activity.[53]

Developing a new drug is a lengthy and complex process that requires an extensive range of stages and strategies. Drug development research encompasses a set of extended steps and complicated strategies. Modern developments in computational modeling methods, pharmaco-kinetic profile (ADMET), molecular docking, high-throughput virtual screening, and bioavailability evaluations of molecules are considered as highly sophisticated techniques for speeding up the drug development processes.[20] Moreover, combining MD simulation with free-binding energy estimation improves spatial fitting accuracy, interaction stability, and ligand binding affinity at the active site of proteins.[54] In the present study,100 compounds were examined and screened for their ability to inhibit the DprE1 protein (PDB ID: 4FEH) of *Mtb,* as this enzyme is an oxidoreductase that is involved in the biosynthesis of cell wall.[55] The Rv3790 gene product DprE1 has been shown to be the target for two different classes of anti-tubercular drugs, namely benzo-thiazinones (BTZ) and the dinitro-benzamide derivatives (DNB); this enzyme is not present in humans, and represents a useful tool for the identification of new potent anti-tubercular inhibitors.[56] To screen the compounds with higher binding affinities, molecular docking was used in addition to toxicity testing and ADMET screening.[55]

Our study selected the top six compounds with a maximum binding affinity with the receptor protein, DprE1, with a binding score ranging from -8.5 to -10.5 cal/mol. Later, we assessed pharmacokinetic characteristics because compound optimization is essential for substances to pass the standard clinical trial and emerge as potential drug candidates.[57] All six compounds fit the criteria for being considered as potential drug candidates, according to our prediction of ADMET properties on docked compounds. A toxic substance is capable of causing harm to an organism.[58,59] The results indicate that toxicity is responsible for the failure of late-stage drug development. *In silico* toxicity analysis is highly effective because it overcomes all the drawbacks of conventional methods, such as the need for animals, expense, and length of time required for *in vivo* testing.[60]

Consequently, we also analyzed the toxicity profiles of the top six substances using an *in-silico* method. The data obtained from the toxicology server indicated that none of the compounds are carcinogenic. The ability of the compounds to cause reverse mutation was evaluated using the AMES test through a computational approach, and it was revealed that all of the compounds tested negative.[62] The compounds were found to be weak hERG inhibitors by the toxicity prediction test. The result analysis of all these parameters prompted us to perform the M D simulation analysis of the top-scored compound along with the receptor protein.

As MD simulation analyses the physical movements of atoms, it has become an indispensable tool for CADD.[62] Using MD simulation, the stability of drug candidates towards the target protein of interest is determined. Our six selected compounds were validated using RMSD, RMSF, and Rg values and calculated hydrogen bonds using MD simulation. Upon observation, amino acids including LYS 37, GLN 30, and HIS 85 were found to possess ligand residues.[62] Lysine acetylation plays a regulatory role in the pathogenesis of *Mtb,* including cell cycle regulation and apoptosis, while histidine is vital for the growth of *Mtb*. Molecular Dynamic Simulations corroborate the conformational

stability of the proposed novel drug molecules.[63] However, additional *in vivo* experiments are required as it may take years to undergo many clinical trials to prove themselves as potent drugs and be available for humankind.

CONCLUSION

In conclusion, we explored the structural-based virtual screening to identify promising chemical entities as the inhibitors of DprE1 from various databases, including PubChem and ZINC databases, and obtained compounds from other published sources. One hundred (100) compounds were chosen for their anti-microbial activity after the initial sorting of the compounds. The multiple steps, including ADMET screening, molecular docking, and toxicity analysis, led to selecting six bioactive (6 molecules) hit molecules. The detailed analysis of pharmacokinetics and drug-like properties using ADMET toxicity implied that six chemical entities (C1-C6) might be investigated as potential candidates for the lead optimization against the DprE1 protein of *Mtb*. Multiple M D simulations of the complex of DprE1 with lead molecules were performed to ascertain the conformational stability of hit-molecules at the active site of the DprE1 receptor in an aqueous environment. From the findings of the current studies, it can be stated that 3,3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) can be developed as a novel anti-TB drug candidate as it is effective against DprE1 by performing the inhibition of the DprE1 activity which subsequently prevents the biosynthesis of the cell wall in *Mtb*. Therefore, our computational studies suggested that the chosen compounds (C1-C6) in general and 3,3'-((3-nitrophenyl) methylene) bis (2H-chromen-2-one) (C2) in particular could be investigated further as novel lead molecules for the rational drug designing of DprE1- inhibitors in the therapy of tuberculosis.

ACKNOWLEDGEMENT

The authors are thankful to the School of Biological Sciences, University of Kashmir. Mr. Bashir A. Sheikh is a recipient of a Departmental Fellowship from the University of Kashmir, Govt. of India to this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

Author contribution

M.A.M and B.A.S conceptualization/design of the research work and analysis of data. B.A.S. and B.A.B collected the data and wrote the manuscript. B.A.S, B.A.B, M.A, ZA, MA, AA and MAM reviewed, and edited the manuscript, Figures/tables and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

This work was financially supported by grants to MAM by the Science and Engineering Research Board, Department of Science and Technology (SERB-DST) Govt. of India, New Delhi; vide Project Grant No: TAR/001213/2018.

ABBREVIATIONS

TB: Tuberculosis; ***Mtb*:** *Mycobacterium tuberculosis;* **MD simulation:** Molecular dynamic simulation; **DprE1:** Decaprenylphosphoryl-β-D-ribose 2'-epimerase; **PTB:** Pulmonary tuberculosis; **ADME:** Absorption, Distribution, Metabolism and Excretion; **TLRs:** Toll-like receptors; **XDR-TB:** Extremely drug resistant tuberculosis; **MDR-TB:** Multi-drug resistant tuberculosis; **TDR-TB:** Totally drug-resistant TB.

REFERENCES

1. Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P, *et al*. Global Tuberculosis Report 2020 – Reflections on the Global TB burden, treatment and prevention efforts. Int J Infect Dis. 2021;113;Suppl 1:S7-S12. doi: 10.1016/j.ijid.2021.02.107, PMID 33716195.
2. Jeremiah C, Petersen E, Nantanda R, Mungai BN, Migliori GB, Amanullah F, *et al*. The WHO Global tuberculosis 2021 Report–not so good news and turning the tide back to End TB. Int J Infect Dis. 2022.
3. World health, O. Global tuberculosis report. 2020: Executive summary;2020.
4. Tiberi S, Zumla A, Migliori GB. Multidrug and extensively drug-resistant tuberculosis: Epidemiology, clinical features, management and treatment. Infect Dis Clin North Am. 2019;33(4):1063-85. doi: 10.1016/j.idc.2019.09.002, PMID 31668191.
5. Khawbung JL, Nath D, Chakraborty S. Drug resistant tuberculosis: A review. Comp Immunol Microbiol Infect Dis. 2021;74:101574. doi: 10.1016/j.cimid.2020.101574, PMID 33249329.
6. Kalscheuer R, Palacios A, Anso I, Cifuente J, Anguita J, Jacobs WR Jr, *et al*. The *Mycobacterium tuberculosis* capsule: A cell structure with key implications in pathogenesis. Biochem J. 2019;476(14):1995-2016. doi: 10.1042/BCJ20190324, PMID 31320388.
7. Garcia-Vilanova A, Chan J, Torrelles JB. Underestimated manipulative roles of *Mycobacterium tuberculosis* cell envelope glycolipids during infection. Front Immunol. 2019;10:2909. doi: 10.3389/fimmu.2019.02909, PMID 31921168.
8. Daffé M, Marrakchi H. Unraveling the structure of the mycobacterial envelope. Microbiol Spectr. 2019;7(4):7-4. doi: 10.1128/microbiolspec.GPP3-0027-2018, PMID 31267927.
9. Grzelak EM, Choules MP, Gao W, Cai G, Wan B, Wang Y, *et al*. Strategies in anti-*Mycobacterium tuberculosis* drug discovery based on phenotypic screening. J Antibiot (Tokyo). 2019;72(10):719-28. doi: 10.1038/s41429-019-0205-9, PMID 31292530.
10. Qadri H, Shah AH, Mir M. Novel strategies to combat the emerging drug resistance in human pathogenic microbes. Curr Drug Targets. 2021;22(12):1424-36. doi: 10.2174/1389450121666201228123212, PMID 33371847.
11. Sheikh BA, Bhat BA, Ahmad Z, Mir MA. Strategies employed to evade the host immune response and the mechanism of drug resistance in *Mycobacterium tuberculosis*: in search of finding new targets. Curr Pharm Biotechnol. 2021. doi: 10.2174/1389201023666211222164938, PMID 34951359.
12. Cohen KA, Manson AL, Desjardins CA, Abeel T, Earl AM. Deciphering drug resistance in *Mycobacterium tuberculosis* using whole-genome sequencing: Progress, promise, and challenges. Genome Med. 2019;11(1):45. doi: 10.1186/s13073-019-0660-8, PMID 31345251.
13. Sheikh BA, Bhat BA, Mehraj U, Mir W, Hamadani S, Mir MA. Development of new therapeutics to meet the current challenge of drug resistant tuberculosis. Curr Pharm Biotechnol. 2021;22(4):480-500. doi: 10.2174/1389201021666200628021700, PMID 32600226.
14. Mir MA. Developing costimulatory molecules for immunotherapy of diseases.

Academic Press; 2015.
15. Piton J, Foo CS, Cole ST. Structural studies of *Mycobacterium tuberculosis* DprE1 interacting with its inhibitors. More Medicines for Tuberculosis: Fuelling Drug Discovery against *Mycobacterium tuberculosis*. 2017 Chapter A1;22(3):189. doi: 10.1016/j.drudis.2016.09.014, PMID 27666194.
16. Mir MA. Costimulation immunotherapy in allergies and asthma. Sydney: Association of Clinical Pathologists Press; 2015.
17. Sheikh BA, Bhat BA, Alshehri B, Mir RA, Mir WR, Parry ZA, *et al*. Nano-drug delivery systems: Possible End to the rising threats of tuberculosis. J Biomed Nanotechnol. 2021;17(12):2298-318. doi: 10.1166/jbn.2021.3201, PMID 34974855.
18. Qadri H, Shah AH, Mir MA. Role of immunogenetics polymorphisms in infectious diseases. A Molecular Approach to Immunogenetics. Elsevier; 2022. p. 169-91.
19. Bhat BA, Mir WR, Sheikh BA, Alkanani M, Mir MA. Metabolite fingerprinting of phytoconstituents from *Fritillaria cirrhosa* D. Don and molecular docking analysis of bioactive peonidin with microbial drug target proteins [sci rep]. Sci Rep. 2022;12(1):7296. doi: 10.1038/s41598-022-10796-7, PMID 35508512.
20. Niranjan Kumar N, Srivastava R, Prakash A, Lynn AM. Virtual screening and free energy estimation for identifying *Mycobacterium tuberculosis* flavoenzyme DprE1 inhibitors. J Mol Graph Model. 2021;102:107770. doi: 10.1016/j.jmgm.2020.107770, PMID 33065513.
21. Ghattas MA, Mansour RA, Atatreh N, Bryce RA. Analysis of enoyl-acyl carrier protein reductase structure and interactions yields an efficient virtual screening approach and suggests a potential allosteric site. Chem Biol Drug Des. 2016;87(1):131-42. doi: 10.1111/cbdd.12635, PMID 26259619.
22. Imran M, A S A, Thabet HK, Abida M, Afroz Bakht M. Synthetic molecules as DprE1 inhibitors: A patent review. Expert Opin Ther Pat. 2021;31(8):759-72. doi: 10.1080/13543776.2021.1902990, PMID 33709862.
23. Ezquerra-Aznárez JM, Degiacomi G, Gašparovič H, Stelitano G, Sammartino JC, Korduláková J, *et al*. The veterinary antiparasitic selamectin is a novel inhibitor of the mycobacterial DprE1 enzyme. bioRxiv. 2021.
24. Mir MA, Bhat BA, Sheikh BA, Rather GA, Mehraj S, Mir WR. Nanomedicine in human health therapeutics and drug delivery: Nanobiotechnology and nanobiomedicine. Advances in Chemical and Materials Engineering. 2021:229-51. doi: 10.4018/978-1-7998-5563-7.ch013.
25. Ma Z, He S, Yuan Y, Zhuang Z, Liu Y, Wang H, *et al*. Design, synthesis, and characterization of TNP-2198, a dual-targeted rifamycin-nitroimidazole conjugate with potent activity against microaerophilic and anaerobic bacterial pathogens. J Med Chem. 2022;65(6):4481-95. doi: 10.1021/acs.jmedchem.1c02045, PMID 35175750.
26. Riccardi G, Pasca MR, Chiarelli LR, Manina G, Mattevi A, Binda C. The DprE1 enzyme, one of the most vulnerable targets of *Mycobacterium tuberculosis*. Appl Microbiol Biotechnol. 2013;97(20):8841-8. doi: 10.1007/s00253-013-5218-x, PMID 24037308.
27. Dhameliya TM, Bhakhar KA, Gajjar ND, Patel KA, Devani AA, Hirani RV. Recent advancements and developments in search of anti-tuberculosis agents: A quinquennial update and future directions. J Mol Struct. 2022;1248:131473. doi: 10.1016/j.molstruc.2021.131473.
28. G AC, Gondru R, Li Y, Banothu J. Coumarin–benzimidazole hybrids: A Review of Developments in Medicinal Chemistry. Eur J Med Chem. 2022;227:113921. doi: 10.1016/j.ejmech.2021.113921, PMID 34715585.
29. Shawky AM, Ibrahim NA, Abourehab MAS, Abdalla AN, Gouda AM. Pharmacophore-based virtual screening, synthesis, biological evaluation, and molecular docking study of novel pyrrolizines bearing urea/thiourea moieties with potential cytotoxicity and CDK inhibitory activities. J Enzyme Inhib Med Chem. 2021;36(1):15-33. doi: 10.1080/14756366.2020.1837124, PMID 33103497.
30. Chakraborti S, Chakraborty M, Bose A, Srinivasan N, Visweswariah SS. Identification of potential binders of Mtb Universal Stress Protein (Rv1636) through an *in silico* approach and insights into compound selection for experimental validation. Front Mol Biosci. 2021;8:599221. doi: 10.3389/fmolb.2021.599221, PMID 34012976.
31. Alagumuthu M, Rajpoot S, Baig MS. Structure-based design of novel peptidomimetics targeting the SARS-CoV-2 spike protein. Cell Mol Bioeng. 2021;14(2):177-85. doi: 10.1007/s12195-020-00658-5, PMID 33072222.
32. Sumsion GR, Bradshaw MS, Beales JT, Ford E, Caryotakis GRG, Garrett DJ *et al*. Diverse approaches to predicting drug-induced liver injury using gene-expression profiles. Biol Direct. 2020;15(1):1. doi: 10.1186/s13062-019-0257-6. PMID 31941542.
33. Mehraj U, Aisha S, Sofi S, Mir MA. Expression pattern and prognostic significance of baculoviral inhibitor of apoptosis repeat-containing 5 (BIRC5) in breast cancer: A comprehensive analysis. Advances in Cancer Biology - Metastasis. 2022;4:100037. doi: 10.1016/j.adcanc.2022.100037.
34. Bhakhar KA, Gajjar ND, Bodiwala KB, Sureja DK, Dhameliya TM. Identification of anti-mycobacterial agents against mmpL3: Virtual screening, ADMET analysis and MD simulations. J Mol Struct. 2021;1244:130941. doi: 10.1016/j.molstruc.2021.130941.
35. Singh S, Das T, Awasthi M, Pandey VP, Pandey B, Dwivedi UN. DNA topoisomerase-directed anticancerous alkaloids: ADMET-based screening, molecular docking, and dynamics simulation. Biotechnol Appl Biochem. 2016;63(1):125-37. doi: 10.1002/bab.1346, PMID 25594242.
36. Bhowmik R, Nath R, Roy R. Screening of zinc database against streptococcal cysteine protease enzyme for identification of novel Group A streptococcus inhibitors. JPRI. 2021:401-12. doi: 10.9734/jpri/2021/v33i43A32504.
37. Abdullahi M, Adeniji SE. *In-silico* molecular docking and ADME/pharmacokinetic prediction studies of some novel carboxamide derivatives as anti-tubercular agents. Chem Afr. 2020;3(4):989-1000. doi: 10.1007/s42250-020-00162-3.
38. Kumar R, Garg P, Bharatam PV. Shape-based virtual screening, docking, and molecular dynamics simulations to identify Mtb-ASADH inhibitors. J Biomol Struct Dyn. 2015;33(5):1082-93. doi: 10.1080/07391102.2014.929535, PMID 24875451.
39. Noorulla KM, Suresh AJ, Devaraji V, Mathew B, Umesh D. Molecular modeling of drug-pathophysiological Mtb protein targets: Synthesis of some 2-thioxo-1, 3-thiazolidin-4-one derivatives as anti-tubercular agents. J Mol Struct. 2017;1147:682-96. doi: 10.1016/j.molstruc.2017.07.009.
40. Mukerjee N, Das A, Maitra S, Ghosh A, Khan P, Alexiou A, *et al*. Dynamics of natural product Lupenone as a potential fusion inhibitor against the spike complex of novel Semliki Forest Virus. PLOS ONE. 2022;17(2):e0263853. doi: 10.1371/journal.pone.0263853, PMID 35213606.
41. Castrosanto MA, Mukerjee N, Ramos AR, Maitra S, Manuben JJP, Das P, *et al*. Abetting host immune response by inhibiting *Rhipicephalus sanguineus* Evasin-1: An *in silico* approach. PLOS ONE. 2022;17(9):e0271401. doi: 10.1371/journal.pone.0271401, PMID 36099243.
42. Naz S, Farooq U, Khan S, Sarwar R, Mabkhot YN, Saeed M, *et al*. Pharmacophore model-based virtual screening, docking, biological evaluation and molecular dynamics simulations for inhibitors discovery against α-tryptophan synthase from *Mycobacterium tuberculosis*. J Biomol Struct Dyn. 2021;39(2):610-20. doi: 10.1080/07391102.2020.1715259, PMID 31937192.
43. Kalaivanan C, Sankarganesh M, Suvaikin MY, Karthi GB, Gurusamy S, Subramanian R, *et al*. Novel Cu(II) and Ni(II) complexes of nicotinamide based Mannich base: Synthesis, characterization, DFT calculation, DNA binding, molecular docking, antioxidant, antimicrobial activities. J Mol Liq. 2020;320:114423. doi: 10.1016/j.molliq.2020.114423.
44. Arvind A, Kumar V, Saravanan P, Mohan CG. Homology modeling, molecular dynamics and inhibitor binding study on MurD ligase of *Mycobacterium tuberculosis*. Interdiscip Sci Comput Life Sci. 2012;4(3):223-38. doi: 10.1007/s12539-012-0133-x.
45. Oso BJ, Adeoye AO, Olaoye IF. Pharmacoinformatics and hypothetical studies on allicin, curcumin, and gingerol as potential candidates against COVID-19-associated proteases. J Biomol Struct Dyn. 2022;40(1):389-400. doi: 10.1080/07391102.2020.1813630, PMID 32876538.
46. Billones JB, Carrillo MCO, Organo VG, Macalino SJ, Sy JB, Emnacen IA, *et al*. Toward antituberculosis drugs: *In silico* screening of synthetic compounds against *Mycobacterium tuberculosis* l, d-transpeptidase 2. Drug Des Devel Ther. 2016;10:1147-57. doi: 10.2147/DDDT.S97043, PMID 27042006.
47. Kb S, Kumari A, Shetty D, Fernandes E, Dv C, Jays J *et al*. Structure based pharmacophore modelling approach for the design of azaindole derivatives as DprE1 inhibitors for tuberculosis. J Mol Graph Model. 2020;101:107718. doi: 10.1016/j.jmgm.2020.107718, PMID 32949960.
48. Bleicher KH, Böhm HJ, Müller K, Alanine AI. Hit and lead generation: Beyond high-throughput screening. Nat Rev Drug Discov. 2003;2(5):369-78. doi: 10.1038/nrd1086, PMID 12750740.
49. Andrews N, Stowe J, Kirsebom F, Toffa S, Rickeard T, Gallagher E, *et al*. Covid-19 vaccine effectiveness against the Omicron (B.1.1.529) Variant. N Engl J Med. 2022;386(16):1532-46. doi: 10.1056/NEJMoa2119451, PMID 35249272.
50. Van der Westhuyzen R, Mabhula A, Njaria PM, Müller R, Ngumbu Muhunga D, Taylor D, *et al*. Benzoheterocyclic oxime carbamates active against *Mycobacterium tuberculosis*: Synthesis, structure–activity relationship, metabolism, and biology triaging. J Med Chem. 2021;64(13):9444-57. doi: 10.1021/acs.jmedchem.1c00707, PMID 34138573.
51. Berdigaliyev N, Aljofan M. An overview of drug discovery and development. Future Med Chem. 2020;12(10):939-47. doi: 10.4155/fmc-2019-0307, PMID 32270704.
52. Abrahams KA, Besra GS. Mycobacterial drug discovery. RSC Med Chem. 2020;11(12):1354-65. doi: 10.1039/d0md00261e, PMID 34085044.
53. Vamathevan J, Clark D, Czodrowski P, Dunham I, Ferran E, Lee G, *et al*. Applications of machine learning in drug discovery and development. Nat Rev Drug Discov. 2019;18(6):463-77. doi: 10.1038/s41573-019-0024-5, PMID 30976107.
54. Rajkishan T, Rachana A, Shruti S, Bhumi P, Patel D. Computer-aided drug designing. Adv Bioinformatics. 2021:151-82.
55. Mounika G, Khuntia A, Nayak S, Kumar BS, Yellasubbaiah N, Sabbi TK, *et al*. *In-silico* Analysis of the Inhibitory Activities of Novel AZO Derivatives of benzimidazole on *Mycobacterium tuberculosis* DPRE1.
56. Manina G, Pasca MR, Buroni S, De Rossi E, Riccardi G. Decaprenylphosphoryl-β-

D-ribose 2′-epimerase from *Mycobacterium tuberculosis* is a magic drug target. Curr Med Chem. 2010;17(27):3099-108. doi: 10.2174/092986710791959693, PMID 20629622.

57. Balganesh TS, Bhat JJ, Ugarkar B. Modern drug discovery and development for TB: The India narrative. Drug Discovery and Drug Development. Springer; 2021. p. 37-61.
58. Raveesha R, Kumar KY, Raghu MS, Prasad SBB, Alsalme A, Krishnaiah P, *et al*. Synthesis, *in silico* ADME, toxicity prediction and molecular docking studies of N-substituted. J Mol Struct. 2022;1(2, 4) triazolo [4, 3-a] pyrazine derivatives as potential anticonvulsant agents:132407.
59. Bhat BA, Mir WR, Sheikh BA, Rather MA, Dar TUH, Mir MA. *In vitro* and *in silico* evaluation of antimicrobial properties of *Delphinium cashmerianum* L., a medicinal herb growing in Kashmir, India. J Ethnopharmacol. 2022;291:115046. doi: 10.1016/j.jep.2022.115046, PMID 35167935.
60. Mehraj U, Mushtaq U, Mir MA, Saleem A, Macha MA, Lone MN, *et al*., editors. Chemokines in triple-negative breast cancer heterogeneity: new challenges for clinical implications. Elsevier; 2022.
61. Kwofie SK, Dolling NNO, Donkoh E, Laryea GM, Mosi L, Miller WA Iii, *et al*. Pharmacophore-Guided Identification of Natural Products as Potential Inhibitors of *Mycobacterium ulcerans* Cystathionine γ-synthase MetB. Computation. 2021;9(3):32. doi: 10.3390/computation9030032. Note s. MDPI stays neutral with regard to jurisdictional claims in published … ; 2021.
62. Shukla R, Tripathi T. Molecular dynamics simulation in drug discovery: Opportunities and challenges. Innov Implement Computer Aided Drug Discov Strateg Ration Drug Des. 2021:295-316.
63. Tabti K, Elmchichi L, Sbai A, Maghat H, Bouachrine M, Lakhlifi T, *et al*. *In silico* design of novel PIN1 inhibitors by combined of 3D-QSAR, molecular docking, molecular dynamic simulation and ADMET studies. J Mol Struct. 2022;1253:132291. doi: 10.1016/j.molstruc.2021.132291.

Cite this article: Sheikh BA, Bhat BA, Rizvi MA, Ahmad Z, Almilaibary A, Alkhanani M, *et al*. Computational Studies to Identify Potential Inhibitors Targeting the DprE1 Protein in *Mycobacterium tuberculosis*. Int. J. Pharm. Investigation. 2023;13(1):129-38.