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Review RRJODDD

In-Silico Repurposing of Antipsychotic Drugs Against Novel Protein Targets in Multidrug-Resistant *Mycobacterium Tuberculosis*

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Abstract

Multidrug-resistant TB is a growing worldwide health concern, particularly in high-burden areas. Resistance to frontline drugs like isoniazid and rifampicin is caused by mutations in key Mycobacterium tuberculosis proteins. Given the difficulty of developing new antibiotics, medication repurposing offers a good alternative. The potential use of molecular docking to target resistance-associated TB proteins with FDA-approved antipsychotic drugs is investigated in this work. Three proteins were selected: InhA (PDB ID: 5W07), which is involved in the manufacture of mycolic acid; KatG (PDB ID: 1U2J), which activates isoniazid; and Rv0678 (PDB ID: 4NB5), a transcriptional repressor that regulates efflux pumps linked to drug resistance. Docking was done with AutoDock Vina. The results showed that Fluspirilene and Ziprasidone interacted strongly with KatG, but Lurasidone and Bifurprunox demonstrated favourable binding to both InhA and Rv0678. Interestingly, pimozide showed a broad spectrum of inhibitory potential since it interacted with all three proteins. These findings provide credence to the idea that certain antipsychotics may be repurposed as adjuvant treatments by disrupting several pathways of resistance in MDR-TB. More experimental validation is required to confirm their efficacy and safety.

Keywords: *Mycobacterium tuberculosis*, InhA, KatG, Rv0678, Molecular Docking, Antipsychotic drugs, Drug repurposing, Multidrug-resistance (MDR)

INTRODUCTION

Drug repurposing, also called drug refocusing, has become very important in tackling tough and resistant diseases such as tuberculosis (TB). With drug repurposing, experts do not need to spend years and lots of money creating new drugs. Rather, they use familiar products with proven safety to search for possible new medicinal uses [1]. It helps a lot in the current effort to overcome anti-drug resistance that poses a serious challenge in treating TB. By repurposing drugs, it is possible for them to skip the initial clinical trial stages, reduce the development process, deal with resistant TB varieties, and enhance the outcome for patients [2].

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According to estimates, every year about 10 million cases of TB are reported and 1.5 million individuals die because of it [3]. In TB, there is a close interaction happening between the infectious pathogen and the immune system of the host. During inhalation, *M. tuberculosis* infects alveolar macrophages and keeps the phagosomes in an immature form to avoid getting eliminated by the immune system [4]. Therefore, the infection passes into a dormant period or develops into actual disease that causes granulomas and cell death [5, 6]. The cell wall of bacteria prevents drugs from entering the cells, and their slow metabolism as

well as the activity of drug pumping help cells resist the drugs. Resistance most often develops as a result of drug target mutations like spontaneous changes in chromosomes, changes in key pathways, or enhancing efflux mechanisms/pumps, formation of biofilms and it becomes stronger when treatment is not thorough, management is not well done, and resistant strains are sent to others [7]. In addition, strategies M. tuberculosis uses to avoid being killed by the host's immune cells enable the infection to last a long time and continue a hidden state, even when treated with many drugs. Multidrug-resistant TB (MDR-TB) causes difficulty both in treating and managing the disease across the globe [8]. Thus, treating this bacterium requires strategies that focus on the pathogen as well as on the host's immune response.

To treat drug-sensitive TB, people are usually given a combination of first-line antibiotics like isoniazid, rifampin, pyrazinamide, and ethambutol for at least six months [9]. MDR-TB endangers many people across the globe by causing these commonly used anti-TB drugs to become ineffective. Because of resistant strains, treatment is usually longer using second line drugs like fluoroquinolones, bedaquiline, linezolid, and clofazimine, which is more difficult to carry out, and can involve harmful side effects, so scientists must search for new therapies [10, 11]. To give an example of a treatment plan, WHO has introduced updates with new 6-month treatments using bedaquiline, delamanid, and bedaquiline, added to other medications like levofloxacin or clofazimine, in order to help people with MDR and rifampicin-resistant TB, but quickly, resistance to these agents has been reported. Another example is the all-oral treatment plans such as BPaL and BPaLM (bedaquiline, pretomanid, linezolid, with or without moxifloxacin) have made a difference, but they are not yet able to handle all the challenges related to drug resistance [12, 13].

Renewed interest in several existing drugs for anti-infective, anticancer, or neuropsychiatric use is focused on their effect against TB for their antimycobacterial effects by numerous studies [14, 15, 16]. Some of the non-neuroleptic phenothiazines could fight *M. tuberculosis* in mice and solutions without causing damaging side effects [17, 18]. Other potential antipsychotic medications, for example pimozide and fluspirilene, were found worth exploring by using in silico techniques [19]. Such findings may open up new methods of TB treatment by focusing on new proteins in *M. tuberculosis* instead of the ones targeted by the current batch of TB drugs [20]. Some research areas are still missing for the full identification and checking of these new targets, as well as understanding the exact action happening when antipsychotics are used to treat tuberculosis. Besides, molecular docking and similar computational approaches for drug repurposing have yet to be maximized in drug safety related studies.

One important way to solve the problem is molecular docking, which helps forecast binding between molecules and proteins on a molecular level [21]. It can model how FDA-approved medications are expected to connect and bind to new targets found in the tuberculosis bacterium. The aim of molecular docking is to spot protected combinations of a molecule with a target that give hints on therapy and reveal new uses for drugs. The main advantages are saving time and resources, early screening relying less on many tests in the lab, and the possibility to target multiple targets at once [22]. So far, well-known docking algorithms and software, such as AutoDock, GOLD, and Discovery Studio, have given researchers the ability to accurately assess if antipsychotic drugs bind with the target protein.

Using what was learned from these advances, this study looks into the interactions between various antipsychotic drugs and three multidrug-resistant (MDR) targets in M. tuberculosis: InhA, KatG, and RV6078. There is evidence that they play a role in causing drug resistance, so they stand out as candidates for treating such problems in the future [23, 24, 25]. In order to find molecules that might overcome resistance, we perform molecular docking simulations and examine how antipsychotic drugs bind with their targets and what patterns of interactions occur. In short, the use of virtual screening supports the development of new treatments for MDR-TB by identifying additional drugs from existing ones.

METHODOLOGY

Retrieval of Proteins

The three proteins KatG, InhA and RV6078 involved in the multidrug resistance (MDR) in Mycobacterium tuberculosis were retrieved using the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https://www.rcsb.org) in pdb format. The structures of the proteins were retrieved via RCSB PDB Search API. KatG is a bifunctional catalase-peroxidase enzyme which activates the frontline tuberculosis prodrug isoniazid (INH), and is an important part of its bactericidal action. This activation is impaired by mutations or modifications in KatG, which confers resistance and subsequently KatG has been established as a key molecular target in MDR research [26, 27]. InhA an enoyl-acyl carrier protein reductase, is required in the synthesis of mycolic acids, which is a major constituent of the mycobacterial cell wall; inhibition of InhA causes cell wall synthesis to be impaired and resistance occurs largely by mutations which decrease drug binding efficacy, making it an important confirmed drug target [28, 29]. RV6078 is a transcriptional regulator of an efflux pump system, MmpS5-MmpL5, which expels several anti-tubercular drugs decreasing their intracellular level, and thus increases drug resistance. This protein is a good target to reversal of efflux-based MDR mechanisms [30, 31].

Retrieval of Ligands

Around 24 ligands were downloaded from the DrugBank database (https://go.drugbank). To perform repurposing analysis, the drugs of interest were selected because they fall under the category of antipsychotics in DrugBank. The associated two-dimensional (2D) chemical structures were, in turn, downloaded via PubChem database (https://pubchem.ncbi.nlm.nih.go). The PubChem 2D structures were downloaded in 2D-SDF formats that could be used in molecular docking investigations. This combined retrieval strategy warranted the presence of well-characterized antipsychotic drugs with definite structural representations to use in the computational analysis.

Protein Purification and Protein Validation

The retrieved KatG, InhA and RV6078 protein structures were refined in a way that would render them suitable in performing molecular docking studies. The protein models were next introduced into BIOVIA Discovery Studio (https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/visualization), where they were made ready to publication-quality structures. The water molecules, heteroatoms, ligands bound to the protein and ions that are likely to interfere with the precision of docking were removed. The reason behind this step of purification is to enhance the precision of the docking process by making the protein environment simpler and eliminating the elements that may result in the distortion of ligand binding [32]. Then polar form of hydrogen atoms and polar groups were added in order to more closely model the physiological conditions to allow the more realistic simulation of the interaction of the molecules under aqueous conditions. To ensure that these refined proteins were structurally valid, Ramachandran plot analysis was carried out to retrieve the backbone dihedral angles (phi and psi) of the amino acids residues. This analysis was done using the PDBsum webserver of EMBL-EBI (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) since it gave the opportunity to assess the stereochemical quality of the proteins on which basis the molecular docking could be carried out [33].

Molecular Docking

In silico method of molecular docking was used to investigate the binding mode and affinity of the selected multidrug-resistant proteins (InhA, KatG and RV6078) with the 24 selected antipsychotic ligands. The computation procedure is used to predict the ligand fitting in active sites of target proteins and measures the strength and stability of the interactions. A key measure used to characterize these interactions is binding affinity, a measurement of the energy of the interaction; the smaller the binding affinity, the stronger and more stable the protein-ligand complex. Another standard important evaluation measure is Root Mean Square Deviation (RMSD) where lower RMSD values indicate more conformational stability of docking poses. The molecular docking workflow was performed in three significant steps: initially, molecular structures of the purified proteins and ligands were subjected to

the required preparation that involved energy minimization and the conversion of formats (.pdb to. pdbqt) using PyRx version 0.8 that incorporates OpenBabel to perform this task [34]. The ligands were assigned Kollman charges and torsional degrees of freedom in order to increase the accuracy of docking. Then, AutoDock Vina docking was done in PyRx, with nine conformations per ligand-protein pair. The best pose was chosen according to such criteria as minimum binding affinity and zero RMSD. The grid box was set so as to include the regions that bind the ligands in order to avoid atomic displacement during the docking procedure. Lastly, the three best ligand-protein complexes with binding affinity -9 kcal/mol or less and RMSD 0 were further visualized and analyzed through the BIOVIA Discovery Studio Visualizer [32] in order to describe the active site interactions in detail. The coordinates and interaction profiles of these complexes were properly documented ready to be interpreted.

Molecular Visualization

The docked conformations with maximum binding affinities which were the result of molecular docking procedure were downloaded in PDB format to be analyzed in detail. The top binding poses in both 2D and 3D were modeled in the BIOVIA Discovery Studio environment to allow an easy exploration of the protein-ligand interactions. This visualization was carried out by extensive exploration of non-bonded interactions which include hydrogen bonds, hydrophobic interactions, and electrostatic interactions and also by examining the bond distances and types to gain more insight into the binding modes and affinity of the complexes.

In-Silico Pharmacology Studies

In silico pharmacology of the top three ligands with the highest binding affinities to each of the proteins KatG, InhA, and RV6078 was performed by a series of computational analyses that examined the pharmacokinetic and pharmacodynamic attributes of the ligands. First, the physicochemical properties of these ligands were described with the aid of SwissADME (http://www.swissadme.ch/), which estimates the main parameters that include lipophilicity, molecular size, polarity, flexibility, saturation, and solubility, which are useful in predicting drug-likeness and oral bioavailability. Subsequently, a stringent filtration of the ligands was conducted based on the Lipinski on the Rule of Five, which is a criterion that guarantees desirable absorption and permeation properties.

These ligands were retrieved in PubChem and their canonical SMILES strings determined to further explain the ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties that are imperative in drug development by thoroughly analyzing them using ADMETlab 3.0 (https://admetlab3.scbdd.com/server/screening). This platform gave detailed predictions on medicinal chemistry friendliness, pharmacokinetics, and toxicity risks, thus ligands with best drug-like characteristics and minimal toxicity were identified.

The integrative utilization of SwissADME and ADMETlab 3.0 enabled a prioritization systematizing in regard to physicochemical and toxicological parameters, thereby enabling suitable candidates to be chosen in regard to further development against the MDR-related M. tuberculosis proteins.

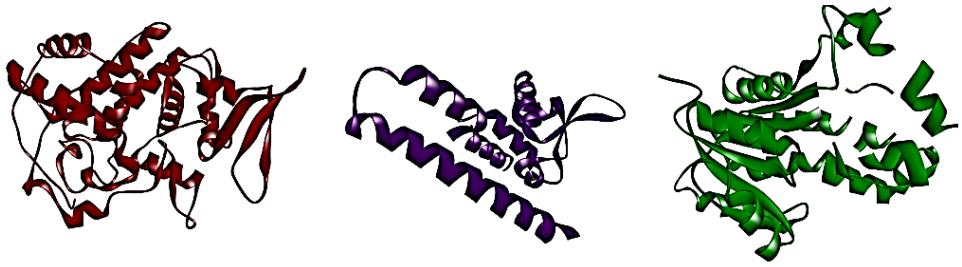
RESULTS

Protein Structure Analysis

The three proteins KatG, InhA and RV6078 were resolved using the X-ray diffraction method. The resolution of each protein are: KatG [2.3 Å], Rv0678 [1.641 Å], InhA [2.65 Å]. The purified proteins (as shown in Figure 1) were brought into the biologically active chains by eliminating all the heteroatoms, water molecules and the ligand groups and ions in order to make them optimal to serve in the docking studies by keeping only the A chain, the most active portion of the protein. To increase the polarity of the proteins, polar groups and hydrogen atoms were added to facilitate the correctness of the molecular interactions in the docking simulation.

The structural validation of the purified protein structures was good with most of the amino acid torsion angles falling in the energetically favorable Ramachandran plot analysis using PROCHECK.

Based on a set of established criteria, with reference to analysis of 118 high-resolution structures (resolution ≤ 2.0 Å, R-factor ≤ 20.0), a model is of good quality when over 90% of residues are found in the most favored regions of the Ramachandran plot.

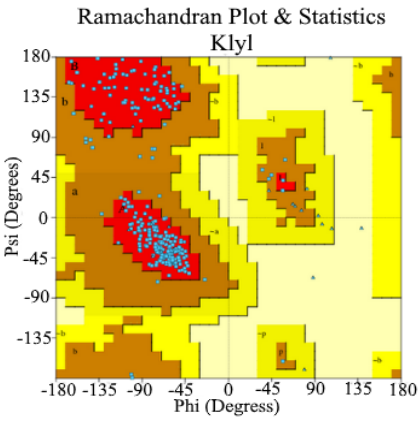


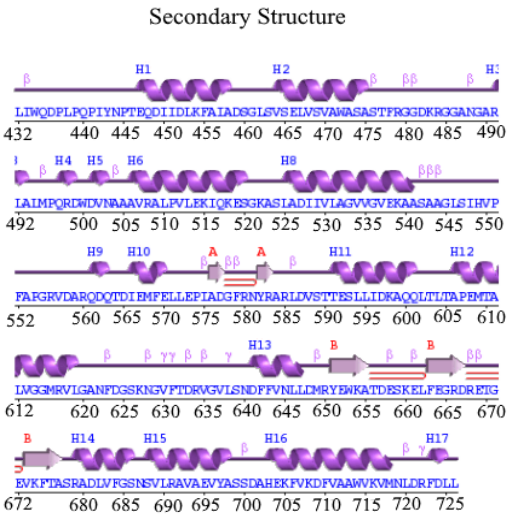
Purified-KatG (PDB ID: 1u2j) | Purified-Rv0678 (PDB ID: 4nb5)) | Purified-InhA (PDB ID: 5w07))

Figure 1. Purified 3-D structures of KatG, Rv0678, InhA.

As shown in Figure 2, the PROCHECK analysis of KatG exhibits a good stereochemical quality profile. The Ramachandran plot statistics says that 90.8% of non-glycine and non-proline residues are in the most favored regions [A, B, L]. Further, 9.2% of residues are located in the additional allowed regions and more significantly, there are no residues in the generously allowed or disallowed regions which highlights the reliability of the structure. The overall number of residues are 295 residues (21 glycines and 10 prolines) and only 2 end residues are excluded.

Purified-KatG (PDB ID: 1u2j)





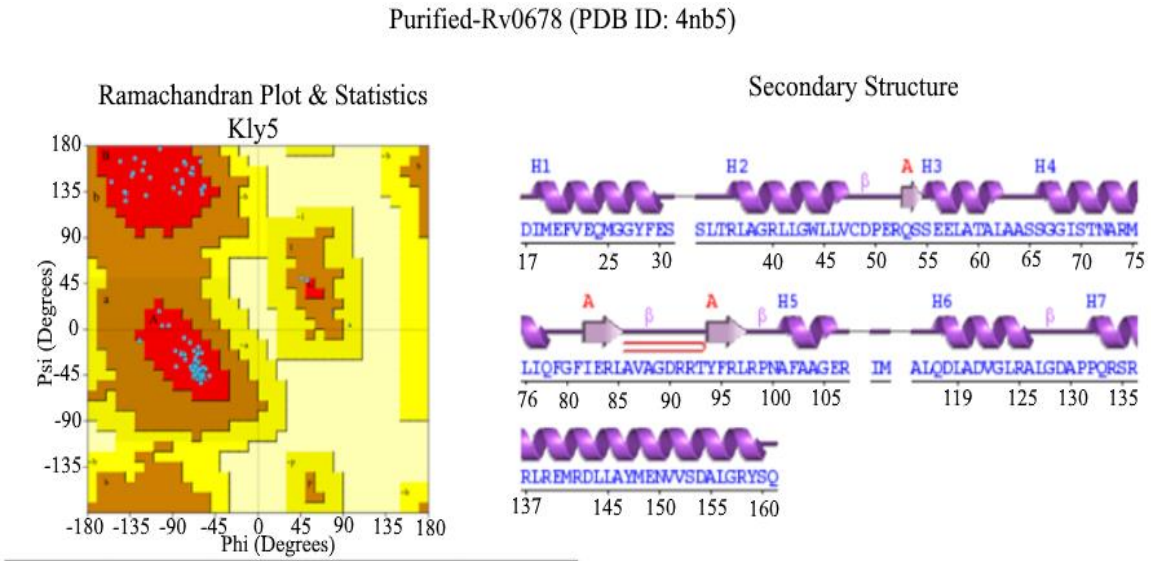
Protein	Favoured Regions	Additional Allowed Regions	Generously Allowed Regions	Disallowed Regions
KatG	90.8%	9.2%	0%	0%

Figure 2. Ramachandran plot and secondary structure of KatG obtained using PDBsum.

There are 114 non-glycine and non-proline residues in Rv0678 (as shown in Figure 3), 98.2% (112 residues) are in the most favored regions (A, B, L). Moreover 1.8% (2 residues) are located in the extra permitted areas (a, b, l, p) and importantly no residues were found in either the generously permitted or disallowed areas. The model contains in total 139 residues, 12 glycine residues and 4 proline residues, and 9 end-residues not included in the core Ramachandran plot analysis.

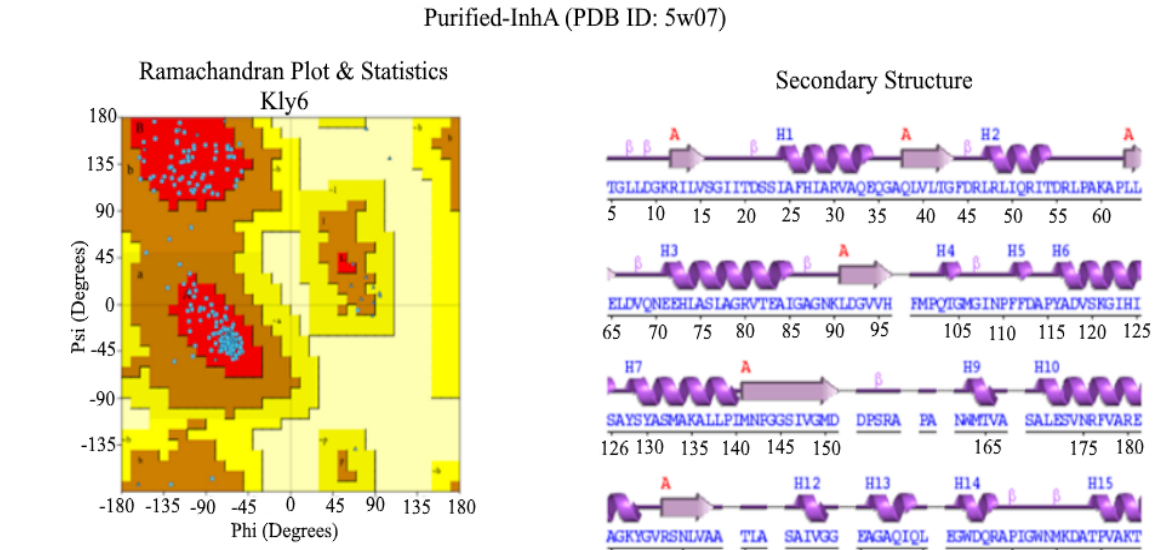
The InhA protein (as shown in Figure 4) analysis shows that of 195 non-glycine and non-proline residues, 181 residues (92.8 percent) are found in the most favoured regions [A, B, L] of the

Ramachandran plot. Another 14 residues (7.2%) are allowed in the additionally allowed regions [a,b,l,p], and none in either the generously allowed or disallowed regions. Its structure contains 250 residues in total, 25 Glycine residues, 12 proline residues, and 18 C-terminal residues without glycine and proline.



Protein	Favoured Regions	Additional Allowed Regions	Generously Allowed Regions	Disallowed Regions
Rv0678	98.2%	1.8%	0%	0%

Figure 3. Ramachandran plot and secondary structure of Rv0678 obtained using PDBsum.



Protein	Favoured Regions	Additional Allowed Regions	Generously Allowed Regions	Disallowed Regions
InhA	92.8%	7.2%	0%	0%

Figure 4. Ramachandran plot and secondary structure of InhA obtained using PDBsum.

The three proteins have good stereochemical quality and fit very well within known PROCHECK Ramachandran plot criteria thereby proving that they are all reliable and well-modeled structures. The suitability of the next structural and functional analyses is supported by their high percentages of residues in the most preferred regions.

Molecular Docking Analysis

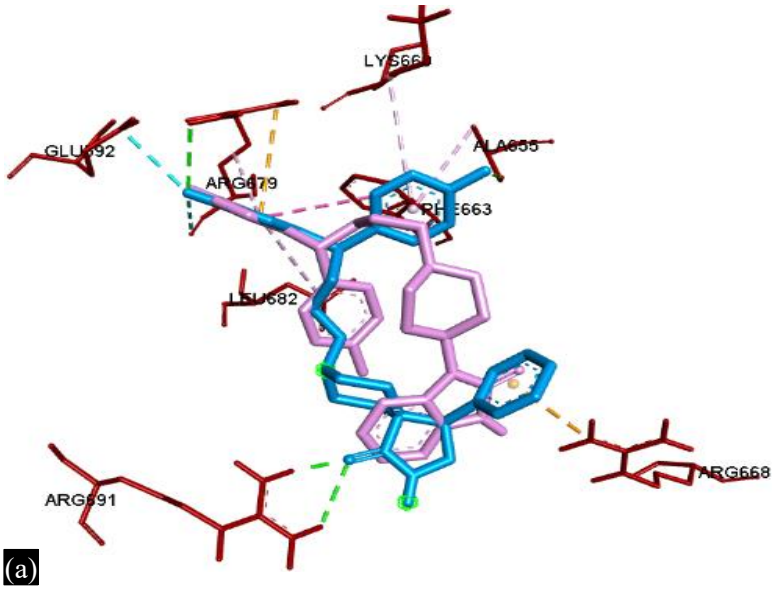
The docking simulations produced a number of models per protein-ligand interaction, and the best docking poses were taken on the basis of the most negative binding affinity scores, and with RMSD values of zero indicating a good fit. The 24 ligands were ranked by their best results, and the top three, which had the strongest affinity to the targets, were selected, with all of them having binding affinities less than -8.5 kcal/mol, suggesting the strong binding potential. The ligands that were characterised as high-affinity ones were Fluspirilene, Ziprasidone, Lurasidone, Bifeprunox and Pimozide and subjected to further detailed structural and interaction analysis and visualisation, as they show the better predicted inhibitory activity on the proteins of interest, KatG, Rv0678 and InhA.

Molecular Visualization

The visualization offered an interactive and thorough environment to view the details of binding modes, pattern of interaction and spatial orientation of the ligands of the active sites of the proteins. The findings revealed hydrophobic and aromatic interactions that additionally strengthen the ligand fit in the binding pocket and validated the docking scores generated earlier. 2D interaction diagrams and receptor-ligand interaction surfaces allowed a concise depiction of these intricate molecular interactions, which allowed identifying the residues key to ligand binding.

Visualization of the docked complexes of the KatG protein with the three ligands- Fluspirilene, Pimozide, and Ziprasidone revealed certain binding sites consisting amino acid residues like ARG668, ARG691, ARG679, ALA655, LYS660, PHE663, LEU682, and GLU592, all belonging to chain A as shown in Figure 5. Ziprasidone did not show any evident interactions with KatG in the molecular visualization analysis.

The docking poses of the three ligands, Lurasidone, Bifeprunox, and Pimozide, with the Rv0678 protein (as shown in Figure 6) were visualized, which demonstrated that the amino acids involved in the defined binding sites were ARG139, ARG125, ARG142, SER135, LEU124, and GLY128, belonging to chain A. Conversely, just like observation made in KatG, both Bifeprunox and Pimozide did not show any observable interaction with Rv0678 in the visualization studies despite ranking within the top three ligands in the docking output.



(a)

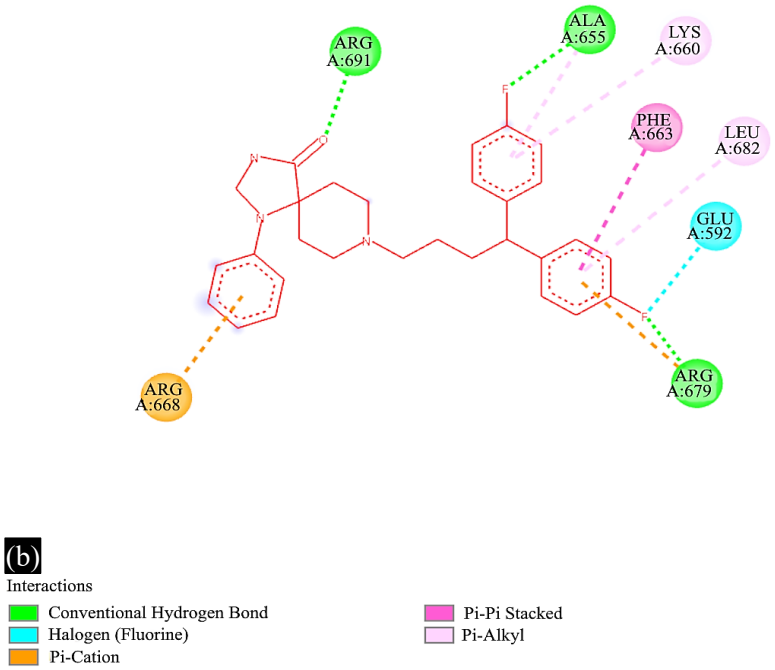
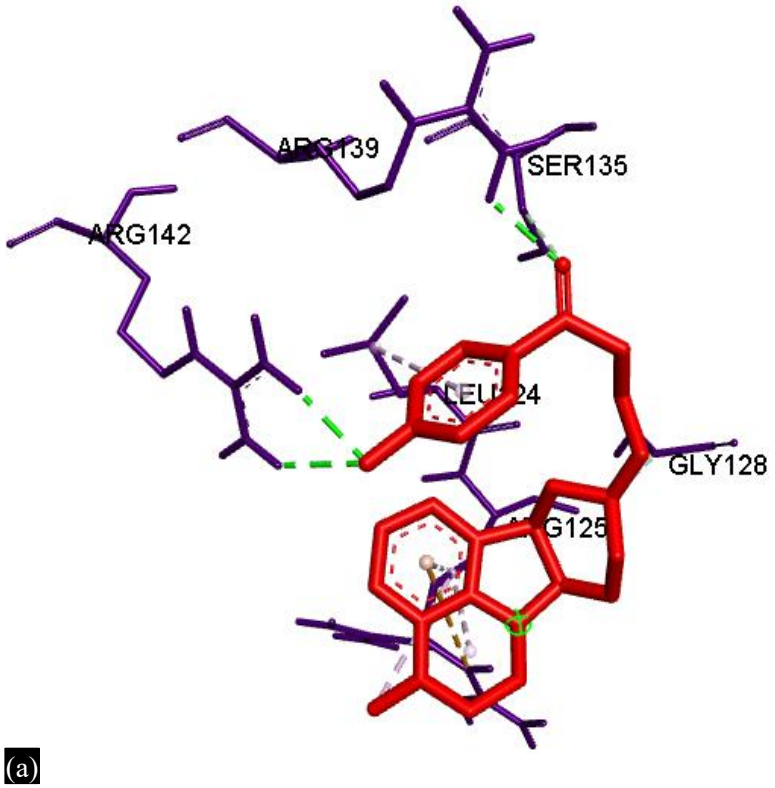


Figure 5. Visualization of molecular interactions of KatG (Red) with the ligands Fluspirilene (blue), Pimozide (pink) a) 3D structure b) 2D structure.

Ziprasidone, Bifeprunox and Pimozide showed lack of apparent binding to the previously determined key residues which implies these ligands may have docked well because of some energetic terms, however its spatial orientation or conformation fit within the KatG and Rv0678 binding pockets are not ideal or specific. Such a lack of connection demonstrates potential shortcomings of docking scoring functions alone and the value of complementary visualization to assess the plausibility of ligand-protein binding.



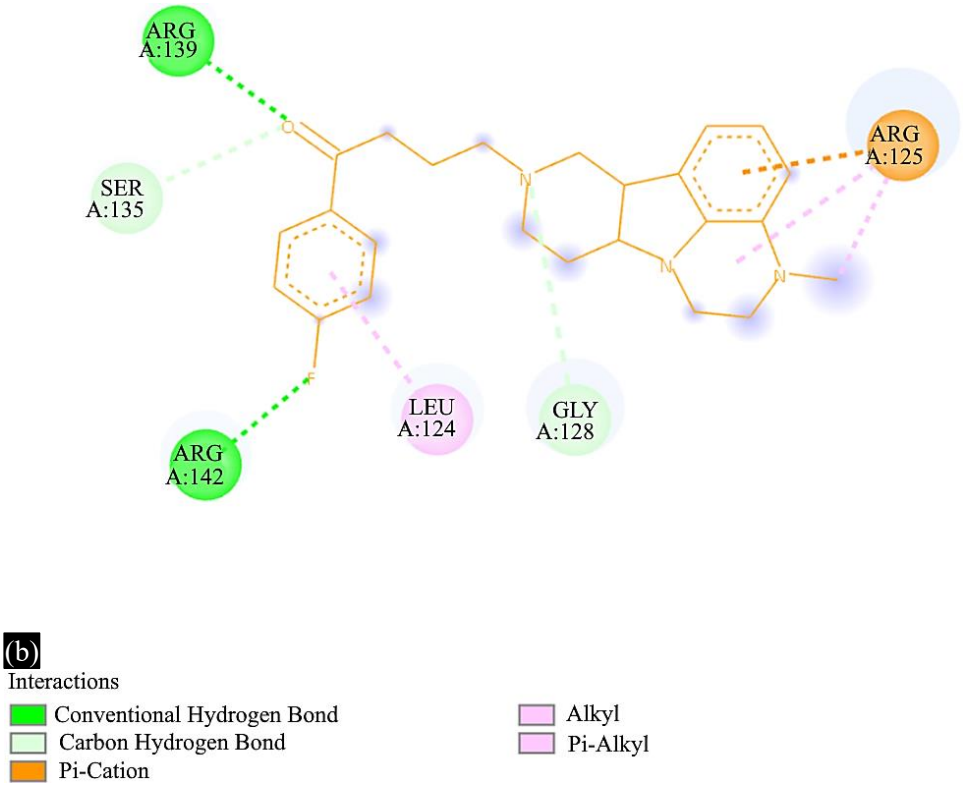
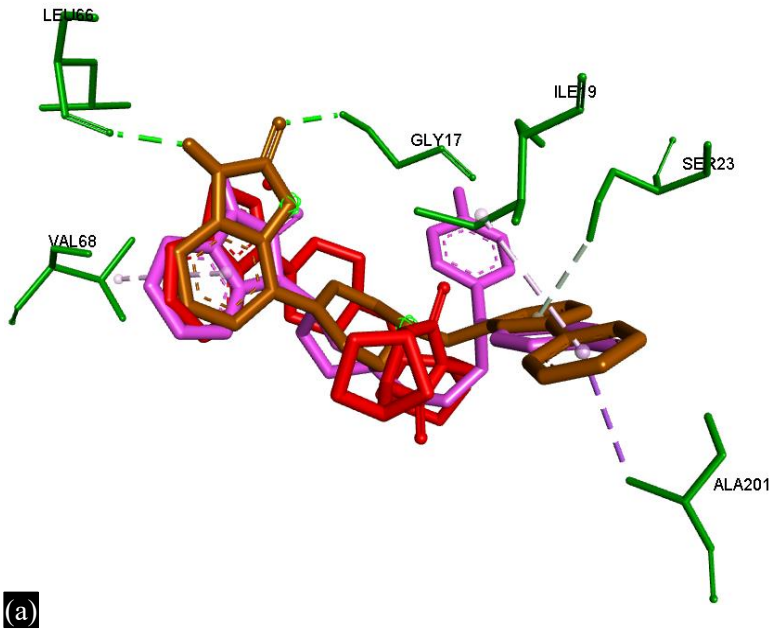


Figure 6. Visualization of molecular interactions of Rv0678 (purple) with the ligands Lurasidone (red) a) 3D structure b) 2D structure.

When the docked complexes of the InhA protein (in Figure 7) with the three ligands, Lurasidone, Bifeprunox, and Pimozide, were visualized, binding sites were observed to be composed of the amino acids residues of VAL68, LEU66, GLY17, ILE19, ALA201, and SER23, all of which belonged to chain A. In contrast to the above findings with KatG and Rv0678 proteins, where not every ligand depicted clear interaction, InhA depicted clear and specific binding interaction with all the three ligands. Such uniform interaction in all three ligands suggests a good spatial orientation and conformational fit in the InhA binding pocket, which implies the suitability of the protein to be targeted by these compounds.



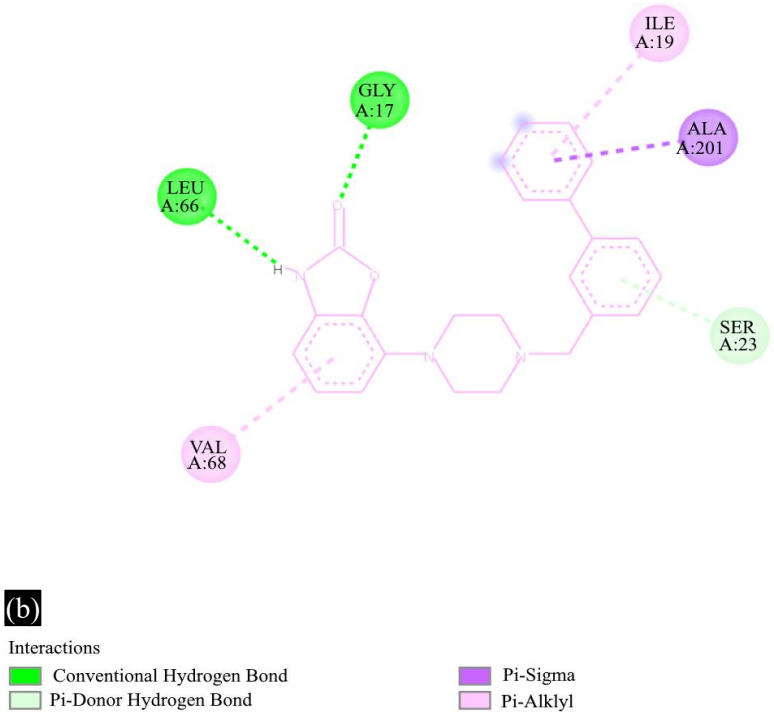


Figure 7. Visualization of molecular interactions of InhA (Green) with the ligands Bifeprunox (brown), Pimozide (pink), and Lurasidone (red) a) 3D structure b) 2D structure.

The interaction of the ligands with the amino acid residues in all the three proteins involved a mixture of stabilizing hydrogen bonds as well as hydrophobic in addition to confirming their binding strong and stable in the active site of the protein.

Based on these results Fluspirilene and Pimozide should be further ADME screened against KatG, Lurasidone against Rv0678 and all three ligands Lurasidone, Bifeprunox and Pimozide against InhA, since their binding pocket profile and visualization-guided interaction would indicate a better pharmacokinetic and pharmacodynamic prospect in the drug development pipelines.

ADME analysis

Fluspirilene (PubChem id: 3396), Bifeprunox (PubChem id: 208951), Lurasidone (PubChem id: 213046), and Pimozide (PubChem id: 16362) were analysed for their physiochemical properties (Table 1), medicinal chemistry (Table 2), absorption, distribution (Table 3), and toxicity (Table 4).

The ADME examination examined the drug's solubility, glycoprotein permeability, human gastrointestinal absorption, and level of blood-brain penetration (as shown in Table 3). The medication candidate's ability to cross the blood-brain barrier is determined by the blood brain barrier (BBB). This information is essential for drug production. Gastrointestinal (GI) adsorption should be high to boost the drug's efficacy. For the best possible medicinal activity, oral medications should in fact have high gastrointestinal absorption and solubility.

Table 1. Physicochemical properties of the ligand molecules.

Ligands	Molecular weight	Fraction Csp3	Rotatable bonds	TPSA	Lipophilicity (iLOGP)
Fluspirilene	475.57	0.34	7	35.58	4.16
Bifeprunox	385.46	0.21	4	52.48	3.46
Lurasidone	492.68	0.68	5	84.99	4.46
Pimozide	461.55	0.32	7	41.03	4.23

Table 2. Data for the properties of the Lipinski rule obtained using Swiss absorption, distribution, metabolism, excretion.

Ligands	Molecular weight	MLogP	Hydrogen donors	Hydrogen acceptors	Molar refractivity
Fluspirilene	475.57	5.11	1	4	145.27
Bifeprunox	385.46	3.35	1	3	123.02
Lurasidone	492.68	4.12	0	4	151.55
Pimozide	461.55	5.45	1	4	135.86

Table 3. Absorption distribution metabolism excretion data obtained using SwissADME.

Ligands	Blood-brain barrier penetration	GI absorption	PGP substrate	Solubility (LOGSw-SILICOS IT)	PAINS	Brenk	Bioavailability
Fluspirilene	Yes	High	Yes	-9.64	0	0	0.55
Bifeprunox	Yes	High	Yes	-7.98	0	0	0.55
Lurasidone	No	High	No	-5.28	0	1	0.55
Pimozide	No	High	Yes	-9.21	0	0	0.55

Table 4. Toxicity categorization data obtained using ADMET 3.0.

Ligands	hERG	H-HT	DILI	Ames	ROA	Carcinogenicity	Respiratory
Fluspirilene	0.992048	0.881137	0.031222	0.243315	0.973077	0.289834	0.964657
Bifeprunox	0.950223	0.828686	0.901551	0.612586	0.689072	0.843523	0.910791
Lurasidone	0.903039	0.891103	0.971753	0.767963	0.702719	0.748491	0.991746
Pimozide	0.98575	0.879877	0.03612	0.347954	0.951022	0.281374	0.97809

DISCUSSION

Tuberculosis (TB), an infection by *Mycobacterium tuberculosis*, has been one of the major health issues worldwide because of its infectious property and the ability to acquire drug resistance [3)] [35]. It is mainly transmitted through air particles of infected persons making it dispersible in vulnerable populations [36]. The current standard care combines first-line medications, namely, isoniazid and rifampicin, yet the incomplete or incorrect treatment promotes the development of multidrug-resistant isolates (MDR-TB), which makes the control of the disease challenging [37, 38]. Molecular investigations have signified important pathogenic proteins such as KatG, Rv0678 and InhA (Figure 1), which are incorporated in the survival as well as drug resistance physiology of the bacteria [7]. The catalase-peroxidase KatG activates isoniazid, and mutations in this site cause high-level resistance; other mutations in InhA, which is involved in mycolic acid synthesis, also cause resistance by target modification. Mutations in Rv0678 affect the regulation of efflux pumps that contributes to resistance to newer drugs such as bedaquiline [39]. The targets have been chosen because these proteins are core to drug susceptibility and pathogenesis, and thus represent good targets of therapeutic intervention.

The growing problem of drug resistance requires new solutions, and drug repurposing is one of the effective and affordable methods. Drug repurposing leverages on FDA-approved drugs to discover new clinical uses of the known drugs, potentially saving years of time, millions of dollars, and risks of the conventional drug discovery. Antipsychotic agents were considered based on their advantaged pharmacokinetic properties and the potential to bind to bacterial proteins targets, which could alter the viability of the pathogens. Fluspirilene, Ziprasidone, Lurasidone, Bifeprunox, and Pimozide have become the potential repurposed agents against TB targets with interesting inhibitory activity in silico as seen in this study. Molecular docking is the highly defining computational method that explains the affinity and mode of binding of these repurposed drugs with the target proteins. It can screen and rank candidate molecules very quickly based on binding energies and conformational fit to aid in the wise choice of molecules to take forward to experimental validation [15, 40].

Docking and visualization studies of the present work showed that the three target proteins had high binding affinity with specific ligands with binding energies of less than -8.5 kcal/mol, which implies a strong binding potential. The top interacting ligands of KatG - Fluspirilene and Pimozide (Figure 5) - interacted with the amino acid residues which shows the particular interactions in the active site. Interestingly, Ziprasidone, although scored high based on the docking scores did not show any binding site interaction on visualization which indicated the docking scoring functions may be limited without structural analysis. In the same way, in the case of Rv0678, Lurasidone showed specific interactions (Figure 6), but Bifeprunox and Pimozide did not show any considerable visible interactions, which highlights the importance of combined docking and visualization methods to conduct accurate screening. In comparison, InhA exhibited specific and steady binding affinity with all the three ligands, namely, Lurasidone, Bifeprunox and Pimozide (Figure 7), with residues indicating a better fit in the conformation of these ligands and InhA, could be considered a strong target of these ligands. These results guide the choice of Fluspirilene and Pimozide in KatG, Lurasidone in Rv0678 and all three ligands in InhA to be taken forward to ADME profiling.

The ligands of choice were subjected to ADME analysis to determine their absorption, distribution, metabolism and excretion. The purpose of this assessment was to predict the pharmacokinetic behaviour and drug-likeness of these compounds to make sure that they are suitable as potential therapeutic agents. These findings (Table 1–5) were in favor of the further pursuit of these ligands in drug development pipelines, in addition to the molecular docking and visualization results.

Table 5. Binding affinity of the ligands with their respective proteins.

Proteins (right)	**KatG**	**Rv0678**	**InhA**
Ligands (down)	*Binding affinity*		
Fluspirilene	-9.1	NIL	NIL
Bifeprunox	NIL	-8.4	-9.0
Lurasidone	NIL	-8.4	-8.8
Pimozide	-8.5	-8.5	-8.7

To address the urgency posed by drug resistance, a comprehensive approach to identifying and optimising repurposed drugs against TB is exemplified by the convergence of computational docking, visualisation, and ADME evaluations. This approach could shorten development times, enhance treatment results, and increase the arsenal against highly and extensively drug-resistant TB strains.

CONCLUSION

The current study revealed how in silico molecular docking methods effectively worked to confirm the binding affinities and interaction patterns of the repurposed antipsychotic drugs towards essential proteins of Mycobacterium tuberculosis KatG, Rv0678 and InhA, thus presenting their suitability as new pharmacological entities. Molecular docking allowed quick and economical screening and identification of potential ligands including Fluspirilene, Pimozide and Lurasidone, which demonstrated high affinity and good spatial orientations, especially towards InhA. Visualization analyses of complements gave important validation of the docking outcomes and showed that in some cases a high docking score did not correspond to correct interaction with the binding site, thereby stressing the relevance of incorporating structural investigation in in silico analyses. Further, ADME profiling of these best candidates further refined their drug-likeness and pharmacokinetic suitability making them better candidates to be taken into drug development pipelines. This work contributes to the increased usefulness of drug repurposing with the help of computational techniques in combating infectious diseases. Further experiments on the in vitro and in vivo verification of these identified compounds are essential to prove their effectiveness, pharmacodynamics and toxicity in a biological environment. Such integrative methodologies that couple experimental validation with computational docking and structural visualization will offer a focused, efficient road-map to hasten the discovery of drugs to cure tuberculosis with increasing drug resistance.

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Abbreviations

ALA	Alanine
ARG	Arginine
BPaL	Bedaquiline, Pretomanid, Linezolid
BPaLM	Bedaquiline, Pretomanid, Linezolid with or moxifloxacin
DILI:	Drug-Induced Liver Injury
EMBL-EBI	European Molecular Biology Laboratory-European Bioinformatics Institute
GLU	Glutamic acid
GLY	Glycine
H-HT	Human Histamine Transporter
hERG	human Ether-à-go-go-Related Gene (potassium ion channel)
ILE	Isoleucine
InhA	Enoyl-Acyl Carrier Protein (ACP) Reductase
KatG	Catalase-Peroxidase
LEU	Leucine
LYS	Lysine
M. tuberculosis	Mycobacterium tuberculosis
MDR	Multi-drug resistance
PAINS	Pan-Assay Interference Compounds
PGP substrate	P-glycoprotein substrate
PHE	Phenylalanine
PROCHECK	Protein Structure Validation Software
RCSB PDB	Research Collaboratory for Structural Bioinformatics Protein Data Bank
RMSD	Root Mean Square Deviation
ROA	Route of Administration
Rv0678	Transcriptional Repressor of MmpL5-MmpS5 Efflux Pump System
SER	Serine
SMILES	Simplified Molecular Input Line Entry System
TB	Tuberculosis
TPSA	Topological Polar Surface Area
VAL	Valine

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