Computational Identification of DrugBank Compounds targeting Dihydrolipoyl Dehydrogenase: Molecular Docking and Dynamics Insights

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Abstract

Tuberculosis (TB), driven by Mycobacterium tuberculosis (Mtb), remains a leading cause of morbidity and mortality worldwide, with the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains posing significant challenges to existing treatment strategies. This project targets dihydrolipoyl dehydrogenase (DLD), a critical enzyme involved in Mtb's central metabolism, virulence and defence against host-induced oxidative and nitrosative stress. DLD's essential role in cellular respiration and detoxification highlights it as a promising drug target to combat resistant TB strains. In this study, molecular docking and molecular dynamics simulations (MDS) were performed to identify and evaluate potential DLD inhibitors sourced from the Drug Bank database. Docking analyses revealed DIQUAFOSOL and DARAPLADIB as the top-performing compounds, with docking scores of -8.7 kcal/mol and -8.5 kcal/mol respectively. DIQUAFOSOL demonstrated significant interactions, forming eight hydrogen bonds and DARAPLADIB exhibited two hydrogen bonds. MDS using GROMACS provided insights into the stability and dynamic behaviour of the ligand-enzyme complexes.

Key parameters, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), hydrogen bonding, Lennard-Jones (LJ) energies and Coulomb interactions, were extracted and analysed to assess the binding stability and conformational integrity of the complexes. The findings underscore the potential of DIQUAFOSOL and DARAPLADIB as promising DLD inhibitors, paving the way for further experimental validation and development of novel anti-TB therapies targeting metabolic and detoxification pathways in Mtb.

Keywords: Tuberculosis (TB) Dihydrolipoyl Dehydrogenase (DLD) Molecular Docking DrugBank Molecular Dynamics Simulations.

Introduction

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), remains one of the most pervasive infectious diseases globally, contributing significantly to morbidity and mortality rates[7]. The increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains poses substantial challenges to existing treatment regimens, rendering many frontline drugs ineffective. This highlights the urgent need for novel therapeutic strategies targeting essential and druggable pathways within the pathogen. One promising target is dihydrolipoyl dehydrogenase (DLD), a key enzyme vital for Mtb's survival, virulence and metabolic resilience. DLD's involvement in central metabolism and its role in detoxifying reactive oxygen and nitrogen species during host immune responses make it a compelling candidate for anti-TB drug development[1].

Dihydrolipoyl dehydrogenase is a flavoprotein enzyme that forms a crucial part of major metabolic complexes including the pyruvate dehydrogenase complex (PDC), alpha-ketoglutarate dehydrogenase complex (KDC) and branched-chain alpha-keto acid dehydrogenase complex (BCKDC). These complexes are fundamental to cellular respiration, energy production and biosynthetic precursor formation. The PDC links glycolysis to the citric acid cycle by converting pyruvate into acetyl-CoA, a precursor for oxidative phosphorylation and ATP synthesis. KDC catalyses the conversion of alpha-ketoglutarate to succinyl-CoA, ensuring energy homeostasis and generating reducing equivalents such as NADH and FADH2. Similarly, the BCKDC mediates the breakdown of branched-chain amino acids, producing acyl-CoA derivatives that fuel the citric acid cycle[11].

Dihydrolipoyl dehydrogenase (DLD) is essential for maintaining metabolic stability and energy equilibrium in Mycobacterium tuberculosis (Mtb) through its involvement in critical metabolic complexes related to cellular respiration and energy production[5]. In addition to its metabolic roles, DLD is essential for protecting Mtb from the host's immunological response. It neutralises detrimental reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced during infection, enabling Mtb to endure oxidative and nitrosative stress, which are critical elements of the host's immunological response[14].

The enzyme's dual role in metabolism and detoxification underscores its importance as a genetically validated and promising target for anti-TB therapeutics[15]. Advancements in computational drug discovery have accelerated the identification of potential inhibitors targeting DLD[9].

Molecular docking and virtual screening enable researchers to predict small molecules' binding affinities and orientations within the enzyme's active site. These *in silico* methods efficiently screen large chemical libraries, including natural products and pharmacological compounds, expediting the drug discovery pipeline.

In this study, compounds from DrugBank were screened through molecular docking to identify potential DLD inhibitors. Targeting DLD offers a novel mechanism of action that circumvents existing resistance pathways by disrupting the citric acid cycle, impairing ATP production and hindering the synthesis of essential precursors. Additionally, by inhibiting DLD's detoxification function, these compounds enhance Mtb's susceptibility to host immune responses. Integrating DrugBank compounds with distinct pharmacological profiles into this discovery pipeline introduce structurally diverse molecules that may overcome traditional resistance mechanisms. The identification of DIQUAFOSOL and DARAPLADIB as promising DLD inhibitors marks a critical step towards developing innovative therapeutics to combat MDR and XDR TB strains, addressing the ongoing global tuberculosis crisis.

Material and Methods

Protein Preparation: The three-dimensional structure of dihydrolipoyl dehydrogenase (DLD) was retrieved from the Protein Data Bank (PDB ID: 8U0Q) for molecular docking investigations. AutoDock Tools prepared the protein, ensuring optimal conditions for ligand interaction analysis[12]. Preprocessing steps included the removal of water molecules, ligands and heteroatoms that might interfere with accurate docking results. Polar hydrogens were added to simulate the realistic protonation states of the protein and Gasteiger charges were assigned to stabilize the electrostatic environment[17]. As determined through previous structural analyses and relevant literature, the docking grid was carefully set up to target the active site.

Compound Dataset Preparation: The DrugBank database was used to source a comprehensive library of compounds for molecular docking studies targeting DLD. Compounds were retrieved in SDF format and converted to PDBQT format, required for docking with AutoDock Vina 1.2.x using Open Babel-3-1-1[2]. The pre-processing workflow involved multiple steps to ensure structural accuracy and readiness for docking.

Initially, hydrogen atoms were added to each compound to accurately reflect physiological protonation states followed by the assignment of partial charges. Energy minimisation was performed using the MMFF94 force field to optimise molecular geometries ensuring each compound attained its most stable conformation.

This minimization step mitigates potential steric clashes and improves the accuracy of docking predictions by stabilizing ligand structures. Compounds were then saved in PDBQT format which retains torsional flexibility and partial charges enabling integration into the docking pipeline.

Molecular Docking: Molecular docking analyses were conducted with AutoDock Vina to assess the binding affinities and interaction processes of DrugBank drugs with dihydrolipoyl dehydrogenase (DLD). The protein structure (PDB ID: 8U0Q) was obtained from the Protein Data Bank and processed by eliminating water molecules, ions and heteroatoms to prevent interference. Polar hydrogens were incorporated and Gasteiger charges were allocated to emulate authentic electrostatic interactions.

The active site was delineated using structural data and interactions with the natural ligand and essential residues chosen for docking studies. The grid box was designed to completely enclose the active site guaranteeing thorough coverage of catalytically significant areas. The centre was aligned with the centroid of the active site residues for accuracy. The docking methodology was refined by comprehensively augmenting the exhaustiveness parameter to sample ligand conformations and binding poses.

At the same time, essential active site residues were permitted to have restricted flexibility to accommodate induced fit effects. Ligands from the DrugBank database underwent preprocessing through Open Babel for format conversion, which was succeeded by energy minimization to achieve optimal geometries. The docking approach was validated by re-docking the native ligand (U4I), verifying its accuracy by reproducing the established binding position. The highest-ranked compounds were chosen according to docking scores and examined for their interaction profiles, encompassing hydrogen bonds, hydrophobic interactions and π-π stacking. PyMOL was employed to analyse binding orientations. This comprehensive protocol guarantees dependability and strong predictions for subsequent analysis[10].

Molecular Dynamics Simulations: GROMACS was employed to perform molecular dynamics (MD) simulations, investigating the stability and dynamics of protein-ligand interactions for the top DrugBank compounds[8]. The CHARMM force field was utilized to accurately model atomic interactions and molecular motions. Three systems were prepared for simulation: the apo form of the target protein, the protein complexed with the reference ligand U4I and the protein bound to the two lead DrugBank compounds. Each system was solvated in a TIP3P water box to mimic the biological aqueous environment. Sodium (Na^+) and chloride (Cl^-) ions were added to neutralise the systems and to maintain physiological ionic strength[3]. Energy minimization was performed to ensure structural stability and remove steric clashes. The systems underwent two equilibration phases. First, the systems were equilibrated under an NVT ensemble (constant Number of particles, Volume and Temperature) to stabilize the temperature.

This was followed by equilibration under an NPT ensemble (constant number of particles, pressure and temperature), replicating *in vivo* conditions. During these phases, positional restraints were applied to the heavy atoms of the protein and ligands, ensuring the integrity of the complexes as the solvent and ions adjusted to the system. Subsequently, 100-nanosecond production MD simulations were performed for each system. This duration allowed for observing potential conformational changes and long-term interaction dynamics between DLD and the DrugBank compounds, providing valuable insights into their stability and binding processes.

Results and Discussion

Molecular Docking: The docking scores obtained from molecular docking simulations highlight the binding efficiencies of the tested compounds within the active site of dihydrolipoyl dehydrogenase (DLD). DIQUAFOSOL, the top-performing compound, achieved the most favourable docking score of -8.7 kcal/mol reflecting strong binding affinity. This score indicates significant stabilising interactions within the binding pocket primarily driven by a network of eight hydrogen bonds with key residues suggesting it as the most potent inhibitor among the tested compounds. DARAPLADIB, the second-ranked compound, demonstrated a docking score of -8.5 kcal/mol, indicating a slightly lower binding affinity than DIQUAFOSOL. Despite this, its score remains highly favourable, supported by its interaction with critical active site residues through two hydrogen bonds.

These findings underscore DARAPLADIB's potential as a competitive DLD inhibitor. The reference compound, U4I, recorded a docking score of -5.5 kcal/mol, serving as the benchmark for comparison. The significantly higher scores of DIQUAFOSOL and DARAPLADIB compared to U4I emphasise their superior binding potential. The docking scores align well with the interaction profiles suggesting that the identified compounds could be promising candidates for further computational and experimental studies. The molecular interaction analysis revealed distinct binding mechanisms for the lead DrugBank compounds and the reference inhibitor U4I within the DLD active site.

Native ligand U4I high binding affinity was attributed to several key interactions including a hydrogen bond with His258, which stabilised the protein-ligand complex. Additionally, U4I formed a π-π stacking interaction with Phe147, indicative of strong aromatic interactions and a hydrophobic interaction with Val352, reinforcing its overall stability within the binding pocket. These interactions established U4I as a practical reference for evaluating the performance of other compounds.

The docking scores and interaction analyses revealed that the DrugBank-derived compounds demonstrated binding affinities comparable to or greater than the reference inhibitor U4I. With its superior binding affinity and extensive interaction network, DIQUAFOSOL emerged as the most promising candidate. Its diverse hydrophobic contacts, hydrogen bonds and π-π stacking interactions suggested a favourable binding conformation and high stability within the enzyme's active site. These characteristics position DIQUAFOSOL as a potent inhibitor of DLD with potential therapeutic applications. DARAPLADIB also showed competitive binding scores, reinforcing that DrugBank compounds represent a valuable resource for identifying novel DLD inhibitors.

Simulation Analysis and Results: The RMSD values for the Native, DIQUAFOSOL (Top1) and DARAPLADIB (Top2) systems were compared over a 100 ns simulation. The Y-axis represents RMSD values in Å (0–0.35 Å), while the X-axis denotes simulation time in ps. All systems exhibited an initial sharp rise in RMSD, stabilizing after approximately 10 ns indicating structural equilibrium. The native system showed lower RMSD values, suggesting higher structural stability without ligand binding. Conversely, DIQUAFOSOL and DARAPLADIB demonstrated greater RMSD values, reflecting increased protein flexibility upon ligand interaction. DIQUAFOSOL exhibited the most dynamic changes, highlighting ligand-induced structural alterations [Fig. 3(A)].

The RMSF graph compared the flexibility of residues across DIQUAFOSOL, DARAPLADIB and U4I systems. The X-axis represents residue indices, while the Y-axis shows RMSF values (0–0.7 nm). Peaks indicate regions of flexibility, typically loops or dynamic segments. U4I displayed the lowest RMSF values indicating greater stability. In contrast, DIQUAFOSOL showed higher flexibility, particularly around residues 2000 and 6000. DARAPLADIB exhibited intermediate behaviour, with varying flexibility aligned with U4I in certain regions. These RMSF profiles reveal the effects of ligand binding on protein flexibility and dynamics [Fig. 3(B)].

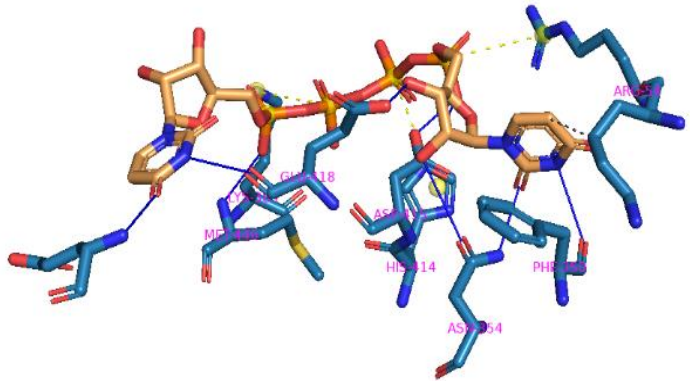
Hydrogen bonding patterns were analysed for native, DIQUAFOSOL (Hb Top1) and DARAPLADIB (Hb Top2) systems during the 100 ns simulation. The native system displayed a consistently low number of hydrogen bonds (0–2), indicating minimal interactions. DIQUAFOSOL showed dynamic variations, with bond counts peaking between 4–10, highlighting significant contributions to stability, particularly during 60–80 ns. DARAPLADIB maintained minimal bonds (0–1), suggesting weaker or transient interactions. These findings emphasise the crucial role of non-native hydrogen bonds (DIQUAFOSOL) in stabilising the system [Fig. 3(C)].

The Rg values for Native, DIQUAFOSOL and DARAPLADIB systems were analysed over 100 ns to assess structural compactness. The Y-axis represents Rg in nm (2.34–2.46 nm), while the X-axis denotes simulation time in ps. All systems stabilized after the initial phase. The native system exhibited the lowest Rg values, indicating a more

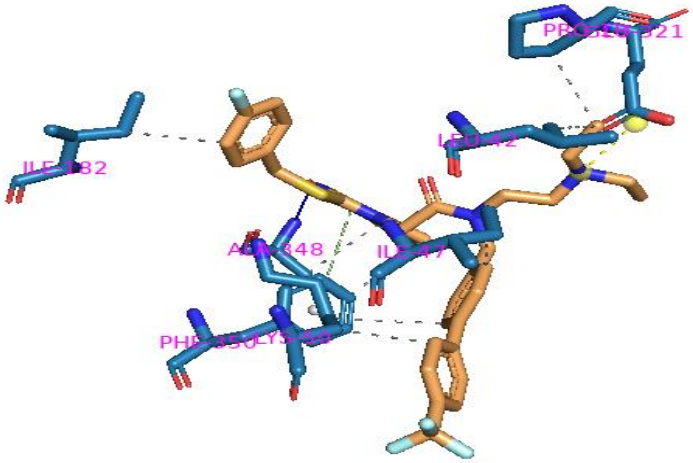
compact and stable conformation. In contrast, DIQUAFOSOL and DARAPLADIB had higher and more variable Rg values with DARAPLADIB being the least compact, reflecting increased structural flexibility upon ligand binding [Fig. 3(D)].

Table 1
Binding Energy and Molecular Interactions of DLD with Inhibitors

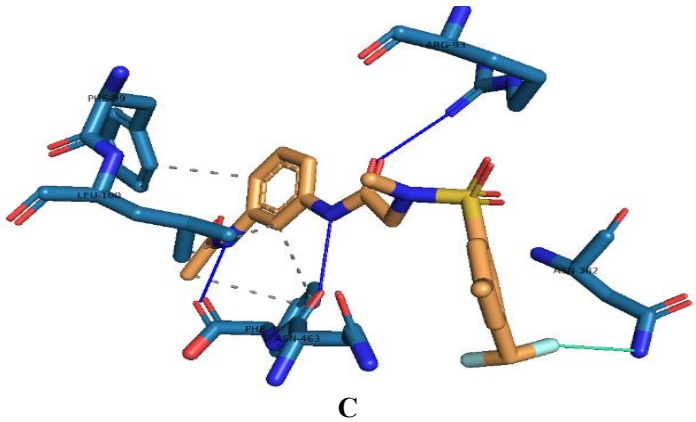
S.N.	Compound Name	Docking Score	Interactions (H-bonds)
Top 1	DIQUAFOSOL	-8.7 kcal/mol	Phe-350(1), Asn-354(2), His-414(1), Asp-415(1), Glu-418(2), Glu-422(1)
Top 2	DARAPLADIB	-8.5 kcal/mol	Glu-321, Ala-348
Native (control)	U4I	-5.5 kcal/mol	Asn-463, Phe-464



A

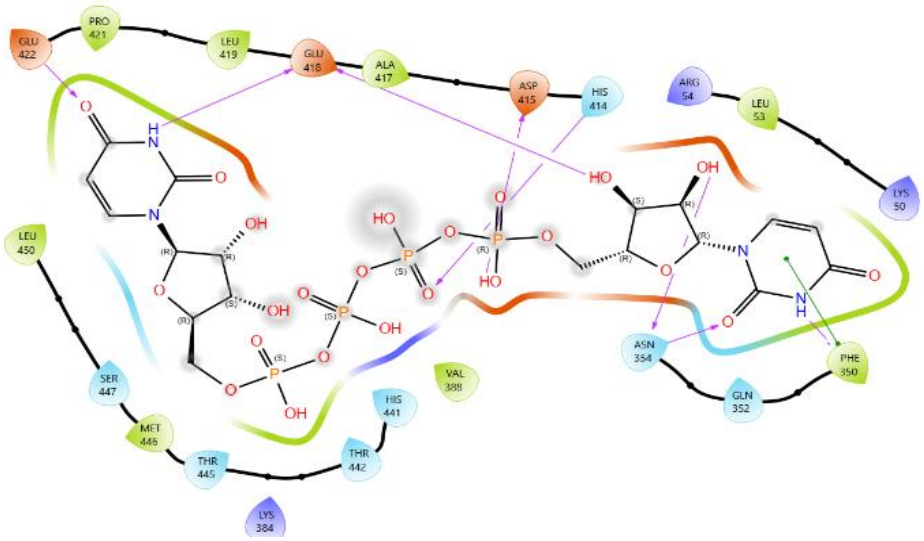


B

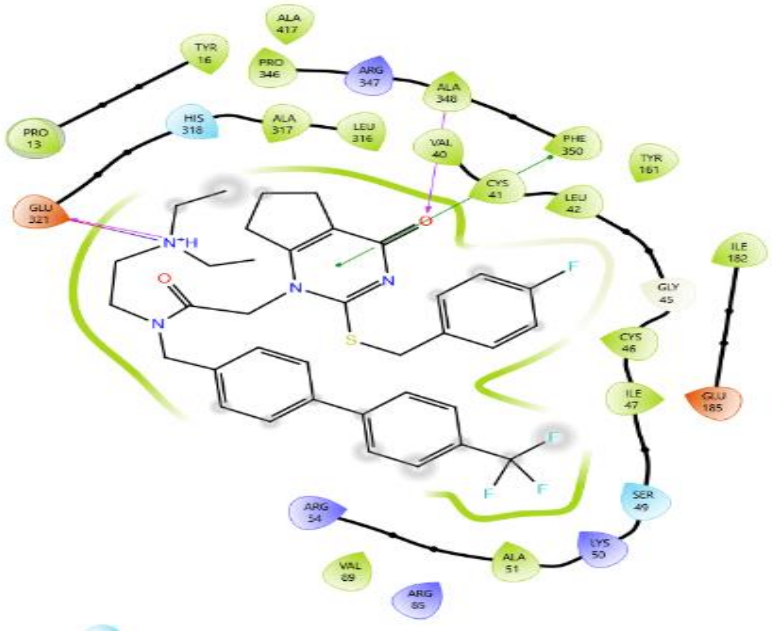


C

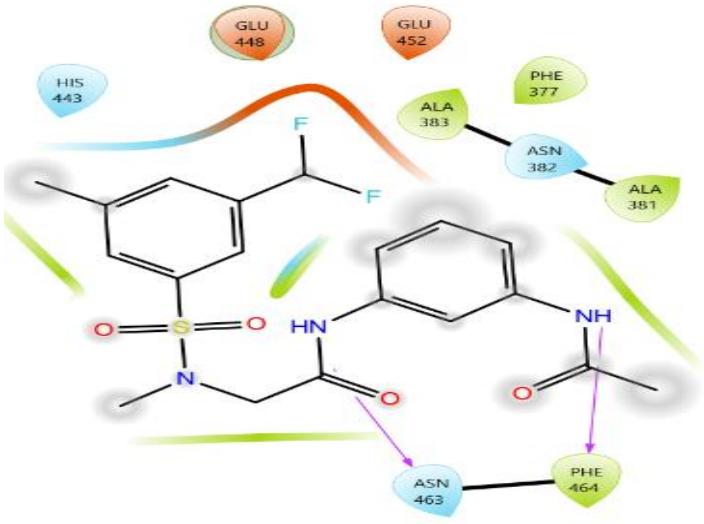
Figure 1: Interactions of Molecular Docking in 3D



A

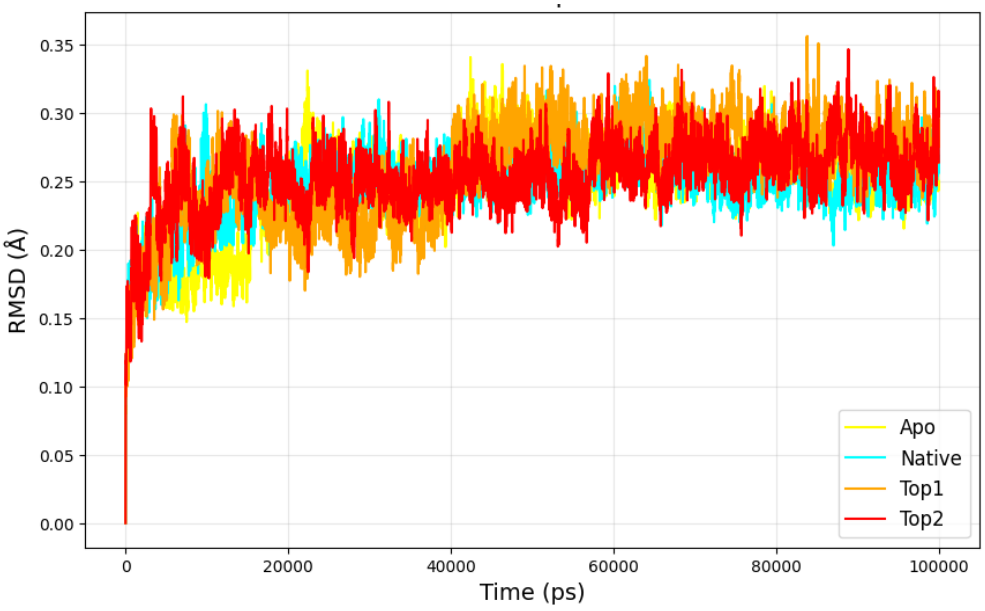


B

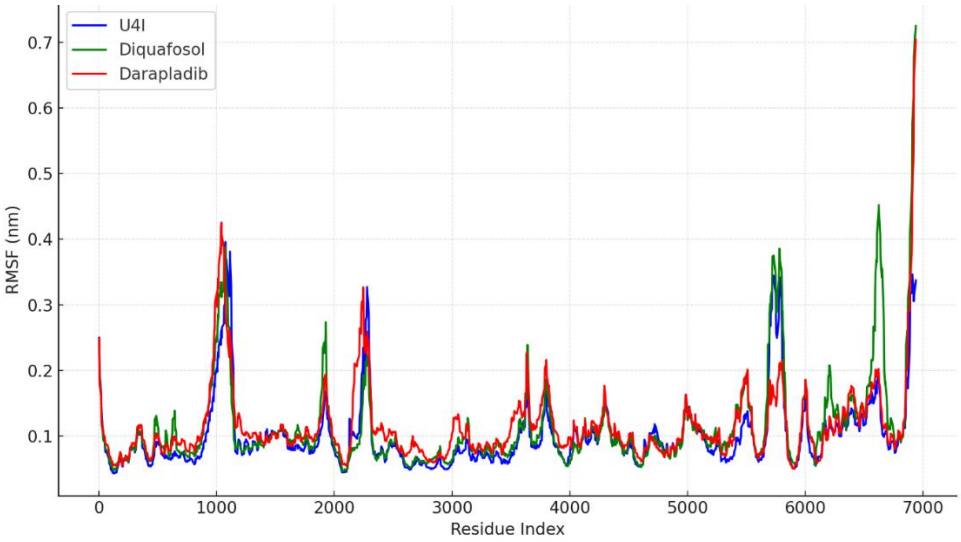


C

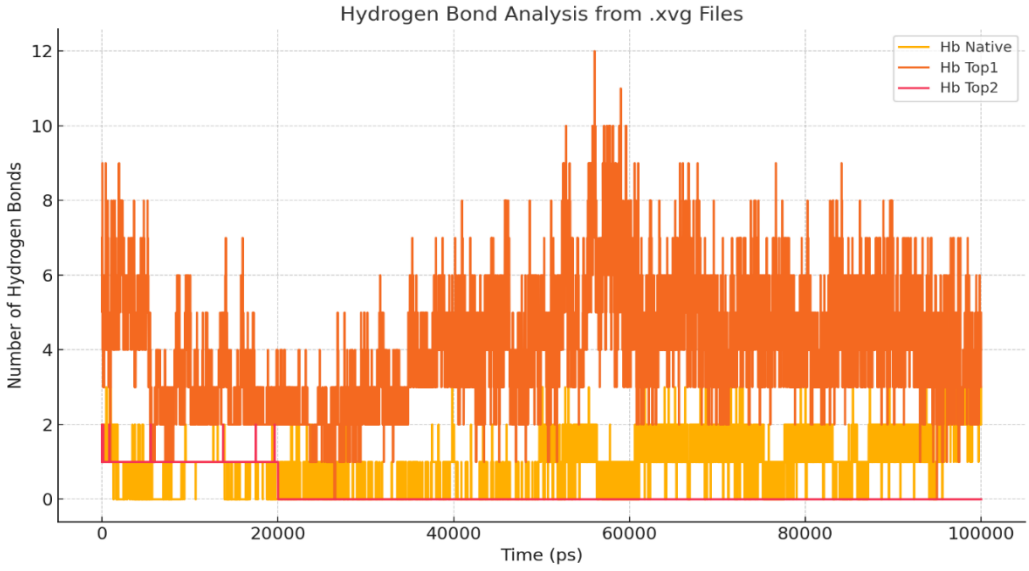
Figure 2: Interactions of Molecular Docking in 2D


RMSD (Å)
Time (ps)
Apo
Native
Top1
Top2

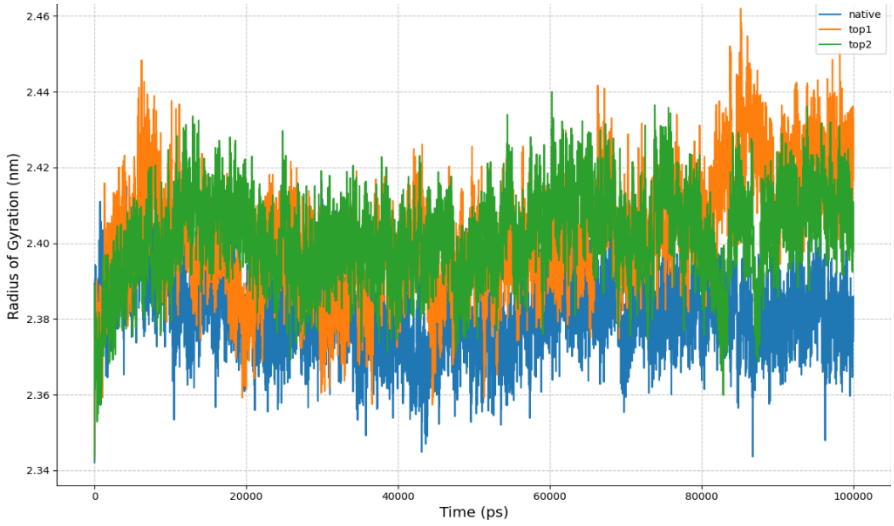
A


U4I
Diquafosol
Darapladib
RMSF (nm)
Residue Index

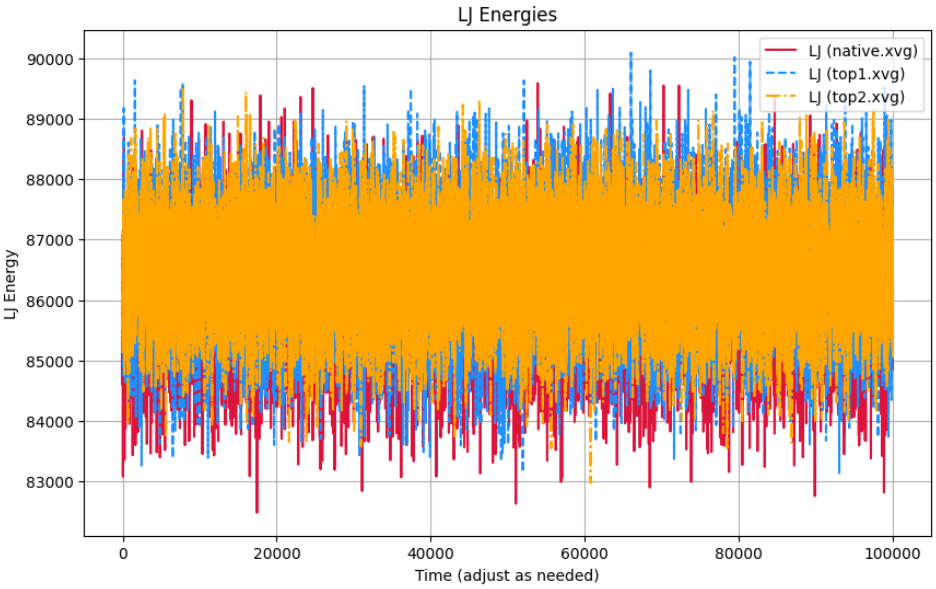
B


Hydrogen Bond Analysis from .xvg Files
Hb Native
Hb Top1
Hb Top2
Number of Hydrogen Bonds
Time (ps)

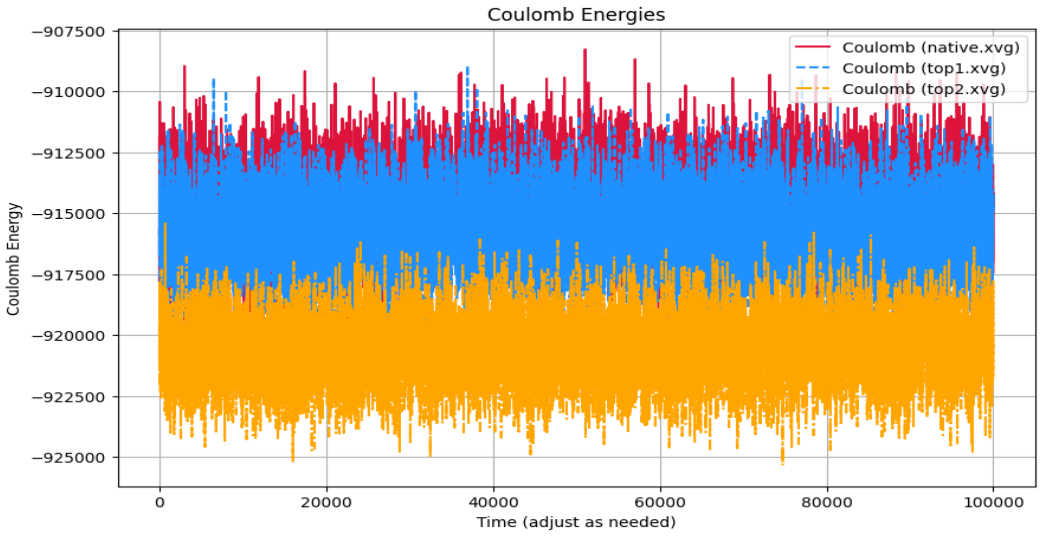
C



D

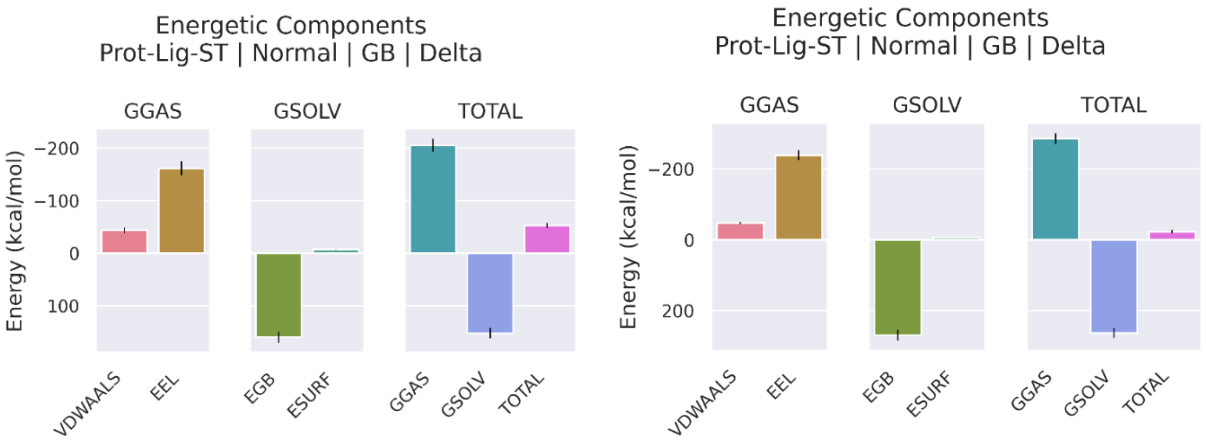


E



F

Figure 3: (A) RMSD, (B) RMSF, (C) Hydrogen Bonds, (D) Radius of Gyration, (E) LJ Energies, (F) Coulomb Energies



A **B**

Figure 4: Energetic contributions to the binding affinity of the (A) Top1 protein-ligand complex and (B) Top2 protein-ligand complex as determined by MMPBSA analysis.

Two energy plots, Lennard-Jones (LJ) and Coulomb energies, highlighted non-bonded interaction stability during the simulations. LJ energies fluctuated between ~83000 kcal/mol and ~90000 kcal/mol, with the native system showing the smallest amplitude, indicating stable van der Waals interactions. DIQUAFOSOL and DARAPLADIB exhibited more significant fluctuations suggesting ligand-induced variations. Coulomb energies ranged from -907500 kcal/mol to -925000 kcal/mol with the native system having the least negative energy and DARAPLADIB, the most reflecting higher electrostatic contributions. The overall stability and equilibration of simulations were confirmed, while differences in energy profiles offered insights into ligand-dependent molecular interactions [Fig. 3(E), Fig. 3(F)].

The comparative analysis of the DIQUAFOSOL (Top1) and DARAPLADIB (Top2) protein-ligand complexes was illustrated in figures 4(A) and 4(B) and highlighted significant insights into their binding affinities as determined by MMPBSA analysis. Both complexes exhibit similar contributions from van der Waals energy (VDWAALS) with approximately -50 kcal/mol in both systems. This indicates that hydrophobic interactions consistently stabilise ligand binding reflecting effective interactions with the protein's hydrophobic binding pocket in DIQUAFOSOL and DARAPLADIB. Electrostatic energy (EEL) contributions differ notably between the two systems.

In figure 4(B), DARAPLADIB demonstrates a stronger electrostatic interaction of approximately -200 kcal/mol, compared to -150 kcal/mol in DIQUAFOSOL, as shown in figure 4(A). This highlights DARAPLADIB's enhanced complementarity with the charged residues of the protein leading to stronger stabilising interactions. The polar solvation energy (EGB) acts as a destabilising factor in both complexes with a more significant contribution in DARAPLADIB (200 kcal/mol) compared to DIQUAFOSOL (100 kcal/mol). This difference suggests that DARAPLADIB is less shielded from the solvent's polar environment resulting in higher destabilisation. However, the non-polar solvation energy (ESURF) remains negligible in both systems contributing minor positive values that do not significantly impact the overall binding affinity.

Gas-phase energy (GGAS), representing the combination of van der Waals and electrostatic interactions without solvent effects is approximately -200 kcal/mol in both systems as shown in figure 4(A) and figure 4(B). This emphasises the stabilising impact of these interactions in the absence of solvation. Conversely, the solvation energy (GSOLV), summing polar and non-polar solvation components, acts as a destabilising factor with DARAPLADIB experiencing slightly higher destabilisation due to its more incredible polar solvation energy.

The total binding energy (TOTAL), shown in the final bars of figure 4(A) and figure 4(B), reflects the net energetic contributions. DARAPLADIB demonstrates a more favourable total binding energy of approximately -200 kcal/mol compared to -100 kcal/mol for DIQUAFOSOL. This difference underscores the stronger binding affinity of DARAPLADIB, primarily driven by its enhanced electrostatic interactions and gas-phase stability, despite the higher destabilisation from polar solvation. As depicted in figure 4, these findings provide a detailed comparison of the energetic profiles and establish DARAPLADIB as the energetically superior ligand in this analysis.

Conclusion

This study applied computational methods to identify potential dihydrolipoyl dehydrogenase (DLD) inhibitors, a vital enzyme in *Mycobacterium tuberculosis* (Mtb) involved in metabolic and detoxification pathways critical for bacterial survival. Molecular docking of DrugBank compounds, including DIQUAFOSOL and DARAPLADIB, evaluated binding efficiencies while molecular dynamics (MD) simulations assessed the stability of protein-ligand complexes. DIQUAFOSOL demonstrated the highest binding affinity with a docking score of -8.7 kcal/mol

attributed to strong hydrophobic, electrostatic and π-π stacking interactions within the DLD active site.

DARAPLADIB also exhibited significant binding, interacting with key residues such as Asn 266, Glu 330, Ser 182 and Gly 184. RMSD and RMSF analyses revealed ligand-induced structural flexibility with DIQUAFOSOL eliciting the most pronounced conformational changes. Persistent hydrogen bonding further confirmed DIQUAFOSOL's stability, while Lennard-Jones and Coulomb energy analyses highlighted stable non-bonded interactions across all systems. MMPBSA analysis reinforced DIQUAFOSOL's superior binding affinity, driven by robust van der Waals and electrostatic forces. DARAPLADIB showed strong electrostatic complementarity but experienced minor destabilisation from polar solvation energy.

Overall, DIQUAFOSOL emerged as the lead candidate with DARAPLADIB as a promising alternative. This research integrates molecular docking, MD simulations and binding energy analysis to identify novel anti-TB agents targeting DLD. Future enzyme inhibition and cellular assays are essential to validate these findings, addressing the global challenge of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB. This computational approach underscores the potential for discovering next-generation TB therapies targeting essential enzymes like DLD.

Acknowledgement

Authors are highly thankful to VFSTR (Deemed to be University) for providing faculty seed grant (F.No. VFSTR/REG/A6/30/2023-24/01 dated 16-05-2023).

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(Received 08th January 2025, accepted 10th March 2025)