Chapter- 6

Summary and Future prospects

Summary

Leishmaniasis affects millions of people worldwide, with VL being the most severe form, causing significant morbidity and mortality. Effective treatment is crucial to reducing the disease burden and preventing fatalities. Despite its prevalence, treatment options remain limited, often toxic, and increasingly ineffective due to rising drug resistance. Resistance to first-line treatments like pentavalent antimonials and miltefosine has been reported, undermining their efficacy. Effective treatment is crucial to reducing the disease burden and preventing fatalities. However, current therapies are often associated with severe side effects and require prolonged administration, which can lead to non-compliance and treatment failures. The traditional methods of drug discovery, while successful in some cases, have limitations in terms of speed, cost, and efficiency. Modern drug discovery leverages high-throughput screening, computational drug design, and drug repurposing to identify potential therapeutic candidates more rapidly and accurately. Identifying specific molecular targets within the *Leishmania* parasite allows for the development of drugs that can specifically disrupt critical biological processes, increasing the chances of successful treatment.

This study aims to uncover the protein target profiles of *Leishmania donovani*, distinctly identifying key proteins and pathways involved in the development of visceral leishmaniasis. The essential proteins in this pathogen were primarily linked to critical pathways such as the purine pathway, pyrimidine pathway, vitamin B6 salvage pathway, and lanosterol pathway, which are necessary for their survival.

Chapter 3 details a study aimed at targeting and inhibiting essential enzymes of *Leishmania donovani* using a multi-target approach. Infections occurring due to VL possess a serious public health concern and till date, there is no established vaccine against it. In the context of this work, a multi-target approach utilizing various screening strategies was used to obtain promising ligands that can inhibit more than one target. The dataset of molecules included derivatives of reported inhibitors, and various filtering parameters were used to screen the most ideal ones. Lipinski's rule of five, Veber's rule, ADMET and PASS analysis were performed on the dataset of 6220 compounds. After the screening process, a total of 15 compounds were selected which were later used to construct docked complexes with APRT and DHODH, the drug target proteins of *Leishmania donovani* i.e., a total of 30 protein-ligand complexes. And based on the docking scores 4 complexes having comparative good docking score were selected. Additionally, short simulations of 20ns were done to filter out

best protein-molecule complexes displaying stability in simulation trajectory. The short run led to the selection of 2 protein-ligands complexes, which were later analyzed by long 100ns of MD simulations. Moreover, various MD investigations showed distinct and valuable outputs to interpret the complex stability. Finally, MM/PBSA calculations for obtaining the binding energies were carried out for Lig_2, Lig_3 and inhibitors with both proteins which showed Lig_3 exhibited the greatest binding energy among others. The electrostatic energy and van der Waals energy are observed to be the key forces for the interaction of Lig_3 with respective protein targets. Overall, Lig_3 is emerging as the most promising inhibitor based on MD simulation and MMPBSA analysis. The binding of Lig_3 does not cause any significant changes in the protein's conformation in both cases. Lig_3 [2-(3,4-dihydroxyphenyl)-7-hydroxy-4-oxo-4H-chromen-5-ylisobutyrate] is also a plant derivative compound and natural products have the potential to show antiprotozoal activities. The selected compound from this multi-target approach may offer improved therapeutic options for combating VL.

Chapter 4 focuses on evaluating approved drug molecules against specific drug targets for VL. Within this investigation, a computational framework was employed to identify two druggable targets of *L. donovani*. The study identified two key therapeutic targets, pyridoxal kinase (PK) and sterol alpha-14 demethylase (SDM), which are crucial enzymes in the vitamin B6 salvage and sterol biosynthesis pathways in *L. donovani*. Drugs were sourced from DrugBank and Drug Central databases, with 325 anti-parasitic compounds screened using PASS analysis. Three ligands (Lig_1, Lig_2, and Lig_3) were selected based on their high Pa values, docking scores, and medicinal relevance. Subsequent MD simulations and MMPBSA analysis uncovered that Lig_1 (Nitazoxanide) not only preserves the structural integrity of both proteins but also enhances the stability essential for inhibiting PK and SDM targets. In general, the study underscores the importance of stability, interactions, and binding energies between the compounds and selected crucial proteins in altering the functions of both targets and ultimately leading to their inhibition. In a nutshell, the findings indicate that obtain compound may exhibit structural mechanism of inhibition against critical PK and SDM proteins of *L. donovani,* highlighting a promising therapeutic approach for VL.

Chapter 5 employed subtractive genomics and structure-based methodologies to identify shared drug targets and potential inhibitors across five *Leishmania* species strains. The subtractive genomics approach identified Glutamate Dehydrogenase (GDH) as a promising

drug target. The study utilized established methodologies, including orthologous group analysis and druggability tests, to validate GDH as a therapeutic target. Multiple sequence alignment revealed conserved GDH sequences, while phylogenetic analysis provided insights into its evolutionary relationships across *Leishmania* species. Using a structure-based approach, the molecular interactions between GDH and three ligands (Bithionol, GW5074, Hexachlorophene) were explored through molecular docking and 100ns MD simulation. GW5074 demonstrated a strong affinity for GDH, indicated by stable RMSD values, a more compact conformation, and more hydrogen bonds compared to Bithionol. Quantitative MMPBSA analysis affirmed the higher binding energy of the GW5074-GDH complex compared to other ligand-bound complexes, highlighting its potential as a potent ligand for drug development. Overall, GW5074 emerges as a promising candidate for inhibiting GDH in *Leishmania* species. This multidimensional approach establishes a solid groundwork for future studies aiming to develop effective therapeutics against *Leishmania* infections.

Overall, the thesis encompasses computational studies on the screened protein targets of *Leishmania donovani*, the pathogen responsible for VL. The research further extended to the selection of potential molecules capable of inhibiting essential proteins vital for the pathogen's survival. Additionally, the proteomes of various *Leishmania* species were meticulously investigated using established methodologies to identify a crucial common protein target among them. Overall, proposed compounds may assist to obtain new antileishmanial drugs against the essential proteins for prevention transmission and eradication of the disease. Further experiments and investigation are required for a lead molecule to show inhibitory effects against the proteins leading to the death of the microorganism.

Future prospects

The research conducted in this thesis opens up further avenues for exploration, which include:

1. The ligand identified in this study can be further evaluated across other *Leishmania* species to develop therapeutic drugs specifically for leishmaniasis patients.
2. Investigating hypothetical proteins across various *Leishmania* species proteomes can identify new and essential drug targets, potentially aiding the development of precision medicine.
3. *In vitro* and *in vivo* experiments are required for establishing ligand as inhibitor against the specific drug targets of *Leishmani*