Abstract

The evolution of computational biology has provided extensive opportunities for researchers to explore and identify efficient drug molecules using computational tools and techniques. This integration of various computational approaches signifies a paradigm shift in drug design and development, ushering in a new era of research. This interdisciplinary collaboration offers a multifaceted approach to drug discovery, enabling a comprehensive exploration of potential therapeutic agents. By leveraging computational methods, scientists can analyze vast datasets of genomes and proteomes, model complex interactions at molecular and systemic levels, and predict the behavior of potential drug candidates. This integrated approach not only accelerates the drug discovery process but also enhances the precision and efficiency of identifying promising compounds for further experimental validation.

Leishmaniasis, a disease transmitted by female sandflies, has emerged as a significant global health concern affecting 99 countries, particularly in East Africa, Brazil and the Indian states specifically in Jharkhand, Bihar, West Bengal, and Uttar Pradesh are among the places in which the problem is notably severe. The disease manifests in various clinical forms, including visceral leishmaniasis (VL), muco-cutaneous (MCL), and diffuse cutaneous leishmaniasis (DCL). Visceral leishmaniasis, caused by *Leishmania donovani*, stands out as the most severe form with a high fatality rate.

Although drugs for treatment were introduced decades ago, their efficacy is limited due to variations in drug sensitivity among *Leishmania* species. Additionally, drug resistance poses a significant challenge, rendering pentavalent antimony treatment ineffective in the various regions. Consequently, the emergence of resistance underscores the urgency of exploring novel drug options through diverse approaches such as multi-target, repurposing, subtractive genomics, system biology, etc. In summary, identifying an effective cure for this leishmaniasis disease remains a major challenge, necessitating the search for new drug targets.

Due to the escalating pathogenicity of the disease and the importance for novel targets, our focus shifted to developing a computational strategy aimed at identifying drug target proteins of *Leishmania donovani* and other *Leishmania* species. Additionally, we applied computational strategies to investigate the interaction of a specific protein with potent ligands. Various approaches were used to explore the enzymatic activity and functional

aspects of the selected protein, providing valuable insights into its potential as a therapeutic target. By employing computational techniques, we aim to contribute to the identification and characterization of promising targets for combating *Leishmania donovani*, thereby addressing the urgent need for innovative strategies in managing this disease.

In our pursuit, we endeavored to pinpoint potential lead molecules targeting crucial drug targets in *Leishmania* species. Through this endeavor, we uncovered new agonists showing potential as promising drug candidates for inhibiting the pathogen. Employing a spectrum of computational techniques, we systematically screened compounds against specific protein targets. The selected ligands, distinguished by their superior binding scores, high affinity, and a substantial number of interactions, emerge as prudent candidates for experimental validation. These compounds hold the potential to serve as instrumental agents in experimental verifications, representing a significant step forward in the quest for efficacious drug molecules against *Leishmania* species.

In the initial study of the thesis, a multi-target approach was employed, focusing on the APRT and DHODH proteins. Using a virtual screening strategy, potential ligands were identified and subsequently docked with the selected proteins. Molecular dynamics (MD) simulations were then performed and the resulting analyses identified the ligand [2-(3,4-dihydroxyphenyl)-7-hydroxy-4-oxo-4H-chromen-5-ylisobutyrate] as a promising antileishmanial inhibitor effective against both targets.

In the second study, protein drug targets and essential genes of *L. donovani* were identified, leading to the selection of two targets: pyridoxal kinase (PK) and sterol alpha-14 demethylase (SDM). Potential compounds were gathered from the DrugBank and DrugCentral databases. These compounds underwent a molecular docking process, followed by MD simulations and MMPBSA (Molecular Mechanics Poisson-Boltzmann Surface Area) analysis. This comprehensive approach identified Nitazoxanide as a promising compound due to its effective interaction mechanisms with the selected drug targets, presenting a potential therapeutic strategy for combating VL.

In the final study, subtractive genomics was utilized to identify common drug targets across five strains of *Leishmania* species. Through various screening methods, Glutamate Dehydrogenase (GDH) was selected as a protein target. Subsequently, a structure-based approach identified the ligand GW5074, which demonstrated interactions with the shared GDH targets, highlighting its potential as an inhibitor.

Scope of the work

Despite decades of research efforts, effective treatments for leishmaniasis remain limited, and drug resistance has emerged as a significant challenge. The development of new drugs against *Leishmania donovani* is imperative to combat the disease effectively and address the growing threat of drug resistance. As resistance continues to emerge, the pathogenicity of Leishmaniasis is on the rise, underscoring the need for new targets and potent ligands to counteract this trend. The scope of work on drug discovery for *Leishmania donovani* and other *Leishmania* species underscores a multidisciplinary approach that integrates diverse scientific disciplines, encompassing multitarget strategies, drug repurposing, and subtractive genomics. The research endeavors to bridge gaps in our current understanding of drug development for leishmaniasis by introducing innovative computational techniques. Our focus areas include identifying novel drug targets, screening compound libraries at high throughput, optimizing lead compounds, and assessing the binding affinity and stability of protein-ligand complexes. Our work focuses on five specific drug targets: APRT, a component of the purine pathway; DHODH, involved in the pyrimidine pathway; PK, part of the vitamin B6 pathway; sterol alpha-14 demethylase (SDM), integral to sterol biosynthesis; and GDH. Throughout our chapters, we emphasize the meticulous selection of these protein targets and the application of computational techniques with diverse methodologies.

The central aim of the thesis is to advance and implement innovative methods that offer a more realistic and nuanced approach to target fishing and drug identification, particularly in the context of a distinct class of diseases. By concentrating on leishmaniasis, the thesis contributes to the broader landscape of infectious diseases, offering insights and methodologies that may have implications for similar protozoan-driven ailments. The interdisciplinary nature of the research, combining various methods, underscores the commitment to advancing the field and potentially paving the way for more efficacious treatments for leishmaniasis. By enhancing our knowledge of the parasite's biology and capitalizing on emerging therapeutic targets, we have the potential to create efficient treatments to alleviate the impact of leishmaniasis and enhance public health worldwide.

Outline of the Thesis

Chapter 1: Introduction provides information about the etiology, epidemiology, leishmaniasis treatment, vaccines development and drug resistance in Leishmaniasis. Next it provides an overview of drug discovery approaches in leishmaniasis. Finally, the chapter ends with briefly about the scope of the work.

Chapter 2: The methodology section offers a meticulous account of the various processes involved in the work. It details the different integrators and force fields used in molecular dynamic simulations, as well as the preparation of diverse systems for various proteins and ligands. Additionally, it includes information on the analyses performed.

Chapter 3: The study describes the multiple proteins of *Leishmania donovani*, leading to the selection of two specific protein targets. Ligands for these target proteins were sourced from a database. Subsequent analyses were conducted to examine the structure and dynamics of the protein-ligand complexes. Additionally, the structural stability and instability of the proteins in the presence of ligands were discussed.

Chapter 4: The study explores repurposed drugs from various databases and tests these ligands on key protein targets of *Leishmania donovani*. Multiple screening studies were conducted to identify essential target proteins and corresponding ligands for interaction studies. The stability and dynamics of the protein-ligand complexes were thoroughly analyzed using a variety of methods.

Chapter 5: The study meticulously examines the proteomes of five *Leishmania* species alongside the human proteome to identify a promising drug target for combating *Leishmania* infections through a series of screening steps. The dynamics of the selected *Leishmania* protein were evaluated both in the presence and absence of small molecules. The ligand GW5074 was found to enhance the stability of the protein and demonstrated favorable binding energy with the protein targets.

Chapter 6: It presents the overall findings and conclusions, and discusses the future scope of the work.