## Abstract

Leishmaniasis, a neglected tropical disease caused by various species of Leishmania, presents a significant global health challenge, especially in tropical and subtropical regions like Latin America, Southeast Asia, and parts of Africa. Annually, there are 700,000 to 1 million new cases, and 25,000 to 26,000 deaths occur worldwide. Factors like climatic shifts, co-infections with diseases such as HIV, and population mobility have altered its distribution, exacerbating its impact. It is also termed a "poverty-associated disease" due to its prevalence among economically disadvantaged populations with limited healthcare access and minimal influence on government policies. Current treatment options are inadequate, marked by several issues such as toxicity, high cost, complex administration, and drug resistance. The pharmaceutical industry's lack of focus on this disease further hampers the development of innovative therapeutics, underscoring the need for new drug targets and the repurposing of existing drugs.

Amastigotes of Leishmania, which reside in the phagolysosomes of host cells, endure low pH, high temperatures, and oxidative stress, often entering a semi-quiescent state to evade host defences. They primarily utilize sugars and fatty acids for survival and growth, with the TCA cycle playing a crucial role in their metabolic pathways. Citrate synthase (CS), the first enzyme of the TCA cycle, was identified as a potential drug target due to its pivotal role in the continuation of the TCA cycle & energy production in mitochondria and cholesterol & fatty acid synthesis.


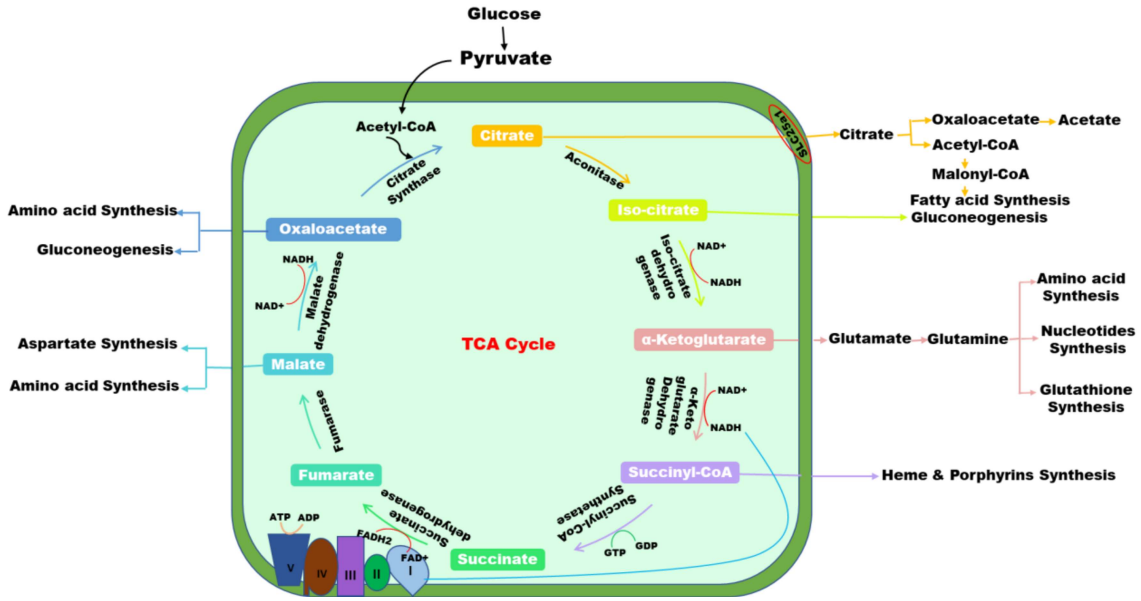


**Figure 1: Biochemical role of TCA cycle intermediate.**

in the cytosol and significant structural and sequential differences from the human version, making it a viable candidate for selective drug targeting.

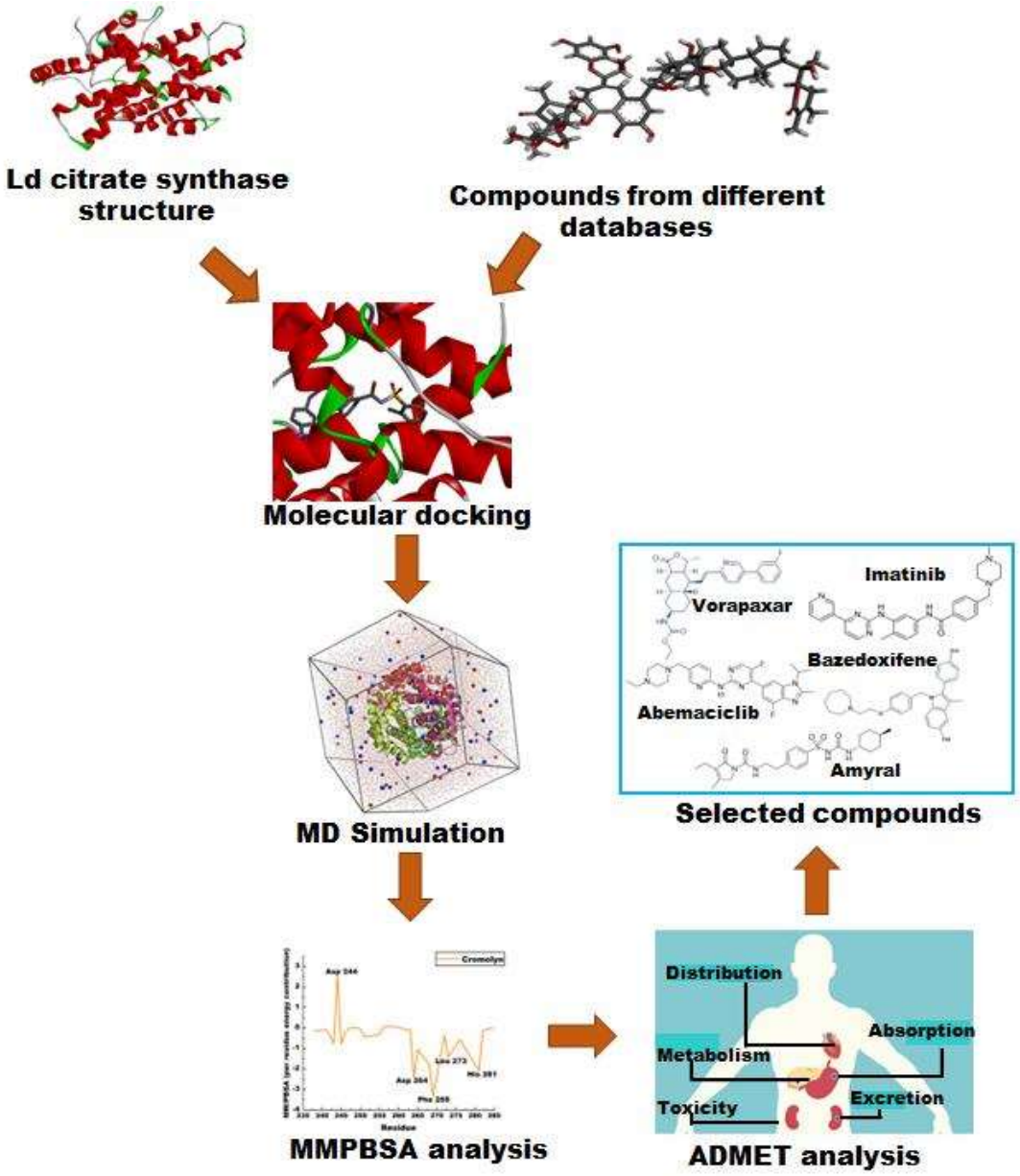


**Figure 2:** ***In silico*** **studies for selecting top hits using LdCS as a drug target.**

In this study, the crystal structure of *Leishmania donovani* citrate synthase (LdCS) was not available; thus, a model was generated using the ITASSER server. The best model, based on C and TM scores, was energy minimized and validated using RAMPAGE and ProSA servers. This validated model was used for molecular docking studies with 1065 natural and 1565 FDA-

approved compounds from the ZINC 15 database. The top five hits—Abemaciclib, Bazedoxifene, Vorapaxar, Imatinib, and Amyral—were selected based on their lower binding energies with LdCS compared to human citrate synthase (HsCS) and interactions with the active site residues of LdCS which analysed through discovery studio analyser. All selected compounds adhered to Lipinski's rule, indicating favourable oral bioavailability. Molecular dynamics (MD) simulations were conducted with GROMACS to assess the stability of the protein-ligand complexes, considering parameters like RMSD, RMSF, radius of gyration, and hydrogen bond analysis. The simulations demonstrated that the ligands formed stable, robust interactions with LdCS. MM-PBSA analysis further confirmed the lower binding energies of these complexes, predominantly driven by hydrophobic contacts. Residue energy decomposition studies identified key residues—Tyr 238, His 242, His 245, Asp 264, Phe 269, Leu 273, and Asn 285—crucial for ligand interactions. Pharmacokinetic studies based on ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis showed that all selected ligands had good water solubility, strong CaCO-2 permeability, good intestinal absorption, and minimal toxicity. These properties suggest favourable pharmacokinetic profiles and potential for effective therapeutic application.

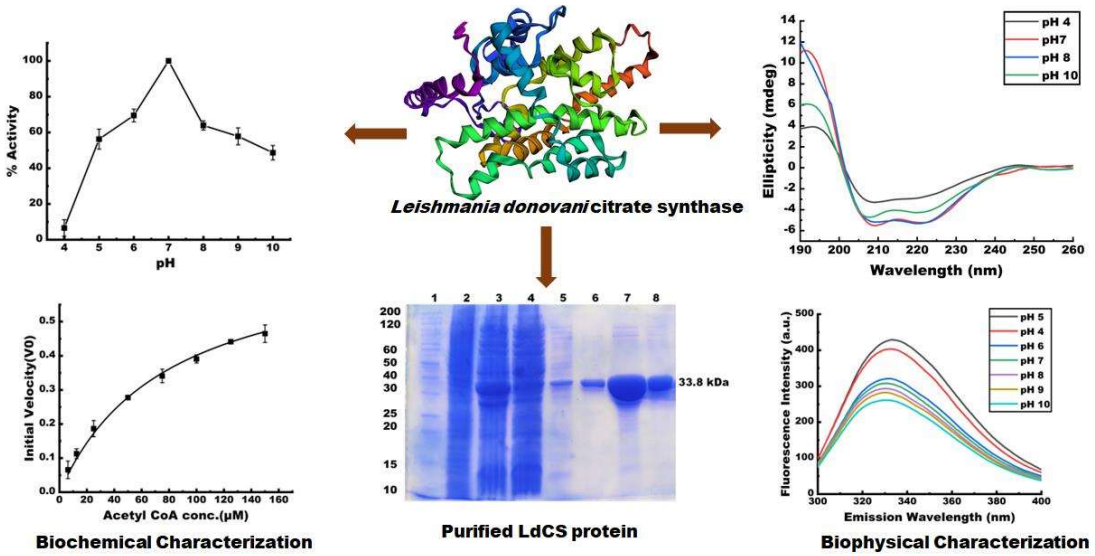


**Figure 3: Biochemical and Biophysical characterization of LdCS.**

To further characterize LdCS, biophysical and biochemical studies were performed. The Ldcs gene was cloned and expressed in a bacterial system, and its protein, purified through affinity chromatography, was confirmed to be pure and of the correct size via SDS-PAGE. Circular dichroism fluorescence spectroscopy revealed that LdCS is predominantly alpha-helical and

maintains a highly folded structure at physiological pH, with changes in secondary structure at extreme pH levels. Thermal denaturation studies indicated that LdCS resists structural changes upon unfolding, with a melting temperature of 44 ± 0.31°C. Fluorescence spectroscopy also indicated that LdCS contains tryptophan residues buried in its core, which are sensitive to environmental changes. Quenching studies with potassium iodide and acrylamide confirmed that these residues are accessible to quenchers, suggesting their strategic location within the protein structure. Urea and GnHCl-mediated unfolding studies provided insights into the stability of LdCS, with transition concentrations indicating a relatively stable protein structure. Functional characterization of LdCS included enzyme activity assays, revealing optimal catalytic activity at pH 7.0. Kinetic studies showed a higher Km for acetyl-CoA than OAA, indicating a lower affinity for acetyl-CoA.

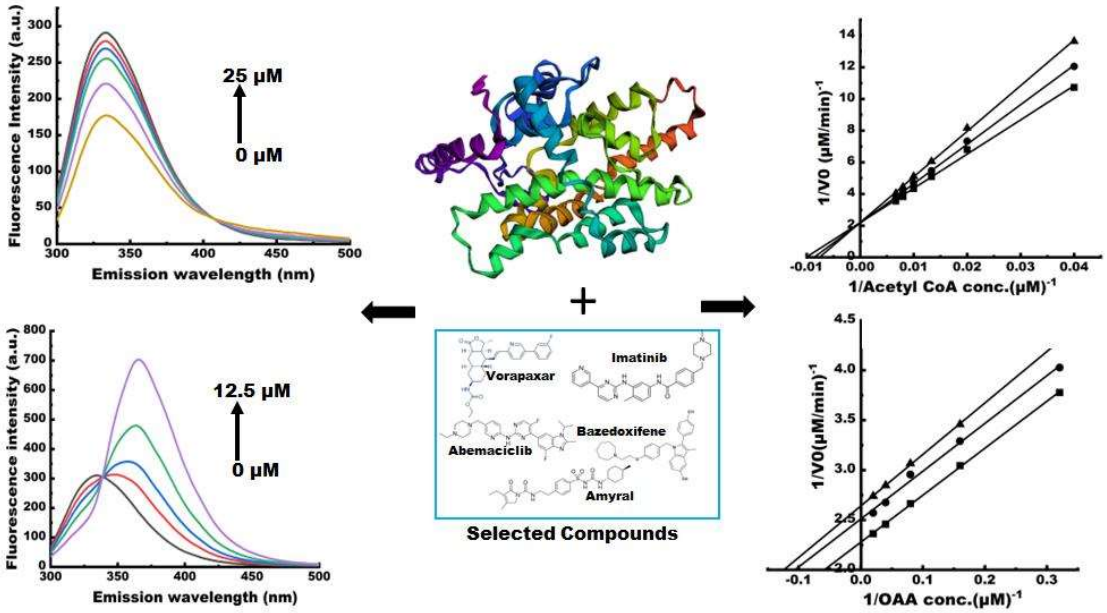


**Figure 4: *Invitro* studies with selected compound.**

Binding studies with selected compounds through fluorescence spectroscopy show good binding affinity, and inhibition studies with selected compounds demonstrated competitive inhibition for acetyl-CoA and uncompetitive inhibition for OAA, with inhibition constants in the micromolar range. Antileishmanial and cytotoxicity studies on promastigote and amastigote forms of Leishmania, as well as murine macrophages, revealed that the selected compounds effectively reduced cell viability and infection rates, with $IC_{50}$ and $EC_{50}$ values lower than those of the standard drug Miltefosine.

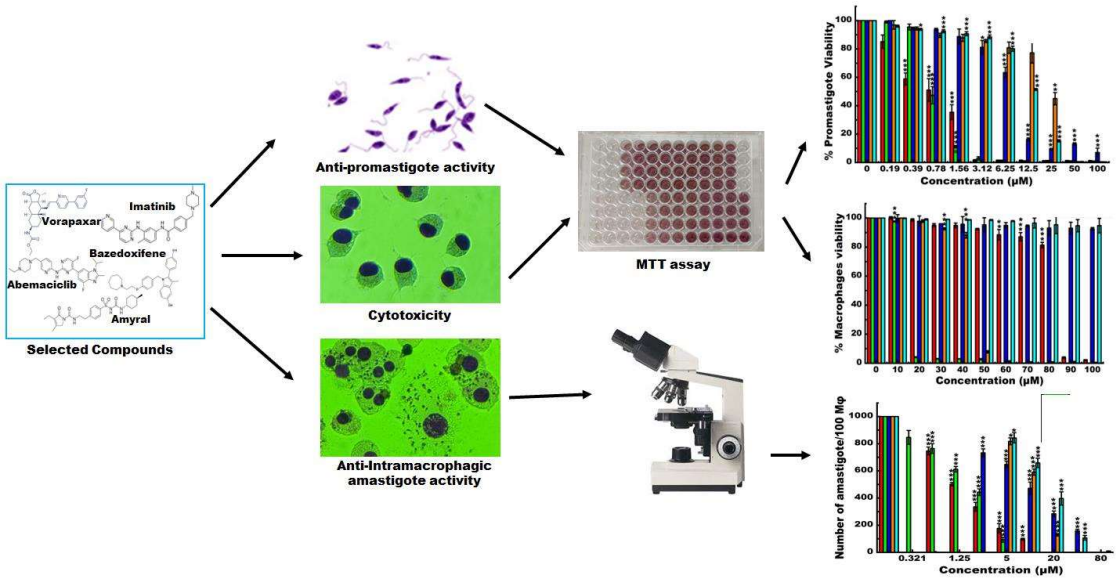


**Figure 5: Antileishmanial and cytotoxicity studies with selected compounds.**

Vorapaxar showed, till 500 μM, no cytotoxic effects on macrophages, while other compounds exhibited low cytotoxicity, indicating a favourable therapeutic index. Further investigations into the mechanism of action of abemaciclib, based on its potent *in-silico* and *in-vitro* activity, revealed morphological alterations in promastigotes, mitochondrial damage, increased ROS production, depolarization of the mitochondrial membrane, nuclear condensation, increase in Sub G1 cell population in cell cycle assay and DNA fragmentation, indicating its action on mitochondria and an apoptotic mode of death. The apoptotic mode of death was further confirmed with Annexin V and PI, indicating that 64% of cells are in an apoptotic state.

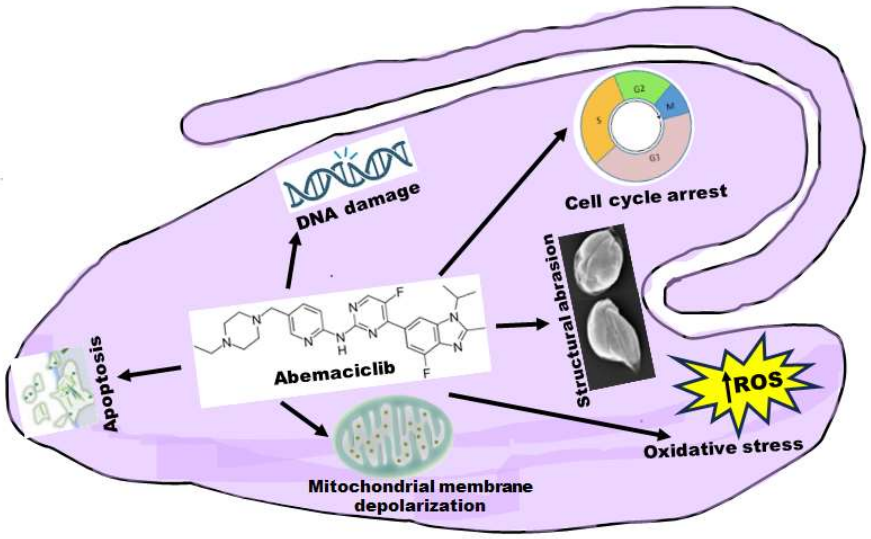


**Figure 6: Mode of action of Abemaciclib on *Leishmania donovani*.**

These findings suggest that Abemaciclib and other selected compounds are promising therapeutic agents for leishmaniasis, warranting further development and clinical evaluation.