DRUG REPURPOSING FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS USING ARGINASE INHIBITORS – AN IN SILICO APPROACH

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Introduction

Leishmaniasis is a neglected tropical disease that significantly impacts public health in developing countries, accounting for approximately 1.6 million new infections each year. Current treatments rely on drugs that induce severe adverse effects, toxicity, and resistance. Therefore, there is an urgent need to identify and develop new agents for the treatment of leishmaniasis. In this scenario, the arginase enzyme from *Leishmania spp*. has emerged as a promising therapeutic target due to its essential role in parasite growth and proliferation. One alternative to reducing the costs and time associated with discovering new bioactive molecules is drug repositioning, which leverages existing knowledge of the pharmacokinetic and toxicological properties of drugs already in clinical use. As a result, the repositioning of existing drugs can significantly shorten the development time of new therapies, which has proven effective in managing leishmaniasis, as evidenced by miltefosine, amphotericin B, and paromomycin.

Material and Methods

This study involved constructing and validating a theoretical model for *L. amazonensis* arginase (LamARG) using the sequence retrieved from the UniProt database (code O96394). The Protein BLAST algorithm was employed to align the sequence with known structures from the Protein Data Bank database. The SwissModel server was used for comparative modeling considering the template indicated by Protein BLAST. The model validation included quality assessment using ProSa-Web, QMEAN, PROCHECK, and VoroMQA, as well as structural alignment with TM-Align. Molecular docking simulations were conducted using the GOLD software to predict the binding modes of 20 active inhibitors of *Lam*ARG, with the aim of constructing pharmacophore hypotheses. For this purpose, ChemScore scoring function was used to evaluate ligand-receptor interactions, as it accounts for the Mn^{+2} metals present in the protein's active site. The binding site was defined by centering a grid box with dimensions of 10 Å, centered on the crystallographic ligand, with coordinates corresponding to: x = -9.328; y = -22.064; z = 8.027. The corresponding pharmacophoric hypotheses were developed and validated using a test set consisting of 20 inhibitors and decoy obtained from the DUDe-E server. Finally, pharmacophore screening was performed in the e-Drug 3D database to identify potential LamARG inhibitors for drug repositioning.

Results and Discussion

The theoretical *Lam*ARG model exhibited 90.9% of residues in the most favorable regions of the Ramachandran's plot, aligning with expected values for high-resolution protein structures in PROCHECK. ProSa-Web calculates and integrates the Z-score of the target protein model within this graphical representation, and comparing the estimated Z-score of -8.45 for the theoretical model shows that its overall quality score falls within the empirical distribution of PDB crystallographic structures. The predicted

overall quality score for the LamARG model, 0.77, also falls within the range of QMEAN scores observed in high-resolution protein structures. VoroMQA determined that the theoretical model achieved a global quality score of 0.56, which falls within the expected quality range for high-resolution protein structures, *i.e.*, scores ≥ 0.4. The structural alignment return an root-mean-square deviation (RMSD) of 0.80 Å, thus, reaffirming its similarity with the crystallographic template structure (PDB ID: 4IU0). Pharmacophore models were constructed based on the predicted docking poses of 20 active compounds compiled from the literature and databases for LamARG. Then, 995 corresponding decoy compounds obtained from the DUDe-E server were added to create the test set. The model with the highest performance on the test set, based on compound 18, was selected for virtual screening (VS) on the e-Drug 3D database. This model demonstrated a Matthew's correlation coefficient (MCC) of 0,68, indicating satisfactory predictive performance for VS campaigns. Overall, 22 commercially available compounds were selected from the pharmacophore screening. Among these, Protokylol and Dabigatran were prioritized for further analysis, showing promising binding modes with *Lam*ARG. Protokylol, in particular, demonstrated a better fit, potentially making it a strong candidate for drug repurposing against cutaneous leishmaniasis.

Conclusion

This study generated a complete and validated theoretical model of LamARG. A literature and database search for active LamARG compounds yielded 20 known enzyme inhibitors, alongside 995 corresponding decoys. Based on molecular docking results, key interactions between known inhibitors and the active site of LamARG were identified, which served as the foundation for individual pharmacophore hypotheses. The pharmacophore model derived from compound 18 demonstrated significant predictive performance and was therefore used as a reference for virtual screening (VS).

VS of the e-Drug database identified 22 compounds with significant stereoelectronic fit to the validated pharmacophore model, with potential to inhibit LamARG. Among them, only Protokylol and Dabigatran were selected for repurposing, as both are orally available in their active, non-metabolized forms. Molecular docking results confirmed the potential of these drugs as LamARG inhibitors, with Protokylol showing the most promising interaction profile.

The identified compounds offer opportunities to improve the quality of life for individuals affected by cutaneous leishmaniasis. Experimental assays are necessary to validate their activity and confirm their repurposing potential. Successful validation could lead to the development of more specific and less toxic treatments, crucial for ensuring efficacy and patient adherence.

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