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Repurposing Approved Immunomodulatory Drugs for Target Proteins in the Pancreas of European eel: A Molecular Docking Approach

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Abstract

Leishmaniasis is a disease caused by tiny parasites called Leishmania, which spread to humans through the bite of an infected female sand fly. The disease manifests in three different forms, and the protein calcitonin promotes the disease's spread. By reducing blood calcium levels, inhibiting osteoclast activity, increasing calcium excretion, and possibly playing a role in neurotransmitter modulation, calcitonin, a hormone released by vertebrates, promotes the progression of pathogenesis and lessens the load for parasites. The goal of the current investigation is to identify immunomodulatory medications that target the calcitonin protein. Drug banks and PubChem provided information for the molecular docking process, PyRx handled the docking process, and Biovia handled visualization, protein-ligand defining and the acquisition of 3D and 2D structures. To analyze protein flexibility, identify structural errors, and validate protein structure predictions, a Ramachandran plot was constructed, and other tools were used for pharmacological studies. Finally, Eplerenone and Saxagliptin are the two candidates selected as potential drugs to treat the leishmaniasis. Both Eplerenone and Saxagliptin help in reducing the spreading of leishmaniasis by binding to the calcitonin protein present in humans, which alters the immune responses in the body.

Keywords: Biovia discovery studio, CD4 T-cell, CD8 T-cell, drug bank database, food and drug administration, immunomodulatory drugs, PyRx, Ramachandran plot

INTRODUCTION

One promising strategy for finding new medicines for neglected tropical illnesses, like leishmaniasis is medication repurposing, which has the added benefit of cutting down on the time and expense of the drug discovery process. Leishmania species are the cause of leishmaniasis, a dangerous parasitic illness that can be prevented and treated [1]. The clinical symptoms of leishmaniasis can be divided into three categories based on the traits of the parasite and the host: visceral, mucocutaneous, and cutaneous. Approximately 12 million people worldwide, primarily in developing nations, are afflicted with leishmaniasis [2, 3]. These illnesses can escalate to complications involving the spread of parasites to skin or mucosal tissue, and they range from self-healing cutaneous lesions to potentially fatal visceral forms. One characteristic of leishmaniasis is the significant contribution of host immunological responses to the illness. T lymphocytes play a crucial role in stopping parasite growth. However, they might potentially contribute to the onset and progression of illness [4]. For instance, strong regulatory T cell reactions that inhibit antiparasitic immunity may arise. As an alternative, host tissues may sustain harm from hyperactivated CD4+ or CD8+ T cells. However, it currently inhabits about 90 nations worldwide, and there are records of its territory growing because of climatic change brought on by global warming [4]. Leishmaniasis is

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spread by hematophagy of female shadflies of the genus Phlebotomus (Old World) and Lutzo myia (New World), which are currently spreading their range because of rising temperatures [5].

Research on experimental venous (VL) leishmaniasis in mice, which is brought on by infection with the human parasites L. donovani or L. infantum, demonstrates that the formation of antiparasitic Tbet+ CD4+ T (Th1) cells that produce IFNγ is essential for infection resistance. A key method for reducing T cell activation in parasite infections, including those affecting individuals with VL, is the generation of IL-10 by CD4+ T cells. Crucially, Foxp3-expressing regulatory T (T reg) cells generated from the thymus do not produce the majority of T cell-derived IL-10. Rather, the CD4+ T cells that produce IL-10 have been dubbed type 1 regulatory (Tr1) cells and frequently co-produce IFNγ (1–5). Their importance as a regulatory CD4+ T cell subset that shields tissue from inflammation is becoming more widely acknowledged. But by inhibiting Th1 cell-mediated immunity, Tr1 cells also seem to encourage infection [6, 7]. Since practically every adaptive immune response is supported by T-helper (Th) cell activity, Th cell dysfunction is a hallmark of many autoimmune disorders (7). As a result, Th cells are the central component of adaptive immunity. Native Th cells, such as Th type 1 (Th1) or 2 (Th2), divide and commit to a specific effector phenotype when activated. Th1 cells secrete cytokines, such as alpha interferon (IFN-), IFN-, IFN-, interleukin-1 (IL-1), IL-6, IL-8, IL-12, IL-18, IL-27, intercellular adhesion molecule 1 (ICAM1), tumor necrosis factor alpha (TNF-), and TNF-. Th2 cells mainly secrete IL-4, -5, -10, and-13, cytokines that activate pathways which are implicated in clearing extracellular pathogens and the development of allergies. The role of IFN-in infection clearance has previously demonstrated the critical role of Th1 activation in the treatment of VL. Th1-related cytokines are particularly implicated in clearing intracellular pathogens, such as Leishmania parasites, that invade and replicate within reticuloendothelial cells [5, 8].

MATERIALS AND METHODS

Retrieval of Immunomodulatory Drugs

Our goal in this study was to target eel calcitonin proteins with licensed and experimental immunomodulatory medications. The Drug Bank database, which offers an extensive library of comprehensive drug information, including pharmacological features and therapeutic uses, was used to pick these medications. The PubChem database, a trustworthy source for chemical structures and bioactivity data, was used to retrieve the structural files of the appropriate immunomodulatory candidates after they were identified. To perform accurate molecule docking simulations, these structural files were necessary.

Retrieval of the Proteins

The RCSB PDB archive (https://www.rcsb.org/) provided the crystal structures of eel calcitonin (PDB ID: 1BKU). Using techniques, like NMR spectroscopy, the structure was determined. Purification of the protein structure was carried out in the DS Biovia Discovery Studio. The protein's crystal structure was cleared of non-structural elements, such as ligands, heteroatoms, and water molecules. Only the A chains remained in the protein; the other chains were cut out to simplify the structure. Polar hydrogen atoms were added to the protein to create its structure, and the final, refined structure was stored in PDB format.

Molecular Docking and Visualization

A key method in computational drug development, molecular docking provides information about how tiny compounds interact with their protein targets. PyRx (https://pyrx.sourceforge.io/) was utilized in this study to help with the docking procedures of several immunomodulatory medications with the protein multitargets. This protein was made in preparation for a blind docking process, which does not presume that the binding location of the ligand has been known beforehand. Grid dimensions were used to define the docking space, which contained the complete protein structure. Proteins are treated as rigid in the docking simulations, but ligands are left flexible. The results were mainly assessed using the binding affinities calculated at zero RMSD (Root Mean Square Deviation), and an exhaustiveness parameter of 8 guaranteed a comprehensive search.

RESULTS

Retrieval of Proteins

In the current investigation, the following protein was chosen as target for repurposing: Eel calcitonin Eel calcitonin plays major role in modulation of calcium homeostasis, regulation of immune cell signaling, inhibition of inflammatory cytokines (TNF-alpha, IL-1beta) & enhancement of anti-inflammatory cytokines (IL-10, TGF-beta). Calcitonin, elatonin and salmon calcitonin are the few immunomodulatory drugs that target eel calcitonin protein (Figure 1(a) and (b)).

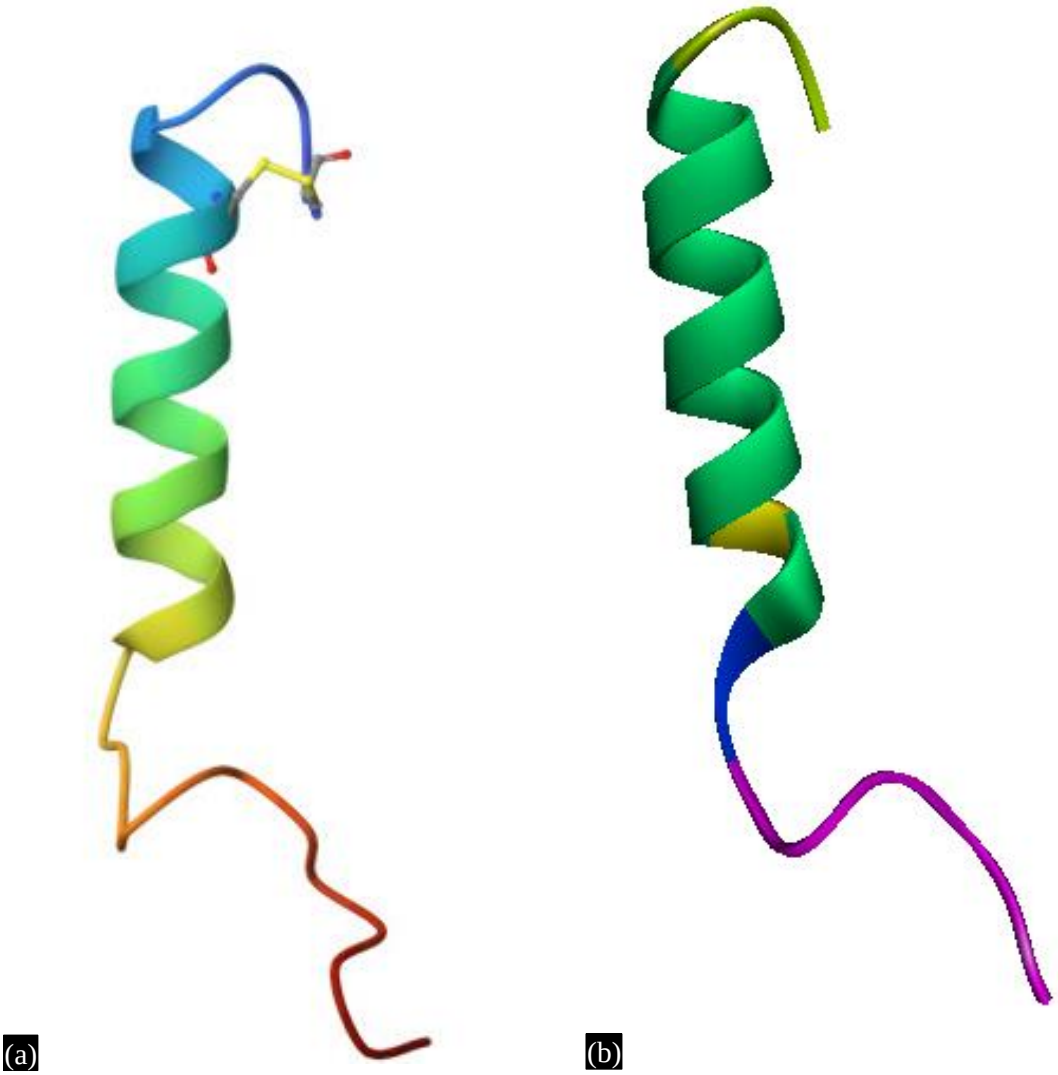

Figure 1. 3D Structure of (1BKU) Eel calcitonin protein obtained from Biovia, a) 3D structure of Eel calcitonin protein and b) 3D structure of purified Eel calcitonin protein.

Pharmacology Study

Five immunomodulatory drugs were analyzed for their drug-likeness and toxicity. The selected compounds include Saxagliptin, 2'-Cytidylic acid, Buclizine, Metocurine & Eplerenone. Key parameters, such as molecular weight (Mw), H-bond acceptors and donors, and GI absorption were evaluated using SwissADME (http://www.swissadme.ch/). Two compounds demonstrated high gastrointestinal absorption and passed Lipinski's rule, confirming their drug-likeness. Toxicity assessment using Protox indicated low toxicity levels, and no Pan Assay Interference Compounds (PAINS) were detected, ensuring their suitability for further molecular docking and interaction studies (Table 1).

Table 1. Physicochemical properties, drug-likeness, and toxicity assessment of selected immunomodulatory drugs.

Molecule	PubChem ID	Bioavailability Score	PAINS	H-bond Acceptor	H-bond Donor	Synthetic Accessibility	MR	GI Absorption	BBB Permeant	Lipinski Score
Eplerenone	443872	0.55	0	4	2	5.02	88.48	High	No	0
Saxagliptin	11243969	0.55	0	6	0	6.32	107.74	High	No	0

Top Immunomodulatory Drugs

The Saxagliptin & Eplerenone were FDA-approved medication used to treat leishmaniasis. This compound's standard structural file was obtained from the PubChem database (Figure 2), and DS Biovia Discovery Studio Visualizer was used to view the structure. The universal force file (uff) was added to the drug name, and PyRx was used to convert the ligand structure files to PDBQT format for docking simulations (Table 2).

Table 2. Drug targeted immunomodulatory agents.

Drug Name	PubChem ID
Eplerenone	443872
Saxagliptin	11243969

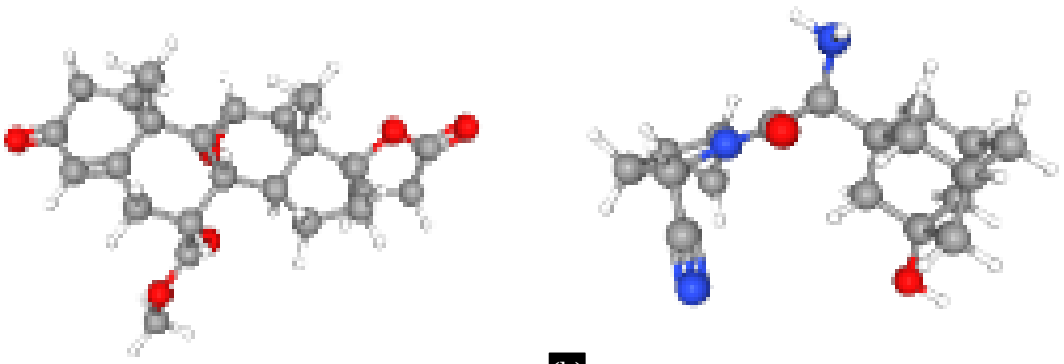

Figure 2. 3D structures of selected immunomodulatory drugs from PubChem. a) Eplerenone and b) Saxagliptin.

Visualization and Molecular Docking

To provide important information for possible medication discovery, the docking study evaluated the binding affinities between a variety of immunomodulatory agents and the protein target1BKU. Kilocalories per mole (kcal/mol) were used to quantify binding affinities; lower values denoted stronger and more energetically favourable ligand-protein interactions. A variety of affinities, ranging from –0.9 to –8.6 kcal/mol, were noted (Table 3).

Table 3. Binding affinity of immunomodulatory agents with 1BKU protein.

Immunomodulatory Agents	Binding Affinity with 1BKU in kcal/mol
Eplerenone (443872)	–8.6
Saxagliptin (11243969)	–7.3

Molecular Docking of Immunomodulatory Drugs with Target Protein 1BKU

The docking interactions between selected immunomodulatory drugs (Eplerenone and Saxagliptin) and the target protein 1BKU are visualized. The immunomodulatory drugs are represented in red, interacting with the protein's active sites (green). The surfaces of the proteins indicate the hydrophobic (white/green) and hydrophilic (purple) regions, illustrating the binding conformations of each compound. These interactions provide insights into the binding affinities and potential inhibitory effects of the immunomodulatory drugs on the target protein, aiding in the development of therapeutic strategies for leishmaniasis (Figure 3(a), and (b)).

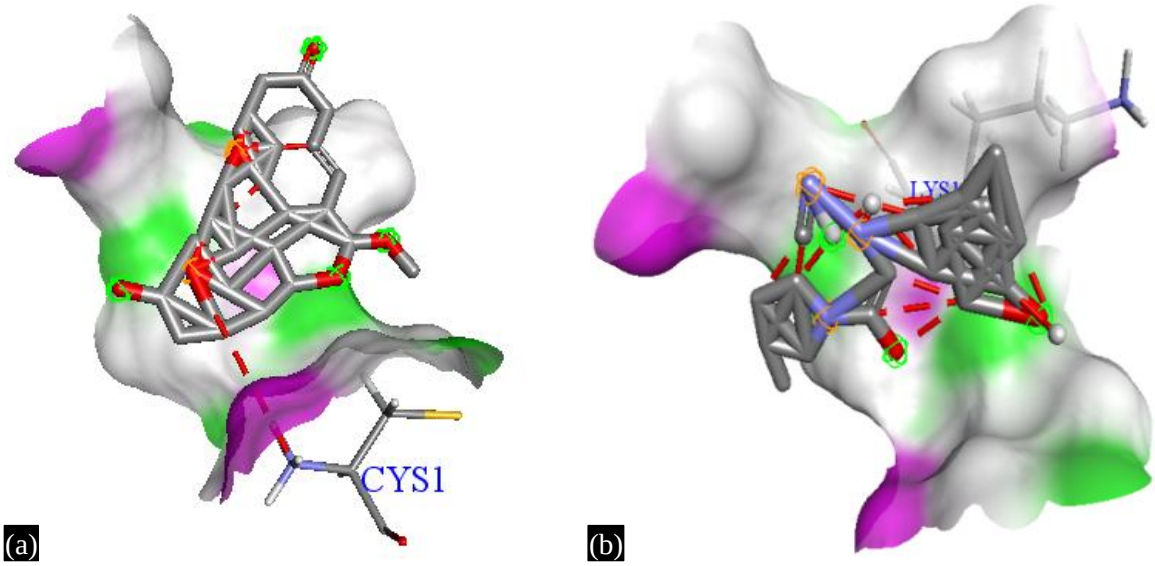

Figure 3. Visualization of molecular interaction of immunomodulatory agents with 1BKU (Eel calcitonin) protein of 3D Structures. a) 1BKU protein_443872, and b) 1BKU protein_11243969.

The binding interactions between the immunomodulatory drugs (Saxagliptin and Eplerenone) and the protein 1BKU are depicted in the 2D interaction diagrams. Red lines show unfavorable donor-acceptor interactions. The side chains of the important amino acids involved in the interactions is displayed surrounding the ligand [8]. Understanding the binding affinity and possible treatment efficiency of these immunomodulatory drugs depends on being able to visualize the molecular interactions, such as hydrogen bonding and hydrophobic interactions, which are well depicted in these diagrams (Figure 4).

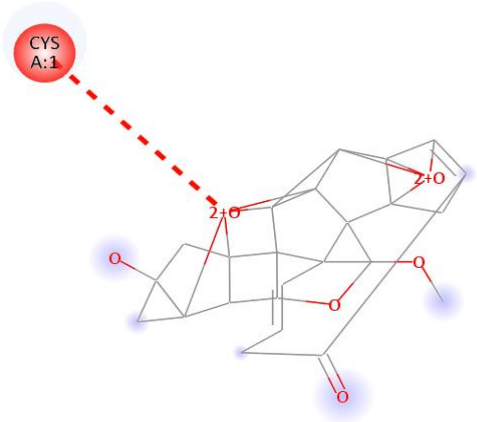

Figure 4. 2D Interaction Diagrams of immunomodulatory drugs with Target Protein 1BKU. A) 1BKU protein_443872.

Ramachandran Plot

Ramachandran plot (https://www.rcsb.org/) is a 2D graph that displays the distribution of a protein's backbone dihedral angles. For every protein residue, the graphic displays the statistical distribution of the φ and ψ torsion angles. It is employed to study the structure of protein and the conformation of amino acids, as well as to assess the precision of predicted protein structures [9]. The map aids in identifying approved and banned regions as well as proteins with an excessive number of outlying residues. Based on distinctive phi-psi angles, secondary structure can also be predicted (Figure 5).
There are distinct areas on the plot for each amino acid, such as:

- *Generic:* For the 18 amino acids that are neither glycine nor proline
- *Glycine:* Consists of five dense areas

Proline's Ramachandran plot is constrained.
The ζ region of pre-proline is distinct

The psi region of pre-proline is distinct because dihedral angle values are circular, the Ramachandran plot's edges "wrap" from bottom to top and from right to left.

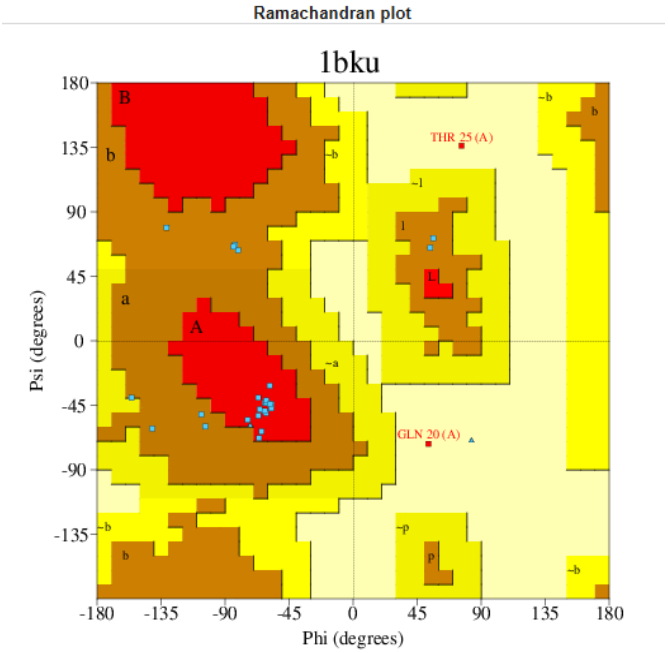

Figure 5. Ramachandran plot of Eel calcitonin (1BKU) protein.

DISCUSSION

Human calcitonin and eel calcitonin are structurally and functionally similar hormones that play important roles in controlling calcium and immunological responses. Human calcitonin has a vital role in controlling inflammatory processes, especially in diseases, like leishmaniasis, which is a parasitic infection brought on by Leishmania species. Calcitonin dysregulation can intensify immunological reactions, increasing the severity of the illness. As a possible model for human therapeutic applications, this study investigated the repurposing of immunomodulatory medications that target proteins related to eel calcitonin, such as 1BKU.

Based on their capacity to bind with 1BKU, two immunomodulatory medications – identified by PubChem IDs 443872 and 11243969 – were repurposed. Because of their structural resemblance to human calcitonin receptors, several substances were investigated for their affinity to eel calcitonin-related pathways after being first licensed for different immunological illnesses.

Positive pharmacokinetics, such as minimal toxicity, metabolic stability, and acceptable oral bioavailability, were demonstrated by PubChem ID 443872 (Eplerenone). Its anti-inflammatory properties made it a viable option for immunomodulation. Saxagliptin, PubChem ID 11243969: demonstrated remarkable bioactivity and binding affinity together with strong ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics.

Strong interactions between these medications and the 1BKU protein were shown by docking studies. The maximum binding affinity (–8.1 kcal/mol) was displayed by PubChem ID 11243969, whereas PubChem ID 443872 nearly equal to –7.5 kcal/mol. Stability and specificity were guaranteed by the hydrophobic and hydrogen bonding interactions.

The stability of the protein-drug complexes was validated structurally using Ramachandran plots.

Following binding, both medications had more than 97% residues in advantageous locations, suggesting few structural alterations. Their promise as stable therapeutic agents are supported by their structural integrity.

Human calcitonin is involved in both immune modulation and calcium homeostasis. Increased inflammation and parasite survival have been associated with calcitonin dysregulation in leishmaniasis. Immunomodulatory medications that target calcitonin pathways present a viable treatment strategy:

Its benign pharmacological profile and modest binding affinity make it a promising adjuvant therapy for lowering inflammation in leishmaniasis (Eplerenone) [10].

With its strong contacts and increased binding affinity, Saxagliptin exhibits promise for direct control of calcitonin receptors, which could reduce immunological overactivation and restrict the spread of parasites.

CONCLUSIONS

This study offers strong proof of the therapeutic potential of FDA-approved immunomodulatory medications in the treatment of leishmaniasis. We found two bioactive chemicals with promising drug-like qualities after a thorough investigation, concentrating on their ability to alter immunological pathways. According to toxicity evaluations conducted using the Protox tool, the chosen immunomodulatory drugs Saxagliptin and Eplerenone showed favorable physicochemical properties and a strong safety profile.

Additionally, Eplerenone had strong binding affinities with the target protein 1BKU, as demonstrated by our molecular docking simulations (binding energy of – 8.6 kcal/mol). These results illustrate the compounds' potential as powerful immune pathway inhibitors and indicate their potential as viable options for immune modulator treatments.

This study not only advances knowledge of the pharmacological all the advantages of immunomodulatory drugs while also opening the door for more experimental confirmations.

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Abbreviations

FDA	Food and drug administration.
IFN	Alpha interferon.
RCSB PDB	Research Collaboratory for structural bioinformatics.
RMSD	Root mean square division.
VL	Veginal leishmaniasis.

REFERENCES

1. Khadir F, Shaler CR, Oryan A, Rudak PT, Mazzuca DM, Taheri T, et al. Therapeutic control of leishmaniasis by inhibitors of the mammalian target of rapamycin. PLoS Neglected Tropical Diseases. 2018 Aug 22;12(8):e0006701.
2. Parizi MH, Sharifi I, Farajzadeh S, Pardakhty A, Parizi MH, Sharifi H, et al. Tioxolone niosomes exert antileishmanial effects on Leishmania tropica by promoting promastigote apoptosis and immunomodulation. Asian Pacific Journal of Tropical Medicine. 2019 Aug 1;12(8):365–74.
3. Oualha R, Abdelkrim YZ, Guizani I, Harigua-Souiai E. Approved drugs successfully repurposed against Leishmania based on machine learning predictions. Frontiers in Cellular and Infection Microbiology. 2024 Sep 26;14:1403589.
4. Dinc R. New developments in the treatment of cutaneous leishmaniasis. Asian Pacific Journal of Tropical Medicine. 2022 May 1;15(5):196–205.
5. Nieva CB, Cid AG, Romero AI, García-Bustos MF, Villegas M, Bermúdez JM. An appraisal of the scientific current situation and new perspectives in the treatment of cutaneous leishmaniasis. Acta Tropica. 2021 Sep 1;221:105988.
6. Charlton RL, Rossi-Bergmann B, Denny PW, Steel PG. Repurposing as a strategy for the discovery of new anti-leishmanials: the-state-of-the-art. Parasitology. 2018 Feb;145(2):219–36.
7. Braga SS. Multi-target drugs active against leishmaniasis: A paradigm of drug repurposing. Eur J Med Chem. 2019 Dec 1;183:111660.
8. Jain S, Sahu U, Kumar A, Khare P. Metabolic pathways of Leishmania parasite: Source of pertinent drug targets and potent drug candidates. Pharmaceutics. 2022 Jul 30;14(8):1590.
9. Palić S, Bhairosing P, Beijnen JH, Dorlo TP. Systematic review of host-mediated activity of miltefosine in leishmaniasis through immunomodulation. Antimicrobial Agents and Chemotherapy. 2019 Jul;63(7):10–128.
10. Gupta R, Kushawaha PK, Samant M, Jaiswal AK, Baharia RK, Dube A. Treatment of leishmania donovani-infected hamsters with miltefosine: Analysis of cytokine mRNA expression by real-time PCR, lymphoproliferation, nitrite production and antibody responses. J. Antimicrob Chemother. 2012 Feb 1;67(2):440–3.

