Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

Leishmania Proteomics: an in silico perspective †

Carlos A. Padilla,‡ Maria J. Alvarez,¶ and Aldo F. Combariza*,‡

‡*in silico Molecular Modelling and Computational Simulation Research Group, Sciences and Education School, Biology and Chemistry Department, University of Sucre, Sincelejo, Colombia*

¶*in silico Molecular Modelling and Computational Simulation Research Group, Education and Sciences School, Biology and Chemistry Department, University of Sucre, Sincelejo, Colombia*

E-mail: aldo.combariza@unisucre.edu.co

Abstract

We report on the state of the art of research on proteins recognized as potential targets for the development of *Leishmania* treatments and the search of active chemical species. We have reviewed information from experimental *in vitro*, *in vivo*, or *in silico* sources. We classify the gathered information on: a) vector taxonomy and geographical distribution, b) parasite taxonomy, geographical distribution, c) enzymatic function of proteins related to the parasite/host in any of its development states, *id. est.*, oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases and cytokines, and d) information on standard and non-standard treatments from bioactive chemical species. Our aim is to provide a much needed reference layout for research efforts aimed to understand the interaction mechanisms of ligand-protein activation/inactivation processes, specifically related to *Leishmania*, thus, we focus on enzymes known to be part of the biochemical molecular pathways initiated following a *Leishmania* infectious episode.

†Corresponding email aldo.combariza@unisucre.edu.co

© 2022 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

1 Overview

Leishmaniasis is a tropical and subtropical group of neglected zoonotic diseases, caused for species of the *Leishmania* genus,[1–4] which mainly affects mammalians and it is transmitted through the bite of infected female sandflies.[5,6] Leishmaniasis is classified as a Neglected Tropical Disease (NTD) by the World Health Organization (WHO), due to the lack of financial investment in treatments research and development.[3,7,8] Consequently, around the world, Leishmaniasis disease keeps expanding year after year, for both, its Cutaneous (CL) and Visceral (VL) clinical forms, recognized as the main forms. Another clinical form is the Mucocutaneous (MCL), but the WHO only reports the Cutaneous and Visceral forms.[9]

The most common form is the cutaneous Leishmaniasis (see Figure 1), however, the visceral form is comparatively more dangerous, even fatal, due to its impact on internal organs (spleen, liver, etc.). In the year 2020, Brazil reported the higher number of cases per year for both, the visceral and cutaneous forms, followed by Colombia (https://www.who.int/). Specifically, in the 2020 year Brazil reported 16056 cutaneous and 1954 visceral cases. On the other hand, Colombia reported 6124 cutaneous and 8 visceral cases. Brazil's and Colombia's reports add up to 71% of all Leishmaniasis cases in the New World. However, comparing Leishmaniasis prevalence between these two countries, Colombia's prevalence (0.012%) is larger than Brazilian's (0.0075%), *i.e.*, Brazil reports 7.53 cases cases per 100000 people and Colombia reports 12.00 cases per 100000 people.

Leishmania species are grouped and classified taxonomically in two main divisions called "sections": Euleishmania and Paraleishmania.[10,11] These divisions were proposed by Cupolillo *et al* (2000) from molecular and phylogenetic studies of *Leishmania* species, using the *Endotrypanum* genus as an external phylogenetic group to build a Dendrogram. The phylogenetic results showed the evolutionary separations between some established *Leishmania* species (Nei's genetic distance (D) = 1.04). The molecular techniques used by Cupolillo to compare and verify their results was Multilocus Enzyme Electrophoresis (MLEE), analysis

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

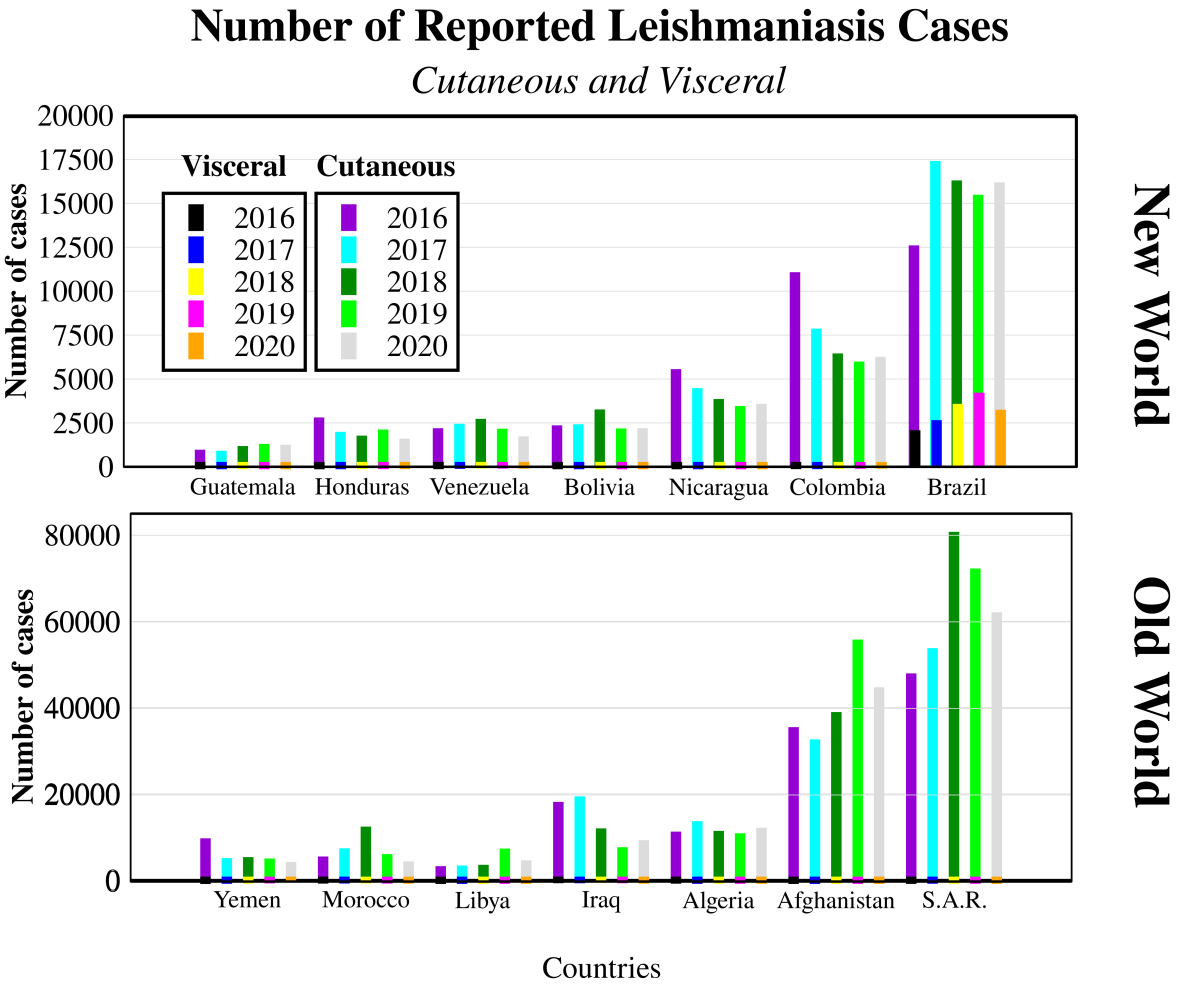

Figure 1: Number of Visceral and Cutaneous Leishmaniasis cases reported by the World Health Organization (WHO). The figure shows the most relevant reports in the world (new and old world).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

of the rRNA gene cluster by Restriction Fragment Length Polymorphism (RFLP) of the intergenic transcribed spacers (ITSrRNA), measurement of sialidase activity and primary DNA sequencing of the small subunit (SSU) rRNA gene.[2,10,12]

From Cupolillo and coworkers, more species were included in the two proposed divisions. Currently, Euleishmania groups the following subgenus: *L. (Viannia)*, *L. (Leishmania)* and *L. (Sauroleishmania)* (Fig. 3).[10,13–15] In turn, Paraleishmania groups the *L. (Endotrypanum)* subgenus, containing the *E. schaudinni* and *E. monterogeii* species (See Fig. 3).[16–19] Specifically for Colombia, we have found reports of *L. amazonensis*, *L. braziliensis*, *L. mexicana*, *L. colombiensis*, *L. guyanensis*, *L. panamensis*, *L. chagasi*, *L. lainsoni* and *L. equatoriensis* as transmission parasites of leishmaniasis.[20,21]

Nowadays, standard active compounds against *Leishmania* parasites are Amphotericin B, Miltefosine, Pentamidine, Antimonials, Paromomycin, Sitamaquine, Pamidronate, Azoles, and Nucleoside analogues. However, these drugs are inefficient and extremely toxic for patients undergoing clinical treatments. These factors, inefficiency and toxicity, depend on parameters such as the immunological state of the infected host, or specifically on the drug pharmacokinetic features. For instance, drugs based on Miltefosine are teratogenic and some treatments based on antimonials generate secondary effects like arthralgia, nausea, abdominal pain, pancreatitis and cardiotoxicity.[22–24] Experimental development and efficiency testing of potential antileishmanial compounds is proven to be a hard and expensive task with low successful rate. The development of new drugs, is no doubt, a complex business. As alternative, computational approaches such as Molecular Dynamics and Molecular Docking are better suited to study new potential antileishmanial active compounds and protein targets. These approaches allow the analysis of a myriad of ligand-protein pairs with relatively low computational cost and closed-up atomistic/molecular description compared with experimental assays.

The development of new drugs based on alternative chemical sources, such as secondary metabolites and peptides extracted from plants and animal species, could represent the basis

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

for more efficient and more beneficial treatments compared to the traditional ones. Our aim is to review general aspects of the *Leishmania* infectious disease in terms of bioactive chemical compounds (standard and alternative) used for therapeutic treatment and the proteins involved on the steps of the *Leishmania* parasitization process.

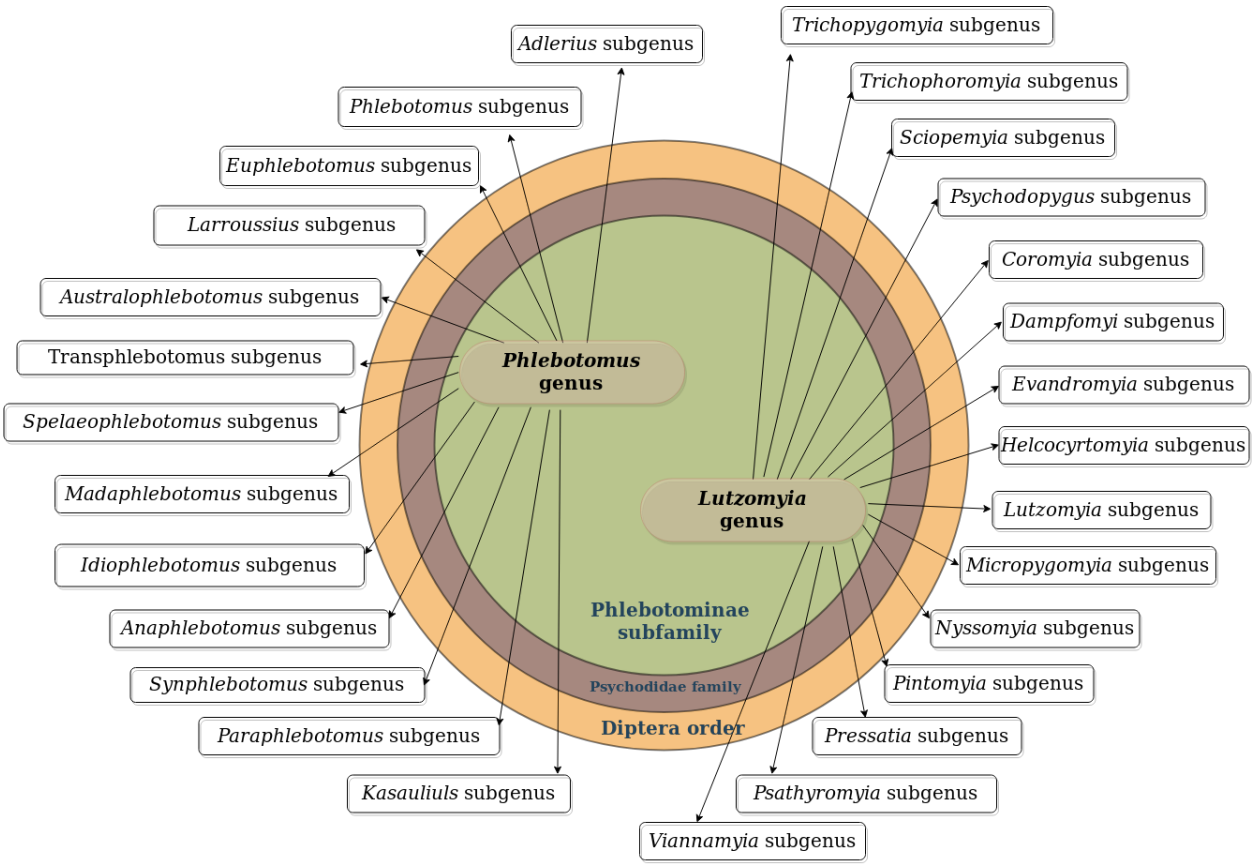

Figure 2: Taxonomic distribution of *Leishmania* vector: Diptera order and Psychodidae family, phlebotomiae subfamily. Gographical distribution: *Phlebotomus* genus for the old world and *Lutzomyia* genus for the new world.[2,17]

2 *Leishmania* vectors

Sandflies are vectors of several dangerous parasites, such as *Leishmania*, *Bartonella bacilliformis* bacteria and some viruses, which can even end up with the death of the host.[25,25–27] Sandflies belong to the Diptera order, Psychodidae family, Phlebotominae subfamily. There are approximately 900 recognized species of sanflies, taxonomically divided in five genus:

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

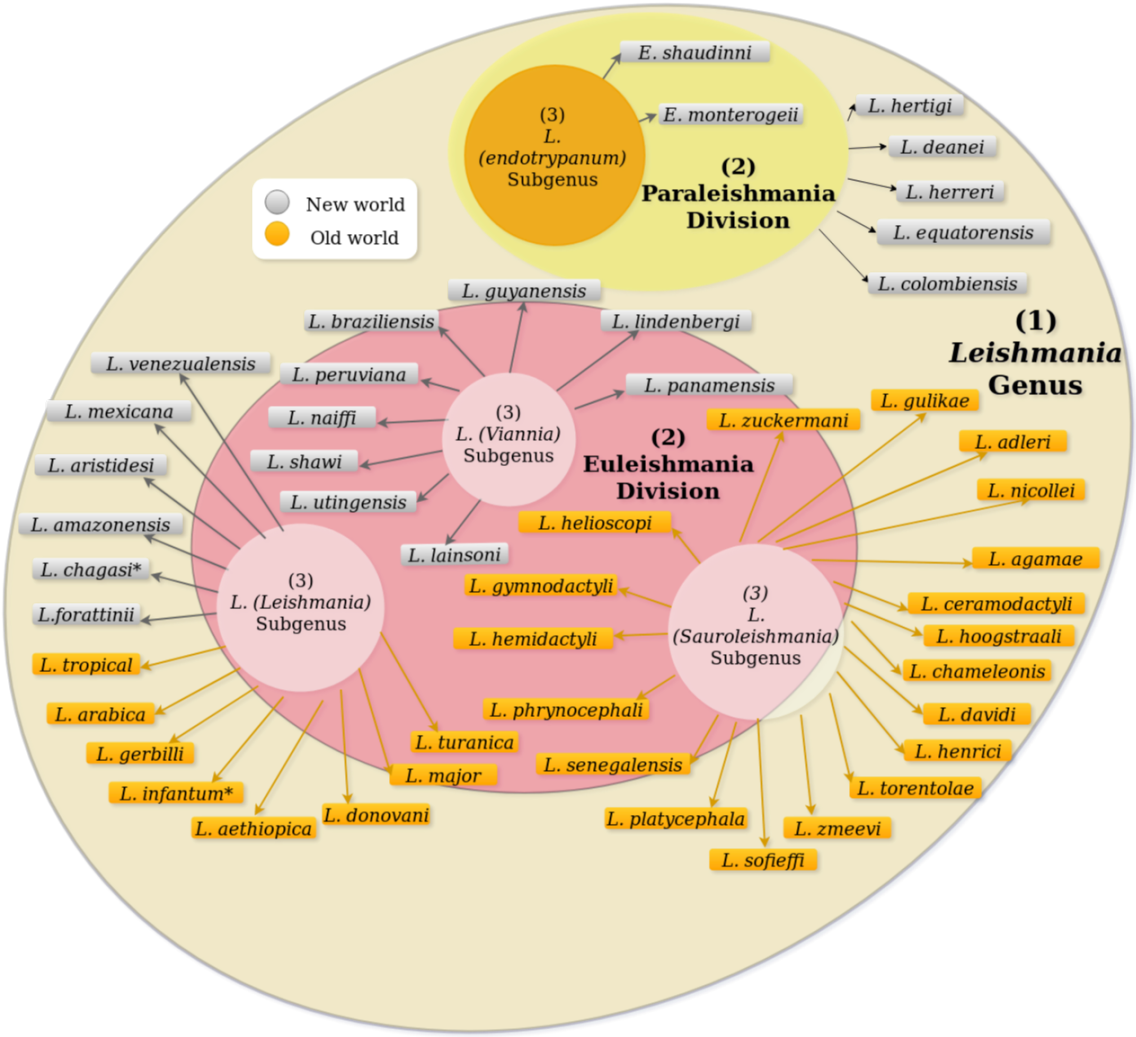

Figure 3: *Leishmania* taxonomic classification and geographical distribution. *Leishmania* genus: euleishmania and paraleishmania. Paraleishmania is conformed by *L. (endotrypanum)* subgenus and euleishmania by *L. (viannia)*, *L. (leishmania)* and *L. (sauroleishmania)* subgenus.[10,13–15]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

Phlebotomus and *Sergentomyia*, which are prevalent in the old world and *Lutzomyia*, *Brumptomyia* and *Warileya* prevalent in the new world.[28–30] *Phlebotomus* and *Lutzomyia* genus are responsible for the transmission of the *Leishmania* parasites.[28] (See Fig. 2).

Nine species of the *Leishmania* genus and fourteen species belonging to the *Lutzomyia* genus have been reported for Colombia.[21,31] Parasite species reported for Colombia are *L. amazonensis*, *L. braziliensis*, *L. mexicana*, *L. colombiensis*, *L. guyanensis L. panamensis*, *L. infantum*, *L. lainsoni* and *L. equatoriensis* (ver Fig. 3). *Lutzomyia* species Colombia reported are *L. flaviscutellata*, *L. colombiana*, *L. spinicrassa*, *L. pia*, *L. towsendi*, *L. hartmanni*, *L. umbratilis*, *L. longiflocosa*, *L. trapidoi*, *L. panamensis*, *L. yuli yuli*, *L. cruciata*, *L. columbiana* and *L. gomezi.*[31]

3 *Leishmania* life cycle and host immune response

Leishmania parasites invade, develop and replicate inside the host Mononuclear Phagocyte System (MPS), attacking macrophages and dendritic cells.[32–35] Incubation time of *Leishmania* parasite from promastigote to amastigote takes between two and three months, time range when the host immune system response activates and leads to an favorable or unfavorable outcome.[33] The parasite cycle begins with the bite of an infected female sandfly, carrier of promastigote *Leishmania* parasites in a meta-cyclic state (see Fig. 4).[36,37] At this stage, neutrophils and macrophages are the first line activated by the immune system, being neutrophils the initiators of the inflammatory response.[35,37,38]

The *Leishmania* parasite has two ways to enter the macrophage: a direct path, via the macrophage, and an indirect path, by attacking the neutrophils.[39] The direct path, occurs when the promastigote is directly endocited by the macrophage phagosome, or parasitophorous vacuole, which undergoes a biochemical transformation into phagolysosome.[40] The indirect path goes through the neutrophil mediated phagocytosis of the parasite, followed by a subsequent macrophage phagocytosis step (see Fig. 4).[39] The promastigote

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

parasite state survives inside the phagolysosome vacuole by producing Lipophosphoglycan (LPG), gp63 protein and glutathione transferase.[36,41]

Following the initial macrophage attack, the biochemical response of the host immune system focus on the production of cytokines by specialized T_H $CD4^+$ cells, either T_H1 or T_H2. Among the T_H1 secreted cytokines we found gamma interferon (IFN-γ), which activates and stimulates the macrophages, increasing its microbiocide activity. Interleucine (IL)-12 and IL-2, cytokines also secreted by T_H1 cells, help T_H $CD4^+$ transformation into T_H1, which is the more suitable form to respond to the exogenous attack, in our case, the metacyclic promastigote form of *Leishmania*.[42,43]

The immune T_H2 response produces IL-4, IL-5, IL-10, the Transforming Growth Factor Beta (TGF-β) and other cytokines. These compound are perfectly suited for facing allergenic diseases or helmintic infections, therefore, is more desirable the T_H1 response. Moreover, the T_H2 response inhibits the T_H1, favoring the propagation and survival of the *Leishmania* parasite.[42,44]

4 Leishmania Proteomics and Metabolomics Analysis

We have made a deep review into databases and reported scientific literature about *Leishmania* metabolic pathways. From the retrieved information, pathways and proteins involved were analyzed, arranged and correlated as shown in figure (see Fig. 5). All schematized proteins in the figure 5 are involved in vital metabolic pathways (glycolysis, PPP, citric acid cycle, among others) and play essential roles into the host infection and immune evasion processes, being critical for *Leishmania* survival.[45] Some of these proteins, perform one or more functions in different metabolic pathways, for instance, Arginase participates in arginine biosynthesis, proline and arginine metabolism and secondary metabolite and antibiotic biosynthesis pathways.[46] On the other hand, the proteins 1E92, 2XSE, 3VIA, 4F2N, 4F40, 4UCM, 2XE4, 3S4O, 5NTH, 4S1E, 3P0I, 3KFL y 5ZWS do not appear in the reviewed

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

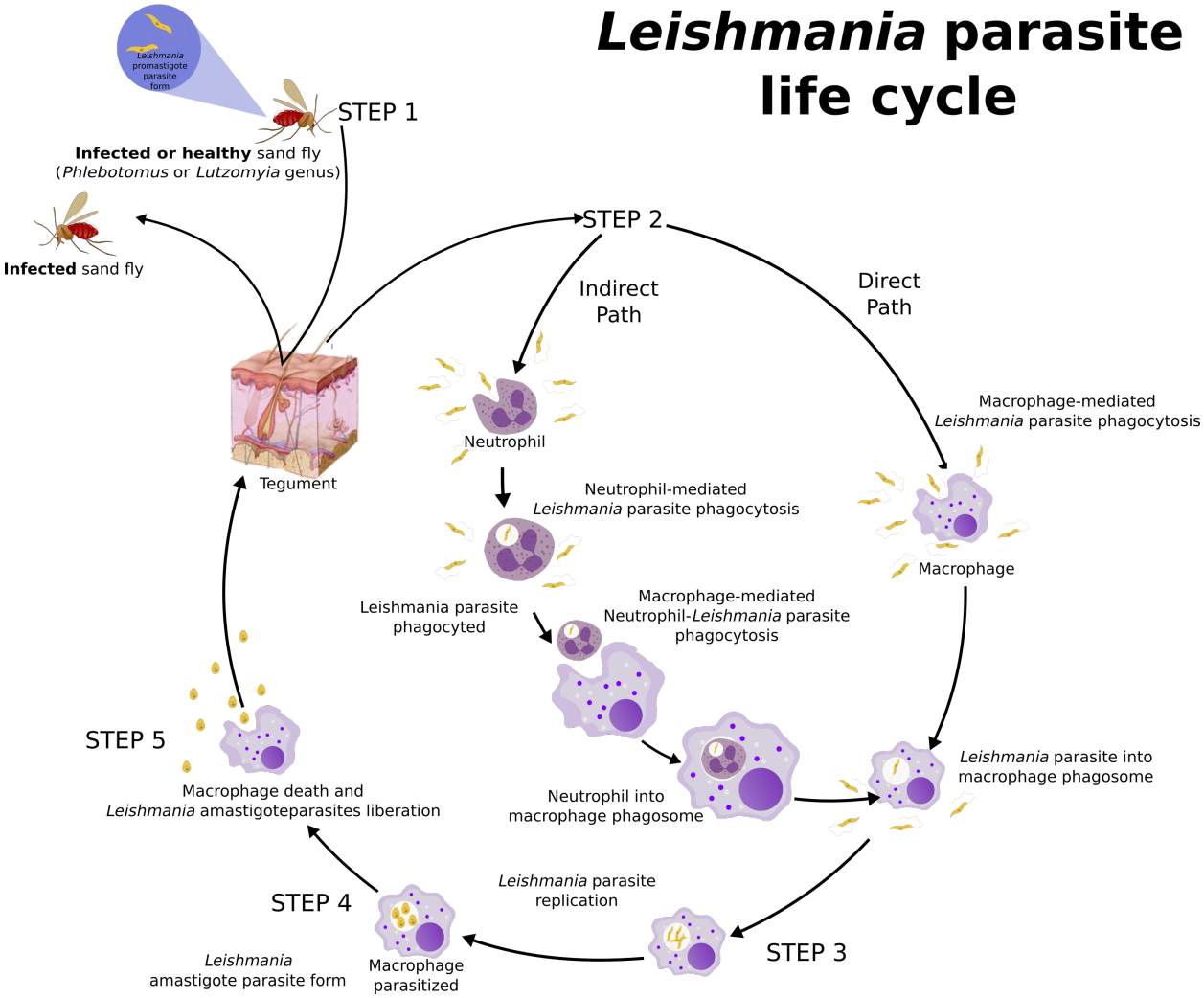

Figure 4: *Leishmania* life cycle can be divided in five steps. **Step 1**: Virulent metacyclic promastigotes are egested when a female sandfly carrier bites a possible host. **Step 2**: Here there are two possible paths. Direct path: promastigotes are phagocytized by macrophage cells. Indirect path: promastigotes are phagocytized by neutrophil cells, subsequently, neutrophils are phagocytized by macrophage. At the end of step 2, promastigotes end up inside a macrophage phagolysosome. **Step 3**: The promastigote parasite produces glutathione transferase, a protein that protects it from the acidic conditions of the phagolysosome, then initiating the replication process . **Step 4**: Transformation of vector promastigote into amastigote takes place. *Leishmania* amastigotes are experts at exploiting host cell machinery to thrive. **Step 5**: Amastigotes are then taken out when a sand fly, either infected or not, bites the host, closing the cycle.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

metabolic databases, hence these are not included into any specific path in figure 5. Nevertheless, the functions of these proteins were reviewed and analyzed in this paper. Finally, the proteins 3B64, 1LML and 4P4M are structural macromolecules and play different roles into *Leishmania* parasite, being excluded of a specific metabolic pathway too. It is important to highlight that they are considered for structural analysis.

Figure 6 shows protein-pathway correlations as well, highlighting protein structures and schematizing the proteins not presented in figure 5. In this figure, proteins not associated to any path, are highlighted with the caption "Not included in pathway", And their EC functions are discriminated by colors, then, proteins with two or more colors have two or more EC functions.

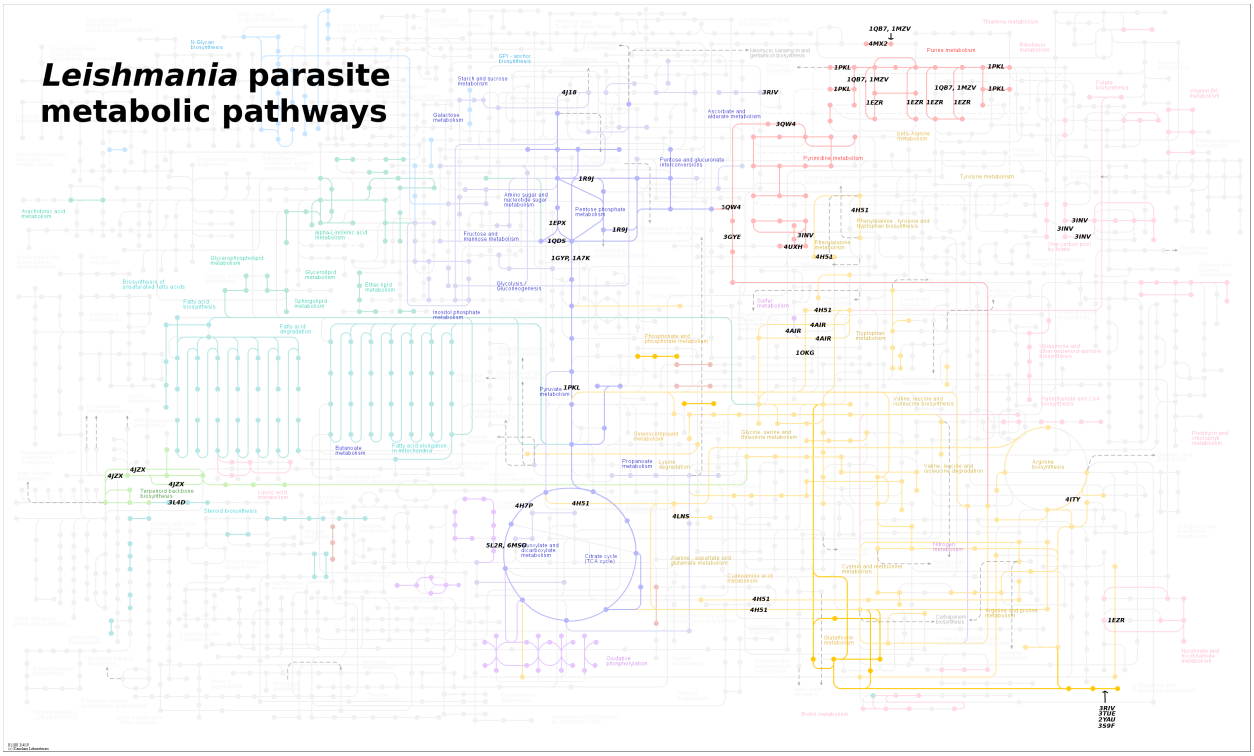

Figure 5: This figure shows *Leishmania* metabolic pathways related with retrieved proteins from databases. The PDB IDs 1E92, 2XSE, 3VIA, 4F2N, 4F40, 4UCM, 2XE4, 3S4O, 5NTH, 4S1E, 3P0I, 3KFL and 5ZWS, do not appear in metabolic pathway databases

Crystal structures presented here were retrieved from the Protein Data Bank (PDB).[47] The PDB search process was carried using the keywords "*Leishmaniasis*" and "*Leishma-*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

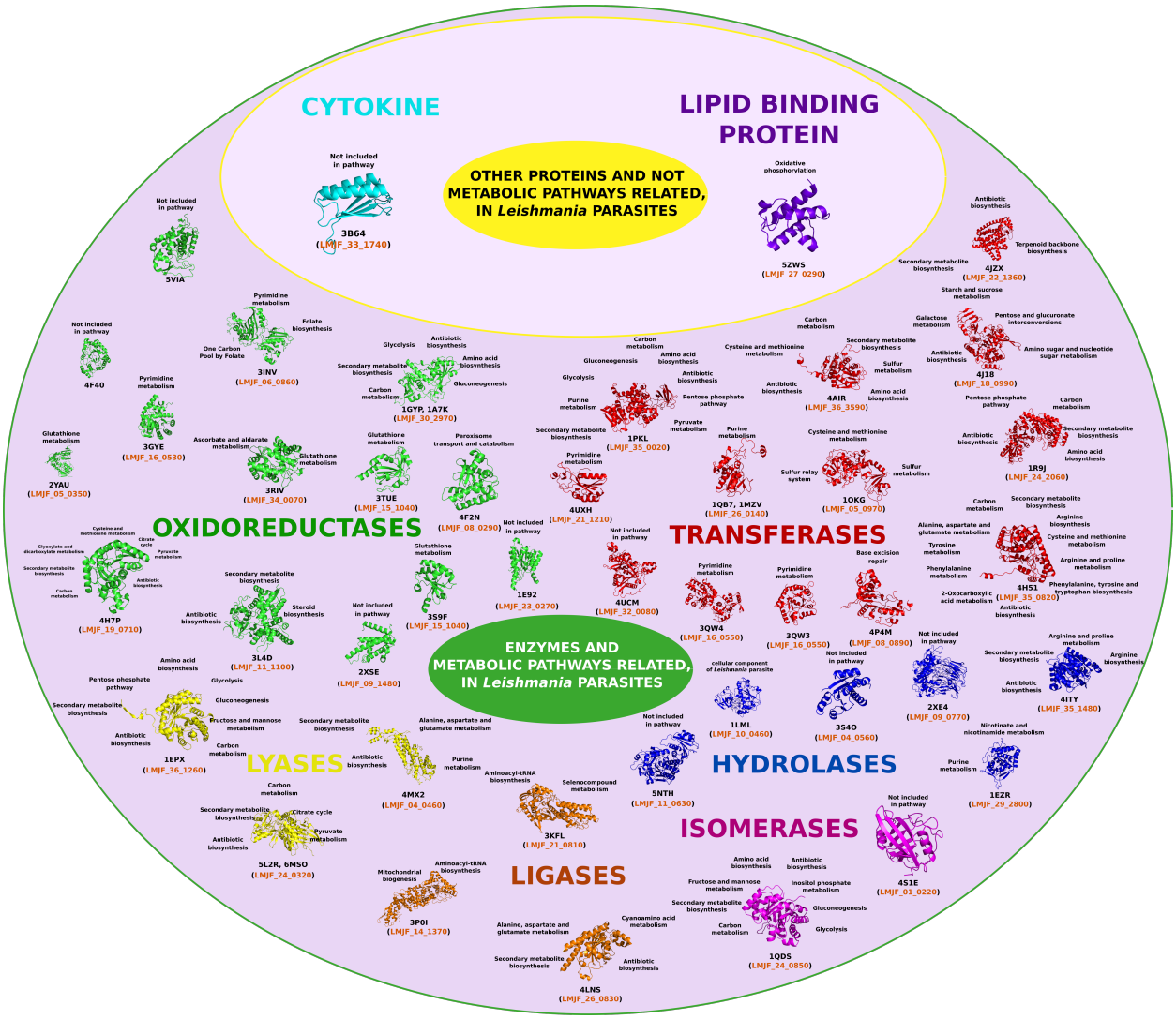

Figure 6: Proteins linked with specific *Leishmania* metabolic pathways and proteins with no metabolic pathway association.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

nia", providing 425 items until 2021, after which we classified the selected proteins according to the parasitization cycle. Other selection criteria were: proteins with different PDB-codes but same structure and proteins with equal structures but elucidated from different organisms. An example of proteins that belong to different groups are: Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS) (PDB ID: 3INV), which is an oxidoreductase and a transferase. A total of 49 proteins comprised the final study group (see Fig. 7). From the selected proteins, the richest group belongs to oxidoreductases (Enzyme Commission Number - EC 1) and transferases (EC 2), with 15 subjects each one, 6 hydrolases (EC 3), 5 lyases (EC 4), 3 isomerases (EC 5), 3 ligases (EC 6), one cytokine and one Lipid Binding Protein (see Fig. 7).

4.1 Oxidoreductases (EC 1)

This group is composed by oxidation-reduction enzymes that catalyse reactions in which a substrate donates one or more electrons to an electron withdrawing species, becoming oxidized in the process.[48] The Glycolysis and Pentose Phosphate Pathways (PPP) are key paths of cellular metabolism in Trypanosomatids and, in turn, are dependent of several oxidoreductase enzymes.[49] In these processes mentioned above, glucose and other hexoses are critical cellular nutrients for *Leishmania* parasites, and also, these parasites (either in its promastigote or amastigote form) are able to extract sugar from extracellular environment or synthesize it *de novo*, via gluconeogenesis.[50] The promastigote parasites are capable of perform both processes, but the amastigote parasites only carry out the gluconeogenetic pathway.[50]

Glyceraldehyde 3-phosphate dehydrogenase *Leishmania (major) mexicana* (PDB ID: 1GYP and 1A7K, see Fig. 7) belongs to the oxidoreductases group and takes part of the Pentose Phosphate and glycolysis metabolic pathways.[49] These are key glycolytic homotetrameric enzymes of 156 kDa that catalyzes the conversion of glyceraldehyde 3-phosphate (G3P) into 1,3-biphosphoglycerate (1,3-BPG) with reduction of NAD^+ to NADH, through

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

the NAD^+ cofactor.[51,52] Malate dehydrogenase (PDB ID: 4H7P) participates in the gluconeogenic process by conversion of oxaloacetate (OAA) and malate, using the NAD/NADH coenzyme system.[53,54] 4H7P have two isoforms in eukaryotes, differing in their subcellular localization and their specifity for the coenzyme NAD (all types of malate dehydrogenases) or NADP (only malate dehydrogenases from chloroplast cells).[54]

Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS) (PDB ID: 3INV) and Pteridine reductase (PTR1) (PDB ID: 1E92) elucidated from *L. major* and *T. cruzi*, respectively, are responsible of protein salvaging in parasites belonging to the Trypanosomatidae family. These salvage pathways are need for normal metabolic processes in *Leishmania* parasites, because theses microorganism are auxotrophics for folate compounds, which are required in critical *Leishmania* metabolic pathways, including nucleic acid and protein biosynthesis.[55,56]

The DHFR enzyme structure diverges largely between hosts and parasites, which has allowed the synthesis of several specific DHFR inhibitors known as antifolates.[57] Dihydroorotate dehydrogenase (DHODH) (PDB ID: 3GYE) is a flavoprotein enzyme involved in the *de novo* pyrimidine biosynthesis pathway.[58,59] The pyrimidine biosynthesis pathway in *Leishmania* parasites is important for DNA and RNA biosynthesis, protein glycosylation, membrane lipid biosynthesis and strand break repair.[58] The DHODH enzymes are divided in the major class 1 (A and B) and 2, with this division correlating with the subcellular location of the protein.[60,61] The class 1 proteins have cytosol subcellular location and are found in Gram-positive bacteria, in the anaerobic yeast *Saccharomyces cerevisiae* and in all trypanosomatids species.[58,61] The 3GYE protein belongs to the class 1, and catalyzes the (S)-dihydroorotate oxidation to orotate in a redox reaction.[61]

Eukaryotic unicellular kinetoplastid flagellates, such as *Trypanosoma* and *Leishmania* species, contain a unique hypermodified base in their nuclear DNA, called J base or β-D-glucosylhydroxymethyl-uracil.[62–64] J-base is associated with the silencing of telomeric expression sites for the variant surface glycoprotein (VSG) genes, but not in actively transcribed VSG genes.[62,63] Currently, it is known that the JBP1 protein is required for J-Base biosyn-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

thesis and maintenance, also, this enzyme is indispensable for *Leishmania* parasites growth and survival.[65,66] Specifically, JBP1 have the DNA-Binding JBP1 domain (DB-JBP1) (PDB ID: 2XSE) that binds to J-DNA making it a potential drug target.[65,67]

Macrophages defense mechanism against *Leishmania* parasites produce peroxynitrite, hydroxyl radicals, hydrogen peroxide, hydroperoxide, superoxide radicals species, among other.[68–70] These compounds are toxic to *Leishmania* parasite metabolism and affect its survival, but these parasites have a trypanothione mediated hydroperoxide metabolism to eliminate endogenous or exogenous oxidative agents.[71] Tryparedoxin (PDB ID: 3S9F) and Tryparedoxin peroxidase I (PDB ID: 3TUE) (TXN/TXNPx) reduce macrophages-hydroperoxides generated species to water.[72] These proteins stay in a cytosolic form and act on the detoxification pathway, an essential process for parasite survival.[72] Pseudoperoxidase *L. major* (*Lm*PP) (PDB ID: 5VIA) is a detoxify heme protein expressed by *Leishmania* parasites against Reactive Nitrogen Species (RNS).[73,74] Heme peroxidases use peroxides to oxidize a variety of physiologically important molecules, for example, ascorbate peroxidase (APX) (PDB ID: 3RIV), which is considered a potential drug target.[73]

Superoxide dismutase enzyme (FeSODA) (PDB ID: 4F2N) protects the *Leishmania* parasite against macrophage toxic radicals. When the amastigote is phagocytized, macrophage cells produce a respiratory burst generating Reactive Oxygen Species (ROS) intermediates, such as hydrogen peroxide, OH^-, $O_2{}^-$ radicals and peroxynitrite, as part of the macrophage mechanism to fight invasive microorganisms.[75–77] Thus, 4F2N acts as the first line of defense against those ROS. This fact, makes 2F2N a suitable enzymatic target for *Leishmania* drug development.[75] Tryopanothione reductase (TR) (PDB ID: 2YAU) is homodimeric enzyme extracted from *Leishmania infantum* and it is essential for parasite survival.[78] This enzyme catalyses the reduction of trypanothione by NADPH, protecting the parasite against oxidative damage and toxic heavy metals.[78–80] TR reduces trypanothione disulfide (TS_2), a bis (γ-L-glutamyl-L-cysteinylglycine) spermidine or bis (glutathionyl) spermidine conjugate, to the di-thiol form [$T(SH)_2$].[79] The enzyme 9,11-endoperoxide prostaglandin H2 reductase

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

from *L. major* (PDB ID: 4F40) is involved in the lipid metabolic pathway, acting through a NADP cofactor.[81,82] Sterol 14α-demethilase (CYP51) *L. infantum* (PDB ID: 3L4D) is a enzyme that catalyzes the removal of the 14α-methyl group from sterol precursors. This reaction is essential for membrane cell biosynthesis, specifically, CYP51 relates to the ergosterol pathway, and it is believed to be decisive for the survival of *Leishmania* parasite.[83]

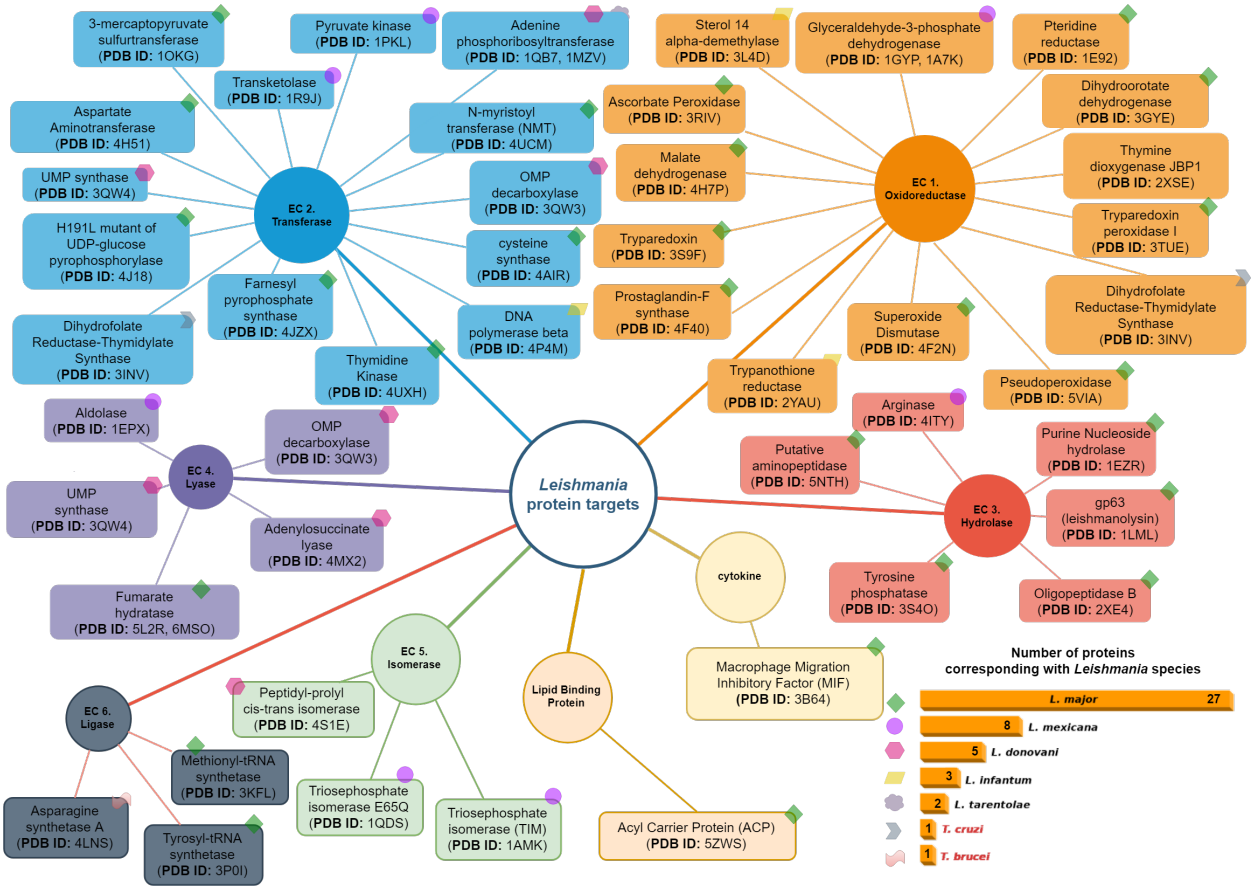

Figure 7: *Leishmania* protein classification by EC group. Oxidoreductases (orange), transferases (blue), hydrolases (red), lyases (violet), isomerases (green), ligases (dark blue), cytokines (yellow) and Lipid Binding protein (light orange). The chart at the bottom right of the figure shows the number of proteins found for each species. *T. cruzi* and *T. brucei* do not belong at the *Leishmania* protein group, but they have been used in some studies as homologous proteins.[56,84]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

4.2 Transferases group (EC. 2)

A transferase catalyzes reactions in which a chemical group is transferred from a electron/proton donor substrate to an electron/proton withdrawing substrate.[48] These proteins catalyze key cellular processes in all kingdoms of life, such as, DNA repair, RNA editing, and activation of metabolites used in catabolic pathways.[85] Purine nucleotide salvage process by *Leishmania* is important to carry parasite viability and growth, due to these parasites are strict purine nucleotide auxotrophs.[86,87] Therefore, these parasites have protein arsenal and some molecular mechanism to purine nucleotide acquisition.[87,88] Adenine phosphoribosyltransferase (APRT) (PDB ID: 1QB7 and 1MZV) belongs to the phosphoribosyltransferase family type I (PRTs)[89] and is involved in purine-salvaging process, catalyzing adenines to adenosine-5-monophosphate (AMP) compound.[87,88] The two most common forms of protein fatty acylation are modification with myristate molecule and other.[90] Modifications with myristate, know as myristoylation, have been implicated in targeting protein to membrane locations, stabilizing protein structures, mediating protein-protein interactions and substrate activation.[90,91] Proteins that are destined to become myristoylated begin its primary sequence with the Methionine-Glycine (Met-Gly) sequence group, where Met- amino acid is removed by methionine amino-peptidase protein and myristate molecule is linked via an amide bond.[90] N-myristoyltransferase (NMT) protein (PDB ID: 4UCM) catalyzes the co-translational transfer of myristic acid (myristate) from myristoyl-CoA to the N-terminal glycine.[91,92] It is important by *Leishmania* parasites and a potential drug target.[93]

The *de novo* pyrimidine biosynthesis pathway involves six enzymatic steps carried to the synthesis of Uridine 5'-monophosphate (UMP), and the final two enzymatic steps are mediated by Orotate Phosphoribosyltransferase (OPRT) and Orotidine 5'-monophosphate decarboxylase (OMPDC) enzymes.[59,94] These two enzyme are expressed as a bifunctional protein, know as UMP synthase.[59] PDB-database have bifunctional UMP synthase protein (PDB ID: 3QW4) and OMP decarboxylase single protein (PDB ID: 3QW3) crystal struc-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

tures. Trypanothione compound play an important role in maintaining intracelullar redox homoeostasis and providing defence against oxidative stress in *Leishmania* parasites.[95–97] The Trypanothione biosynthesis process, indirectly depend on the availability of cysteine and cysteine *de novo* biosynthesis pathway depend of Serine Acetyltyltransferase and Cysteine Synthase (PDB ID: 4AIR) proteins.[95,98] Amastigote *Leishmania* parasites survive and proliferate within phagolysosome vacuole, under extreme acid environment conditions and several toxic compounds.[99] These extreme host cell conditions cause high levels of DNA damage to parasite.[100] Therefore, DNA Polymerase Beta (PDB ID: 4P4M) is essential to *Leishmania* parasites for maintenance, replication and recombination of DNA.[100] This protein is specially required to amastigote parasite forms.[100]

Process to obtain pyrimidine and purine nucleotides in *Leishmania* parasites, may be through *de novo* biosynthesis and/or salvage pathways[101] *Leishmania* species have both pyrimidine *de novo* biosynthesis and salvage pathways, but are incapable of synthesising purines *de novo*.[101] Thymidine kinase (TK) (PDB ID: 4UXH) is an essential enzyme that initialize the pyrimidine salvage process.[102] This protein catalyzes ATP γ-phosphate transfer to 2'-deoxythymidine (dThd), forming thymidine monophosphate (dTMP). It is a important enzyme because plays a key role in parasitization process.[103] Farnesyl pyrophosphate synthase (FPPS) (PDB ID: 4JZX) is involved in ergosterol synthesis, acting in the early steps of isoprene synthesis and maintainance of lipid bilayer integrity.[104,105] It is a potential enzymatic target, because, it was successful inhibited with bisphosphonate previously.[104] UDP-glucose pyrophosphorylase *L. major* (UGP) (PDB ID: 4J18) catalyzes the reversible conversion of glucose-1-phosphate (Glc-1-P) and uridine 5'-triphosphate (UTP) to UDP-Glc and inorganic pyrophosphate (PPi) in the presence of Mg^{2+}, as part of the glycolytic pathway.[85]

Aspartate aminotransferase (AAT) (PDB ID: 4H51) catalyzes the reversible transfer of the α-amino group of aspartate and glutamate, converting L-aspartate and 2-oxoglutamate to oxaloacetate and L-glutamate (http://brenda-enzymes.info).[106] AAT action depends on the pyridoxal phosphate (PLP) cofactor and therefore, represents a potential drug target.[107]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

Sulfurtransferases are a family enzyme widely distributed on prokaryotes and eukaryotes organism, and the 3-mercaptopyruvate sulfurtransferase (PDB ID: 1OKG) belongs to this family and is involved in cysteine metabolism, polarizing the carboxyl group of 3-mercaptopyruvate through a tiophilic attack.[108,109] Transketolase (PDB ID: 1R9J) is a key enzyme to the nonoxidative branch of the PP pathway, which transfers two-carbon glycolaldehyde units from ketose-donors to aldose-acceptor sugars.[110] Finally, pyruvate kinase (PDB ID: 1PKL) catalyzes the phosphoenolpyruvate-phosphate group transfer to adenosine diphosphate. This enzyme is involved in the glycolytic pathway.[111]

4.3 Hydrolases group (EC. 3)

Hydrolases catalyze reactions in which a bond in any suitable substrate is hydrolyzed to produce two fragments.[48] Due to the *Leishmania* parasite are unable to make *de novo* biosynthesis process of purines, the Purine Nucleoside Hydrolase *L. major* enzyme (PDB ID: 1EZR) is the main responsible of nucleotide salvaging from the host.[112] Therefore, 1EZR is a potential drug target. The *Leishmania* parasite promastigote expresses glycoproteins on its surface, and one of these enzymes expressed is known as Leishmanolysin (*gp63* gene) (PDB ID: 1LML).[113] 1LML protein play an important role in the macrophage infection process, therefore, this enzyme is a potential drug target.[113] Peptidase proteins family play key roles in metabolic pathways, host invasion and parasite immune evasion to most parasites.[114] Oligopeptidase B (OPB) *L. major* (PDB ID: 2XE4) protein belongs to peptidase family, and some studies citated by McLuskey,[115] say that 2XE4 protein is an important virulence factor.[115,116] Another peptidase enzyme is Leucyl aminopeptidase (LAP) *L. major* (PDB ID: 5NTH), which is involved in N-terminus catalysis of proteins.[117]

Tyrosine phosphatase (PRL-1) (PDB ID: 3S4O) is mainly secreted by the promastigote *Leishmania* form, however, the amastigote form produces PRL-1 more efficiently and abundantly during the macrophage infection process, which is important for the parasite survival.[118] Finally, the Arginase protein (PDB ID: 4ITY) catalyzes the first step of polyamine

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

biosynthesis. This process makes part of the cellular growth process, and its of paramount importance for parasite survival.[119]

4.4 Lyase group (EC. 4)

Lyase enzymes are a group of enzymes that catalyzes non-hydrolytic reactions, in which a chemical group is cleaved and removed from any suitable substrate, leaving to create a double bond or a ring structure.[48] Adenylosuccinate lyase (ASL) (PDB ID: 4MX2) is a lyase protein, and have been identified as vital component of purine salvaging in *Leishmania donovani*.[87] Fumarate hydrolase (FH) class 1 enzyme is a protein with two isoforms: a mitochondrial (PDB ID: 6MSO) and a cytosolic ((PDB ID: 5L2R)). 5L2R produces fumarate substrate for the dihydroorotate dehydrogenase. Additionally, this enzyme migrates to the cellular nucleus, playing a key rol in DNA repair processes.[120] 6MSO catalyzes the stereospecific reversible conversion of fumarate to S-malate. This reaction is part of the tricarboxylic acid (TCA) cycle, takes part of the succinic fermentation pathway, participates in DNA repair processes and is proposed to provide fumarate for the *de novo* pyrimidine biosynthetic pathway.[121] Finally, aldolase *L. mexicana* (PDB ID: 1EPX) enzyme, is involved in the glycolytic pathway and catalyzes the Fructose-1,6-bisphosphate conversion to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.[122]

4.5 Isomerases group (EC. 5)

Isomerase enzymes catalyze one-substrate/one-product reactions that can be regarded as isomerization reactions.[48] Triosephosphate isomerase (TIM) (PDB ID: 1AMK) plays a preponderant role in the glycolysis process as catalyst of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (GAP).[123] A TIM E65Q mutant (PDB ID: 1QDS) has been studied and is regarded as more stable than TIM wild-type.[124] Peptidyl-prolyl cis-trans isomerase (PDB ID: 4S1E) accelerates the folding process of proteins. 4S1E catalyzes the cis-trans isomerization of proline imidic peptide bonds in oligopeptides `https:`

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

//www.uniprot.org/uniprot/Q9U9R3.[125]

4.6 Ligases group (EC. 6)

Ligase enzymes catalyze bond formation between two or more macromolecules, it process usually are associated with hydrolysis of a small chemical molecule coupled to the macromolecules.[48] For this review, ligase group has associated to three proteins recognized as potential enzymatic targets: Methionyl-tRNA synthetase (PDB ID: 3KFL)and Tyrosyl-tRNA synthetase (PDB ID: 3P0I), with structural parameters elucidated with MgATP as substrate and methionine as solvent and recognized as essential for biological processes such as gene translation.[126,127] The third enzyme is Asparagine synthetase A (ASNA) (PDB ID: 4LNS), which is an ammonium and glutamine dependent enzyme. In experimental (*in vivo* and *in vitro*) studies, 4NLS protein was recognized as causing of growth delay in parasite and it was catalogued as a potential drug target for *Leishmania* bioactive principles development.[84]

4.7 Cytokines group

The Migration Inhibitory Factor (MIF) from *L. major* (PDB ID: 3B64) has been also recognized as a possible drug development target. This cytokine is an ortholog of human MIF, also known as Lm1740MIF. 3B64 interacts with MIF receptors, such as HLA class II histocompatibility antigen gamma chain (also called invariant chain or CD74) and exhibits an antiapoptotic activity that may facilitate the intracellular persistence of *Leishmania* into macrophages.[128]

4.8 Lipid Binding Protein group

Apo-Acyl Carrier Protein (PDB ID: 5ZWS) plays an important role in the synthesis of fatty acids, non-ribosomal polypeptides and polyketides. The fatty acid pathway, and their more complex forms, recently gained attention in *Leishmania* researches, because it plays a role

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

in protozoan parasites survival inside the host.[129]

5 Leishmaniasis drugs

Pentavalent antimonials (Sb(V)) were the first developed *Leishmania* control bioactive chemical species, however, development of *Leishmania* resistance rendered the Pentavalent Antimmonials highly inefficient.[2] From this critical point, the development of antileishmanial compounds was on the rise and nowadays, standard drugs are based on active compounds such as Amphotericin B, Pentamidine, Miltefosine, Paromomycin, Sitamaquine, etc., but these are inefficient too. Treatments based on these drugs are expensive and generate resistance for *Leishmania* parasites.[2,9,24]

5.1 Standard drugs

Standard Leishmaniasis treatment are based on the following types of chemicals: antimonials (Sb(V)), amphotericin B, Pentamidine, Miltefosine (hexadecylphosphocholine), paromomycin (aminosidine), sitamaquine and pamidronate (see Fig. 8).[22,23] These drugs are used for the treatment of either CL, MCL or VL.[9]

Antimonials were the first antileishmania compounds introduced in the 40s decade and available as meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam).[2,23] These are standard first line drugs for treatment, but emergence of resistance has limited their use.[24,130] Antimonials are used for VL treatment, but, different studies found that *L. donovani* and *L. braziliensis* are more sensitive to sodium stibogluconate than *L. major*, *Leishmania tropica* and *L. mexicana*.[2,22] Amphotericin B is a macrolide antibiotic isolated from *Streptomyces nodosus* in 1956 and widely used since the 80s as amphotericin B deoxycholate.[2,23] It selectively inhibits the membrane synthesis of the parasite and causes holes in the membrane, leading to parasite death.[23] It is used as a second-line treatment, and is present in five formulations: amphotericin B deoxycholate (Fungizone), Emulsification of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

Fungizone in intralipid 20 %, liposomal amphotericin B (AmBisome), amphotericin B lipid complex (ABLC; Abelcet) and amphotericin B cholesterol dispersion (ABCD; Amphotec) (see Fig. 8).[22,23] Amphotericin B and its lipid formulations are used as alternative chemotherapeutic treatments.[130] Lipid formulations of amphotericin B have gained more importance, becoming the established leishmaniasis treatment by the US Food and Drug Administration (FDA).[2]

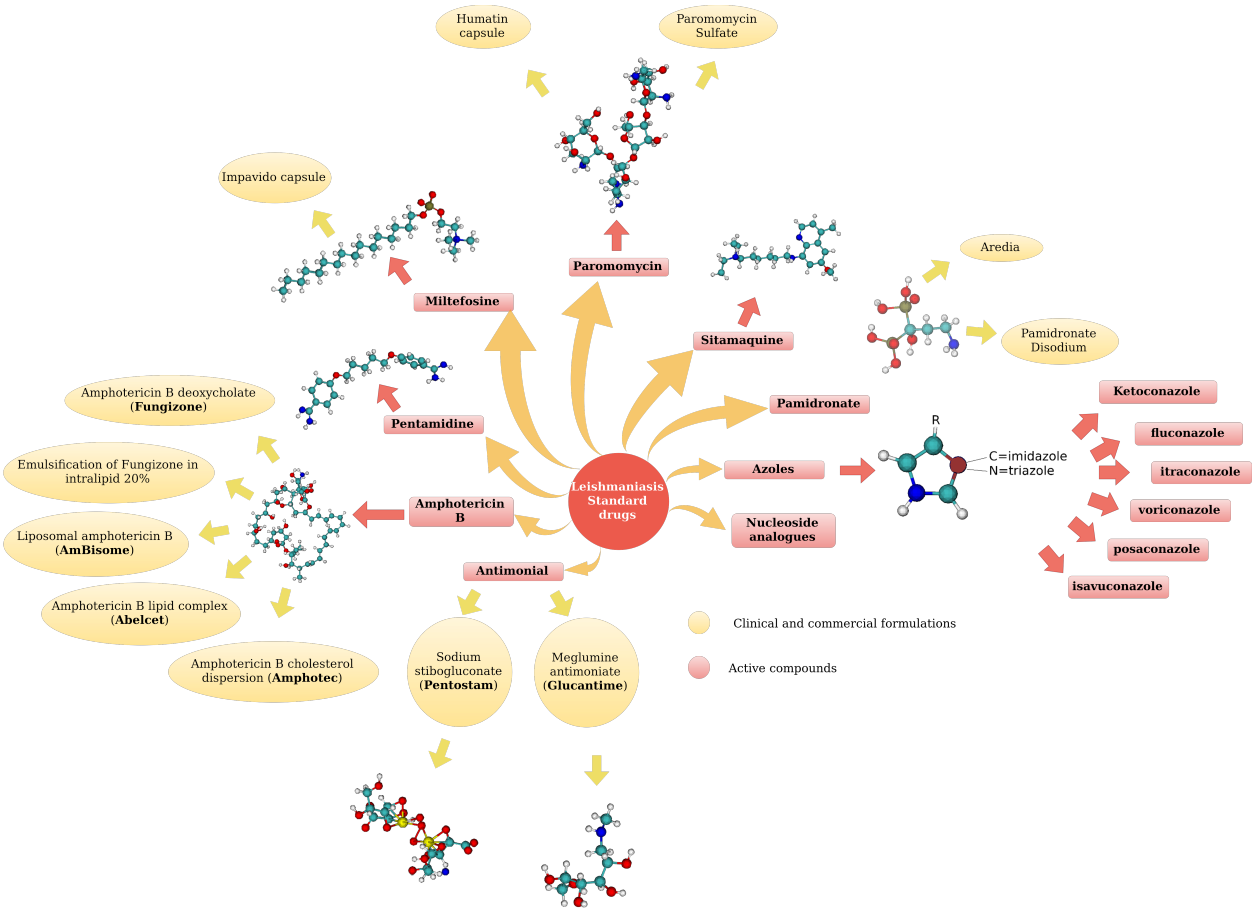

Figure 8: Drugs used against *Leishmania* can be divided in two main groups: standard drugs and alternative drugs. Graphic shows molecular structures of drug active compounds. Structures were obtained from ChemSpider database (http://www.chemspider.com/).

Pentamidine antileishmania activity centers on the parasite polyamines biosynthesis and mitochondrial membrane. They are considered second-line treatment drugs for VL, because its toxicity: myalgia, nausea, headache, hypoglycemia, irreversible insulin dependent diabetes

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

mellitus and death. Also, monetary costs renders them prohibitive.[23] India and East Africa used paromomycin as a cheap alternative treatment, despite its toxicity.[2] Paromomycin remained neglected until the 80s, when topical formulations for VL were developed.[23] One World Health, the Bill and Melinda Gates Foundation, Gland Pharma Limited, IDA Solutions and WHO/TDR partnered to develop Paromomycin as a public health tool to be sold on a not-for-profit basis, at a very low price.[23]

Miltefosine, initially developed as an anticancer drug, currently is the first effective oral treatment of VL.[2,23] Variation in the sensitivities of both, promastigote and amastigote stages of *L. donovani*, *L. major*, *L. tropica*, *Leishmania aethiopica*, *L. mexicana* and *L. panamensis*, were investigated *in vitro*. From these assays, *L. donovani* was recognized as the most sensitive species to this treatment.[22] Miltefosine ED_50 against *L. donovani* was measured in the range of 0.12 to 1.32 μM. Sitamaquine is rapidly metabolized, forming desethyl and 4-CH2OH derivatives, which might be responsible for its activity. Toxicity appears to be relatively mild, as it causes mild methemglobinaemia.[23,131] Finally, pamidronate is a bisphosphonate drug with significant activity against *Leishmania donovani in vitro*. FPPS protein (PDB ID: 4JZX) is potently inhibited by bisphosphonates in the trypanosomatid parasite.[23] Other two type of drugs considered for leishmaniasis treatment are azoles and nucleoside analogues.[22,132]

Within the azole group are, for example, ketoconazole and itraconazole, which inhibits the C14α-demethylase. Nucleoside Analogues, such as allopurinol and pyrazolopyrimidine, are known to inhibit enzymatic processes of the purine salvaging pathway in Leishmania.[22]

5.2 Metabolomics: Non standard drugs

We curated a list of databases of bioactive compounds used to treat the Leishmaniasis disease, alternative to commercial drugs (See Figure 9). Databases in figure 9 are discriminated in metabolome, chemical-tools, chemical-structure, natural-products and medical-literature subjects. Additionally, we have reviewed papers compiling more than 200 chemical species

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

411 and several extracts isolated from plants (see Supporting Information). Assays in these works
412 are based on *in vivo* and *in vitro* techniques, under a variety of physical chemical conditions
413 and *Leishmania* species.[133–135]

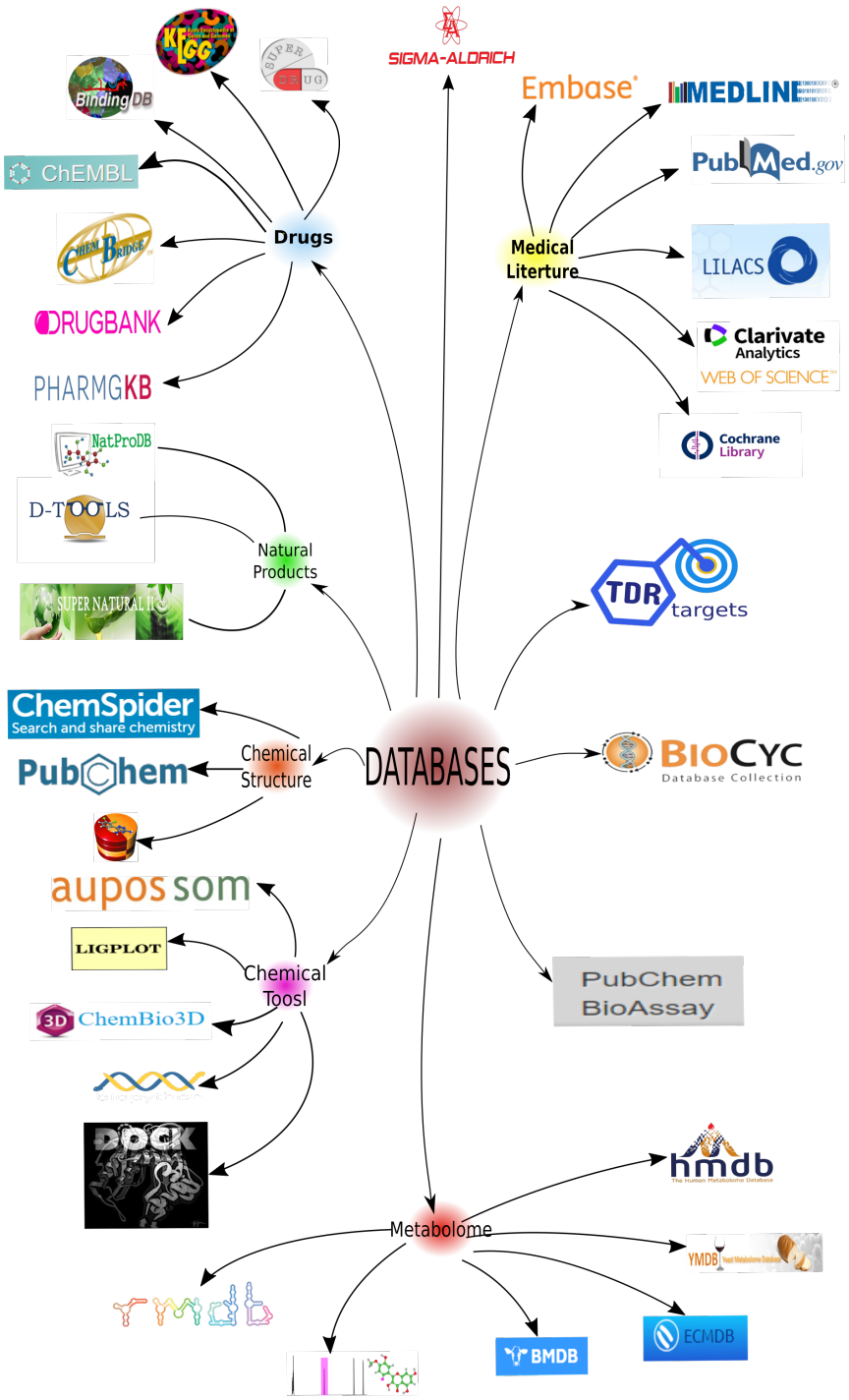

Figure 9: Drug-database review. This figure shows useful information related to general databases and databases related with specific antileishmanial drugs

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

On the other hand, antimicrobial peptides (AMP), currently the major type of compounds used as antibiotics, are being used as treatments against Leishmaniasis (*in vitro* assays, mainly). AMP's are produced by all kinds of living organisms and act on viruses, bacteria, fungi and parasites. In animals, most AMPs are found in tissues and organs, due to the fact that these macromolecules are the first line of innate immune host defense.[136,137]

Is important to highlight that *Leishmania* parasites can develop resistance against these secondary metabolites and AMPs too, therefore, the use of monotherapies is not an good option. The rationale behind combined therapies is to prevent the risk of parasite resistance, to provide increased efficacy resulting from synergistic effects, minimizing dose requirements and to reduce therapy time, eventually leading to a reduction of side toxic effects, or al least to mild toxicity and reduce mortality.[9]

6 Acknowledgement

Authors acknowledge Universidad Nacional de San Agustn (UNSA, Arequipa, Per) for providing access to INKARI (High Performance Computer Facilities, located at the Astronomic Observatory of CHARACATO - UNSA, Arequipa, Per). Also, we acknowledge Dr. Wilson M. Castro Silupu for building the scientific cooperation bridges UNSA-UNISUCRE, and M.Sc. Wilder Nina C. for technological support with INKARI.

References

(1) Torres-Guerrero, E.; Quintanilla-Cedillo, M. R.; Ruiz-Esmenjaud, J.; Arenas, R. *F1000Research* **2017**, *6*, 750.

(2) Bruschi, F.; Gradoni, L. In *The Leishmaniases: Old Neglected Tropical Diseases*; Bruschi, F., Gradoni, L., Eds.; Springer International Publishing: Cham, 2018; pp 1–245.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(3) Grifferty, G.; Shirley, H.; McGloin, J.; Kahn, J.; Orriols, A.; Wamai, R. *Research and Reports in Tropical Medicine* **2021**, *Volume 12*, 135–151.

(4) Maheshwari, K. K.; Bandyopadhyay, D. *Current Medicinal Chemistry* **2021**, *28*, 472–495.

(5) Killick-Kendrick, R. *Clinics in Dermatology* **1999**, *17*, 279–289.

(6) (OMS), O. M. d. l. S. *Control de las leishmaniasis*; 2010; p 200.

(7) J, B.; M, B. M.; Chanda, K. *Frontiers in Chemistry* **2021**, *9*.

(8) Pandian, S. R. K.; Panneerselvam, T.; Pavadai, P.; Govindaraj, S.; Ravishankar, V.; Palanisamy, P.; Sampath, M.; Sankaranarayanan, M.; Kunjiappan, S. *Frontiers in Nanotechnology* **2021**, *3*.

(9) Zulfiqar, B.; Shelper, T. B.; Avery, V. M. *Drug Discovery Today* **2017**, *22*, 1516–1531.

(10) Cupolillo, E.; Medina-Acosta, E.; Noyes, H.; Momen, H.; Grimaldi, G. *Parasitology Today* **2000**, *16*, 142–144.

(11) Momen, H.; Cupolillo, E. **2000**, *95*, 583–588.

(12) Kostygov, A. Y.; Yurchenko, V. *Folia Parasitologica* **2017**, *64*, 1–5.

(13) Lainson, R.; Shaw, J. In *The Leishmaniases in Biology and Epidemiology*; Peters, W., Killick-Kendrick, R., Eds.; Academic Press, 1987; Vol. 1; Chapter 1, pp 1 – 120.

(14) Lainson, R.; Shaw, J. In *Biology of the Kinetoplastida*, 2nd ed.; In WHR Lumsden, D. E., Ed.; Academic Press: London and New York, 1979; p 116.

(15) Corrêa, J. R.; Brazil, R. P.; Soares, M. J. *Memorias do Instituto Oswaldo Cruz* **2005**, *100*, 587–592.

(16) Franco, A. M.; Grimaldi, G. *Memorias do Instituto Oswaldo Cruz* **1999**, *94*, 261–268.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(17) Akhoundi, M.; Kuhls, K.; Cannet, A.; Votýpka, J.; Marty, P.; Delaunay, P.; Sereno, D. *PLoS Neglected Tropical Diseases* **2016**, *10*, 1–40.

(18) Mesnil F, B. E. *C R Soc Biol* **1908**, *65: 587*.

(19) JJ, S. *London School of Hygiene and Tropical Medicine Memoir*, 13th ed.; H. K. Lewis (1969): London, 1969; p 132.

(20) WHO/PAHO, *Leishmaniases: Epidemiological Report in the Americas*; 2018; pp 1 – 7.

(21) Universidad del Rosario, Colombia, el país con más especies de parásitos de Leishmania. 2017; `http://www.urosario.edu.co/UCD/Leishmania/Documento/pdf-fasciculo-leishmaniasis-universidad-del-rosari/`.

(22) Croft, S. L.; Sundar, S.; Fairlamb, A. H. *Society* **2006**, *19*, 111–126.

(23) Kumar, A. *Leishmania and Leishmaniasis*; 2013; Vol. 3.

(24) Ghorbani, M.; Farhoudi, R. *Drug Design, Development and Therapy* **2018**, *12*, 25–40.

(25) Tsirigotakis, N.; Pavlou, C.; Christodoulou, V.; Dokianakis, E.; Kourouniotis, C.; Alten, B.; Antoniou, M. *Parasites & Vectors* **2018**, *11*, 97.

(26) Bates, P. A. *International Journal for Parasitology* **2007**, *37*, 1097–1106.

(27) ESTRADA, L. G.; APONTE, O. A.; BEJARANO, E. E. *Acta Biológica Colombiana* **2015**, *20*, 225–228.

(28) Alemayehu, B.; Alemayehu, M. *Health Science Journal* **2017**, *11*, 1–6.

(29) Pérez-doria, A.; Hernández-oviedo, E.; Elías-Bejarano, E. *Acta Biológica Colombiana* **2009**, *14*, 135–140.

(30) Ready, P. D. *Annual Review of Entomology* **2013**, *58*, 227–250.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(31) OPS/OMS, O. P. d. l. S. *Leishmaniasis: Informe Epidemiológico de las Américas*; 2018; Vol. 6; pp 1 – 7.

(32) Catta-Preta, C. M. C.; Mottram, J. C. *Nature* **2018**, *560*, 171–172.

(33) Chappuis, F.; Sundar, S.; Hailu, A.; Ghalib, H.; Rijal, S.; Peeling, R. W.; Alvar, J.; Boelaert, M. *Nature Reviews Microbiology* **2007**, *5*, 873–882.

(34) Rittig, M. G.; Bogdan, C. *Parasitology Today* **2000**, *16*, 292–297.

(35) Ferreira, C.; Estaquier, J.; Silvestre, R. *Current Opinion in Microbiology* **2021**, *63*, 231–237.

(36) Cunningham, A. C. *Experimental and Molecular Pathology* **2002**, *72*, 132–141.

(37) Nathan, C. *Nature Reviews Immunology* **2006**, *6*, 173–182.

(38) Charmoy, M.; Auderset, F.; Allenbach, C.; Tacchini-Cottier, F. *Journal of Biomedicine and Biotechnology* **2010**, *2010*, 1–8.

(39) Laskay, T.; Zandbergen, G. v.; Solbach, W. *Immunology* **2008**, *213*, 183–191.

(40) Naderer, T.; McConville, M. J. *Cellular Microbiology* **2008**, *10*, 301–308.

(41) Naderer, T.; Vince, J.; McConville, M. *Current Molecular Medicine* **2004**, *4*, 649–665.

(42) Kindt, T. J.; Goldsby, R. A.; Osborne, B. A. In *INMUNOLOGÍA*, 6th ed.; Fraga, J. d. L., Ed.; Mc Graw Hill, interamericana, 2007.

(43) Parham, P. In *The Immune System, Fourth Edition*, 4th ed.; Science, G., Ed.; Taylor and Francis Group, 2014; Vol. 39; p 624.

(44) Kima, P. E. *Microbes and Infection* **2014**, *16*, 721–726.

(45) McConville, M. J.; Naderer, T. *Annual Review of Microbiology* **2011**, *65*, 543–561.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(46) Kanehisa, M. *Nucleic Acids Research* **2006**, *34*, D354–D357.

(47) Berman, H. M. *Nucleic Acids Research* **2000**, *28*, 235–242.

(48) Cornish-Bowden, A. *Perspectives in Science* **2014**, *1*, 74–87.

(49) De Rycker, M.; Baragaña, B.; Duce, S. L.; Gilbert, I. H. *Nature* **2018**,

(50) Rodriguez-Contreras, D.; Hamilton, N. *Journal of Biological Chemistry* **2014**, *289*, 32989–33000.

(51) Seidler, N. W. *GAPDH: Biological Properties and Diversity*; Advances in Experimental Medicine and Biology; Springer Netherlands: Dordrecht, 2013; Vol. 985; pp 1–291.

(52) Kim, H.; Feil, I. K.; Verlinde, C. L. M. J.; Petra, P. H.; Hol, W. G. J. **1995**, *34*, 14975–14986.

(53) Pelley, J. W. *Elsevier's Integrated Biochemistry*; Elsevier, 2007; pp 65–71.

(54) Minárik, P.; Tomásková, N.; Kollárová, M.; Antalík, M. *General physiology and biophysics* **2002**, *21*, 257–65.

(55) Gourley, D. G.; Schüttelkopf, A. W.; Leonard, G. A.; Luba, J.; Hardy, L. W.; Beverley, S. M.; Hunter, W. N. *Nature Publishing Group* **2001**, *8*, 521 – 525.

(56) Vadloori, B.; Sharath, A. K.; Prabhu, N. P.; Maurya, R. *BMC Research Notes* **2018**, *11*, 1–7.

(57) Chang, C.-f.; Papadopoulou, B.; Wang, J.; Bray, T.; Whiteley, J. M.; Lin, S.-x.; Ouellette, M.; Al, W. E. T. *Archives of Biochemistry and Biophysics* **1997**, *342*, 197–202.

(58) Feliciano, P. R.; Cordeiro, A. T.; Costa-Filho, A. J.; Nonato, M. C. *Protein Expression and Purification* **2006**, *48*, 98–103.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(59) Jones, M. E. *Annual Review of Biochemistry* **1980**, *49*, 253–279.

(60) Björnberg, O.; Rowland, P.; Larsen, S.; Jensen, K. F. *Biochemistry* **1997**, *36*, 16197–16205.

(61) Cordeiro, A. T.; Feliciano, P. R.; Pinheiro, M. P.; Nonato, M. C. *Biochimie* **2012**, *94*, 1739–1748.

(62) Gommers-Ampt, J. H.; Van Leeuwen, F.; de Beer, A. L.; Vliegenthart, J. F.; Dizdaroglu, M.; Kowalak, J. A.; Crain, P. F.; Borst, P. *Cell* **1993**, *75*, 1129–1136.

(63) van Leeuwen, F.; Taylor, M. C.; Mondragon, A.; Moreau, H.; Gibson, W.; Kieft, R.; Borst, P. *Proceedings of the National Academy of Sciences* **2002**, *95*, 2366–2371.

(64) Yu, Z.; Genest, P. A.; ter Riet, B.; Sweeney, K.; DiPaolo, C.; Kieft, R.; Christodoulou, E.; Perrakis, A.; Simmons, J. M.; Hausinger, R. P.; van Luenen, H. G.; Rigden, D. J.; Sabatini, R.; Borst, P. *Nucleic Acids Research* **2007**, *35*, 2107–2115.

(65) Heidebrecht, T.; Christodoulou, E.; Chalmers, M. J.; Jan, S.; Ter Riet, B.; Grover, R. K.; Joosten, R. P.; Littler, D.; Van Luenen, H.; Griffin, P. R.; Wentworth, P.; Borst, P.; Perrakis, A. The structural basis for recognition of base J containing DNA by a novel DNA binding domain in JBP1. 2011.

(66) Genest, P. A.; ter Riet, B.; Dumas, C.; Papadopoulou, B.; van Luenen, H. G.; Borst, P. *Nucleic Acids Research* **2005**, *33*, 1699–1709.

(67) Kryshtafovych, A. et al. *Proteins: Structure, Function, and Bioinformatics* **2011**, *79*, 6–20.

(68) Barr, S. D.; Gedamu, L. *Journal of Biological Chemistry* **2003**, *278*, 10816–10823.

(69) Murray, H. W.; Nathan, C. F. *The Journal of experimental medicine* **1999**, *189*, 741–6.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(70) Nathan, C.; Shiloh, M. U. *Proceedings of the National Academy of Sciences of the United States of America* **2000**, *97*, 8841–8848.

(71) Mutlu, O. *Brazilian Archives of Biology and Technology* **2014**, *57*, 244–252.

(72) Fiorillo, A.; Colotti, G.; Boffi, A.; Baiocco, P.; Ilari, A. *PLoS Neglected Tropical Diseases* **2012**, *6*, e1781.

(73) Bose, M.; Saha, R.; Sen Santara, S.; Mukherjee, S.; Roy, J.; Adak, S. *Free Radical Biology and Medicine* **2012**, *53*, 1819–1828.

(74) Chreifi, G.; Dejam, D.; Poulos, T. L. *Journal of Biological Inorganic Chemistry* **2017**,

(75) Phan, I. Q. H.; Davies, D. R.; Moretti, N. S.; Shanmugam, D.; Cestari, I.; Anupama, A.; Fairman, J. W.; Edwards, T. E.; Stuart, K.; Schenkman, S.; Myler, P. J. *Acta Crystallographica Section F Structural Biology Communications* **2015**, *71*, 615–621.

(76) GHOSH, S.; GOSWAMI, S.; ADHYA, S. *Biochemical Journal* **2003**, *369*, 447–452.

(77) Slauch, J. M. *Molecular Microbiology* **2011**, *80*, 580–583.

(78) Ilari, A.; Baiocco, P.; Messori, L.; Fiorillo, A.; Boffi, A.; Gramiccia, M.; Di Muccio, T.; Colotti, G. *Amino Acids* **2012**, *42*, 803–811.

(79) Fairlamb, A. H.; Cerami, A. *Annual Review of Microbiology* **1992**, *46*, 695–729.

(80) Bernardes, L.; Zani, C.; Carvalho, I. *Current Medicinal Chemistry* **2013**, *20*, 2673–2696.

(81) Kabututu, Z.; Martin, S. K.; Nozaki, T.; Kawazu, S.-i.; Okada, T.; Munday, C. J.; Duszenko, M.; Lazarus, M.; Thuita, L. W.; Urade, Y.; Kubata, B. K. *International Journal for Parasitology* **2002**, *32*, 1693–1700.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(82) Moen, S. O.; Fairman, J. W.; Barnes, S. R.; Sullivan, A.; Nakazawa-Hewitt, S.; Van Voorhis, W. C.; Staker, B. L.; Lorimer, D. D.; Myler, P. J.; Edwards, T. E. *Acta Crystallographica Section F Structural Biology Communications* **2015**, *71*, 609–614.

(83) Hargrove, T. Y.; Wawrzak, Z.; Liu, J.; Nes, W. D.; Waterman, M. R.; Lepesheva, G. I. *Journal of Biological Chemistry* **2011**,

(84) Manhas, R.; Tripathi, P.; Khan, S.; Sethu Lakshmi, B.; Lal, S. K.; Gowri, V. S.; Sharma, A.; Madhubala, R. *Journal of Biological Chemistry* **2014**, *289*, 12096–12108.

(85) Führing, J.; Cramer, J. T.; Routier, F. H.; Lamerz, A.-C.; Baruch, P.; Gerardy-Schahn, R.; Fedorov, R. *ACS Catalysis* **2013**, *3*, 2976–2985.

(86) Martin, J. L.; Yates, P. A.; Boitz, J. M.; Koop, D. R.; Fulwiler, A. L.; Cassera, M. B.; Ullman, B.; Carter, N. S. *Molecular Microbiology* **2016**, *101*, 299–313.

(87) Boitz, J. M.; Strasser, R.; Yates, P. A.; Jardim, A.; Ullman, B. *Journal of Biological Chemistry* **2013**, *288*, 8977–8990.

(88) Silva, M.; Silva, C. H.; Iulek, J.; Oliva, G.; Thiemann, O. H. *Biochimica et Biophysica Acta - Proteins and Proteomics* **2004**,

(89) Phillips, C. L.; Ullman, B.; Brennan, R. G.; Hill, C. P. *EMBO Journal* **1999**, *18*, 3533–3545.

(90) Resh, M. D. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1999**, *1451*, 1–16.

(91) Robinson, D. A.; Wyatt, P. G. *Acta Crystallographica Section F Structural Biology Communications* **2015**, *71*, 586–593.

(92) Selvakumar, P.; Kumar, S.; Dimmock, J.; Sharma, R. *Atlas of Genetics and Cytogenetics in Oncology and Haematology* **2012**, 1–9.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(93) Price, H. P.; Menon, M. R.; Panethymitaki, C.; Goulding, D.; McKean, P. G.; Smith, D. F. *Journal of Biological Chemistry* **2003**, *278*, 7206–7214.

(94) French, J. B.; Yates, P. A.; Soysa, D. R.; Boitz, J. M.; Carter, N. S.; Chang, B.; Ullman, B.; Ealick, S. E. *Journal of Biological Chemistry* **2011**,

(95) Williams, R. A. M.; Westrop, G. D.; Coombs, G. H. *Biochemical Journal* **2009**, *420*, 451–462.

(96) Oza, S. L.; Shaw, M. P.; Wyllie, S.; Fairlamb, A. H. *Molecular and Biochemical Parasitology* **2005**, *139*, 107–116.

(97) Krauth-Siegel, R. L.; Comini, M. A. *Biochimica et Biophysica Acta (BBA) - General Subjects* **2008**, *1780*, 1236–1248.

(98) Fyfe, P. K.; Westrop, G. D.; Ramos, T.; Müller, S.; Coombs, G. H.; Hunter, W. N. *Acta Crystallographica Section F Structural Biology and Crystallization Communications* **2012**, *68*, 738–743.

(99) McConville, M. J.; de Souza, D.; Saunders, E.; Likic, V. A.; Naderer, T. *Trends in Parasitology* **2007**, *23*, 368–375.

(100) Mejia, E.; Burak, M.; Alonso, A.; Larraga, V.; Kunkel, T. A.; Bebenek, K.; Garcia-Diaz, M. *DNA Repair* **2014**, *18*, 1–9.

(101) Hammond, D. J.; Gutteridge, W. E. *Molecular and Biochemical Parasitology* **1984**, *13*, 243–261.

(102) Al-Madhoun, A. *Mini Reviews in Medicinal Chemistry* **2004**, *4*, 341–350.

(103) Timm, J.; Bosch-Navarrete, C.; Recio, E.; Nettleship, J. E.; Rada, H.; González-Pacanowska, D.; Wilson, K. S. *PLOS Neglected Tropical Diseases* **2015**, *9*, e0003781.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(104) Aripirala, S.; Gonzalez-Pacanowska, D.; Oldfield, E.; Kaiser, M.; Amzel, L. M.; Gabelli, S. B. *Acta Crystallographica Section D: Biological Crystallography* **2014**,

(105) de Mattos Oliveira, L.; Araújo, J. S. C.; Bacelar Costa Junior, D.; Santana, I. B.; Duarte, A. A.; Leite, F. H. A.; Benevides, R. G.; Coelho dos Santos Junior, M. *Journal of Molecular Modeling* **2018**, *24*, 314.

(106) Schomburg, I.; Jeske, L.; Ulbrich, M.; Placzek, S.; Chang, A.; Schomburg, D. *Journal of Biotechnology* **2017**, *261*, 194–206.

(107) Abendroth, J.; Choi, R.; Wall, A.; Clifton, M. C.; Lukacs, C. M.; Staker, B. L.; Van Voorhis, W.; Myler, P.; Lorimer, D. D.; Edwards, T. E. *Acta Crystallographica Section F:Structural Biology Communications* **2015**,

(108) Alphey, M. S.; Williams, R. A.; Mottram, J. C.; Coombs, G. H.; Hunter, W. N. *Journal of Biological Chemistry* **2003**, *278*, 48219–48227.

(109) Williams, R. A. M.; Kelly, S. M.; Mottram, J. C.; Coombs, G. H. *Journal of Biological Chemistry* **2003**, *278*, 1480–1486.

(110) VEITCH, N. J.; MAUGERI, D. A.; CAZZULO, J. J.; LINDQVIST, Y.; BARRETT, M. P. *Biochemical Journal* **2004**, *382*, 759–767.

(111) Rigden, D. J.; Phillips, S. E.; Michels, P. A.; Fothergill-Gilmore, L. A. *Journal of Molecular Biology* **1999**, *291*, 615–635.

(112) Shi, W.; Schramm, V. L.; Almo, S. C. *The Journal of Biological Chemistry* **1999**, *274*, 21114–21120.

(113) Schlagenhauf, E.; Etges, R.; Metcalf, P. *Structure* **1998**, *6*, 1035–1046.

(114) McKerrow, J. H.; Caffrey, C.; Kelly, B.; Loke, P.; Sajid, M. *Annual Review of Pathology: Mechanisms of Disease* **2006**, *1*, 497–536.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(115) McLuskey, K.; Paterson, N. G.; Bland, N. D.; Isaacs, N. W.; Mottram, J. C. *Journal of Biological Chemistry* **2010**, *285*, 39249–39259.

(116) Teixeira, P. C.; Velasquez, L. G.; Lepique, A. P.; de Rezende, E.; Bonatto, J. M. C.; Barcinski, M. A.; Cunha-Neto, E.; Stolf, B. S. *PLOS Neglected Tropical Diseases* **2015**, *9*, e0003411.

(117) Timm, J.; Valente, M.; García-Caballero, D.; Wilson, K. S.; González-Pacanowska, D. *mSphere* **2017**, *2*.

(118) Leitherer, S.; Clos, J.; Liebler-Tenorio, E. M.; Schleicher, U.; Bogdan, C.; Soulat, D. *Infection and Immunity* **2017**,

(119) D'Antonio, E. L.; Ullman, B.; Roberts, S. C.; Dixit, U. G.; Wilson, M. E.; Hai, Y.; Christianson, D. W. *Archives of Biochemistry and Biophysics* **2013**, *535*, 163–176.

(120) Feliciano, P. R.; Drennan, C. L.; Nonato, M. C. *Proceedings of the National Academy of Sciences* **2016**, *113*, 9804–9809.

(121) Feliciano, P. R.; Drennan, C. L.; Nonato, M. C. *ACS Chemical Biology* **2019**, *14*, 266–275.

(122) Chudzik, D. M.; Michels, P. A.; De Walque, S.; Hol, W. G. *Journal of Molecular Biology* **2000**, *300*, 697–707.

(123) Williams, J. C.; Zeelen, J. P.; Neubauer, G.; Vriend, G.; Backmann, J.; Michels, P. a.; Lambeir, a. M.; Wierenga, R. K. *Protein engineering* **1999**, *12*, 243–250.

(124) Lambeir, A. M.; Backmann, J.; Ruiz-Sanz, J.; Filimonov, V.; Nielsen, J. E.; Kursula, I.; Norledge, B. V.; Wierenga, R. K. *European Journal of Biochemistry* **2000**, *267*, 2516–2524.

(125) Bateman, A. et al. *Nucleic Acids Research* **2017**, *45*, D158–D169.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2022 doi:10.20944/preprints201902.0122.v4

(126) Larson, E. T.; Kim, J. E.; Zucker, F. H.; Kelley, A.; Mueller, N.; Napuli, A. J.; Verlinde, C. L.; Fan, E.; Buckner, F. S.; Van Voorhis, W. C.; Merritt, E. A.; Hol, W. G. *Biochimie* **2011**, *93*, 570–582.

(127) Larson, E. T.; Kim, J. E.; Castaneda, L. J.; Napuli, A. J.; Zhang, Z.; Fan, E.; Zucker, F. H.; Verlinde, C. L. M. J.; Buckner, F. S.; Van Voorhis, W. C.; Hol, W. G. J.; Merritt, E. A. *Journal of Molecular Biology* **2011**,

(128) Kamir, D. et al. *The Journal of Immunology* **2008**, *180*, 8250–8261.

(129) Arya, R.; Sharma, B.; Dhembla, C.; Pal, R. K.; Patel, A. K.; Sundd, M.; Ghosh, B.; Makde, R. D.; Kundu, S. *Biochimica et Biophysica Acta - Proteins and Proteomics* **2019**, *1867*, 163–174.

(130) Chawla, B.; Madhubala, R. *Journal of Parasitic Diseases* **2010**, *34*, 1–13.

(131) Soto, J.; Soto, P. *Biomédica* **2012**, *26*, 207.

(132) Ramón Azanza, J.; García-Quetglas, E.; Sádaba, B. *Rev Iberoam Micol* **2007**, *24*, 223–227.

(133) Tiuman, T. S.; Santos, A. O.; Ueda-Nakamura, T.; Filho, B. P. D.; Nakamura, C. V. *International Journal of Infectious Diseases* **2011**, *15*.

(134) Sen, R.; Chatterjee, M. *Phytomedicine* **2011**, *18*, 1056–1069.

(135) Gutiérrez-Rebolledo, G. A.; Drier-Jonas, S.; Jiménez-Arellanes, M. A. *Asian Pacific Journal of Tropical Medicine* **2017**, *10*, 1105–1110.

(136) Zahedifard, F.; Rafati, S. *Expert Review of Anti-infective Therapy* **2018**, *16*, 461–469.

(137) Patiño-Márquez, I. A.; Manrique-Moreno, M.; Patiño-González, E.; Jemioła-Rzemińska, M.; Strzałka, K. *The Journal of Antibiotics* **2018**, *71*, 642–652.