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REVIEW

CHEMISTRY & BIODIVERSITY

Exploring Innovative Leishmaniasis Treatment: Drug Targets from Pre-Clinical to Clinical Findings

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Leishmaniasis is a group of tropical diseases caused by parasitic protozoa belonging to the genus *Leishmania*. The disease is categorized in cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). The conventional treatment is complex and can present high toxicity and therapeutic failures. Thus, there is a continuing need to develop new treatments. In this review, we focus on the novel molecules described in the literature with potential leishmanicidal activity, categorizing them in pre-clinical (*in vitro*, *in vivo*), drug repurposing and clinical research.

Keywords: leishmaniasis, new targets, pre-clinical, clinical, drug repurposing.

1. Introduction

Leishmaniasis is a group of tropical diseases caused by protozoan parasites belonging to the genus *Leishmania*.[1] There are more than 20 species of *Leishmania* distributed worldwide and each species can cause different clinical manifestations, which can be grouped into three main clinical forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL).[2,3] CL is the most common form of the disease and it is generally painless and chronic.[4] In general, the lesions occur at the sites bitten by the infected sand fly, such as the face, legs and arms. MCL is characterized by lesions that can partially or totally destroy the mucous membranes of the cavities of the nose, mouth and throat, which can lead to disfigurement of the patient with consequent social exclusion.[5] VL is the most serious manifestation of the disease and it is characterized by fever, weight loss, enlargement of liver and spleen, pancytopenia and hypergammaglobulinemia and it may be fatal if left untreated.[6,7] Leishmaniasis predominantly affects the poor population of countries in Africa, Asia and Latin America. Recent estimates indicate that about 350 million of people live in a vulnerable situation with the risk of contracting leishmaniasis. In a global perspective, the disease currently affects around 12 million people worldwide.[8] According to WHO, there are currently 98 countries that are endemic for leishmaniasis; nine of them are endemic only for VL, 21 only for CL and 68 countries are endemic for both leishmaniasis forms.[7]

Leishmania spp. are digenetic parasites, presenting two main evolutionary forms during their life cycle: promastigote, present in the invertebrate host and amastigote (present in the vertebrate host). The promastigote form is transmitted to the vertebrate

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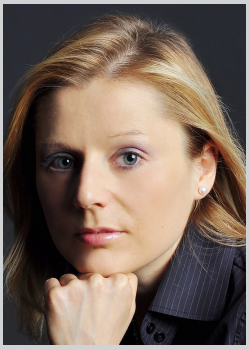
Simone Santiago Carvalho de Oliveira, Postdoctoral Researcher – Summary: I work as a postdoctoral researcher and I have experience with the following topics: peptidases, cytotoxicity, mechanisms of action of chemotherapy, pathogen-host interaction and Leishmania spp. My studies are currently focused on discovering new bioactive compounds that have anti-Leishmania activity and in the study of the mechanism of action of new antimicrobials.

Mariana is studying Pharmacy at Tiradentes University, Aracaju, Brazil, and has an undergraduate research scholarship to work on the in vivo toxicity of isopentyl caffeine for the treatment of leishmaniasis.

Silvio Dolabella is a Pharmacist with Ph.D. in Parasitology from the Federal University of Minas Gerais, Brazil. He has experience in the area of Parasitology, with emphasis on Human Protozoology.

André Luis Souza dos Santos, full Professor – My research group is distinguished by its multidisciplinary nature, with direct involvement of different research institutions from Brazil and from abroad countries, generating productive and effective collaborations in the discovery of novel compounds with promising antimicrobial action as well as in deciphering the molecular mechanisms related to the antimicrobial resistance events.



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host by the bite of infected female phlebotominae sandflies. The promastigotes differentiate into amastigotes in the vertebrate's host, multiply intensely and spread infection (*Figure 1*).[9–11]

Currently, chemotherapy is the basis of antileishmanial treatment. Different drugs are available for use, the choice of medication varying according to the disease form and the geographic region.[12,13] The available drugs are however not ideal, showing high toxicity and low efficacy in some endemic regions.[12,14] Moreover, therapeutic failure, patient recurrence and deaths due to the disease complications are often observed, requiring new efficient treatment options that are less toxic. In this review, we bring together information regarding new molecules with potential leishmanicidal activity. We first describe the drugs currently in use for the treatment of leishmaniasis and highlight their limitations. Then, we relate the new molecules with good potential, classifying them according to the drug development timeline (*Figure 2*) into pre-clinical (*in vitro*, *in vivo* analysis) and clinical phase, also including an extra topic on drug repurposing. Finally, we discuss future perspectives to address the current limitations.

2. Current Treatment Options and Limitations

The drugs of choice for the treatment of CL and VL are pentavalent antimonials, commercially available in the forms of meglumine antimoniate (Glucantime®) or sodium stibogluconate (Pentostam®).[12] Pentavalent antimonials are registered and licensed in Southeast Asia, Latin America and some Mediterranean and African countries.[12] Although these compounds are highly effective in different parts of the world, like Africa, they have been ineffective in India, due to the emergence of resistant strains.[15] Antimonials have low cost and can be administered intravenously (Pentostam) or intramuscularly (Glucantime), and they have also been applied intralesionally for the treatment of CL.[12,16] Although effective, reports of side effects are common in therapy with antimonials, such as nephrotoxicity, cardiotoxicity (arrhythmia, prolongation of the Q–T interval, ventricular tachycardia), hepatotoxicity and elevated levels of pancreatic enzymes.[12,14] HIV-*Leishmania* co-infection has also been associated with an increase in the number of recurrence cases after treatment with antimonials, as well as with the increase in the number of deaths.[17] Moreover, treatment with antimonials is long, which,

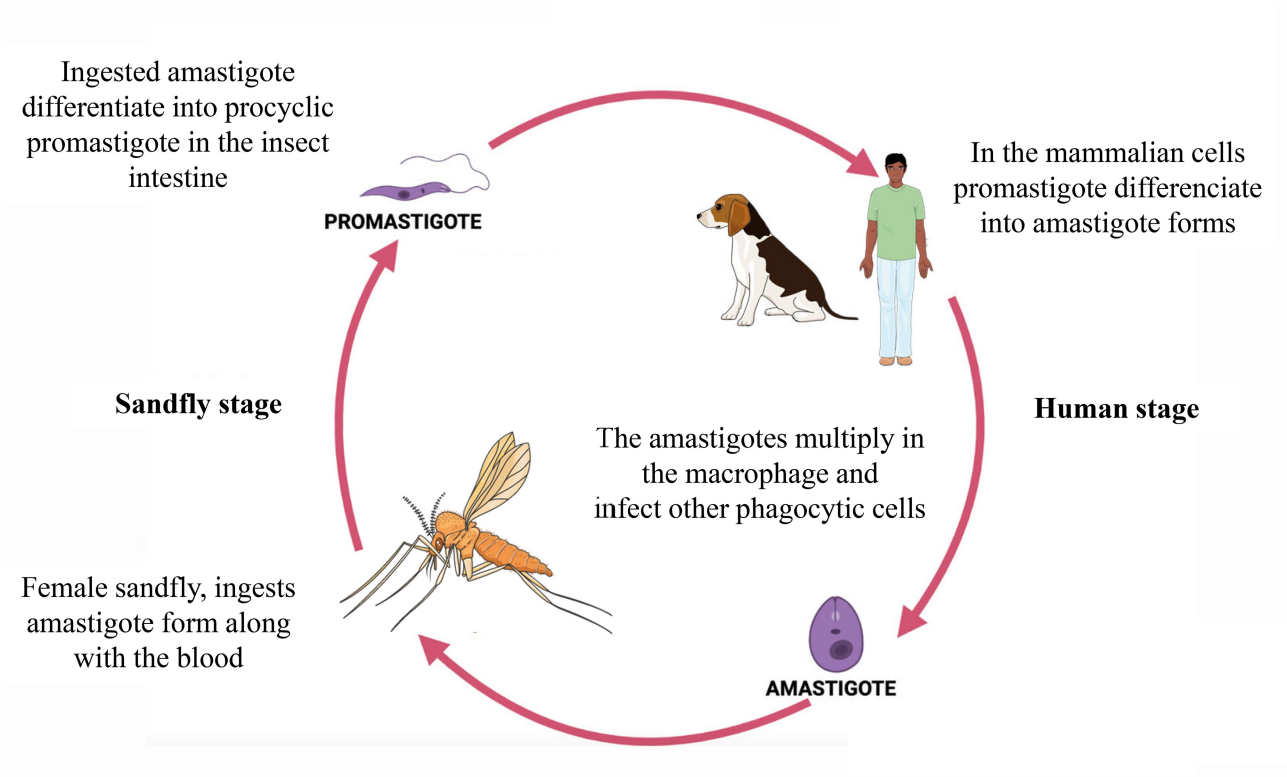

Figure 1. Life cycle of Leishmania in the invertebrate and vertebrate hosts.

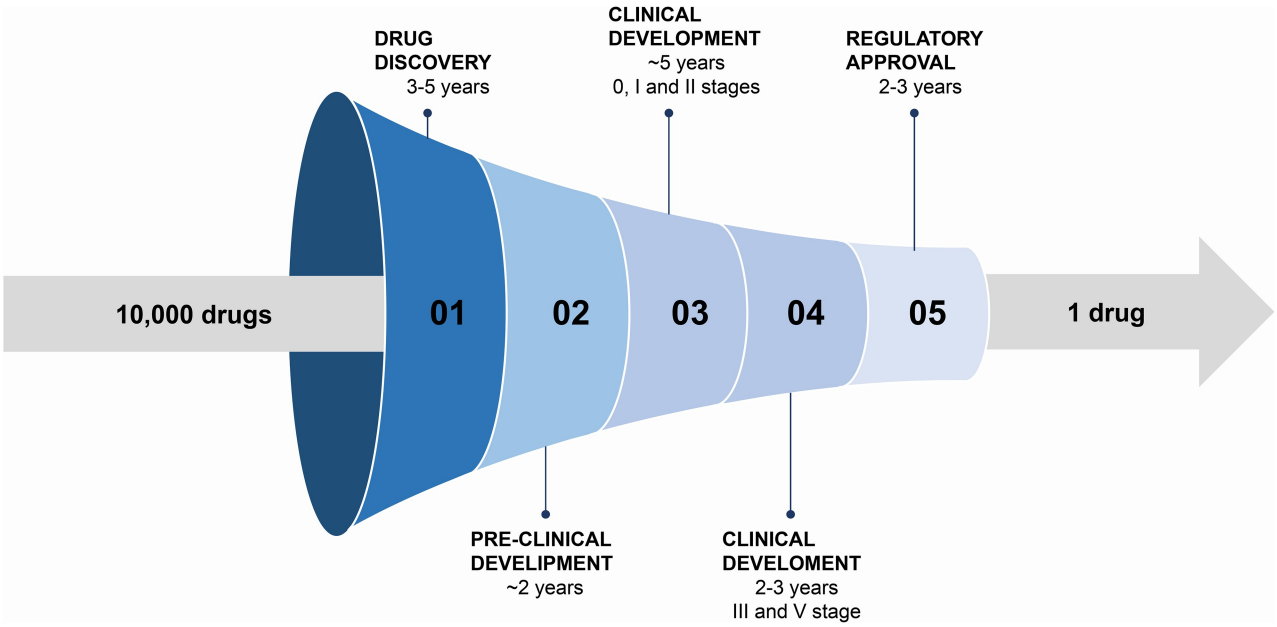

Figure 2. Stages of drug development. The development of a new drug follows several stages such as drug discovery, pre-clinical development, clinical development, and regulatory approval. Researchers begin the discovery of new drugs with potential for the treatment of the disease (for example leishmaniasis) and initiate tests in cells and animals. Then, it proceeds to the clinical research phase with the objective of testing the safety and effectiveness in humans. The development of a new medicine lasts for approximately 15 years before reaching the consumer market.

together with the high toxicity, makes it difficult for patients to adhere to the treatment.[15]

Other drugs have also been widely used in the treatment of leishmaniasis, such as amphotericin B deoxycholate (AmB).[12] AmB is a polyene antifungal that has been used as alternative drug in the treatment of leishmaniasis caused by antimonial-resistant strains.[18] Although highly effective, AmB also has a number of side effects, including nephrotoxicity, electrolyte abnormalities, hypokalemia and myocarditis, requiring hospitalization of the patient and increasing the cost of treatment.[18] In order to minimize these toxic effects, lipid formulations of AmB have been developed, such as liposomal AmB (L-AmB) (AmBisome®), AmB colloidal dispersion (ABCD) (Amphocil®) and AmB lipid complex (ABLC) (Abelcet®).[18,19] These new formulations are just as effective as the original AmB, while at the same time showing better tolerability and less toxicity.[16] Liposomal AmB has been the drug of choice in the treatment of patients with HIV co-infection.[20] However, a study in Bihar, India, showed that the use of this drug in the treatment of these patients did not show any reduction in rates of recurrence and mortality compared to HIV-negative patients.[21] Lipid-based formulations of AmB are quickly targeted at the organs where the disease develops, so that other organs, such as the kidneys, are saved, which reduce their adverse side effects.[18] However, although AmB liposomal formulations are effective and better tolerated, they still need to be administered by intravenous infusions, which require hospitalization of the patient for treatment. Along with this, the high cost of treatment has restricted its use in therapy.[22]

Another drug available for the treatment of leishmaniasis is paromomycin, an aminoglycoside-aminocyclitol antibiotic, used for the first time in the treatment of leishmaniasis in 2002, in the form of paromomycin sulfate.[22] It has been used to treat both forms of the disease, being used in parenteral formulations in the treatment of VL and in topical and parenteral formulations in the treatment of CL.[6] Since leishmaniasis mainly affects low-income populations, the low cost is one of the main advantages of paromomycin, although its parenteral administration makes it difficult for patients to adhere to treatment.[18] Paromomycin has high efficacy and excellent tolerability; however, side effects have also been reported, such as nephrotoxicity, ototoxicity, reversible increase in liver transaminases and pain at the injection site.[11,12] Furthermore, although cases of resistance have not yet been reported, the develop-

ment of *in vitro* resistance to paromomycin has already been reported in some species of *Leishmania*.[19]

Pentamidine is an aromatic diamidine used as a second-line medication in the treatment of leishmaniasis, being administered intramuscularly and its main advantage is short-term therapy.[14,19] On the other hand, pentamidine presents serious adverse reactions, such as nephrotoxicity, cardiotoxicity, pancreatitis, hypoglycemia, leukopenia and anemia, which require constant monitoring of the patient.[12] In addition, compared to other drugs, its effectiveness varies between *Leishmania* species, which has led to a reduction of its use in clinical treatment.[14,19]

Miltefosine is an alkylphosphocholine drug, originally developed as an anticancer agent, was the first oral drug approved for the treatment of VL and CL. In general, it is a well-tolerated drug, but some adverse effects have already been reported, such as nephrotoxicity, gastrointestinal toxicity, and occasional liver toxicity, which makes it necessary to monitor the patient during treatment. Miltefosine also has teratogenic effects and abortive properties, which limits its use during pregnancy; in addition, its high half-life (about 1 week) can stimulate the development of resistance.[14,18]

Sitamaquine (8-aminoquinoline) is another medication that has the advantage of oral administration route. The main adverse effect observed was nephrotoxicity, and other effects were also seen, for example, vomiting, dyspepsia, glomerulonephritis, and headache. However, at doses well tolerated by the body, the drug showed low efficacy. The development of resistance has not yet been reported, although promastigote forms of *L. donovani* resistant to sitamaquine have been selected in the laboratory.[19]

3. New Molecules with Potential for Treatment

3.1. In Vitro Studies

The efficacy of treatment for leishmaniasis depend on a number of factors linked to the host, the environment, the type of drug administered and the parasite.[23] The scientific community is always searching for new therapeutic strategies, and new low cost, less toxic and more effective compounds for the treatment of leishmaniasis. Thus, over the years, new molecules that inhibit the survival of *Leishmania* have been extensively investigated. Initially, to evaluate the potential of new drugs and their toxicity, laboratory studies are conducted as *in vitro* tests. These tests are generally used to evaluate cell toxicity and viability and have several advantages such as low cost, limited toxic waste, and controlled testing conditions.[24,25] In this topic we describe the novel molecules which have shown potential antileishmanial effect in the *in vitro* tests.

Oliveira *et al.* reported the use of five thiazole derivatives of the thiazopyridines class (TP) and five of the thiazoacetylpyridines class (TAP) to evaluate *in vitro* activity against *L. infantum*.[26] Thiazoles are a class of compounds that exhibit broad range of biological activity, including antitumor, antibacterial, and anti-inflammatory activities. In this study, all the tested compounds inhibited the growth of promastigotes and presented low cytotoxicity. However, TAP-01, TAP-04 and TAP-06 presented higher potential with lower IC_{50} values against amastigote (0.99, 0.43 and 0.59 μM, respectively). TAP-04 (*Figure 3*, Structure 1, S1) showed better activity against both forms of the parasite, as well as lower cytotoxicity, and high selectivity index (SI/amastigote = 137.37) which is about seven times higher than the minimum established for a promising drug. The authors also investigated the possible intracellular targets, as well as the effect of the compounds on the parasite's cell membrane. The promastigotes without the addition of TAP-04 presented regular morphology, with a well-preserved nucleus, occupying the most central part of the cytoplasm, and chromatin associated with the internal nuclear membrane. After exposure to 1/2×IC50 TAP-04, the promastigotes presented swollen mitochondria with intense disorganization in the mitochondrial ridges, in addition to changes in the shape of the cell body, presenting rounded morphology and loss of the flagellum, together with loss of cell volume with intense wrinkles, suggesting cell death via apoptosis.

Chauhan *et al.* analyzed the leishmanicidal activity of *trans*-dibenzalacetone (DBA, S2), a low cost and easily synthesizable monoketone analog of curcumin.[27] The authors observed an IC_{50} of 7.43 ± 1.88 μg/mL and 17.80 ± 1.42 μg/mL for amastigotes and promastigotes, respectively. The treatment of promastigotes with DBA caused morphological changes, such as cell reduction and rounding, in addition to extensive cytoplasmic vacuolization after 24 h of treatment. In addition, features associated with apoptosis such as cell arrest in the G0/G1 phase, depolarization of the mitochondrial membrane potential and an increase in cytosolic Ca^{2+} levels were observed. The authors also reported reduced levels of glutathione (GSH) in the treated cells compared to untreated cells. Kinetoplastids like *Leishmania* and *Trypanosoma* show an unusual form of glutathione

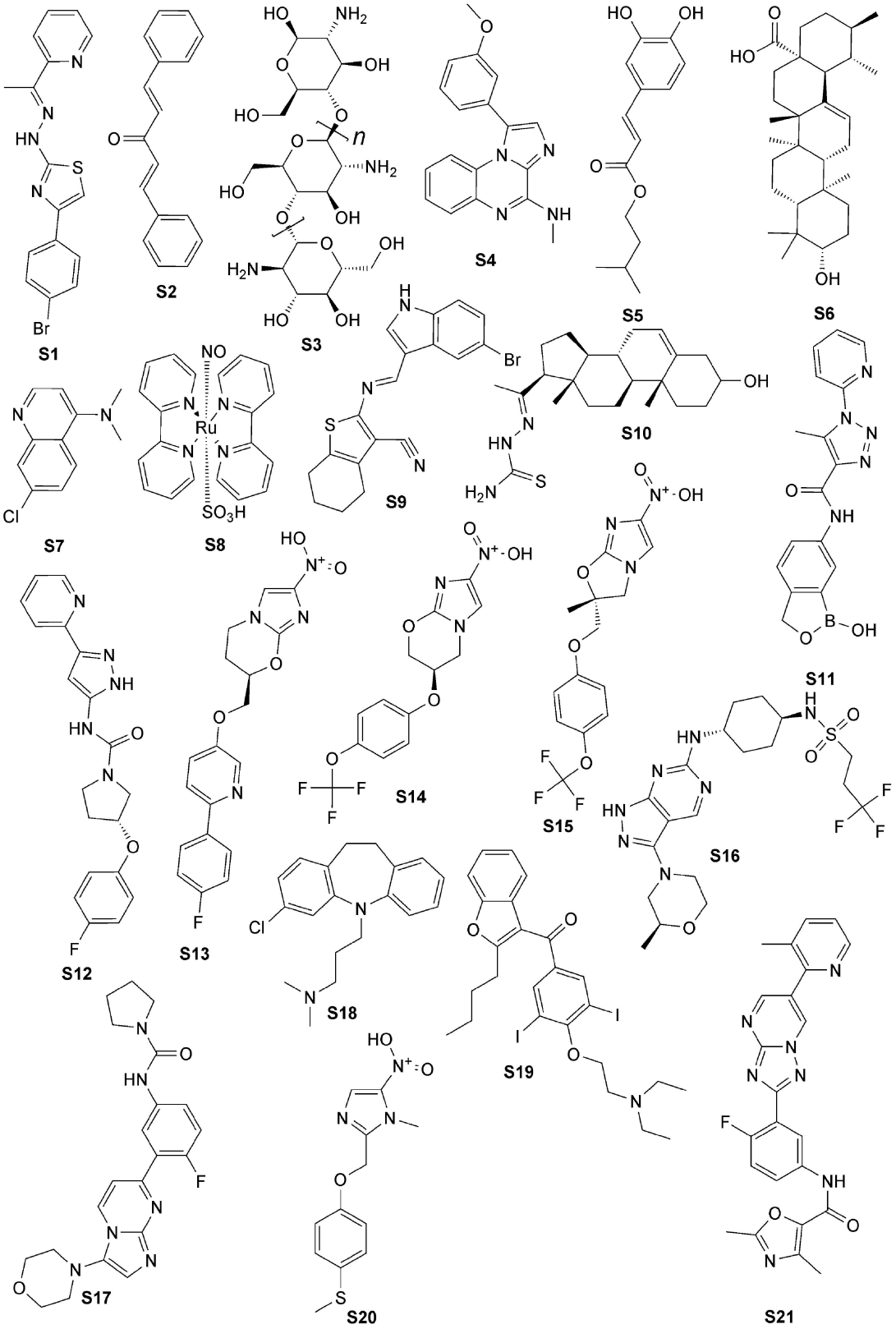

Figure 3. Chemical structures of the molecules cited in this work. S1-TAP-04, S2-DBA, S3-chitosan, S4-EAPB0503, S5-Isopentyl caffeate, S6-ursolic acid, S7-GF1059, S8-Ruthenium nitrosol complex, S9-SB-83, S10-Compound 8, S11-DNDI-6148, S12-DNDI-1044, S13-DNDI-0690, S14-DNDI-8219, S15-DNDI-VL-2098, S16-DDD853651/GSK3186899, S17-GSK3494245/DDD01305143, S18-Clomipramine, S19-Amiodarone, S20-Fexinidazole, S21-LXE408.

(trypanothione) essential for the survival of the parasite and which is the target for the development of new drugs.

Chitosan (S3) is a biodegradable cationic polysaccharide, with proven antimicrobial, antileishmanial and immunostimulating activities.[28] Previous studies have shown its *in vitro* antileishmanial activity with IC_{50} ranging from 70 to 240 μg/mL against both promastigotes and amastigotes forms of *L. infantum*, *L. amazonensis* and *L. chagasi*.[29–31] Recently, Riezk *et al.* evaluated the *in vitro* antileishmanial activity of various forms of chitosan, including low, medium and high molecular weight chitosan and its derivatives, against extracellular promastigotes and intracellular amastigotes of *L. major* and *L. mexicana*.[32] In this study, the authors demonstrated for the first time a pH dependent antileishmanial activity of chitosan and its derivatives, which may justify the great variability of the antileishmanial activity of this compound in the literature. The authors reported minimal cytotoxicity against human squamous carcinoma cells (KB cells), with CC_{50} of 750 μg/mL at pH 7.5 or 6.5. Chitosan and its derivatives were approximately 7 to 20 times more active at pH 6.5 than at pH 7.5, with high molecular weight chitosan being the most potent (except carboxymethyl chitosan). This increased chitosan activity at a lower pH of 6.5 may be due to its greater ionization. It is believed that the positive charge can increase the antimicrobial activity of chitosan due to the interaction with the negatively charged microbial membrane. The authors concluded that chitosan and its water-soluble derivatives have antileishmanial activity against *L. major* and *L. mexicana* promastigotes and amastigotes in a pH-dependent manner and that their antileishmanial activity is related to the direct uptake of chitosan in the parasitophore vacuole by pinocytosis.

El Hajj *et al.* characterized the effect of imiquimod, an immunomodulatory drug, against *L. tropica* and *L. major*, and elucidated the molecular mechanisms that dictate antileishmanial efficacy against both the strains.[33] In addition, they also investigated the potency and molecular mechanisms of an imiquimod analog, EAPB0503 (S4), against these two strains. Both drugs reduced the replication of amastigotes, however, the compound EAPB0503 was more potent, particularly against amastigote forms of *L. tropica*. Toll-like receptor 7 (TLR7) was found to be positively regulated, mainly by imiquimod, and to a lesser extent by EAPB0503. Furthermore, the drugs activated the canonical route of NF-κB, generating an immune response and the superregulation of i-NOS in infected macrophages. The authors conclude that imiquimod and EAPB0503 could be strong candidates for the treatment of *L. tropica*.

Our group has been studying isopentyl caffeate (ICaf, S5) for its antileishmanial activity. ICaf is derived from caffeic acid and has shown good activity against the promastigote and amastigote forms of cutaneous (*L. amazonensis*) and visceral (*L. chagasi*) leishmaniasis.[34,35] The hydrophobic nature of ICaf limit its bioavailability. To overcome its low solubility an inclusion complex of ICaf in β-cyclodextrin (β-CD) was developed. The ICaf/β-CD with improved solubility retained high antileishmanial activity *in vitro* (<10 μg/mL) against both *L. amazonensis* and *L. chagasi*.

3.2. In Vivo Studies

The safety assessment of new molecules requires animal studies before advancing for clinical trials. These *in vivo* studies aim to obtain information on several aspects of the new molecule with respect to its functioning in a biological system, before its testing in humans.[36,37] The selection of the animal model to be used is one of the most important steps, since the choice of the species will give the best correlation with the human trials. Differences in the gut, enzyme activity, circulatory system, or other characteristics make certain models more suitable than others for a given goal. For example, differences in metabolism between two species will affect both toxicology and efficacy of the substance.[37,38] It is also important to mention that animal models of human diseases not only facilitate compound selection and early assessment of the mechanism of action, but also provide a better understanding of the therapeutic index and therefore improve clinical-dose selection for human trials.[39]

Bilbao *et al.* evaluated the efficacy of ursolic acid (UA, S6),[40] a multifunctional triterpenoid with proven antitumor, antioxidant, antimicrobial and antifungal activities.[41,42] In this study, *in vivo* UA activity was evaluated in both acute and chronic models of VL. In addition, topical formulations were also developed, and their efficacy was tested in a chronic model of CL. The authors first reported the antileishmanial activity *in vitro* against amastigotes of *L. amazonensis* and *L. infantum*, being six and three times greater than the promastigotes, respectively. Also, the selectivity index of UA was found to be higher (three and eight-fold higher depending on the strain) compared to miltefosine. The authors explained greater activity of UA observed in the amastigotes compared to the promas-

tigotes by their dual mode of action. UA showed activity not only on the parasite, but also stimulated the immunological response of the host cell, mainly by increasing NO production in macrophages. In acute and chronic model of *L. infantum* (VL), UA was tested via intraperitoneal route administering 5 mg/kg daily for seven consecutive days. A 99,83% and 99,78% reduction in the parasitic load was observed in the spleen and liver in the acute model, while a 58% and 79% reduction in the number of parasites in the spleen and liver was observed in the chronic model. In the chronic model for CL, the lesion progression decreased by approximately 42% and 50% (at weeks 10 and 15) during topical administration of AU ointment (0, 2%) for 28 days. However, it can be noted that the parasites were not fully eradicated after topical administration, since inflammation increased at week 15. AU was shown to modulate the Th1 response in the immune system, resulting in the death of the parasite, by increasing of IFN-γ and decreased IL-4. Furthermore, no evident signs of toxicity were observed in the animals, which suggests that ursolic acid was well tolerated in the administered dose, making it a promising candidate against visceral and cutaneous leishmaniasis.

In 2019, in an *in vitro* and *in vivo* study against *L. infantum* and *L. amazonensis*, Soyer *et al.* evaluated the activity of a chloroquinoline derivative (GF1059, S7) by means of cytotoxicity analysis in macrophages, hemolytic potential in human red blood cells, and efficacy of the compound against infected macrophages.[43] The data showed IC_{50} of 4.23 ± 0.34 and 7.53 ± 1.04 μM against promastigotes, and 4.73 ± 1.09 and 8.75 ± 1.10 μM against amastigotes of *L. infantum* and *L. amazonensis*, respectively. Macrophages treated with GF1059 prior to infection with *L. amazonensis* and *L. infantum* showed 79% and 80% reduced infection. The authors also reported a reduction in the parasitic load in infected tissue (50%), spleen (29%), liver (50%) and lymph node (59%). GF1059 showed low toxicity and did not induce hemolysis in human red blood cells. The compound also resulted in decreased membrane potential, increased autophagic vacuolos and excessive production of reactive oxygen species, all of which were probably responsible for causing death of the parasite. In addition, phosphatidylserine markers and membrane integrity were also evaluated, and, despite mitochondrial damage, the results did not show any type of alteration in these evaluations.

Nascimento *et al.* evaluated the leishmanicidal potential of the ruthenium nitrosol complex (RuNO, S8) against *L. brasiliensis* by means of an *in vitro* (mice dermal fibroblasts) and an *in vivo* study (using hamsters).[41] The nitrosyl ruthenium complexes have proven biological activity, such as antiparasitic, anti-angiogenic, analgesic, gastroprotective and cerebral neuroprotection.[44–49] In this study, the authors showed that RuNo had no cytotoxic effect on dermal fibroblasts at any of the concentrations tested. In the *in vitro* tests, 4 h treatment with RuNO complex (100 μM) could completely reduce the number of infected cells. Similarly, in the hamster model, after the first week it was possible to observe a reduction in lesion size. The number of parasite decreased 99.9% with 300 μg/kg/day of the compound. Despite good leishmanicidal action, this study did not report any possible mechanism of action, however, the authors discuss that NO is one of the crucial molecules in the control of the parasitic load during the development of cutaneous leishmaniasis. Ruthenium complexes have shown to function as NO donor and associated with antileishmanial activity by NO release and intracellular elimination of the parasite.[44,50]

Rodrigues *et al.* analyzed the acute toxicity, genotoxicity and oral efficacy of SB-83 (S9) (a 2-aminotiophene derivative) *in vivo* against *L. amazonensis*.[51] Thiophene belong to a group of aromatic heterocyclic compounds and present biological properties such as anti-inflammatory, antitumor, antinociceptive, anticonvulsant and antiarrhythmic activities.[52] The authors demonstrated that in the ninth week of the treatment with SB-83 (200 mg/kg), there was a reduction in lesion size by $52.47\% \pm 5.32$. The parasitic load was reduced in the lymph node ($42.57\% \pm 3.14$) and in the spleen (100%). Th1 and Th2 responses were analyzed by measuring IFN-γ and IL-10 levels, respectively. An increase in IFN-γ (5.67 times) was observed without any alteration in the IL-10 levels. The increase in IFN-γ confirms the immunomodulatory activity of SB-83 because the production of IFN-γ by Th1 lymphocytes is one of the main activators of macrophages responsible for the control of *Leishmania*. The SB-83 toxicological study concluded that there were no changes in biochemical and hematological parameters, suggesting the safety of the compound. The genotoxicity was analyzed via micronucleus tests, which assesses the ability of a substance to cause clastogenicity and aneugenicity. The results showed that SB-83 did not cause an increase in the number of micronuclei in the peripheral blood of the treated animals, suggesting absence of genotoxic effect.

Aguilera *et al.* evaluated the potential of a new class of steroids against *L. infantum* and

L. amazonensis.[53] The authors synthesized and chemically characterized a series of nineteen steroidal arylideneketones and thiazolidenehydrazines and explored biological activity against *L. infantum* and *L. amazonensis* (both *in vitro* and *in vivo)*, in addition to analyzing the genotoxicity and acute toxicity *in vitro* and in mice. The data showed that compound 8 (ID 1260, a steroidal thiosemicarbazone compound) (S10) was most active, with an IC_{50} of less than 200 nM in human macrophages infected with *L. infantum*. In the genotoxicity test, compound 8 used in a single dose of 150 mg/kg did not induce chromatin damage in bone marrow cells, thus not presenting toxicity.

Recently, Drugs for Neglected Diseases initiative (DNDi) reported three classes of lead-derived chemicals: benzoxaboroles (S11), aminopyrazoles (S12) and nitroimidazoles (S13, S14, and S15), which have great antileishmanial activity. These compounds have shown favorable pharmacokinetic profiles ensuring bioavailability after oral administration and high levels of activity against murine visceral leishmaniasis.[54] Van Bocxlaer *et al.* evaluated the effectiveness of these compounds in the *in vitro* and *in vivo* models for cutaneous leishmaniasis.[55] The *in vitro* activity of the compounds was evaluated against intracellular amastigote forms of *L. major*, *L. aethiopica*, *L. amazonensis*, *L. panamensis*, *L. mexicana and L. tropica*. Drugs that demonstrated potent activity ($EC_{50} < 5$ μM) against at least 4 of 6 species were evaluated *in vivo* in mouse models. The animals received treatment for 5 or 10 days with oral or topical formulations. Due to the cutaneous form of the disease, the efficacy was expressed by the size of the lesion and the parasitic load. The results of this study showed that all the drugs tested showed marked levels of potency against the species of the Old and New World. In the *in vitro* peritoneal assay, the aminopyrazoles showed the most potent antileishmanial activity with EC_{50} values in the nanomolar range (like amphotericin B). It is noteworthy that 3 nitroimidazoles, 1 benzoxaborole and 3 aminopyrazoles showed consistent and potent activity against a variety of *Leishmania* species. Moreover, all the compounds were well tolerated with no obvious signs of toxicity observed in mice after oral administration. In some cases, there was a complete reduction in the size of the lesion, that was correlated with the reduction of the parasite load and its effectiveness was dependent on the dose and duration of treatment.

Wyllie *et al.* reported GSK3186899/DDD853651 (S16), a pyrazolopyrimidine derived from trifluoropropyl sulfonamide.[56] This compound was selected as a preclinical candidate based on the general properties of the molecule (potency, efficacy in the mouse model, pharmacokinetics, and safety profile) against VL. In addition to its favorable physico-chemical and pharmacokinetic properties, it presented adequate toxicological results for its use in clinical trials. Detailed studies on the action indicated that the compounds in this series act mainly by inhibiting the parasite cdc-2 related kinase 12 (CRK12), that is essential in the cell cycle of the parasite,[57] thus is also a potential pharmacological target for visceral leishmaniasis. Treatment with this compound resulted in the accumulation of parasitic cells in the G1 and G2 phases of the cell cycle together with a decrease in the proportion of cells in the S phase, suggesting a cell cycle arrest in the G1/S and G2 phases.[56] In another study carried out by the same research group, the compound GSK3494245/DDD01305143 (S17) (belonging to the same series), also demonstrated potential to treat leishmaniasis. The compound was shown to have promising pharmacokinetic properties and *in vivo* efficacy in the mouse model when compared to miltefosine. Furthermore, it was confirmed that it worked mainly by inhibiting chymotrypsin-like activity catalyzed by the β5 subunit of the *L. donovani* proteasome.[58]

During a study to discover anti-tuberculosis agents, Thompson *et al.* discovered a new class of 7-substituted oxazines, with potent antileishmanial activity.[59] After identification and susceptible efficacy tests, seeking better solubility and safety, the bioisosteres formed by replacing a phenyl with pyridine or pyrimidine showed improved solubility and potency, while more hydrophilic lateral chains reduced the activity against visceral leishmaniosis. Evaluation of a representative set of nine racemic compounds in the visceral leishmaniasis (*L. donovani*) mouse model showed phenylpyridines 79 (or R form 71) and 93 (or R form 94) as the most effective, with compound 93 showing 50% inhibition at 1.56 mg/kg. In the chronic infection (*L. infantum*) hamster model, compound 71 (at 12.5 mg/kg) achieved reductions exceeding 99% in parasite burden for all target organs. Subsequent synthesis and assessment of the enantiomers of both lead compounds identified the R forms as superior, and in the case of 71, this outcome was reinforced by excellent results in the *L. infantum* hamster model and favorable pharmacokinetics data in the hamster and mouse.

4. Drug Repurposing

Currently, the need to obtain a drug more quickly and with lower cost is essential for both the pharmaceutical industry and the population. Thus, the repurposing of drugs has been gaining space within this market, allowing reduction in expenses, and at the same time optimizing the release time by health regulatory agencies.[60] The reuse of drugs is neither uncommon nor less profitable for companies and, given the global health situation, it presents a reliable and safe option to design an efficient therapy.[61] In the United States, for example, drug reuse accounted for about 30% of drug approvals in the recent years.[62] Despite having some limitations, the reuse of clinical trials represent a good strategy, because it facilitates the reuse of drugs already approved, thus reducing the time and bureaucracy to reach the market.[63] Drug repurposing helps to develop new therapies with drugs that are already used clinically and whose tests with patients are already known.[64]

Using this strategy, Rodrigues *et al.* demonstrated that clomipramine (S18) had potential against *L. amazonensis*.[65] This medication is a tricyclic antidepressant used to treat psychiatric disorders, such as obsessive-compulsive disorder.[66] In this study, the parasites were incubated in the presence or absence of different concentrations of clomipramine for 72 h. The results demonstrated that the antidepressant was a selective inhibitor of the extracellular and intracellular forms of the parasite, with IC_{50} of 8.31 ± 3.29 μM and IC_{90} of 21.58 ± 3.44 μM against promastigotes. There was also greater selectivity against parasites than host cells, with a selectivity index of 11.72 for amastigotes and 21.81 for promastigotes. Clomipramine has been reported to act via mitochondrial pathway in *L. amazonensis*, in addition to inhibiting the function of the trypanothione reductase.[67] In this study, Rodrigues *et al.* showed that both in promastigotes and amastigotes, the drug induced oxidative stress by increasing the levels of reactive oxygen species and H_2O_2.[65] In promastigotes, the reduction in cell size, membrane shrinkage, DNA fragmentation and exposure to phosphatidylserine, suggests cell death via apoptosis. Despite changes in mitochondrial physiology, ATP levels were not affected in promastigotes and amastigotes, and no loss of membrane integrity was observed.

Bemani *et al.* evaluated amiodarone (S19) (a antiarrhythmic class III drug) against *L. major*.[68] The authors reported good potential in both *in vitro* and *in vivo* analyzes, through tests related to wound healing, immune response, activities of antioxidant enzymes and malondialdehyde (MDA), ferric reducing ability of plasma (FRAP) and adiponectin levels in infected mice. An IC_{50} of 1 μM (promastigotes) and 0.7 μM (amastigotes) was observed. There was a decrease in the size of lesions treated with amiodarone (40 mg/kg) after 12 days. The number of macrophages and neutrophils were lower in the lesions of animals treated with the drug, so that they demonstrated adequate formation of granulation tissue, well vascularized and infiltrated by fibroblasts and mature fibrocytes, different from untreated groups that presented necrosis, infiltration by polymorphonuclear cells, macrophages, and lymphocytes, as well as small amounts of fibrous connective tissue with few newly regenerated blood vessels. Greater angiogenesis was observed in lesions treated with amiodarone, probably due to the decrease of levels of tumor necrosis factor-α (TNF-α) and interleukin cytokines such as IL-6. Although the presence of TNF-α has an advantage over the host's defense mechanism against pathogens, an excessive release results in impaired healing. Moreover, it is necessary to have a balance in the release of IL-6, since this interleukin mediates the transition from acute to chronic inflammation. Despite the potential of amiodarone, total wound healing was not observed. The parasitic load decreased after 28 days of treatment. There was no significant increase in adiponectin and MDA, important in the wound healing process. Amiodarone caused the formation of autophagosomes, and the presence of lipid bodies and vacuoles in the cytoplasm could be observed. However, the long duration or the administration of high doses of amiodarone have been reported to cause toxicity in several organs.[69–73]

Morais-Teixeira *et al.* evaluated fexinidazole (S20) *in vitro* and *in vivo* studies against *L. infantum*, *L. amazonensis*, *L. braziliensis* and *L. guyanensis*.[74] Fexinidazole is a nitroimidazole that was developed in the 1980s against trypanosomes. The authors observed that after 23 days of oral treatment with fexinidazole (200 and 300 mg/kg/day), 80% of the animals infected with *L. amazonensis* presented complete healing of the lesion. On the other hand, 100% of the animals infected with *L. guyanensis* and treated orally with fexinidazole (200 mg/kg/day) presented a completely healed lesion with 98.4% reduction in the alterations observed in the liver and no significant loss of animal body weight. Fexinidazole thus presented good potential without toxicity in spite of high doses required for the treatment in the animal model.

5. Clinical Trials

Leishmaniasis is an important neglected tropical disease, despite it being one of the six most frequent parasitic diseases in the world. Very few advances have been made for its treatment, with the same pharmacological treatments in use since 1940s. The drugs currently in use for the treatment are associated with low therapeutic indices and significant side effects.[75] Thus, the search for more effective therapies that present fewer side effects for the treatment of leishmaniasis is necessary.

Vast majority of clinical studies in process involve improving the efficacy of drugs already in use for the treatment of leishmaniasis such as pentavalent antimonials (sodium sybogluconate and meglumine antimoniate), amphotericin B deoxycholate, lipid formulations of amphotericin B (e.g., AmBisome@, ABLC, Amphocil@), miltefosine, paromycin and pentamidine,[76] and are related, more specifically, to improve the dose regimen. There are very few potential new drugs in the clinical phase for the treatment of leishmaniasis.

Four drugs (DNDI-0690, DNDI-6148, LXE408 and GSK3494245) in which preclinical development has already been completed have started the clinical studies since 2019. DNDI-0690 belongs to the class of nitroimidazole (a class known for its broad spectrum of antiparasitic properties) which was developed from fexinidazole (structural improvement).[77,78] The preclinical development of DNDI-0690 (S13) has already been completed after observing that it is highly effective in mice and hamsters VL models.[79,80] Its nitro group is believed to be bioactivated by the enzyme nitroreductase NTR2 in *Leishmania* parasites,[81] leading to reactive intermediates that kill the parasite. Under the title "Single Oral Dose Escalation Study of DNDI-0690 in Healthy Subjects", the project was approved for the phase I clinical studies (NIH, 2019; identifying number: NCT03929016).

Another drug in clinical trial for the treatment of *Leishmania* is DNDI-6148 (S11) belonging to the class of oxaborole. It was observed that after 5 to 10 days of treatment, this drug could reduce high levels of parasitic load in VL mice and hamster models. Its mechanism of action is not yet well understood, but it is thought to be distinct from the antileishmanial therapies currently used, since it maintains activity against strains resistant to these drugs. The pharmacological safety studies for this drug have already been completed.[80,82,83] DNDI-6148 was approved at the end of 2018 for phase I clinical studies, with the objective to evaluate its safety and tolerance in increasing single doses (10 mg, 20 mg, 40 mg, 80 mg, 160 mg, 260 mg, 380 mg, and 500 mg) in healthy male volunteers compared to the placebo group. In addition, the phase I study is also being used to study the pharmacokinetic and pharmacodynamic properties of DNDI-6148.[84]

Nagle *et al.* showed that the chemical optimization of GNF6702 successfully led to the compound LXE408 (S21) which, when administered orally, demonstrated excellent efficacy in mice model with a good safety profile.[85] Based on these positive results, LXE408 was approved for clinical trials. In February 2020, the Novartis pharmaceutical industry and the Drugs for Neglected Diseases initiative (DNDi), a non-profit research and development (R&D) organization signed a collaboration and license agreement to jointly develop the LXE408. In this agreement, Novartis was responsible for completing phase I clinical trials. While DNDi was responsible for Phase II and Phase III trials, with the first Phase II study scheduled to begin in early 2021 in India, with additional testing planned to take place in East Africa.[86]

GSK3494245 (S17), which was initially identified as a proteasome inhibitor, is a candidate for the treatment of leishmaniasis due to its potential preclinical leishmanicidal effect, in addition to demonstrating a desirable safety profile and pharmacokinetics.[87] The phase I clinical study titled "Safety, Tolerability and Pharmacokinetics Investigation of GSK3494245 in Healthy Participants" began in September 2020 is a randomized, double-blind, placebo-controlled study to evaluate in humans the safety, tolerability and pharmacokinetics of single doses of GSK3494245 in healthy participants, and consists of 3 cohorts (cohorts 1, 2 and 3), conducted in sequential order.[88] *Table 1* summarizes the novel molecules described in this study. Despite efforts, the number of drugs reaching human trials is still extremely low.

6. Future Perspectives

Currently, there is no fully effective therapy for leishmaniasis. Treatment is based on chemotherapy; however, most drugs are expensive and have toxic side effects. Although advances are limited, the scientific community has been searching for new strategies and compounds that have little or no toxic effect in relation to the current treatment, such as the use of immunotherapies, nanoparticles, vaccines, and substances for topical use (*Figure 4*). The development

Table 1. Recent pre-clinical and clinical studies, described for leishmaniasis.

Drug	Tested Species	Study Phase	Refs.
Thiazopyridine (TP) Thiazoacetylpyridine (TAP)	*L. infantum*	Pre-clinical (*in vitro*)	[26]
trans-dibenzalacetone (DBA)	*L. infantum* *L. amazonensis* *L. chagasi*	Pre-clinical (*in vitro*)	[27]
Imiquimod	*L. tropica* *L. major*	Pre-clinical (*in vitro*)	[89]
Ursolic acid (UA)	*L. amazonensis* *L. infantum*	Pre-clinical (*in vivo*)	[40]
Chloroquinoline derivative (GF1059)	*L. infantum* *L. amazonensis*	Pre-clinical (*in vivo*)	[43]
Ruthenium nitrosyl complex (RuNO)	*L. brasiliensis*	Pre-clinical (*in vitro* and *in vivo)*	[41]
SB-83 (a 2-amino- thiophene derivative)	*L. amazonenses*	Pre-clinical (*in vivo*)	[51]
Steroidal arylideneketones and thiazolidenehydrazines	*L. infantum* *L. amazonenses*	Pre-clinical (*in vitro* and *in vivo)*	[53]
Benzoxaboroles, Aminopyrazoles and Nitroimidazoles	*L. major* *L. aethiopica* *L. amazonensis* *L. panamensis* *L. mexicana* *L. tropica*	Pre-clinical (*in vitro* and *in vivo)*	[80]
DDD853651/GSK3186899	*L. donovani*	Pre-clinical (*in vivo*)	[56]
6-nitro-2,3-dihydroimidazo-[2,1-*b*][1,3]-thiazoles and related -oxazoles	*L. donovani* *L. infantum*	Pre-clinical (*in vitro*)	[59]
Clomipramine	*L. amazonensis*	Pre-clinical (*in vivo*)	[51]
Voriconazole	*L. major*	Pre-clinical (*in vitro* and *in vivo)*	[90]
Fexinidazole	*L. chagasi* *L. amazonensis* *L. braziliensis* *L. guyanensis*	Pre-clinical (*in vitro* and *in vivo)*	[74]
DNDI-0690	*L. mexicana* *L. major*	Phase 1 clinical trials	[80]
DNDI-6148	*L. major* *L. donovani*	Phase 1 clinical trials	[84]
LXE408	*L. donovani*	Phase 1 has been completed	[85]
GSK3494245	*L. donovani*	Phase 1 clinical trials	[87]

process normally occurs through the Target Product Profile, a planning tool that describes the result of research and development of desired drugs and allows the selection, progression, and management according to well-defined decision matrices. Accordingly, some parameters are defined for the choice of the Pre-clinical Target Candidate Profile, in which the physico-chemical, pharmacokinetic, safety and formulation properties of the selected compound are described for pre-clinical development, functioning as a prologue for clinical studies.

Studies have highlighted the use of nanoparticles to reduce drug resistance. The use of drug liberation systems based on nanotechnology, loaded with anti-leishmania agents, can protect the drugs against oxidation reduction and enzymatic reactions, increase bioavailability through controlled drug release and increase efficacy. The scientific community has also investigated the drug-immunotherapy relationship to optimize the treatment of leishmaniasis. Despite promising results, this treatment cannot be recommended due to its high cost. Topical therapies have proven to be less toxic option compared to systemic options, however, limited to less severe forms of treatment, without the risk of dissemination and development of the disease. These methods are recommended for individuals with few and small injuries, more effective in infections by *L. mexicana* or *L. major*. Synergistic drug combinations are another option for a more effective treatment, as combinatorial therapies are also relatively cheaper and present a minimal risk to patients due to the access to previous

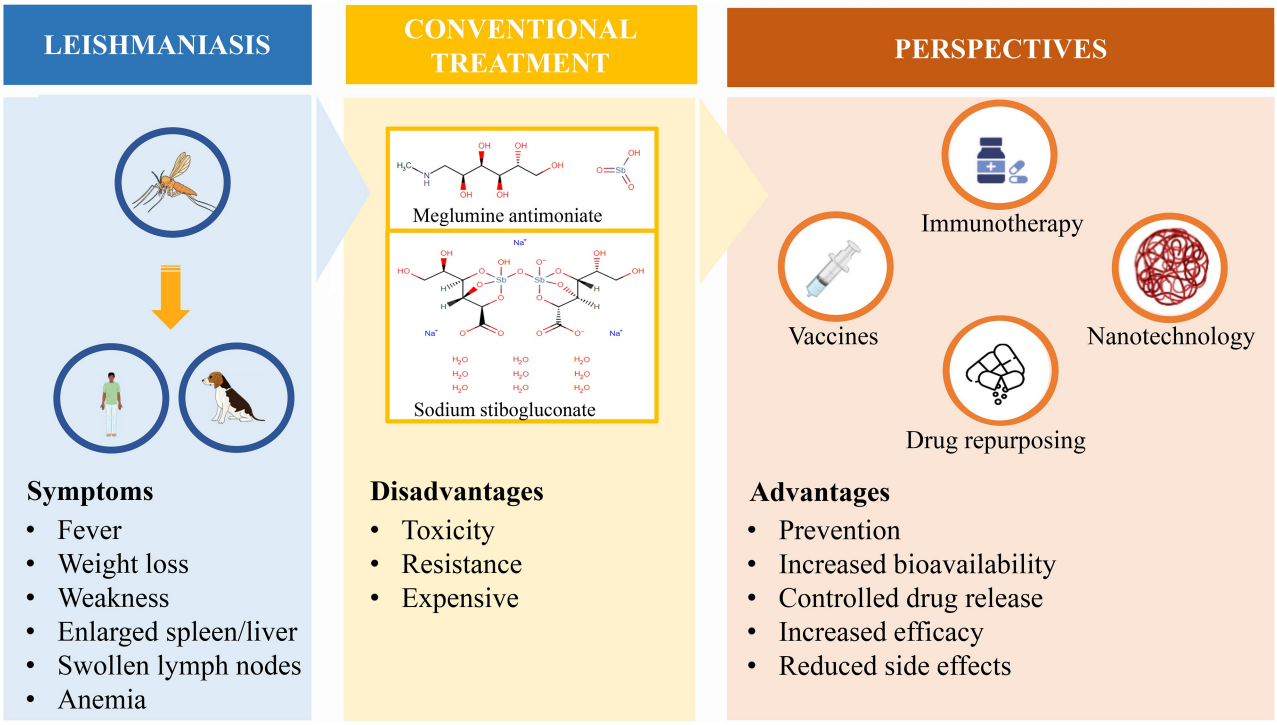

Figure 4. New strategies to overcome the disadvantages associated with the current treatment.

pharmacological, safety and toxicological data. Vaccines have shown excellent results, especially in prevention, however, they are not widely researched and developed. It is believed that its commercialization is less lucrative, when compared to the development of drugs, making research inclined to immunotherapeutic formulations. It is also worth to mention that, due to the complexity of the immune responses necessary for protection against *Leishmania*, the development of vaccines has been a challenging task. Finally, the control of this disease is done exclusively with medications and follows the therapeutic protocol of each country, respecting its regionality and the guidelines of the World Health Organization. The scarcity of new anti-leishmania agents highlights the need to search for newer molecules with anti-leishmania activity, mainly due to the emergence of strains resistant, complications created due to co-infections and toxicity shown by the conventional drugs. Thus, although there have been countless advances, treatment against leishmaniasis is still considered a challenge and the search for ideal treatments continue.

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Author Contribution Statement

WS, SSCO, MHR, ALSS and SSD contributed for the conceptualization, methodology, validation, formal analysis, and investigation, and writing – original draft preparation. EBS, PS and SJ contributed for the methodology, supervision, writing – review and editing, project administration, resources, and funding acquisition. All authors have made a substantial contribution to the work. All authors have read and agreed to the published version of the manuscript.

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