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Leishmaniasis treatment: update of possibilities for drug repurposing

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1. ABSTRACT

The leishmaniases represent a public health problem in under-developed countries and are considered a neglected disease by the World Health Organization (WHO). They are cuased by *Leishmania* parasites with different clinical manifestations. Currently, there is no vaccine, and treatment is inefficient and is associated with both serious side effects often leading to resistance to the parasites. Thus, it is essential to search for new treatment strategies, such as drug repurposing, i.e., the use of drugs that are already used for other diseases. The discovery of new clinical applications for approved drugs is strategic for lowering the cost of drug discovery since human toxicity assays are already conducted. Here, we review a broad analysis of the different aspects of this approach for anti-leishmanial treatment.

2. INTRODUCTION

Drug repurposing (or repositioning) is a strategy within the drug development discovery process employed to find new uses for existing drugs. This process has gained momentum in the past several years, mainly due to an increase in popularity and the influence of the pharmaceutical industry (1,2). Drug repositioning offers a possibility to bypass a dilemma faced by Big Pharma in the drug discovery process. This dilemma involves a productivity gap (discovery of a new chemical entity – NCE), despite the increasing research and investment in of new technologies, such as high-throughput screening (HTS), high-content screening (HCS), and combinatorial chemistry. Often, drug repurposing reduces the risk associated with development, which multiplies throughout the various stages of preclinical and clinical development, because pharmacokinetic and safety profiles have been already determined (3,4) (Figure 1). In recent years, drug repurposing was responsible for 30% of the new drugs and vaccines approved by the Food and Drug Administration (5). Interestingly, drug repurposing may extend the patent period of a drug from three to five years (6). The cost savings of repositioning an approved drug makes it an attractive strategy for neglected tropical diseases

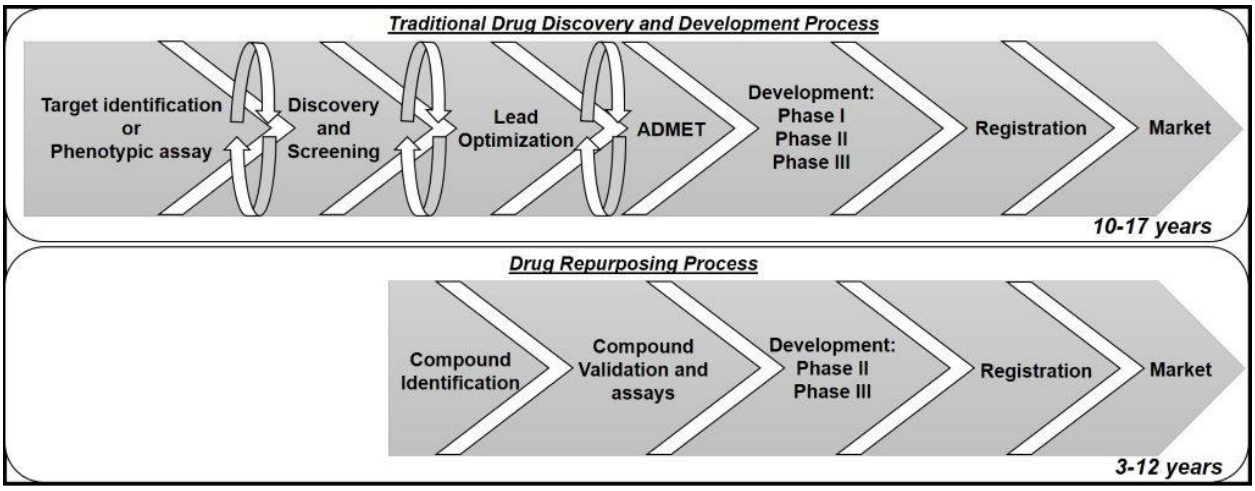

Figure 1. Traditional Drug Discovery and Development process x Drug Repurposing Process.

because of the limited research and development resources in this area. Thus, there have been a number of successful repurposing cases, mainly for the leishmaniases (e.g., pentamidine, amphotericin B, miltefosine and paromomycin were repositioned from other indications) (Figure 2) (7). In this review, different studies of the effectiveness of FDA-approved drugs on leishmaniases will be discussed.

3. LEISHMANIASES

The leishmaniases are a parasitic infectious disease caused by different species (20 species are considered pathogenic for humans) of the genus *Leishmania* and transmitted by *Phlebotomus* and *Lutzomyia* sandflies. The insect vector capable of transmitting the disease to different mammals (including humans) is *Phlebotomus* in the Old World (Europe, Asia and Africa) and *Lutzomyia* in the New World (Americas); in all, there are 30 species capable of transmitting the disease (8,9). The disease affects approximately 300 million people, causes 20 to 30 thousand deaths annually and occurs in 98 countries. Although the leishmaniases have very large geographical coverage, 7 countries account for 90% of the new cases: Bangladesh, Brazil, India, Ethiopia, Kenya, Nepal and Sudan. The number of new cases of the disease is uncertain; however, it is possible estimate an increase of 200,000–400,000 new cases of visceral leishmaniasis (VL) and 700,000–1,200,000 new cases of cutaneous leishmaniasis (10).

3.1. Clinical manifestations

The clinical forms of the disease, i.e., cutaneous, diffuse, disseminated, mucocutaneous and visceral, are categorized by parasite species and the immunological response of patients. ***Localized cutaneous leishmaniasis*** is the most common form found in patients with tegumentary leishmaniasis and is characterized by one or more erythematous papules in the skin (11,12). ***Diffuse cutaneous leishmaniasis***, also called anergic, presents multiple cutaneous lesions without a tendency to form ulcers and with typical lepromatous lesions. With chronic infections, the lesions have abundant parasites and persist for 20 years or more. ***Disseminated cutaneous leishmaniasis*** is characterized by multiple ulcerative or acneiform skin lesions and by a strongly positive leishmanin skin test (13–15). ***Mucocutaneous leishmaniasis*** is more common in the New World, with almost no cases found in the Old World. This clinical form usually occurs after the cutaneous manifestation with destruction of the mucosa and can progress until the destruction of cartilage, leading to the patient's respiratory compromise (16,17). ***Visceral leishmaniasis***, also known as kala-azar, is present both in the New World and in the Old World. This is the most serious form of the disease and can lead to death. There are patients who are asymptomatic, but in general, the symptoms are fever, anorexia, diarrhea, hepatosplenomegaly, enlarged lymph nodes and vascular problems (11). The post-kala-azar form, which occurs only in the Old World, is a complication of the treatment of visceral leishmaniasis. It is characterized by the presence of small nodules on the face and body without pigmentation (18). *Leishmania* species that are pathogenic to humans and their clinical manifestations are listed in Table 1, according to Sereno and collaborators (8).

3.2. Treatment

Currently, the first line of drugs used in the treatment of the leishmaniases is the pentavalent antimonials. In 1912, the physician Gaspar Vianna, after observing the effect of trivalent antimony (tartar emetic) on certain trypanosomes, decided

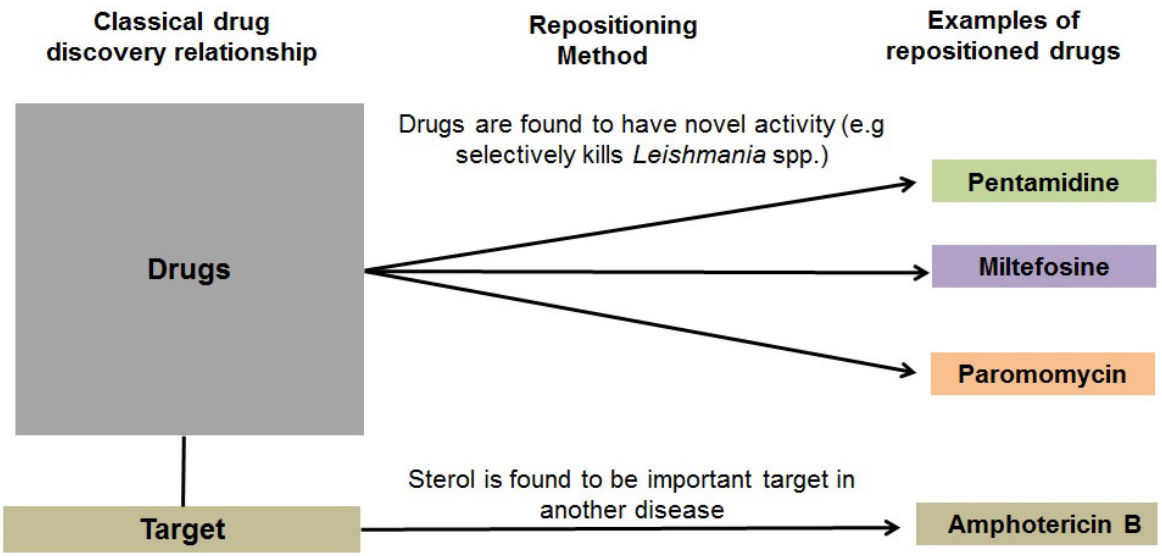

Figure 2. Examples of repositioned drugs for Leishmaniases.

Table 1. Species pathogenic to human, occurrence and clinical manifestation

Complex	Species	Ocurrence	Clinical manifestation
Viannia	*L. shawi*	NW[1] / OW[2]	CL[3]
	L. peruviana	NW[1]	CL[3], MCL[4]
	L. panamensis	NW[1]	CL[3], MCL[4]
	L. lindenbergi	NW[1]	CL[3]
	L. naiffi	NW[1]	CL[3]
	L. braziliensis	NW[1]	CL[3], MCL[4]
	L. guyanensis	NW[1]	CL[3], MCL[4]
	L. lainsoni	NW[1]	CL[3]
Leishmania	*L. venezuelensis*	NW[1]	CL[3]
	L. tropica	OW[2]	CL[3], VL[5]
	L. mexicana	NW[1]	CL[3], DCL[6]
	L. major	OW[2]	CL[3]
	L. infantum	NW[1] / OW[2]	VL[5], CL[3]
	L. donovani	OW[2]	VL[5], PKDL[7]
	L. amazonensis	NW[1]	CL[3], DCL[6], MCL[4], VL
	L. aethiopica	OW[2]	CL[3], DCL[6]
Enrietti	*L. martiniquensis*	NW[1]/ OW[2]	CL[3], VL[5]
	L. siamensis	NW[1] / OW[2]	VL[5], CL[3]
Paraleishmania	*L. colombiensis*	NW[1]	CL[3], VL[5]

[1]New World, [2]Old World, [3]Cutaneous leishmaniasis, [4]Mucocutaneous leishmaniasis, [5]Visceral leishmaniases, [6]Difuse cutaneous leishmaniasis, [7]Post-kala-azar dermal leishmaniasis

to reposition this compound for the treatment of cutaneous leishmaniasis cases diagnosed in São Paulo, Brazil (19). The development of pentavalent antimonials enabled a reduction in treatment toxicity (20). The formulations of the pentavalent antimonials commercially available, sodium stibogluconate (Pentostam®) and N-methyl glucamine antimoniate (Glucantime®) (Figure 3), have been used for the treatment of tegumentary leishmaniasis and visceral leishmaniasis (21). The pentavalent antimonials are registered and licensed in Southeast Asia, Latin America, and some Mediterranean and African countries (22).

In addition, other drugs considered as a second choice have been used, such as amphotericin B, pentamidine, miltefosine and paromomycin (23) (Figure 3). Amphotericin B, an antifungal, has been

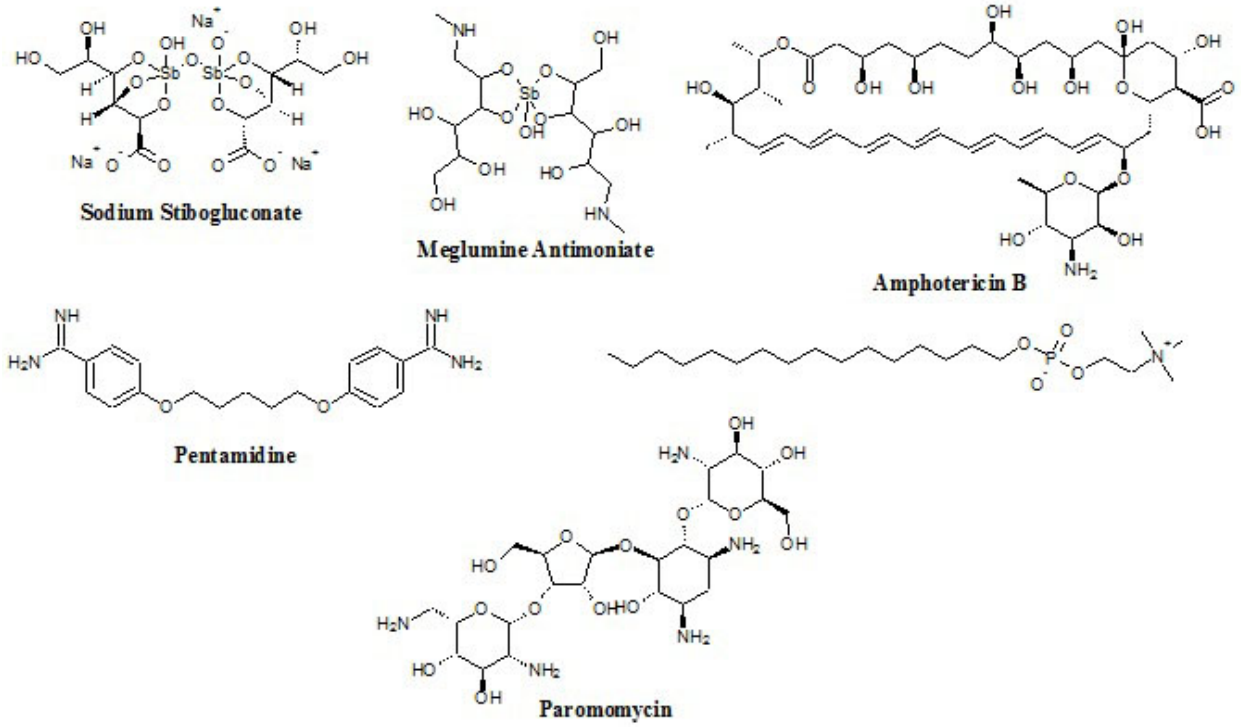

Figure 3. Approved drugs for Leishmaniases.

used as an alternative treatment for leishmaniases but has serious side effects. The liposomal formulation of amphotericin B made the drug less toxic and more effective, despite the high cost, and is the most used formulation for the treatment of visceral leishmaniasis (24). This formulation is licensed to use in India, USA, and Europe for treatment of the visceral form and is used as a second-line drug for the treatment of VL in East Africa and Brazil (22).

Pentamidine, a synthetic derivative of diamidine discovered while searching for hypoglycemic drugs, is used in cases of antimonial resistance as an alternative way of treating the leishmaniases; however, its side effects, which include cardiotoxic and metabolic disorders (diabetes) and cases of poor efficacy and resistance, have made this drug a poor option for the treatment of cutaneous leishmaniasis (25).

The only drug given orally today, miltefosine, was initially developed as an anticancer drug and was used in the treatment of visceral leishmaniasis in India, with patient cure rates of approximately 94%. However, after a decade of use in the country, several cases of recurrence and decreased efficacy of treatment have been described. Despite the advantage of being administered orally, the use of miltefosine is also linked to many side effects that are primarily related to the gastrointestinal tract and occasional hepatic toxicity and nephrotoxicity, in addition to its teratogenic potential (26).

Paromomycin is a broad-spectrum amino-glycoside antibiotic first used in the treatment of leishmaniases in 2002 as paromomycin sulfate. It is used in the treatment of the two forms of the disease and is administered parenterally for visceral leishmaniasis and parenterally and topically for cutaneous leishmaniasis. There are several advantages associated with the use of paromomycin, such as low cost, better efficacy, and fewer side effects. However, there is a likelihood of acquiring resistance with paromomycin monotherapy. Although not described in the clinic, there are reports of *in vitro* resistance in *L. donovani* and *L. tropica* (21). In addition to these drugs commonly used for the treatment of leishmaniases, the World Health Organization also recommends the use of some antifungal azoles as a second choice for the treatment of the disease, but only in specific cases of cutaneous form. For example, the use of fluconazole is recommended for systemic oral therapy of *L. major* infections in the Old World, while ketoconazole is recommended for the systemic treatment of *L. mexicana* infections in adults (27).

4. DRUG REPURPOSING STRATEGIES

Research into new uses for existing drugs is supported by two fundamental scientific concepts: finding new uses of a drug acting through the originally known target (“on-target”) or finding new uses of a drug acting through a novel or unanticipated target (“off-target”) (1, 5, 28). Target-based screening and

phenotypic screening are the two main approaches used in drug discovery, development and repositioning. In fact, the contribution of phenotypic screening to drug discovery has been higher than that of target-based screening (29). Recent partnerships between industry and academia through, for example, high-throughput studies with drug libraries, have brought about a range of advantages and a hope for neglected diseases because the support from organizations such as the Drug for Neglected Diseases initiative (DND*i*), Big Pharma and open access chemical libraries (30–32). HTS campaigns, such as the one published by Peña and coworkers (1.8 million compounds), generate extensive information about compounds of different chemical classes with new biological activities against *L. donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei*; specifically, approximately 200 compounds with activity against each parasite were selected based on the criteria of potency and selectivity (33). However, following this strategy with "premature repositioning" bias, Kaiser and coworkers evaluated 200 drug-like and 200 probe-like compounds from the malaria box library against *L. donovani, L. infantum, T. cruzi* and *T. brucei*, identifying 55 hits for Human African trypanosomiasis, 21 hits for Chagas disease and 8 hits for VL (34). Recently, Kaiser and colleagues evaluated 100 FDA-approved drugs using the repositioning strategy; however, the author reports that the low return on promising drug repurposing options is related to the strategy of repositioning; i.e., it is low risk and high return (35). Despite the recent high-throughput studies on the discovery of drugs for the leishmaniases and other diseases caused by trypanosomatids, the majority of studies on drug repositioning for the leishmaniases have been conducted with classic *in vitro* (promastigote, axenic amastigote and intracellular amastigote) and *in vivo* assays. In this work, a review of studies on the repositioning possibilities of FDA-approved drugs is presented.

4.1. New drug repositioning candidates

4.1.1. Antifungals

Antifungal drugs have been widely used in antileishmanial activity assays, achieving substantial success in experimental animal models (Figure 4). The first class of antifungals described is the polyenic antibiotics. Amphotericin B, a drug belonging to this class, is licensed for the treatment of the leishmaniases. Another polyene drug is nystatin, whose antileishmanial activity was first reported in 1962 against *L. donovani*. Nystatin demonstrated antipromastigote activity against *L. major* with an IC_{50} value of 9.76 µg/mL, inhibiting the entry of promastigotes into macrophages due to the sequestration of macrophage cholesterol and thus indicating the essential role of cholesterol in mediating the entry of the parasite (36–38).

The current treatments of the leishmaniases advocated by the WHO includes the use of some azole drugs as a second choice in the treatment of cutaneous leishmaniasis. However, there are many studies in which several azoles have been developed to treat experimental leishmaniases. Itraconazole and posaconazole were effective against promastigotes and amastigotes of *L. amazonensis*, with IC_{50} values lower than 3 µM (39). Clotrimazole showed antileishmanial activity against promastigotes of *L. infantum*. An organometallic association between ruthenium and clotrimazole resulted in an increase in the antileishmanial activity *in vitro* compared to clotrimazole alone and demonstrated significant efficacy in *in vivo* treatment in the murine model of cutaneous leishmaniasis (40,41). Another azole drug, bifonazole, showed antileishmanial activity in *L. infantum* promastigotes, with an IC_{50} value of 8.97 µM. Econazole also showed antileishmanial activity in promastigotes and intracellular amastigotes of *L. infantum*, and voriconazole showed activity against different species: *L. donovani*, *L. amazonensis* and *L. major* (42,43). The antileishmanial activity of azoles can be influenced by the availability of an exogenous cholesterol source to the parasite. Parasites of *L. amazonensis* treated with ketoconazole and miconazole had decreased ergosterol synthesis accompanied by the accumulation of exogenous cholesterol, compensating for the low levels of endogenous sterols. When the parasites were incubated with delipidated fetal bovine serum, i.e., no availability of exogenous cholesterol, there was a 50% reduction in the IC_{50} values of ketoconazole and miconazole compared to the IC_{50} values of the parasites incubated with complete fetal bovine serum, indicating that this compensatory mechanism of exogenous cholesterol uptake may be a good pharmacological target (44).

Another class of antifungals, the allylamines, is composed of synthetic naphthalene derivatives. Two drugs of this class have been investigated for their antileishmanial activity: butenafine and terbinafine. A recent study showed that butenafine was able to eliminate promastigotes of *L. amazonensis* and *L. braziliensis* with the same efficacy as the reference drug, miltefosine (45). Many studies have also shown the antileishmanial activity of terbinafine. One of the early studies showed that the combination of terbinafine and another antifungal, ketoconazole, produced additive effects on the axenic growth of promastigotes and synergistic effects on the proliferation of intracellular amastigotes of *L. amazonensis*. When treated with terbinafine, a strain of *L. braziliensis* resistant to ketoconazole became more sensitive to azoles since terbinafine interfered in the synthesis of membrane sterols and treatment led to loss of cell viability. Treatment with terbinafine resulted in a decrease in the proliferation of *L. major* promastigotes, but terbinafine

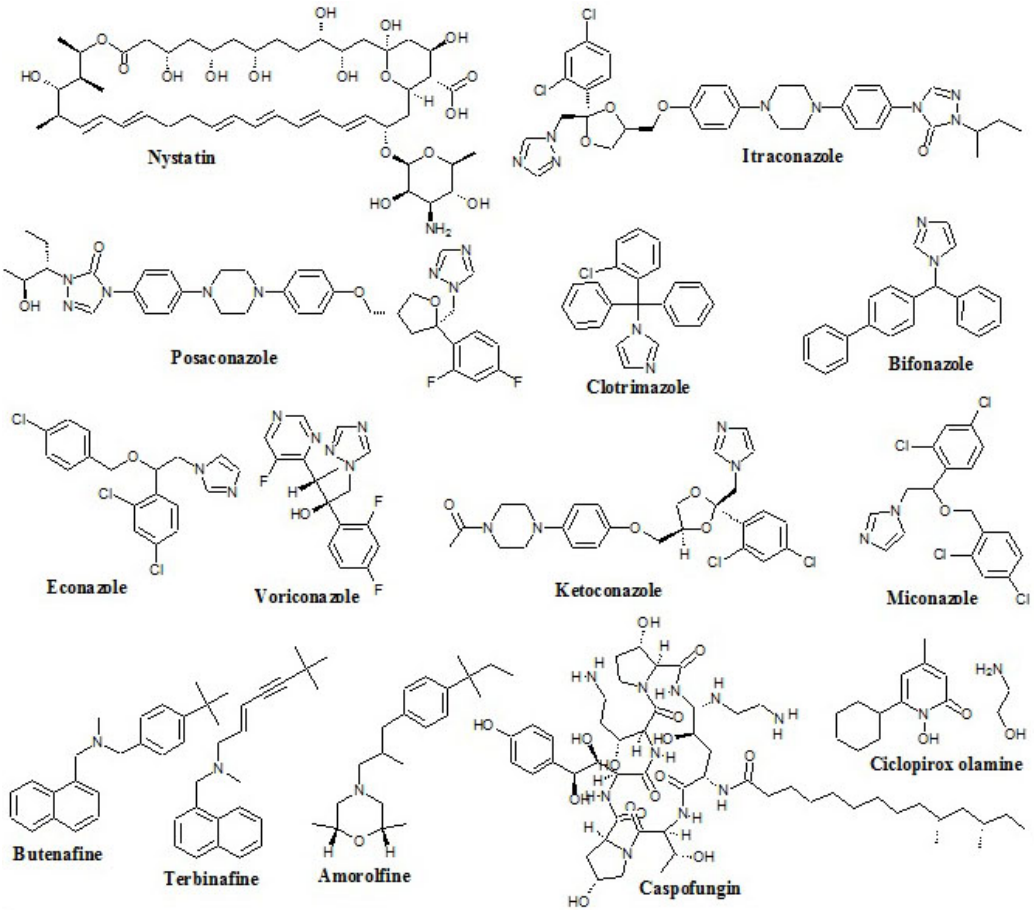

Figure 4. Antifungal drugs

did not induce any inhibitory effect on the proliferation of *L. donovani* or *L. mexicana* promastigotes (46,47).

A controversial result was observed in experimental models of cutaneous leishmaniasis, the treatment with terbinafine of *L. major*-innfected BALB/c mice was effective in reducing lesions, while in the model *L. amazonensis*-infected C57Bl/6 mice, this drug led to no significant reduction of both lesion size or parasite load when compared to the untreated control group (48,49). In the *in vivo* models of visceral infection, treatment of hamsters infected with *L. infantum chagasi* with terbinafine did not cause a difference in weight or spleen parasite load compared to the untreated control, with a significant decrease only observed when the drug was associated with glucantime (50,51).

Studies using other antifungals have been performed; for example, caspofungin, a cyclic lipo-hexapeptide, has been reported to be active against promastigotes of *L. tropica* (52). Amorolfine, which belongs to the morpholine class and is an inhibitor of delta-14-reductase and delta-8-delta-7-isomerase, was tested on intracellular amastigotes of *L. donovani* and demonstrated an IC_{50} lower than the standard antileishmanial drugs sodium stibogluconate and meglumine antimoniate (53). Ciclopirox olamine, a hydroxypyridone and an inhibitor of the enzyme deoxyhypusine hydroxylase, was tested on *L. donovani*, exhibiting an IC_{50} value of 2.14 μM in promastigotes and an IC_{50} value of 1.09 μM in intracellular amastigotes (35).

4.1.2. Antiparasitic drugs

Several antiparasitic drugs have been repositioned for leishmaniases treatment (Figure 5), including antimalarial drugs. Artemisinin is a secondary metabolite of *Artemisia annua* and is considered as one of the reference drug in the treatment of malaria, and some studies showed its efficacy in experimental visceral leishmaniasis caused by *L. donovani*. Macrophages infected with *Leishmania* spp. amastigotes and treated with artemisinin had a lower production of nitrite and nitric oxide synthase mRNA, promoting a greater protection of the host cell (54–57).

Another antimalarial, the aminoquinoline chloroquine, has been studied in a CBA mice model

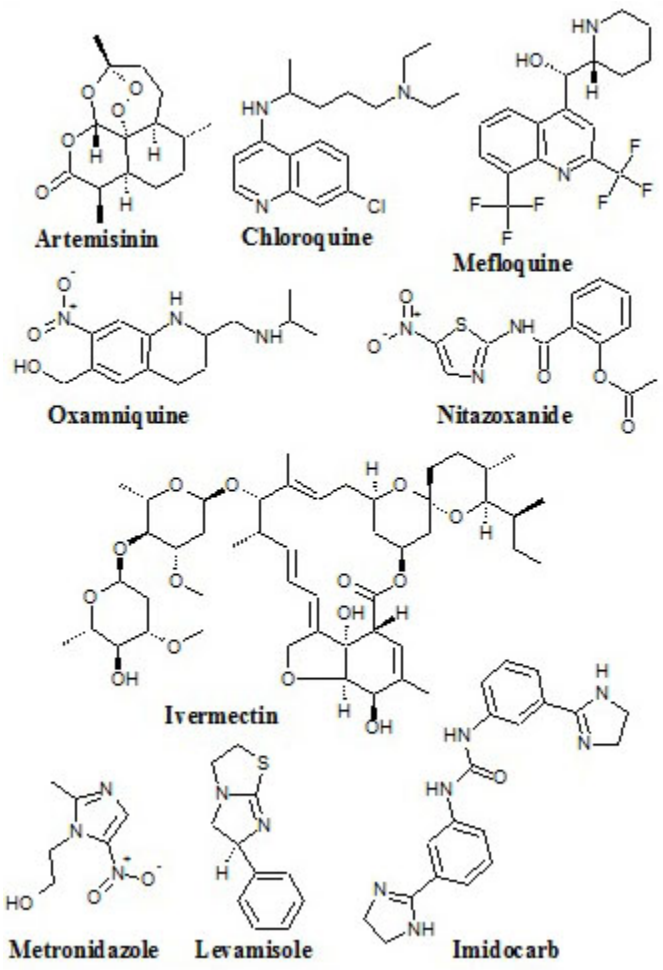

Figure 5. Antiparasitic drugs

infected with *L. amazonensis*. Treatment with chloroquine reduced the size of the lesions and significantly decrease parasitism, showing that it is a promising drug for the treatment of leishmaniases (58). The paper published by Khan *et al.* in 2007 shows that chloroquine analogues are also effective on *L. donovani* promastigotes, including resistant strains (59). Mefloquine, another aminoquinoline, has activity against promastigotes with an IC_{50} of 8.4 μM. However, the use of mefloquine in mice and in the clinic studies does not provide good results, such as non-reduction of the lesions and no effects compared to that of the placebo groups (60–63).

Brazil and Gilbert, in 1976, showed the activity of oxamniquine, used for *Schistosoma mansoni* infections, in hamsters infected with *L. braziliensis* using only 5 doses, suggesting that further studies should be conducted to investigate the action of oxamniquine against *Leishmania* spp.(64).

Ivermectin is a broad-spectrum macrolide used in the treatment of onchocerciasis, strongyloidiasis, ascaridiasis and other diseases caused by worms. In addition, its effectiveness against the promastigote forms of *Leishmania* spp. has already been demonstrated. Studies show that its analogues have specific activity against promastigote and amastigote forms of the parasite and specifically do not affect enzymes in the host (65–67).

Metronidazole is derived from nitroimidazoles and has activity against bacterial infections, such as giardiasis, trichomoniasis and amebiasis. Some studies on the treatment of experimental cutaneous leishmaniasis have demonstrated a significant reduction in lesion size and in the number of parasites via histological examination. It is also a drug without *major* side effects and with easy administration and low cost, which reinforces the idea of using this drug in the treatment of cutaneous leishmaniasis (68–70).

Nitazoxanide is a nitrothiazole that has efficacy in the treatment of giardia and cryptosporidium, and some papers have shown its leishmanicidal activity in the promastigote and amastigote forms of *L. infantum*

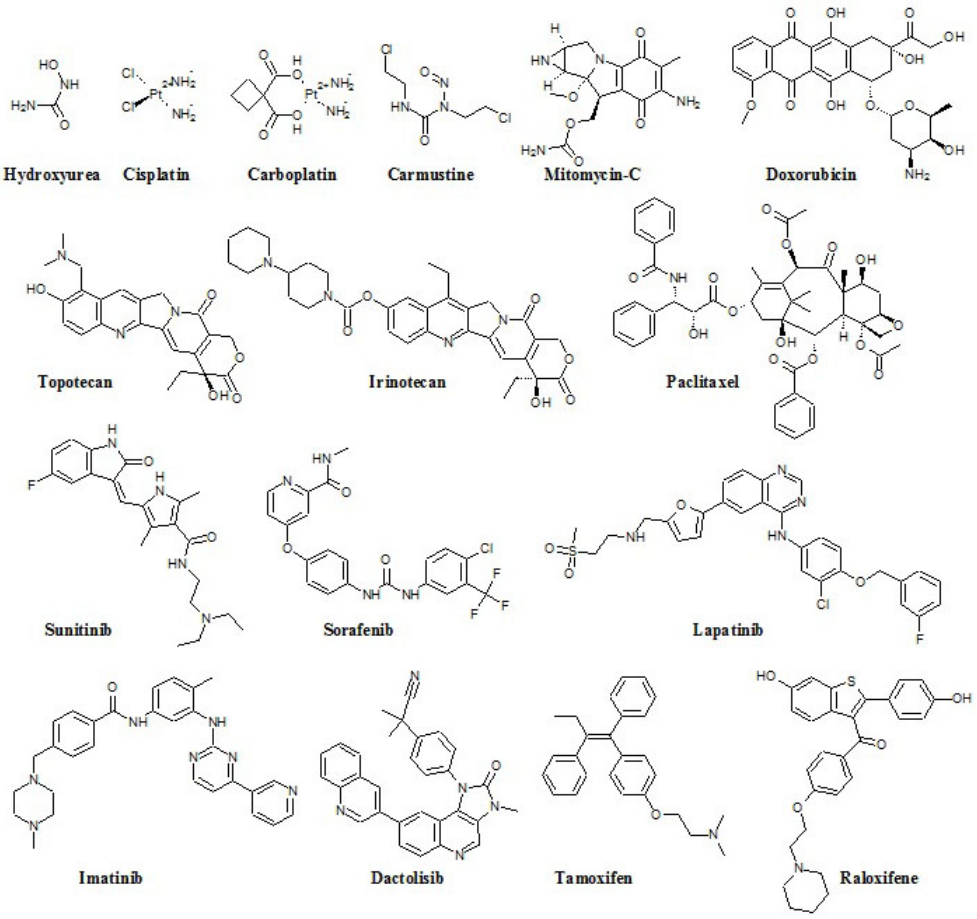

Figure 6. Anticancer drugs

and *L. donovani*. In addition, BALB/c mice infected with *L. donovani* present a significant reduction in liver and spleen sizes in relation to the control group (71,72). In 2013, Mesquita *et al*. suggested that the use of nitazoxanide together with other drugs, such as amphotericin B, miltefosine and glucantime, should be considered as an alternative to the treatment of leishmaniases (73).

Imidocarb (used in the veterinary medicine) approved to treat anaplasmosis and babesiosis was effective alone or combined with levamisole (antihelmintic drug) decrease lesions and parasite loads of *L. amazonensis*-infected BALB/c mice (74).

4.1.3. Anticancer drugs

Antitumorals (Figure 6) also have previously demonstrated the ability to eliminate parasites of the genus *Leishmania*, and the leishmanicidal activity of these drugs will be discussed.

Hydroxyurea (a carbamyl hydroxamate) is used in treatment of myeloid leukemia and melanoma and displays leishmanicidal activity, leading to the death of *L. mexicana in vitro* and inducing alterations in cell cycle by arresting the G_2/M phase in promastigotes. This drug is given orally and has advantages for a possible repositioning for the treatment of leishmaniasis (75).

Cisplatin is used in the treatment of ovarian and testicular cancer and has showed excellent leishmanicidal activity *in vitro* and *in vivo*. This antineoplastic drug induced alterations in the cell cycle in promastigotes and amastigotes with arrest of the S and G2 phases and mitochondrial membrane potential loss. In promastigotes, it also increases the levels of thiols and reactive oxygen species (76,77). Different groups have been using cisplatin in combination with antioxidants and immunomodulatory agents with promising results (78,79). In an attempt to reduce the adverse effects of cisplatin, carboplatin was developed and presented excellent activity against visceral leishmaniasis as well (80).

Carmustine and mitomycin-C, alkylating agents used in treatment of cancer, exhibited leishmanicidal activity *in vitro* against *L. donovani*, with the mechanism of action involving the inhibition of trypanothione reductase. The encapsulation of

mitomycin in nanospheres has been a novel approach used to decrease drug toxicity in the host cell and has shown promising results for repositioning strategies (81–83).

Doxorubicin, an anthraquinone used in the treatment of a wide variety of tumors with a mechanism of action on topoisomerase II and an ability to intercalate in DNA, has potent activity *in vitro* and in an experimental model of visceral leishmaniasis. The development of doxorubicin-loaded microparticles and nanocapsules showed promising results in the experimental model of visceral leishmaniasis (84–86).

Camptothecin is a quinoline and an inhibitor of topoisomerase I used in the treatment of a wide variety of tumors. The camptothecin derivatives topotecan and irinotecan were tested against promastigotes and intracellular amastigotes of *L. infantum*, and only the first drug had activity (87).

Paclitaxel is a tetracyclic diterpene used in the treatment of ovarian, breast and lung cancer. This drug leads to the death of promastigotes and intracellular amastigotes by action on tubulin and alterations in the cell cycle, arresting the G2/M phase of parasites (88,89). Sunitinib is used in the treatment of renal cell carcinoma, sorafenib in hepatocellular carcinoma and breast cancer and lapatinib in breast cancer. All these drugs were evaluated against *Leishmania* spp. and showed excellent activity *in vitro* and in experimental model of visceral leishmaniasis (90). Imatinib is used for the treatment of chronic myeloid leukemia, which inhibits the Abl/Arg kinase, a family of enzymes involved in endocytosis. This drug decreased *in vitro* infection of *L. amazonensis*, indicating that this enzyme is involved in phagocytosis and consequently in *Leishmania* infection. The treatment *in vivo* with imatinib resulted in mice with smaller lesions with fewer parasites (91). Dactolisib, an inhibitor of PI3K and mTOR kinase in clinical study as a possible anti-cancer drug, demonstrated activity *in vitro* against *L. major* and *L. donovani*. However, was ineffective to treat *L.major*-infected mice (92).

Other anticancer agents that could be cited include tamoxifen, which is used in the treatment of breast cancer and is a selective estrogen receptor modulator with activity against *L. amazonensis*, *L. braziliensis, L. infantum chagasi* and *L. major in vitro* and *in vivo*. In parasites, it induces cell death by apoptosis and, in infected macrophages, modifies the alkaline intravacuolar pH and increases drug action against amastigote forms (93–98). The combination of tamoxifen with reference drugs (amphotericin B or miltefosine) used in the treatment of leishmaniasis has also shown promising results (99,100). Raloxifene, an estrogen receptor modulating drug, was tested against the promastigotes of *L. amazonensis*, *L. infantum chagasi*, *L. donovani*, *L. braziliensis*, *L. major* and *L. mexicana*, showing IC_{50} values between 30.2 μM and 38 μM, and against amastigotes of *L. amazonensis* and *L. infantum chagasi*, showing IC_{50} values between 8.8 μM and 16.2 μM, and the parasites exhibited autophagosomes and mitochondrial damage. *In vivo* efficacy trials of raloxifene in *L. amazonensis*-infected BALB/c mice showed a 41.7.% reduction in lesion size of the treated animals compared to the untreated animals and an 89.7.% reduction in parasitic load (101).

4.1.4. Antidepressant drugs

In addition to their characteristic effects, antidepressants of different classes have already demonstrated an excellent ability to eliminate parasites of the genus *Leishmania* (Figure 7).

Tricyclic antidepressants are indicated for the treatment of depression, obsessive compulsive disorder, panic attacks and chronic pain. Three drugs of this class have been studied in relation to their activity and mechanism of action: imipramine, clomipramine and amitriptyline. Imipramine demonstrates leishmanicidal activity, leading to the death of *L. amazonensis in vitro* and *L. donovani in vitro* and *in vivo*. The mechanism of action of imipramine in parasites involves alterations in the sterol profile and proton motive force, inhibition of trypanothione reductase and induction of apoptosis. It also has immunomodulatory capacity, inducing the production of TNF-α and IFN-γ and decreasing that of IL-10 and TGB-β. Other work has demonstrated that imipramine inhibits IL-10 production by upregulating histone deacetylase, which inhibits the acetylation of the IL-10 promoter (102–105). Clomipramine also exhibited activity against *L. donovani* and *L. major*. The mechanism of action in parasites involves the inhibition of L-proline transport, disruption of the plasmatic membrane in promastigotes and inhibition of trypanothione reductase (106,107). Amitriptyline shows activity against *L. donovani* but is less potent than imipramine and clomipramine, causing a decrease in proline transport and depletion in ATP levels in these parasites (105). Interestingly, cyclobenzaprine (CBP), a tricyclic compound structurally related to the amitriptyline and used as muscle relaxer, recently demonstrated activity against *L. infantum in vitro* and *in vivo* by raising ROS levels in promastigotes (108).

Sertraline, a selective serotonin reuptake inhibitor used for the treatment of depression and obsessive compulsive, panic and anxiety disorder, shows promising activity against *L. donovani* with efficacy in oral therapy and induces a reduction in ATP levels and oxygen consumption in promastigotes (109). Mianserin is a tetracyclic antidepressant used in the treatment of depression, anxiety and schizophrenia.

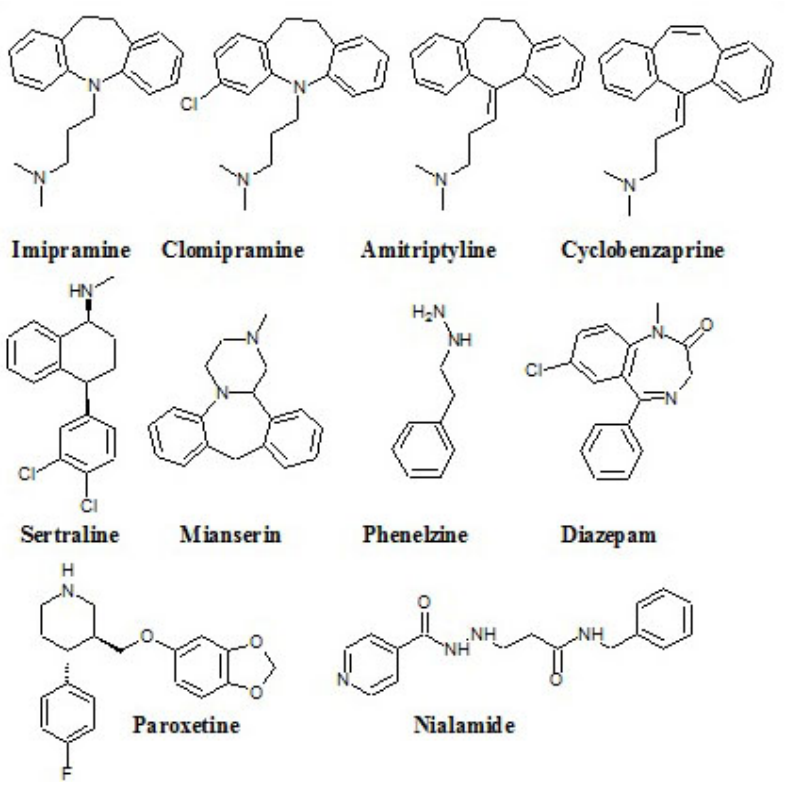

Figure 7. Antidepressant drugs

This drug kills promastigotes and amastigotes of *L. donovani* by inhibiting the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (110).

Phenelzine and nialamide are monoamine oxidase inhibitors used in the treatment of depression and anxiety. Both demonstrated activity against visceral and cutaneous forms of *Leishmania*, but nialamide was more promising in a murine model of cutaneous leishmaniasis (111). Paroxetine is a selective serotonin reuptake inhibitor used in the treatment of depression and anxiety. Recently, it presented activity against promastigotes of *L. infantum* with an IC_{50} equal to 2.2 μM. The mechanism of action of paroxetine in epimastigotes of *T. cruzi* involved the inhibition of the up take of putrescine, but in *Leishmania* spp., the mechanism, which culminates in cell death, has not yet been elucidated (112).

Diazepam is an antidepressant of benzo-diazepines class, was active against promastigotes of *L. mexicana* with IC_{50} equal to 15mM. The treatment for 48h promoted ultrastructural changes indicating interference in cell division (113).

4.1.5. Antihypertensive drugs

Several antihypertensive drugs (Figure 8) have been evaluated against parasites of the genus *Leishmania* in the search for the discovery of new therapeutic options.

Calcium channel blockers are a group of drugs often prescribed for the treatment of high blood pressure (hypertension) that acts by relaxing the arterial smooth muscle (114). Among the calcium channel blockers most used in clinical practice, azelnidipine, cilnidipine, lercanidipine, nicardipine, nitrendipine, nifedipine, nimodipine, and amlodipine were effective against *L. amazonensis*, *L. braziliensis*, *L. infantum chagasi*, and *L. major* (115,116). Different groups have studied using calcium channel blockers against *Leishmania* spp. and observed promising antiparasitic activities (114–118).

Nimodipine, a 1,4-dihydropyridine, was active against the promastigote and intracellular amastigote forms of *L. infantum chagasi*, although the promastigotes were less susceptible. Nimodipine also showed activity against other species of *Leishmania*, yet *L. major* was more susceptible than *L. amazonensis*. The treatment of nimodipine did not block amastigote penetration into the host cells but did cause ultrastructural damages, such as intracytoplasmic vacuoles, membrane blebbing, nuclear membrane bilayer detachment and augmented mitochondria (119).

Nifedipine and verapamil had inhibitory effects on macrophage infection by *L. donovani*,

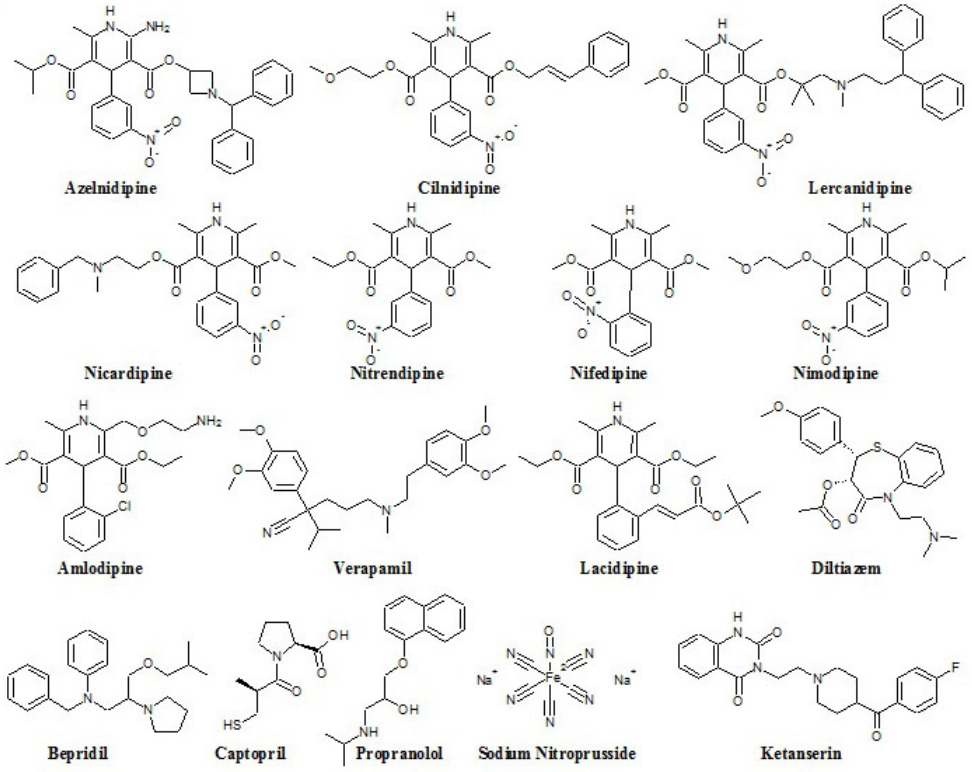

Figure 8. Antihypertensive drugs

suggesting that the Ca^{2+} ion plays a role in the invasion process. In the treatment with verapamil, the number of parasites attached per infected macrophage and the number of infected macrophages was reduced, but no antiparasitic effect was observed. The pretreatment of macrophages with verapamil and nifedipine reduced their ability to bind the parasite (120). Other studies have demonstrated different results, with an increase in the percentage of infected macrophages and in the parasite load in the presence of nifedipine, suggesting that this drug impairs the microbicidal functions of macrophages (121). Another controversial result is related to the activity of verapamil, which inhibited Ca^{2+} uptake by *L. donovani* but did not show leishmanicidal activity against promastigotes and amastigotes (122).

Another interesting use of verapamil, a resistance reversing drug, is inhibiting drug efflux pump proteins such as P-glycoprotein in resistant parasites. Isolates of *L. donovani* resistant to sodium stibogluconate were treated with verapamil, which reversed the drug resistance and showed a significant reduction in IC_{50}. Verapamil alone did not have any effect on the parasites but could reverse the *in vitro* drug resistance of *L. donovani* clinical isolates to sodium stibogluconate (123).

Amlodipine, lacidipine, verapamil and diltiazem inhibited Ca^{2+} uptake by *L. donovani*, but only the first two had leishmanicidal activity against *L. donovani* promastigotes and amastigotes. This result demonstrated that there was no relationship between the leishmanicidal activity and the Ca^{2+} channel blocking action of these drugs because verapamil and diltiazem inhibited Ca^{2+} uptake but did not have activity against the parasites (122). Amlodipine and lacidipine were also effective against infections in BALB/c mice when administered orally, showing significant decreases in the spleen and liver weights and satisfactory reduction in the parasite burdens compared to those of the untreated controls (122). Another calcium-blocking agent, bepridil, demonstrated effectiveness against different visceral and cutaneous forms of *Leishmania* but failed to treat *L. infantum chagasi*-infected hamsters (117).

Goyal *et al.* reported the identification and biochemical characterization of an angiotensin-converting enzyme (ACE)-related dipeptidyl carboxypeptidase (DCP) in *L. donovani*. The ACE inhibitor, captopril, blocked both ACE activities, rat and leishmania, but the sensitivity of parasite's enzyme to this inhibitor is much less than that to the mammalian

one (124). Other studies tested the activity of enalapril and lisinopril against promastigotes and amastigotes of *L. amazonensis*, but no inhibition of parasite growth was observed (125).

In BALB/c mice infected with *L. mexicana*, the β-blocker propranolol demonstrated a 47% inhibition of footpad swelling, a significant reduction of the parasitic burden and a substantial increase in CD4+ and CD8+ splenic T lymphocytes producing IFN-γ, which play an essential role in the elimination of these parasites. It was suggested that β-adrenergic receptor antagonists could be of therapeutic value, either as a treatment or as an adjuvant of vaccines for *L. mexicana* (126). The treatment of *L. amazonensis* promastigotes and amastigotes with other β-blockers, atenolol and metoprolol, did not show efficacy against the parasite (125), but in *L. major* infections, which are characterized by inflammation and hyperalgesia, peripheral hyperalgesia was induced during treatment with atenolol (127).

Sodium nitroprusside (SNP), a potent NO donor, promotes arterial and venous vasodilation and has been used in hypertensive emergencies. The treatment of *L. amazonensis* promastigotes and axenic amastigotes revealed a decrease in parasite number and showed morphological changes with loss of viability (128). SNP had increased leishmanicidal activity against *L. amazonensis*-infected macrophages, reducing the number of amastigotes and stimulating the levels of TNF-α, nitric oxide (NO) and 3-nitrotyrosine, thus suggesting that the drug is an exogenous source of NO (129).

Chlorthalidone and hydrochlorothiazide, two thiazide diuretics, and losartan, an angiotensin II receptor antagonist, were tested against *L. amazonensis* but did not demonstrate activity, showing no activity until the use of concentrations of 100 μM in promastigotes and 25 μM in amastigotes (125).

Ketanserin is an antihypertensive drug but also shows antidepressant action as a 5-HT2A/2C receptor antagonist. This drug leads to the death of promastigotes and intracellular amastigotes of *L. donovani* by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, an important enzyme involved in ergosterol biosynthesis in this parasite (130).

4.1.6. Antibiotics

Antibiotics encompass several classes of molecules possessing leishmanicidal activity (Figure 9), within which is the paromomycin that is approved for the treatment of leishmaniasis in some countries.

Penicillin is used in small concentrations in *Leishmania* spp. cultures in order to suppress bacterial contamination and thus does not have an effect on the parasites. The treatment of cutaneous leishmaniasis caused by *L. tropica* with large doses of penicillin, infiltration typical of leishmaniasis lesions remained, and no recovery was observed over a period of approximately 6 months. The largest dose of penicillin had no effect on lesions of *L. tropica* in humans, but experiments *in vitro* showed that penicillin inhibited the growth of the parasite (131). Oxacillin was also tested against *L. amazonensis* and did not have activity until used at concentrations of 100 μM in promastigotes and 25 μM in amastigotes (125).

Fluoroquinolones, a class of antibacterial agents that act through the inhibition of type II DNA topoisomerases (TOPII), showed leishmanicidal-selective action against intracellular amastigotes of *L. panamensis*. Enoxacin and ciprofloxacin demonstrated to be the most selective agents for the parasites over human macrophages (132). Enoxacin, ciprofloxacin, ofloxacin, lomefloxacin, and norfloxacin displayed significant potencies and selectivity for *L. panamensis* TOPII (133). Marbofloxacin, a third generation fluoroquinolone for veterinary use, was tested *in vitro* against clinical strains of *L. infantum* alone or in combination with allopurinol. The combination between miltefosine and allopurinol caused a significant higher percentage of killed parasites than marbofloxacin plus allopurinol. Marbofloxacin was more efficacious when associated with allopurinol than alone after 72 h of incubation (134). Miltefosine was more efficacious than marbofloxacin, but when compared to meglumine antimoniate and sodium stibogluconate, marbofloxacin was more efficacious, with increases in NO and TNF-α observed after marbofloxacin treatment (135). Dogs naturally infected with visceral leishmaniasis and treated daily with a dose of 2 mg/kg marbofloxacin for different period of times, and the treatment for 28 days lead to clinical improvement and was considered, safe and efficient (136).

Among the aminoglycosides already tested, streptomycin and tobramycin have been shown to inhibit the growth of *L. donovani* parasites, whereas gentamycin did not inhibit the growth *in vitro*. Streptomycin and tobramycin demonstrated activity against intracellular amastigotes, with inhibitions of 63% and 52%, respectively (137).

Azithromycin, a semi-synthetic macrolide antibiotic chemically related to erythromycin and clarithromycin, has provided controversial results. Azithromycin demonstrated activity against promastigotes and amastigotes of *L. amazonensis*, *L. braziliensis*, *L. tropica*, *L. major* and *L. infantum chagasi* (138–140). The *in vivo* studies showed a decrease in the

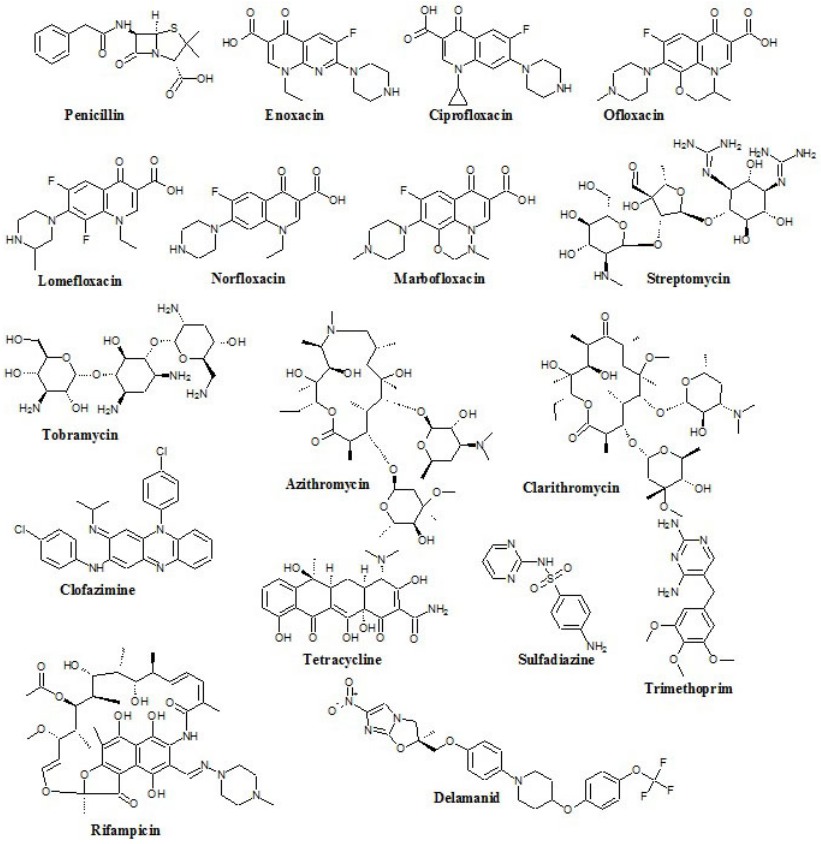

Figure 9. Antibiotics drugs

parasite number following subcutaneous administration of azithromycin in BALB/c mice infected with *L. major* (139). The activity of azithromycin was also evaluated in golden hamsters infected with *L. braziliensis* and *L. amazonensis* using oral administration. NO activity against infections with *L. amazonensis* could be observed, but activity was demonstrated against *L. braziliensis*. However, azithromycin was unable to totally block the development of foot pad swelling, although the lesions were significantly smaller in the treated animals (141). The combination of azithromycin with glucantime or miltefosine was also evaluated. The combination of azithromycin and glucantime was not more efficient than treatment with glucantime alone in the experimental cutaneous leishmaniasis (142). Oral treatment with azithromycin plus miltefosine for a short period of 10 days in *L. major*-infected mice induced a dramatic clinical improvement, but relapse rapidly developed after the cessation of therapy (143). Clarithromycin exhibits antileishmanial properties that were more effective against promastigotes and intracellular amastigotes of *L. tropica* and *L. donovani* than azithromycin at lower concentration with minimal toxicity to mammalian cells (140,144). However, clarithromycin, when tested against promastigotes and amastigotes of *L. amazonensis*, did not demonstrate activity (125). Clofazimine, originally developed for the treatment of tuberculosis and used in combination with rifampicin and dapsone as multidrug therapy (MDT) for the treatment of leprosy (145), demonstrated leishmanicidal activity (146). Clofazimine showed leishmanicidal activity against *L. infantum* and *L. tropical*, with IC_{50} values of 4.48 μM and 2.96 μM, respectively, (147). Another antibiotic, tetracycline, was tested on the promastigote form of *L. major* and demonstrated leishmanicidal activity (148). Cephalexin, a cephalosporin, has been tested against promastigotes and amastigotes but did not show activity up to concentrations of 100 μM in promastigotes and 25 μM in amastigotes (125).

The combination of sulfadiazine, trimethoprim, and metronidazole or tinidazole showed good activity as an oral treatment for 12 to 25 weeks in nine patients with kala-azar, and no side effects were noticed (149). The combinations between trimethoprim and sulfamethoxazole and between isoniazid and rifampicin demonstrated no antileishmanial activity against intracellular amastigotes of *L. tropica* (less than or equal to 40% parasite elimination) but have been reported as being orally efficacious in other studies (146,150,151).

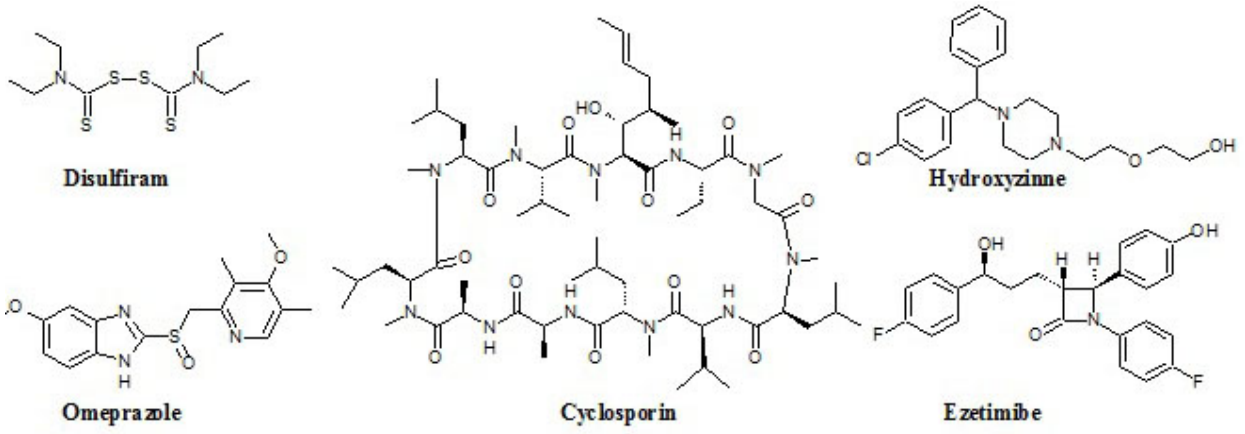

Figure 10. Miscellaneous drugs

Other studies showed that rifampicin, an antibiotic commonly used in the treatment of mycobacterial infections, significantly inhibited the growth of promastigotes and intracellular amastigotes of *L. aethiopica*, *L. donovani*, *L. tropica* and *L. major* (152,153). Rifampicin and amphotericin B were combined *in vitro* in intracellular amastigotes of *L. tropica* and showed a synergistic effect at several cell concentrations (154). Experiments *in vivo* were also conducted, and the treatment of mice infected with promastigotes of *L. amazonensis* with daily doses of 0.5 mg of rifampicin provided a significant reduction in the size of local lesions, but daily doses of 20 mg/kg in children or 1200 mg in adult patients infected with *L. braziliensis* did not bring any sign of improvement after 30 days of treatment (155).

Novel approved drug for for the treatment of MDR tuberculosis, Delamanid showed potent *in vitro* and *in vivo* activity against *L. donovani* and *L. infantum*. BALB/c mice infected with *L. donovani* treated with maximum dose of 30 mg/kg had suppressed infection in 99.5.%, given an estimated ED_{50} and ED_{90} of 7.3. and 21.5 mg/kg, respectively (156).

4.1.7. Other drugs

Other drugs have also been investigated for their antileishmanial activity (Figure 10). Disulfiram, used to support the treatment of chronic alcoholism, showed excellent activity against promastigotes and intracellular amastigotes with IC_{50} values of 0.058 µM and 0.062 µM, respectively (157).

Cyclosporin, an immunosuppressant, is also a drug whose antileishmanial activity has been tested; in promastigotes of *L. donovani*, it caused a reduction in parasite growth in a dose-dependent manner, with a more than 5-fold decrease in parasite growth at the highest concentration tested and with an IC_{50} value between 15 and 20 µM. Axenic amastigotes of *L. donovani* showed high susceptibility to the drug, which exhibited an IC_{50} value between 5 and 10 µM (158).

In another study, cyclosporin was able to reduce the infection rate of *L. major*-infected macrophages to less than 15% compared to that of untreated cultures, which had a 75% infection rate (159).

Hydroxyzine, an antagonist of histamine receptor H1 drug, was tested against *L. infantum* promastigotes and had an IC_{50} value of 59.57 µM (160). Omeprazole is a specific inhibitor of human gastric K^+H^+-ATPase. This drug had an inhibitory effect on K^+H^+-ATPase from *L. donovani*. The activity of this drug was tested on the promastigotes of *L. donovani*, and an IC_{50} value of 50 µM was obtained. Omeprazole also had a significant effect on the survival of intracellular amastigotes: at a concentration of 150 µM, there were few or no parasites within the macrophages in comparison to untreated macrophages (161).

Ezetimibe, an antihypercholesterolemic drug, was active on promastigotes and amastigotes of *L. amazonensis*, with IC_{50} values of 30 µM and 20 µM, respectively, were obtained. Analysis has shown that drug-treated parasites become rounded and that ezetimibe interfered with the ergosterol biosynthesis pathway. Assays of the combination of ezetimibe with the antifungals ketoconazole and miconazole showed a synergistic effect, with a significant decrease in the IC_{90} values for intracellular amastigotes; for ketoconazole, there was a reduction from 11.3. µM to 4.14 µM, and for miconazole, there was a reduction from 11.5 µM to 8.25 µM, representing an improvement in the antileishmanial action of azoles (125).

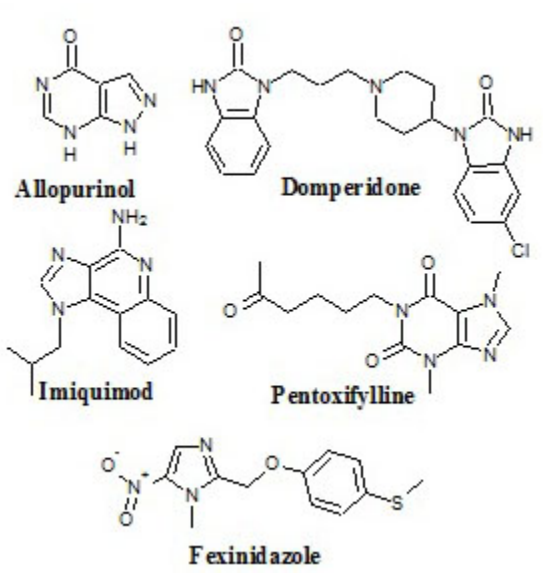

Figure 11. Drugs in clinical trials involving dogs or humans.

5. CLINICAL TRIALS

Several groups have performed clinical trials using patients or dogs infected with different species of *Leishmania*, demonstrating a broad spectrum of clinical manifestations.

The efficacy of allopurinol (Figure 11) was evaluated in the treatment of 45 dogs with clinical signs indicating leishmaniases. In the treated group, a significant improvement in the general body condition was observed, such as reduction in peripheral lymphadenopathy, splenomegaly and cutaneous ulcerations. Comparing the allopurinol-treated group with the placebo group a significant decrease in the amastigotes in lymph node and bone marrow aspiration smears was observed (162). A controversial result was observed after treatment with allopurinol of adult patients with cutaneous leishmaniasis caused by *L. panamensis* and *L. braziliensis*, in which no effect was achieved (163). Another drug that was tested in dogs was domperidone, which is involved in the innate immune response and which could control early infections by *Leishmania* spp. Infected dogs were treated with an oral suspension of domperidone, which reduced the risk of developing clinical canine leishmaniasis (164).

The efficacy of rifampicin (Figure 9) and omeprazole (Figure 10) was determined through a randomized, double-blind, placebo-controlled study conducted on 50 patients with cutaneous leishmaniasis caused by *L. tropica*. The result demonstrated that 20% of patients had unhealed lesions in the group receiving rifampicin and omeprazole, whereas 82% of patients had unhealed lesions at the end of 6 weeks in the placebo group (165).

Treatment with azithromycin (Figure 9) in patients with cutaneous leishmaniasis in two regions of Brazil, Araçuaí and Varzelândia in Minas Gerais state, and Manaus in Amazonas state, demonstrated different results. The first study in patients with mucosal leishmaniasis demonstrated that azithromycin is a good therapeutic option, whereas in the second one the drug showed a very low efficacy for cutaneous leishmaniasis (166,167). The other group that used azithromycin for the treatment of patients with Old World cutaneous leishmaniasis also observed no effect (168). Meanwhile, the effect of azithromycin on patients with cutaneous leishmaniasis caused by *L. braziliensis* was compared with that of meglumine antimoniate. Patients received oral azithromycin at 500 mg/day or intramuscular meglumine antimoniate at 10 mg Sb/kg/day for 28 days. The treatment with azithromycin showed moderate activity and provides evidence about the safety of this therapy (169).

Ergosterol biosynthesis inhibitors, such as itraconazole, ketoconazole, fluconazole and terbinafine, have been studied for many years (Figure 4), and some clinical trials have been carried out (170). A randomized, double-blind, placebo-controlled clinical trial using oral itraconazole evaluated 200 patients infected with *L. major*. After 8 weeks of treatment, no difference was observed compared to the placebo group (171). However, other controlled studies showed that oral itraconazole therapy was safe and effective for the treatment of cutaneous leishmaniasis caused by *L. major* (172).

Oral ketoconazole was tested for the treatment of cutaneous leishmaniasis and compared with intralesional administration of meglumine antimoniate. Ketoconazole treatment demonstrated a cure rate of 89% and a failure rate of 11% and seemed to be safe and effective for treatment, especially in children (173). The combination of intralesional sodium stibogluconate (SSG) and oral ketoconazole was demonstrated to be more effective than intralesional SSG alone, especially in cases with large plaque lesions and mucosal lesions. This combination is easy to use, effective, painless and free of *major* side effects (174). Other studies showed that the outcome of the ketoconazole treatment of cutaneous leishmaniasis was influenced by the parasite species. Patients infected with *L. braziliensis* and treated with ketoconazole showed a cure rate of 30%; however, patients infected with *L. mexicana* showed a cure rate of 89%. These differences highlight the importance of speciation in the treatment of leishmaniasis (175).

High doses of fluconazole were used in two studies: (i) a non-controlled open trial with patients that showed a cure rate of 75–100% for cutaneous leishmaniasis (176), and (ii) a randomized controlled trial to evaluate the efficacy and safety of oral fluconazole in patients infected with *L. braziliensis* that considered the drug ineffective (177). The treatment of patients infected with *L. major* with 400 mg of oral fluconazole daily was more efficient than treatment with 200 mg, but it showed side effects (178). Another antifungal, terbinafine, was tested as a topical formulation. Topical terbinafine used for the treatment of cutaneous leishmaniasis caused by *L. tropica* was more efficient than the placebo group, but the cure rate depended on the lesion type (179).

Imiquimod (Figure 11), which acts as an immunomodulatory agent, was tested in combination with meglumine antimoniate for the treatment of cutaneous leishmaniasis. Imiquimod indirectly stimulates the production of a T-cell type 1 response, and the application of 5% of drug in a cream for 4 weeks did not improve the response to the meglumine antimoniate treatment (180).

Pentoxifylline (Figure 11), a xanthine derivative used to treat peripheral vascular disease, inhibits tumor necrosis factor-α (TNF-α), attenuates the immune response and decreases tissue inflammation (181). This drug has been investigated alone or combined with meglumine antimoniate, mainly in cases of refractory mucocutaneous leishmaniasis or cutaneous leishmaniasis (17,182–184). The lesions of patients with mucocutaneous leishmaniasis caused by *L. braziliensis* receiving the combination treatment healed faster than those of the patients treated with the antimonial alone (185). The combination of glucantime and orally administered pentoxifylline was used in the 10 patients with refractory mucosal leishmaniasis, and a re-epithelization of the mucosa was observed in 90% of patients (186). In another study, a randomized, double-blind trial for mucosal leishmaniasis caused by *L. braziliensis* was also conducted using oral pentoxifylline combined with glucantime. This combination accelerates the healing time of mucosal leishmaniasis with a 58% cure rate compared with control group and thus should be a therapeutic choice for the treatment of refractory mucosal leishmaniasis (187).

Fexinidazole (Figure 11) is a 5-nitroimidazole that has shown activity *in vitro* and *in vivo* against Chagas disease and human African trypanosomiasis (188–190). Currently, fexinidazole is in clinical development for the treatment of human sleeping sickness, with phase I completed (191).

Studies *in vitro* and *in vivo* have demonstrated that fexinidazole and its metabolites showed activity against visceral leishmaniasis caused by *L. donovani* and are activated by nitroreductases (192,193). Subsequently, fexinidazole was included in a clinical trial as an oral drug for the treatment of visceral leishmaniasis in Sudan. According to clinicaltrial.gov, fexinidazole is in a phase II trial to determine its efficacy as an oral treatment of visceral leishmaniasis in Sudanese adult patients. The treatment consists of 600 mg tablets given orally after the main daily meal at a daily dose of 1800 mg (3 tablets) once a day for 4 days, followed by 1200 mg (2 tablets) once a day for 6 days (194). This clinical trial appears as finished on clinicaltrial.gov; however, the results are not yet reported.

6. CONCLUSION

In this work, the main studies on the drug repositioning of FDA-approved drugs for cutaneous and visceral leishmaniasis were summarized. Drug repurposing is an attractive strategy for neglected tropical disease drug discovery and development, particularly for leishmaniases because a large part of the therapeutic arsenal consists of drugs that have been repurposed. This review presents drugs that have been identified by systematically searching the literature and, in figures, shows the distribution of pharmacological classes and available drugs for the purpose of repositioning. Recent studies using HTS and HCS approaches have contributed to the advancement of the classical drug discovery process and repurposing strategy for different diseases. Even with all the available new technologies, most of the studies identified in this review involve classical assays performed by groups worldwide. In Figure 12 are represented the main classes of drugs studied for repositioning, independent of having a promising

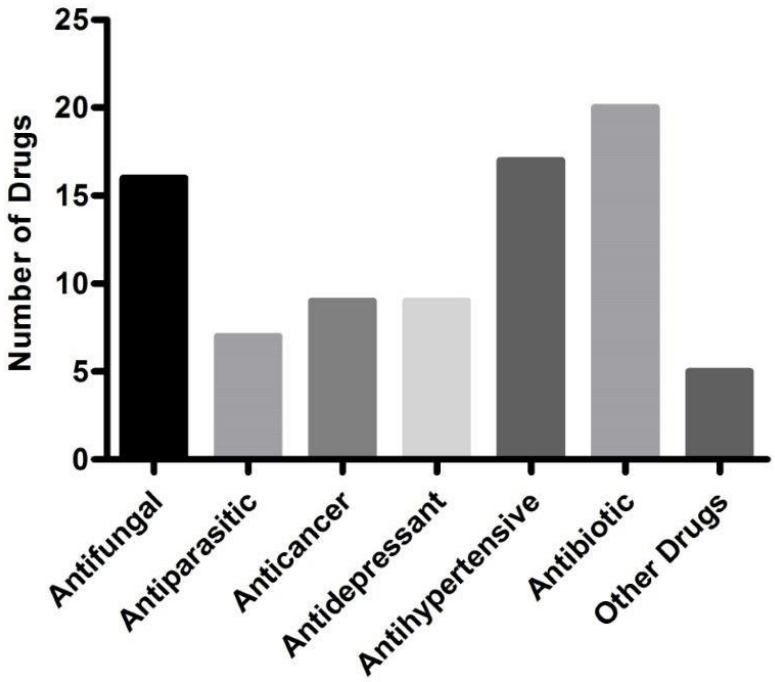

Figure 12. Drug repurposing for leishmaniases studies**.** The graph represents number of FDA-approval drug available for use against *Leishmania* spp. *in vitro* and/or *in vivo.*

result. The most advanced class in preclinical stages with positive results are the antitumorals. Tamoxifen as monotherapy or combinatory therapy (with reference drugs) has *in vivo* efficacy in different models of experimental leishmaniases, and *in vitro* resistance induction studies have shown that the parasites are not prone to develop resistance to tamoxifen. Fexinidazole is in phase II clinical studies for visceral leishmaniasis; however, the result of this trial was still not reported. The last drug repurposed for leishmaniases was 85097uifylline, used as an adjuvant therapy for cutaneous leishmaniasis in New World and Old World. Drug repurposing is a fast and cheap strategy for the discovery and development of new treatments for leishmaniases.

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