EXPERT OPINION

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New perspectives for leishmaniasis chemotherapy over current anti-leishmanial drugs: a patent landscape

Alice Machado-Silva, Pedro Pires Goulart Guimarães, Carlos Alberto Pereira Tavares & Rubén Dario Sinisterra[†]

[†]*Universidade Federal de Minas Gerais, Instituto de Ciências Exatas, Departamento de Química, Belo Horizonte-MG, Brazil*

Introduction: Although leishmaniasis is estimated to cause the ninth largest disease burden among individual infectious diseases, it is still one of the most neglected diseases in terms of drug development. Current drugs are highly toxic, resistance is common and compliance of patients to treatment is low, as treatment is long and drug price is high.

Areas covered: In this review, the authors carried out a patent landscape in search for new perspectives for leishmaniasis therapy. This search encompassed patent documents having priority date between 1994 and 2014. Selected compounds were compared to current anti-leishmanial drugs regarding efficacy and toxicity, when experimental data were available.

Expert opinion: Most patents related to drugs for leishmaniasis have not been produced by the pharmaceutical industry but rather by public research institutes or by universities, and the majority of the inventions disclosed are still in preclinical phase. There is an urgent need to find new ways of funding research for leishmaniasis drugs, incentivizing product development partnerships and pushing forward innovation.

Keywords: amphotericin B, leishmaniasis therapy, meglumine antimoniate, miltefosine, paramomycin, patents, pentamidine

Expert Opin. Ther. Patents [Early Online]

1. Introduction

Leishmaniasis is a wide-spectrum disease, encompassing a variety of clinical syndromes [1]. It is transmitted to mammals via the bite of phlebotomine sand flies infected by any of the 20 or more protozoan species from the genus *Leishmania* [2,3]. The most common clinical forms of the disease are visceral (VL) and cutaneous (CL) leishmaniasis. VL is usually characterized by high fever, weight loss, swelling of the spleen and liver and anemia [4]. It is highly lethal, causing an estimate of over 50 thousand deaths per year. Reported deaths mainly involve non-treated individuals and children [5]. CL, on the other hand, forms self-healing ulcers on exposed skin areas. Recent estimates indicate that it affects 0.7 – 1.2 million people every year [6]. In some cases, it may cause serious deformities.

Classified as a Neglected Tropical Disease, leishmaniasis has strong and complex links with poverty [7]. Other factors that also play a role in the dynamics of the disease include malnutrition, weak immune system, illiteracy, lack of resources and environmental changes such as deforestation, construction of dams and urbanization, as well as the migration of non-immune people to endemic areas, which accompany these processes [8,9].

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Article highlights.

- This review describes new perspectives for leishmaniasis therapy based on patents filed between 1994 and 2014.
- An analysis of patents with experimental *in vivo* or *in vitro* evidence confirming leishmanicidal activity is carried out and the efficacy and toxicity of promising compounds disclosed on such documents are compared to current anti-leishmanial drugs.
- The analysis includes newly synthesized compounds, analogues, prodrugs and new uses of standard drugs such as drug delivery systems.
- Most patents related to leishmaniasis drugs have been produced by public research institutes or by universities.
- The high number of patents that are dead due to failure of payment of maintenance fees (lapsed patents) compared to patents refused for not meeting patentability requirements (revoked) is an indication of the difficulties faced by assignees to reach the final stages of innovation.
- These findings support the notion that more incentives are needed to launch into the market promising new leishmanicidal drugs.

This box summarizes key points contained in the article.

Although leishmaniasis is still considered a 'disease of the poor', it is reaching other regions in the globe. For instance, in the US, it has been detected in American soldiers who return from the Middle East with the disease [10] and in dogs and cats (it is endemic in foxhounds) [11]. It is also considered a threat in south-western Europe, mainly because of risk of spread of endemic species: *L. infantum* and *L. tropica* [12,13].

Treatment efficacy depends on both host and parasite factors, as some therapies and regimens are effective only against certain *Leishmania* species/strains and only in certain parts of the world [1]. Apart from low efficacy, treatment limitations include long duration, high toxicity, side effects and high cost, all of which contribute to low compliance [14]. Both forms of the disease are emerging in patients with HIV infection, implicating in poorer treatment responses, higher relapse rates and higher drug toxicity, thus representing an extra challenge to treatment [5,15].

The principal lines of treatment are based on the pentavalent antimonials sodium stibogluconate and meglumine antimoniate. Their main drawbacks are the need for daily parenteral administration, variable efficacy against VL and CL and the increasing incidence of resistance [2,16-18]. Second-line treatments include: i) amphotericin B, a highly efficient but toxic drug; ii) its liposomal formulations, which are less toxic but expensive [19]. Despite the higher cost, there is evidence in the literature of efficient single dose regimens with such formulations. It has been argued this would render cost of treatment comparable to free amphotericin B and should remove concerns about compliance to treatment [20]. However, the observed efficacy of single doses is not universal [21]; iii) miltefosine (hexadecyl phosphocholine), an orally administered but costly drug [22]. Due to its teratogenicity, it is not safe for pregnant women. In addition, it is not effective against all *Leishmania* strains and its long half-life makes it vulnerable to rapid development of drug resistance [20]; iv) paramomycin, which is not available worldwide, requires injections for 3 weeks and monitoring of serum transaminase. Lack of adequate data regarding its safety in pregnancy and variable results depending on the species and epidemiological situation involved represent further disadvantages [2,20]; and v) pentamidine, a toxic drug of questionable efficacy [2]. There are also combination drugs being evaluated, such as LAmB (Liposomal amphotericin B) and miltefosine, LAmB and paramomycin, among others [22-26]. Compared to VL, proven treatment for CL is even more limited [27].

According to the World Health Organization, "clinical research to evaluate new drugs and combinations of drugs to reduce the duration of treatment for all clinical forms of leishmaniasis remains a high priority". In addition, "efforts should be made to improve access to medicines and reduce their cost" [5]. However, a recent analysis by Golgher *et al.* [28] estimated that only one new drug or formulation against leishmaniasis will be introduced in the market before 2018.

Given the drawbacks of current therapy and WHO's recommendations regarding the priorities for leishmaniasis drug development, the present paper aims to identify new developments on leishmaniasis drugs by analyzing the latest patenting trends.

2. Promising compounds for leishmaniasis treatment: patent landscape

To gather information on compounds with leishmanicidal activity, searches were carried out at the commercial intellectual property portal Questel Orbit (www.orbit.com). The search terms used were: i) *leishman* and (treat* or cure or therap* or curing) in the title, abstract or claims and A61K (A61K - Preparations For Medical, Dental, Or Toilet Purposes) as the International Patent Classification (IPC) or Cooperative Patent Classification (CPC). The data range was set as 1994 to 2014 (priority date).

This search retrieved 561 patent families, 117 of which were considered too broad as leishmaniasis treatment was claimed but there was no experimental evidence specifically confirming leishmanicidal activity. These documents were excluded from our analyses. Remaining documents were then analyzed regarding legal status, country and date of priority (where and when the patent was first filed) and assignee name. From the 444 families left, 294 (66%) are alive patents or applications, while 138 (31%) are already dead and 12 (3%) have unknown legal status. From the patents that are alive, 190 (65%) have been granted and 104 (35%) are still pending (**Figure 1**). Ninety-three per cent of dead patents have lapsed due to failure of payment of maintenance fees, while the remaining 7% have been revoked (protection has been terminated on one or more grounds, such as lack of novelty) (data not shown). Material disclosed on patent

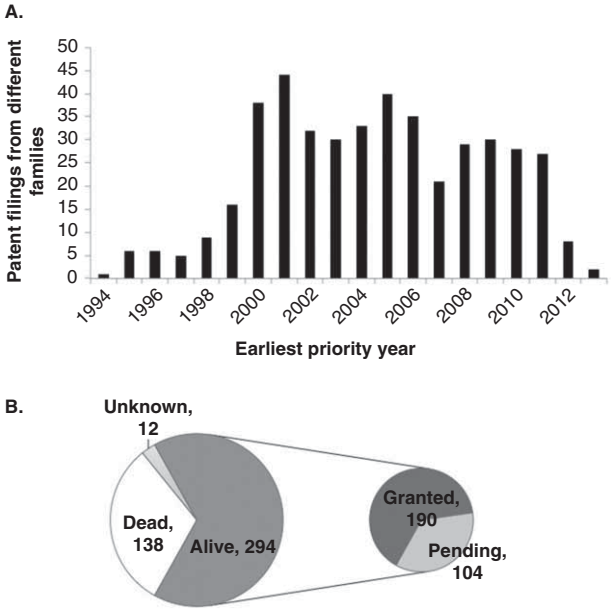

Figure 1. Distribution of patent applications related to leishmaniasis treatment by **(A)** earliest priority year and **(B)** legal status.

documents that are dead can be explored by third parties anywhere in the world.

Most patent documents were first filed in the US (47.2%), followed by Brazil (9.4%), Spain (6.6%), Denmark, France and India (with 6% each). This is a rough indication of where research efforts are concentrated, although it could also represent where a company's headquarters are or the country's strength in patenting. Countries with more than one priority filing are shown in the map (**Figure 2**).

Top assignees are shown in **Figure 3A**. The National Institute of Health – NIH (USA) is the largest patent assignee, with 22 patent families, followed by Council of Scientific and Industrial Research – CSIR (India) with 15, Consejo Superior de Investigaciones Científicas – CSIC (Spain) with 14, Institut de Recherche pour le Devéloppement – IRD (France) with 13 and Universidade Federal de Minas Gerais – UFMG (Brazil) with 11. Further analyses of all assignees indicated that most are universities and research institutes (54%), while 30% are companies. Assignees were classified as *others* when inventor's names were given as assignee name or when it was not possible to classify them in the previous categories. The information in **Figures 2** and **3** could be useful when searching for R&D partnerships in leishmaniasis treatment, especially taking into account that such partnerships are extremely important in the case of neglected diseases.

Patents where then analyzed regarding disclosure of promising compounds for leishmaniasis treatment. In this case, only valid patents or patent applications were considered (294 documents). Specifically in the case of new compounds, experimental evidence of leishmanicidal activity was required for an invention to qualify for inclusion in the present article. Furthermore, documents were only included in our analysis when a reference drug was used as control, so we could have a proper basis for comparison with the state-of-the-art drugs. The main findings of these searches are discussed below.

2.1 New molecules and new uses of old drugs

Among the documents retrieved in the patent search, it was possible to identify new molecules with leishmanicidal activity or new uses for already existing drugs in leishmaniasis treatment. Experimental evidence for each of these molecules is described below. Chosen compounds were grouped according to the standard drugs used as positive controls in the experiments disclosed in the patent document: amphotericin B, pentamidine, meglumine antimoniate or miltefosine. Details of experimental data included in the patent documents are shown on Tables 1, 2 and 3 in Annex 1.

2.1.1 Amphotericin B

Amphotericin B is a highly hydrophobic polyene with antifungal activity produced by the filamentous bacteria *Streptomyces nodosus* and used as a second-line drug in the treatment of leishmaniasis. Although efficient as a chemotherapeutic agent, amphotericin B presents poor bioavailability and high toxicity [29]. Toxic effects can be divided into infusion-related reactions (such as chemical phlebitis, hypoxia, chills, fever and nausea) and nephrotoxicity (manifested by renal insufficiency, hypokalemia, hypomagnesemia, renal tubular acidosis and anemia) [30,31]. In addition to toxicity, a few cases of resistance have been reported [32]. Hence, there is an urgent need for developing other drugs that could substitute for this antifungal polyene.

In the search for alternative therapy, Tunac *et al.* developed novel polyene macrolides which are water soluble and present broad-spectrum antifungal and antiparasitic activity as well as improved bioavailability [33]. One of these molecules, a sodium salt of amphotericin B, was named Corifungin. Studies in rats indicated Corifungin is well tolerated in animals with minimal toxicity. In addition, it completely inhibited *L. donovani* intracellular growth *in vitro* and *in vivo*. Its IC_{50} was comparable to that of amphotericin B, but without toxic effects on THP-1 cells. The advantage of using Corifungin over amphotericin B seems to be its reduced toxicity and high absorption. The patent owner, Acea Biotec, was granted 'orphan drug status' from the US FDA for the indication of Corifungin in the treatment of VL [34]. Corifungin is also active against other diseases caused by fungi or amoeba [35,36].

Another disclosed formulation, a water extract of the mushroom *Agaricus blazei* and its purified fractions, was described by Coelho *et al.* as having potential for topic or oral treatment of leishmaniasis [37]. *In vitro* leishmanicidal activity was demonstrated against several parasite strains. *Leishmania amazonensis* parasite burden and lesion size reduction were comparable or higher than that obtained with amphotericin B treatment. As *A. blazei* is an edible mushroom, low toxicity was expected. Indeed, this extract presented low toxicity in murine macrophages

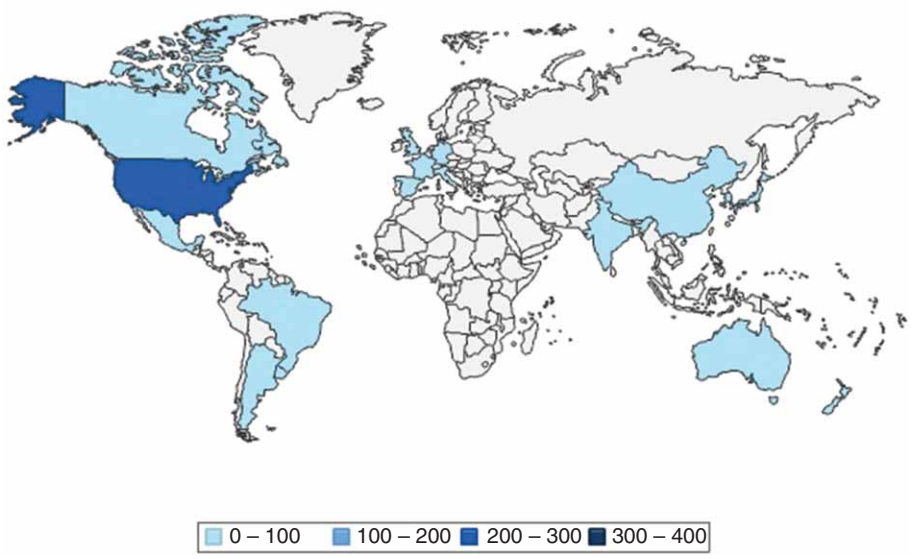

Figure 2. Distribution of patent applications related to leishmaniasis treatment by priority country. The color scheme represents number of priority applications.
Reproduced from Questel.

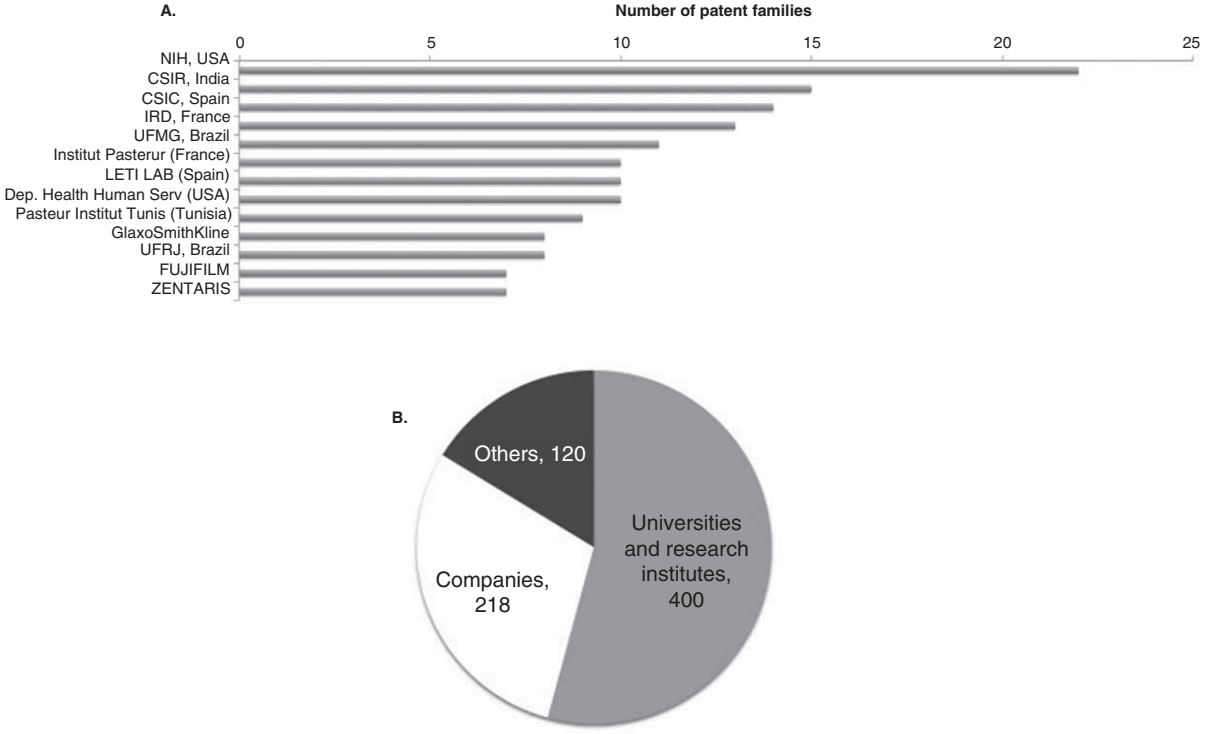

Figure 3. (A) Top assignees and **(B)** Distribution of patents by assignee type.
CSIC: Consejo Superior de Investigaciones Científicas; CSIR: Council of Scientific and Industrial Research; NIH: National Institute of Health; IRD: Institut de Recherche pour le Devéloppement; UFMG: Universidade Federal de Minas Gerais; UFRJ: Universidade Federal do Rio de Janeiro.

and no hemolytic activity in human red blood cells [38]. Later results showed hepatic damage was only observed with amphotericin B treatment and that *in vivo* activity against *L. chagasi*-infected mice is comparable to that of amphotericin B [39,40]. Authors suggest this is a strong candidate for the treatment of VL and indicate a possible use in chemo-prophylactic regimen.

Other interesting compounds for the treatment of leishmaniasis were disclosed by Bilbao *et al.* [41]. These compounds share an oxoisoaporphine structure and some of them, such as 2,3-dihidro-*7H*-dibenzo[*de*,*h*]-quinolin-7-ona and 2,3,8,9,10, 11-hexahidro-*7H*-dibenzo[*de*,*h*]-quinolin-7-ona, were able to inhibit *L. amazonensis* growth *in vitro* more efficiently than amphotericin B. However, the first compound presented a much lower cytotoxic activity towards J774 macrophages than the second. This compound also presented *in vitro* activity against other *Leishmania* strains and was highly efficient in reducing *L. infantum* parasite burden in mice. This is therefore a promising compound for the treatment of leishmaniasis, as it also has low toxic effects and a potential for broad coverage on different genotypes and parasite populations. Although other oxoaporphines have been studied for their leishmanicide activity, they are mostly present in low concentrations in plants [42]. The ones described by Bilbao *et al.*, on the other hand, can be easily synthesized using standard chemical processes.

Dichloromethane and hexane fractions of *Calophyllum brasiliense* leaf extract also present activity against *L. amazonensis* infection both *in vitro* and *in vivo* [43,44]. Topical or intraperitoneal treatment reduced lesion size and parasite number in the lymph nodes of *L. amazonensis*-infected mice, and results were comparable to amphotericin B treatment. In addition, whereas animals treated with amphotericin B presented changes in hair color, increase in urine excretion and weight loss, these changes were less severe in the animals treated with *C. brasiliense* extract and fractions, suggesting less collateral effects. Indeed, the advantages of topical treatment, as proposed by Cortez *et al.,* are the few adverse effects, better compliance by the patient and reduced cost. Another compound isolated from the leaves of *C. brasiliense*, mammea A/BB, a coumarin type mammea, also presented leishmanicidal activity *in vivo*. Results were comparable to meglumine antimoniate treatment, as assessed by measuring footpad lesion size in *L. amazonensis*-infected mice [45]. It has been suggested that this is the active compound in the above-described dichloromethane fraction. Treated animals presented no behavioral alteration and histopathological analyses of the liver, spleen, lung, testicles, esophagus, kidney, heart, duodenum and stomach indicated no treatment-related changes. This is a strong candidate for the topical treatment of CL.

Novel compounds with leishmanicidal activity were also extracted from the brown algae *Sargassum yamadae* by Kimura *et al.* [46]. One of such compounds was able to inhibit *L. major* growth *in vitro* and lesion formation in *L. major*-infected mice, reducing antibody titer against *L. major* and the detection of protozoan DNA in the blood of mice (by PCR) at comparable levels to amphotericin B. Toxicity in mammals still needs to be assessed.

In some cases, a reduction in toxic effects is considered advantageous for a new leishmaniasis drug, even if results demonstrate a higher IC_{50} than that obtained for standard drugs. For instance, Rahman *et al.* described a topical ointment formulation for treatment of CL prepared from methanolic extracts of *Physalis minima*, an herbal plant from the genus *Physalis* [47]. Although the chloroform extract presented the highest leishmanicidal activity *in vitro*, the methanolic extract in petrolatum exhibited the lowest toxicity against 3T3 cells. The later was therefore chosen for the preparation of an ointment formulation for topical treatment of CL, resulting in complete cure of lesions in mice within 12 weeks, without any notable systemic effects. Physalin compounds obtained from another species of *Physalis*, *P. angulata,* also presented antileishmanial activity *in vitro* (against *L. amazonensis*, *L. major*, *L. brasiliensis* and *L. chagasi*) and were able to reduce lesion size, tissue damage and parasite burden in *L. amazonensis*-infected mice after topical treatment [48]. Again, although IC_{50} was higher than for amphotericin B, experiments indicated the concentrated ethanolic extract of *P. angulata* is non-mutagenic in the Ames test up to 3 kg/plate and does not induce chromosome damage in rodents [49].

2.1.2 Pentamidine

Pentamidine, introduced in 1952 in leishmaniasis therapy, is still used as one of the drugs of choice in leishmaniasis treatment [16,50]. However, its use as a leishmanicide agent is restricted due to its high toxicity, which can cause adverse effects such as nausea, vomiting, headache, hypoglycemia and sudden death [51,52].

In an attempt to develop new leishmanicide agents to overcome these side effects, Tidwell *et al.* have synthesized compounds based on an aromatic ring, or multiple aromatic rings linked covalently, which were then branched by adding functional groups or alkyl groups [52]. IC_{50} values obtained with these compounds in *in vitro* experiments with *L. donovani* were significantly lower than that of pentamidine. Moreover, *in vitro* toxicity on L-6 rat mycoblasts was also lower. These results suggest a potential for lower adverse side effects compared to pentamidine. It will be interesting to see the follow-up research, with *in vivo* results.

Concerned with the need for pentamidine to be administered parenterally, causing potentially severe side effects, this group of researchers developed oral prodrugs based on dicationic diaryl thiophenes, selenophenes and bichalcophenes for treating *L. donovani* infection [53,54]. Bichalcophenes were chosen as many studies and patents of synthetic and naturally occurring chalcone derivatives have reported their potential against *Leishmania* [53,55-60]. In the first work by Tidwell *et al.* [53], the IC_{50} obtained for one of these compounds in the *in vitro* experiments with *L. donovani* was significantly less than that obtained for pentamidine. Toxicity against rat mycoblasts *in vitro* was also lower. In the second work [54], most prodrugs exhibited poor *in vitro* activity due to the absence of the enzymes needed for bioconversion to the active diamidines. However, two of these compounds showed much lower IC_{50} values against *L. donovani* than pentamidine, as well as lower toxicity on rat mycoblasts. Moreover, the enhanced solubility and alternative route of administration of the new pentamidine derivatives positively influenced their pharmacokinetic behavior,

resulting in improved absorption and bioavailability in rats and lower toxicity compared to pentamidine [61]. These prodrugs therefore show potential for leishmaniasis treatment, especially as an oral alternative to pentamidine. It will be interesting to see leishmanicidal evidence *in vivo*.

Boechat *et al.* have developed 1,2,3-triazole or imidazole compounds useful for treating or inhibiting leishmaniasis [62]. Several of the synthesized compounds showed higher inhibition of *L. amazonensis* growth *in vitro* at a lower concentration than pentamidine.

Werbovetz *et al.* synthesized derivatives of dinitroaniline sulfonamide compounds for the treatment of parasitic diseases, including leishmaniasis. All synthesized compounds presented higher IC_{50} than pentamidine against *L. donovani*. However, the modifications introduced by this invention resulted in higher inhibition of leishmania tubulin assembly and lower IC_{50} for most of the synthesized compounds compared to oryzalin, representing an improvement of these derivatives over this marketed dinitroaniline compound [63].

2.1.3 Meglumine antimoniate

For many years this was the standard recommended drug for leishmaniasis treatment, together with sodium stibogluconate [64]. However, the major downsides of using this pentavalent antimonial are the need for injectable administration, hospitalization and monitoring, as well as the side effects, among which are: high cardiotoxicity [65,66], pancreatitis [66,67] and nephrotoxicity [68].

Aiming to replace meglumine antimoniate in CL treatment, quaternary ammonium salts containing an *N*-(halomethyl) substituent were developed by Cedeno-Medina *et al.* in 2012 [69]. Some of the disclosed compounds effectively killed axenic amastigotes of *L. (V) panamensis*. Topical application of the halogenated ammonium salts resulted in the healing of cutaneous lesions in infected golden hamsters. Results were similar to the ones obtained with meglumine antimoniate intramuscular injection, although the required dose was smaller.

Beilles *et al.*, on the other hand, disclosed a new use in leishmaniasis therapy for a drug already used in the treatment cardiac arrhythmia, 2-*n*-Butyl-3-[4-(3-di-*n*-butylaminopropoxy)benzoyl]-5-methylsulphonamidobenzofuran, or dronedarone [70]. *In vitro* experiments indicated dronedarone presents potential activity against CL and VL. *In vivo* experiments demonstrated that an aqueous gel formulation of dronedarone, used as topic treatment, was able to efficiently inhibit lesion growth in *L. amazonensis*-infected mice. Parasite loads in the ear were significantly reduced. Results obtained were comparable to that of intralesional meglumine antimoniate treatment. IC_{50} values in *in vitro* experiments were in the micromolar range, placing dronaderone between amphotericin B and miltefosine in terms of activity. IC_{50} values for meglumine antimoniate were much higher. Nontoxic maximum concentration on macrophages was calculated as 12.5 µM for dronaderone, making it a promising candidate for CL treatment. Moreover, additive interactions were observed between dronedarone and meglumine antimoniate or amphotericin, while synergistic interactions were observed with miltefosine. The latter is especially interesting, as it could imply the possibility of dose reduction in the concomitant treatment of dronedarone and miltefosine. Other results indicated that *L. amazonensis* strains that are resistant to meglumine antimoniate respond well to dronedarone and no resistance was detected after 6 months of *in vitro* culture with increasing doses of dronedarone (similar exposition to meglumine antimoniate resulted in an increase of IC_{50} by 200 times). These results, coupled with its low toxicity, synergistic activity with reference drugs and possibility of therapeutic effect on various *Leishmania* strains make it an excellent candidate drug for leishmaniasis treatment, especially in cases of meglumine antimoniate resistance. Dronedarone's activity against *L. mexicana* is shown by Benaim *et al.* [71].

Pollmeier and Blair disclosed a method of treatment for *L. infantum* infection in canines using fexinidazole. A complete curing efficacy was obtained with fexinidazole in *L. infantum*-infected dogs (meglumine antimoniate was used as positive control) [72]. This compound already presented activity against *Trypanosoma cruzi*, *Trichomonas vaginalis*, *Entamoeba histolytica*, and *L. donovani*, as claimed in a previous patent. Moreover, Wyllie *et al.* have demonstrated that fexinidazole significantly inhibited *L. donovani* infection in mice. Percentage inhibition was similar to miltefosine and sodium stibogluconate [73]. Thus, these findings have demonstrated the potential of fexinidazole as an oral drug for leishmaniasis treatment. Fexinidazole is on Phase II clinical trials for VL [74].

2.1.4 Miltefosine

Miltefosine is an alkylphosphocholine drug with well-known activity against leishmaniasis species. It is the first and only oral drug registered for VL and CL therapy. However, due to its long half-life, it is feared that resistance may quickly emerge [75]. In fact, the high relapse rate observed for VL treatment with this drug has been cited as its major limitation [76,77].

Oleyl phosphocholine (OIPC) is a structural analogue of miltefosine [78]. Its use for leishmaniasis treatment was specifically claimed in 2007 [79]. Inventors found that OIPC administration by oral or intravenous route in dogs, at therapeutically effective dosages, do not incur the side effects observed with miltefosine. Moreover, its activity in lower concentrations than miltefosine suggested that toxicity would also be reduced, opening new possibilities for new uses, in particular long-term administration. In fact, OIPC's *in vitro* activity against several *Leishmania* strains was later demonstrated by Fortin *et al.* [78] OIPC's leishmanicidal activity was comparable to miltefosine, with IC_{50} values in the lower micromolar range (lower than the average values obtained for paramomycin and pentavalent antimonial, but higher than the ones for amphotericin B). *In vivo* experiments demonstrated that at a 40 mg/kg dose administered by oral gavage for 5 consecutive days, OIPC/H_20 was significantly more effective than miltefosine in reducing *L. infantum* parasite burden in the liver

of golden hamsters. Results for spleen and bone marrow were similar for both compounds. More importantly, excellent results were obtained with a single dose of 100 mg/kg of OlPC/H_2O or OlPC liposomes. No signs of toxicity or adverse drug-related effects were noted. The possibility of complete cure with a shorter treatment regimen than miltefosine (currently 28 days) is one of the major advantages envisaged for OIPC. In addition, OlPC appears to clear more rapidly than miltefosine, which may be beneficial with regard to the selection potential for resistance.

Searching for an alternative for miltefosine in leishmaniasis treatment, Dafra Pharma developed solid dosage forms of OIPC for oral administration [80]. Previously suggested OIPC formulations were generally solutions, suspensions or emulsions, as OIPC's low melting point, irregular particle size and shape and high hygroscopicity limited the development of solid dosage forms. Dafra Pharma's new formulation, which is stable for 1 year in temperatures ranging from 2 to 75°C, presented higher leishmanicidal activity at equivalent doses when compared to miltefosine after oral administration to hamsters artificially infected with *L. infantum.* OlPC liposome formulations at 40 mg/kg showed similar results to free OIPC. Interestingly, at 20 mg/kg, liposome formulations showed in fact a lower reduction in parasite burden compared to free OIPC. The possibility of using free OIPC instead of a liposome formulation is beneficial to decrease the cost of the final formulation.

A further patent application from the same company discloses ointments and creams containing OIPC as an active ingredient for the treatment of several diseases, including leishmaniasis [81]. The claimed formulation was found to be more efficient than a commercial miltefosine formulation for the treatment of mice subcutaneously infected with *L. major*, resulting in higher lesion reduction and complete elimination of parasite burden in draining lymph nodes and skin upon topical administration. Although treatment resulted in weight loss and strong skin reactions, the efficacy presented by the claimed formulation makes this a promising compound that could substitute miltefosine in the future. A more recent study has demonstrated OIPC's clinical efficacy against *L. infantum* canine leishmaniasis [82].

Ihara *et al.*, on the other hand, synthesized rhodacyanine derivative compounds to treat leishmaniasis, aiming for an effective drug with lower side effects. It is well known that rhodacyanine presents high toxicity against parasitic leishmaniasis [83-85] and low cytotoxicity against mammalian cells. The *in vitro* data showed that the synthesized rhodacyanine derivatives exhibited far higher *L. major* inhibitory activity than standard drug miltefosine and lower cytotoxicity than an allosteric Hsp70 inhibitor compound with anti-*Leishmania* activity, the rhodacyanine dye MKT-077 [86,87], demonstrating effective anti-*Leishmania* activity with potentially less side effects [88].

Another alternative was disclosed by Camarasa Rius *et al.* [89]. This group has synthesized nucleoside derivatives for treating infectious diseases caused by *Leishmania* in humans or animals. In the *in vitro* experiments, one of the synthesized compounds showed far higher cytotoxic activity against *L. infantum* than miltefosine. In addition, toxicity on Jurkat human cells is significantly lower than for miltefosine, indicating that this compound may constitute a potential alternative to the drugs currently used for treatment of the leishmaniasis [89].

Another promising compound in this respect is spinosad, an insecticide comprised of spinosyn A and D [90]. Spinosyns, which are fermentation products produced by the soil bacteria *Saccharopolyspora spinosa*, consist of a 5,6,5-tricylic ring system fused to a 12-membered macrocyclic lactone, a neutral sugar (rhamnose), and an amino sugar (forosamine) [91]. Studies *in vitro* using *L. donovani* demonstrated that Spinosad's selectivity index (CC_{50}/IC_{50}) was more promising than that of miltefosine: 4.73 compared to 39.04, respectively. Studies *in vivo* indicated that spinosad was able to eliminate 82 and 73% of parasites in the spleen and liver of *L. donovani*-infected mice, respectively. Treatment with spinosad had no effect on liver or spleen weight and no sign of toxicity to infected mice. The related patent, which refers to a new use for spinosyns, is owned by a chemical enterprise based in Greece named Entarco.

2.2 Drug delivery

Many amphotericin B formulations exist in the state of the art, some of which are currently commercialized. In the specific time range encompassed by the present patent landscape, we have identified several amphotericin B formulations, including liposomal ones. For instance, Nahid and Antara disclosed *in vivo* data using *L. donovani*-infected mice showing amphotericin B's therapeutic efficacy was synergistically enhanced by using a cationic liposomal formulation. At a dose of 3.5 mg/kg, this formulation completely cleared parasites at the liver and spleen of infected mice, presenting a better efficacy than one of the marketed liposomal formulations, Ambisome. Interestingly, this formulation increased immune activity in mice, suggesting the possibility of prophylactic use. Thus, the invention provides a promising formulation to treat both VC and CL [92].

In the search for new topical formulations with greater efficacy and lower toxicity, Duran *et al.* developed a topical pharmaceutical formulation comprising a complex of amphotericin B-γ-cyclodextrin for treating parasitic infections, including CL. The *in vitro* data against many different *Leishmania* species showed this formulation presented higher safety profile than the conventional formulation of amphotericin B deoxycholate. However, *in vivo* results were modest. Moreover, the topical formulation presented higher physical and chemical stability than conventional formulation of amphotericin B deoxycholate and, unlike the latter, there is no need for this new formulation to be dissolved in dimethyl sulfoxide [93,94].

Aiming to reduce the toxicity of amphotericin B, Duran *et al.* have developed water-dispersible albumin

microspheres containing amphotericin B [95]. Amphotericin B's mode of action relies on its ability to bind ergosterol as well as episterol and 5-dihidroepisterol molecules, altering the structure of the cellular membrane of fungi and some protozoans, such as *Leishmania*. However, amphotericin B presents the disadvantage of also binding cholesterol molecules in the cellular membranes of mammals. Although the affinity for cholesterol is lower than for the above-mentioned molecules, this can still account for undesirable toxic effects to mammals. Indeed, alternative lipidic formulations exist to circumvent this toxicity (for a review see Balasegaram *et al.* [96]). However, these are still costly. The above-mentioned albumin-microspheres formulation has the advantage of reducing amphotericin B toxicity, while being cheaper to produce than liposomal formulations. Results indicate that the albumin formulation was more efficient than amphotericin alone in reducing parasite burden in the spleen of *L. infantum*-infected syrian golden hamsters. Moreover, acute toxicity studies in golden hamsters showed a much lower dose of amphotericin B resulted in 100% animal death. This dose could be increased by 20 times using the albumin formulation and still result in 100% animal survival. These results indicate this is a very promising alternative amphotericin B formulation for leishmaniasis treatment.

Dealbornoz *et al.* disclosed nanoparticles obtained by the micellar polymerization of alkyl cyanoacrylate loaded with antileishmanial drugs such as amphotericin B. The *in vivo* studies showed treatment with polymeric nanoparticles loading amphotericin B resulted in higher parasite load reduction in the spleen of *L. donovani*-infected rats than free amphotericin B treatment. It should be noted that even unloaded polymeric nanoparticles showed a significant parasite reduction [97].

Domb *et al.* disclose three formulations of modified dextran polymer conjugates with amphotericin B as an alternative to the commercially available liposomal and micellar formulations. Although the IC_{50} for the free drug was smaller than the ones obtained with dextran polymer conjugates, toxicity was greatly reduced [98].

Drug delivery systems using a different drug than amphotericin B have also been disclosed. Rezende *et al.* developed drug delivery systems based on liposomes enabling the leishmanicidal drug to reach all *Leishmania*-infected mice. Both conventional and pegylated liposomes loaded with meglumine antimoniate showed higher efficacy against *L. infantum chagasi* present in the liver of infected mice than control groups (saline or unloaded liposomes). However, some of the developed formulations were more effective to target certain organs than others. For instance, pegylated liposomes provided higher targeting of the leishmanicidal drugs to the bone marrow and spleen [99]. On the other hand, the blend of conventional and pegylated liposomes loaded with meglumine antimoniate presented a synergistic ability to significantly decrease *L. infantum chagasi* parasites present in the spleen, when compared to the individual formulations. Hence, depending on the target organ, different formulations can be chosen. Targeted delivery to the bone marrow, spleen and liver are a means to decrease side effects. Therefore, delivery systems such as these are very useful for leishmaniasis treatment.

Blanco-Prieto *et al.* developed lipid nanoparticles loaded with edelfosine or other phospholipid ethers for *Leishmania* treatment by oral route. The drug delivery systems were tested in *L. major*-infected mice. Results showed not only an improvement of the bioavailability over the free phospholipid ether but also a significant decrease in the evolution of infection and reduction of *L. major* parasites. In addition, treatment did not cause weight loss in animals [100].

Finally, Coelho *et al.* developed mono- and multilayered films using polymeric dispersion of chitosan in aqueous organic solutions with activity against leishmaniasis [101]. Although the developed chitosan films did not show such a high *in vitro* cytotoxicity against *L. amazonensis* as amphotericin B, they can still be used against leishmaniasis on their own or, more likely, as a release formulation for other active drugs, including volatile substances, resulting in a synergistic effect against leishmaniasis.

Despite the promising research related to drug delivery systems for improved leishmaniasis treatment, one needs to keep in mind that higher final formulation cost is critical obstacle for drug development in this area. Hence, for such systems to reach the market, the final cost needs to be reduced.

3. Conclusion

The number of patents recovered in the search for promising compounds for leishmaniasis treatment indicates there is quite a lot of international effort for the search of less toxic and efficient alternatives for leishmaniasis treatment. Not all patents retrieved were included in this review. Inclusion criteria were based on the evidence of leishmanicidal activity, except for the section of drug delivery, and on the use of a standard drug as positive control.

Most of the R&D is still in preclinical stage at the time of filing, what is very common in the pharmaceutical field. The difficulty to analyze the follow-up of these patents to determine the present stage of development of the disclosed technology prevents a current scenario to be drawn from these data. A link of patent data to clinical trials is needed to add transparency to patent analyses, this being especially useful in the case of neglected diseases.

The high number of patents that are dead due to failure of payment of maintenance fees (lapsed patents) compared to patents refused for not meeting patentability requirements (revoked) seems to be an indication of the difficulties faced by research institutes and universities, which constitute the majority of patent assignees, in maintaining these patents alive and/or carrying out all the stages necessary for the disclosed compounds to reach the final stages of innovation, entering into the market. Thus, governmental action is crucial to guarantee innovation.

4. Expert opinion

For a long time, the first choice therapy for leishmaniasis treatment was parental administration of pentavalent antimonials. However, increased parasite resistance has been observed and several side effects are experienced by patients. Amphotericin B and its liposomal formulations are effective, although such drugs are expensive and require hospitalization. Treatments using oral miltefosine are encouraging; however, therapies are extensive, lasting in general 28 days and are linked to potential toxicity, resistance and teratogenicity. Therefore, the development of alternative therapeutic strategies to treat leishmaniasis is a high-priority. Several laboratories are investigating natural products, searching for new candidates, and the screening of these materials for antileishmanial activity should continue in the future. Specifically for cutaneous disease, many studies have shown promising results with immunotherapy/immunochemotherapy, aimed to modulate and activate the immune response to obtain a therapeutic cure. Over the past few decades, major emphasis has been given to the identification of new formulations for both oral and topical treatments. This is an interesting approach, offering several advantages in comparison with parental administration, like improved safety, better compliance, decreased pain and reduced cost compared to needle-use treatment. The main problem for the investigation of new drugs is that the disease occurs mainly in poor countries affecting the poorest, so the drugs required should preferably be inexpensive and designed for oral administration. One has to keep in mind that since a low cost final formulation is needed, efficient formulations may be developed and still not solve the problem of lack of adequate therapy.

This review has disclosed many interesting drug candidates for leishmaniasis therapy. Analyzing each of the disclosed compounds further, we have selected the ones we consider as the most promising. This is a difficult prediction, as most of the inventions are still in the early stages of drug development. Furthermore, as most patents came from universities and research institutes and not pharmaceutical companies, it is hard to predict which ones will enter clinical trials. Still, selection criteria were based on the *in vivo* data included in the patent document or in a related scientific publication, on the comparison of leishmanicide activity with a reference drug, on the potential toxicity and on the potential of fulfillment of patient's needs. The main challenge regarding market needs was the reduction in length of treatment, as in most patents it is too early to tell if this will be possible.

For CL, we considered an oral or topic formulation as the ultimate goal of drug development. Hence, we would like to draw attention to:

1) Dronedarone aqueous gel formulation: an excellent candidate for CL topic treatment due to its lower cytotoxicity and IC_{50} compared to meglumine antimoniate and miltefosine. Its synergistic activity with reference drugs, such as miltefosine, brings in the benefits of combined therapy, and *in vitro* experiments have shown its potential in case of meglumine antimoniate resistance. Furthermore, *in vitro* activity has been demonstrated against several *Leishmania* strains, an important advantage if this hold true *in vivo*.
2) Quaternary ammonium salts containing an *N*-(halomethyl) substituent: Topical application showed similar *in vivo* results against *L. (V) panamensis* to the ones obtained with meglumine antimoniate intramuscular injection. However, a much smaller dose was required in comparison to the later. Thus, this drug candidate has the potential of reduced side effects by using a smaller dose and avoiding the systemic effects of intramuscular injection.

For VL, we considered the ultimate goal would be an oral drug with the potential of reducing cost and length of treatment in comparison with the currently marketed amphotericin B formulations. The following compounds were selected:

1) Fexinidazole: Presents potent activity against *L. donovani* and *L. infantum in vitro* and *in vivo* and is on Phase II clinical trials for VL. Thus, it is not a drug candidate on its early stages of development, increasing its chances of being successful. This is an important oral drug alternative for VL treatment.
2) OIPC is a very interesting drug as experimental data indicate it can be used both for VL and CL treatments. This drug candidate presented higher leishmanicidal activity *in vivo* compared to miltefosine after oral administration against *L. infantum*. Also, topical applications of OIPC were more efficient against *L. major in vivo* than miltefosine administered by oral route. Thus, OIPC is has great potential for VL and CL treatments and, if successful, could substitute miltefosine in the future.

Drug delivery is an area of research we consider particularly interesting, as it has the potential of enabling drug targeting, as well as reducing toxicity and length of treatment. However, patent data retrieved in our search did not point out to research in this direction. Still, we would like to draw attention to the following formulation:

1) Water-dispersible albumin microspheres containing amphotericin B: The data indicate this is a very promising alternative amphotericin B formulation for CL and VL treatments, with higher activity and lower toxicity than free amphotericin B and cheaper than liposomal formulations.

As a final consideration, taking into account the number of patents coming from academia, it is urgent to find new ways to make sure research into treatment for neglected diseases

will continue into clinical phases. DNDi (Drugs for Neglected Diseases initiative) has an important role in this direction. After a decade of existence, the initiative has delivered six new treatments for neglected diseases and established a solid drug development pipeline. Over 350 collaborations in 43 countries, including nearly 20 pharmaceutical and biotechnology companies, and over 50 universities and research institutes have been put into action. DNDi can be viewed as a successful model that has both built a robust pipeline of game-changing drug candidates and delivered life-improving and live-saving treatments to millions of patients. One example is the combination therapy of sodium stibogluconate and paromomycin, recommended as first-line treatment of kala-azar in East Africa, which reduces treatment duration by nearly half and decreases total cost, compared with sodium stibogluconate alone. DNDi's portfolio of projects, some now in clinical trials, for example, fexinidazole and oxaborazoles, certainly will result in new drugs for leishmaniasis treatment in near future.

Declaration of interest

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Affiliation

Alice Machado-Silva[1],
Pedro Pires Goulart Guimarães[1],
Carlos Alberto Pereira Tavares[2] &
Rubén Dario Sinisterra[†1]
[†]Author for correspondence
[1]Universidade Federal de Minas Gerais, Instituto de Ciências Exatas,Departamento de Química, Av. Antonio Carlos, 6627, Pampulha, CEP 31270-901, Belo Horizonte-MG, Brazil
Tel: +55 31 3409 5778;
Fax: +55 31 3409 5700;
E-mail: sinisterra@ufmg.br
[2]Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Bioquímica e Imunologia, Av. Antonio Carlos, 6627, Pampulha, CEP 31270-901, Belo Horizonte-MG, Brazil

Supplementary material available online

Tables 1, 2 and 3