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# Investigational Drugs for Visceral Leishmaniasis

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## Abstract

**Introduction—**The armamentarium of antileishmanials is small. It is further being threatened by development of resistance and decreasing sensitivity to the available drugs. Development of newer drugs are sorely needed.

**Areas covered—**Literature search on investigational drugs for visceral leishmaniasis (VL) was done on PubMed. Those candidates with at least in vitro and in vivo activity against leishmania species causing VL were reviewed. Among the investigational drugs the nitroimidazole compound fexinidazole is the one of the few drugs which has reached phase II trials. Although the (S)-PA-824 is in phase II trials for the treatment of tuberculosis its R enantiomer has shown good antileishmanial activity. Development of sitamaquin, which has completed phase II studies has been stopped for VL due to its low efficacy. Many novel delivery system and oral formulations of Amphotericin B which are cheap and less toxic are in investigational stages, and will go a long way in improving the treatment of VL.

**Expert opinion—**Very few new drugs have reached the clinical stage in the treatment of this neglected tropical disease. Thus, there is an urgent need for support from public private partnerships to ensure that drug candidates are promptly taken forward into development.

### Keywords

Visceral leishmaniasis; investigational drugs; therapy

## 1. Introduction

### 1.1 Organism

Leishmaniasis, a vector-borne disease, is caused by an obligate intracellular protozoan of the genus *Leishmania*. It broadly manifests as visceral leishmaniasis (VL; also known as kalaazar), cutaneous leishmaniasis (CL) and mucocutaneous leishmaniasis (MCL). VL is caused by the *Leishmania donovani* complex: *L. donovani*, the causative organism of VL in the Indian subcontinent and Africa; *L. infantum* (*L. chagasi*) which causes VL in the Mediterranean basin, Central and South America. CL is caused by various *Leishmania* species[1].

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In South Asia and the Horn of Africa, the predominant mode of transmission of VL is anthroponotic, and humans with kala-azar or post--kala-azar dermal leishmaniasis (PKDL) provide the major reservoir for transmission[2, 3]. In the Mediterranean, the Middle East and Brazil, VL is zoonotic, with the domestic dog as the most important reservoir host sustaining transmission[3]. Most CL have zoonotic transmission except those caused by *L. tropica*, which is predominantly anthroponotic. The only proven vectors of human disease are sand fly of species *Phlebotomus* in the Old World and *Lutzomyia* in the New World[1].

### 1.2 Disease

VL is the most severe form of leishmaniasis, characterized by prolonged fever, splenomegaly, hepatomegaly, pancytopenia, progressive anemia and weight loss. If untreated, VL is uniformly fatal. After recovery, about 50% of patients in Sudan and 1 to 3% in India develop dermal leishmaniasis characterized by indurated nodules or depigmented macules called PKDL[4, 5]. The clinical features of CL tend to vary between and within regions, reflecting different species of parasite or the type of zoonotic cycle concerned, immunological status and also perhaps genetically determined responses of patients[6]. In CL, Old World species mostly cause benign and often self limiting cutaneous disease, while New World species cause a wide spectrum of manifestation, from benign to severe disease including mucosal involvement.

### 1.3 Epidemiology

Approximately 0.2 to 0.4million VL cases and 0.7 to 1.2million CL cases occur each year. More than 90% of global VL cases occur in just six countries: India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia. CL is more widely distributed, with about one-third of cases occurring in each of three regions, the Americas, the Mediterranean basin and western Asia from the Middle East to Central Asia. The ten countries with the highest estimated case counts, Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica and Peru, together account for 70 to 75% of globally estimated incidence of CL[7].

HIV—VL co-infection is reported from more than 35 countries. Initially, most of these cases were from south-western Europe, but the number of cases is increasing in sub-Saharan Africa, especially Ethiopia, Brazil and South Asia[8-10].

### 1.4 Present treatment guidelines

**Visceral leishmaniasis—**At present, single dose of 10 mg/kg of Liposomal Amphotericin B (L-AmB) or combination therapy consisting of either (single injection of 5 mg/kg L AmB and 7-day 50 mg oral miltefosine or single injection of 5 mg/kg L AmB and 10-day 11 mg/kg intramuscular paromomycin (PM); or 10 days each of miltefosine and PM) are the preferred treatment options in the Indian subcontinent[11, 12]. The combination of Sodium stibogluconate (SSG) with paromomycin (PM) for 17 days is the treatment of choice in East Africa and Yemen, whereas L-AmB up to a total dose of 18 -- 21 mg/kg is the treatment of choice in Mediterranean Basin, Middle East, Central Asia and South America[1, 13].

**Post-kala-azar dermal leishmaniasis—**In India, Amphotericin B 60 -- 80 doses of 1mg/kg over 4 months or miltefosine for 12 weeks are the recommended regimens but the compliance is poor. In East Africa, PKDL is not routinely treated, as the majority of cases (85%) heal spontaneously within 1 year. Only patients with severe or disfiguring disease, those with lesions that have remained for > 6 months, those with concomitant anterior uveitis and young children with oral lesions that interfere with feeding are treated, with either SSG (20 mg/kg/day per day) for up to 2 months or a 20-day course of L-AmB at 2.5 mg/kg/day[1].

**HIV-VL co-infection—**Lipid formulations infused at a dose of 3 -- 5 mg/kg daily or intermittently for 10 doses (days 1-5, 10, 17, 24, 31 and 38) up to a total dose of 40 mg/kg are recommended. Antiretroviral therapy should be initiated and secondary prophylaxis should be given till the CD4 counts are > 200/μL[1].

The armamentarium of antileishmanials is small, consisting of pentavalent antimonials, amphotericin B (AmB) and its lipid formulations, miltefosine and paromomycin. For several decades, pentavalent antimonials ($Sb^v$) have been the standard first- line medicines for visceral leishmaniasis. However, widespread resistance to the drug has developed in North Bihar and neighbouring areas of Nepal[14, 15]. The efficacy of the only oral drug miltefosine has also declined dramatically in the Indian subcontinent[16]. With the dwindling efficacy of drugs, development of newer antileishmanials is the need of the hour. Here we have reviewed the various investigational drugs for the treatment of VL. Literature search was done on Pubmed. As our search revealed a large number of compounds with antileishmanial activity, we reviewed those with at least *in vitro* and *in vivo* activity against *leishmania* species causing visceral disease. Preference to drugs which can be administered orally, already in use for other indications, cheap and stable at different temperatures have been given in this review .

## 2. Investigational drugs

### 2.1 Nitroimidazole compound

**2.1.1 Fexinidazole—**Fexinidazole (1-methyl-2-((p-(methylthio)- phenoxy)methyl)-5-nitroimidazole (Fig: 1), CAS registry number 59729- 37-2) had been in preclinical development in the 1970s and early 1980s as a broad-spectrum antimicrobial agent by Hoechst AG (now sanofi-aventis)[17, 18]. Although the *in vivo* activity of fexinidazole against African trypanosomes was observed in early 80's further development of the drug was halted[19]. It was rediscovered as a promising drug candidate for the treatment of Human African trypanosomiasis (HAT) by the Drugs for Neglected Diseases initiative (DNDi) after extensive compound mining efforts of more than 700 new and existing nitroheterocycles mostly nitroimidazoles[20].

A bacteria-like nitroreductase has been implicated in both the mode of action and the mechanism of resistance to nitro-drugs in the related trypanosomatids, *Trypanosoma brucei* and *T. cruzi*[21-23]. Fexinidazole, is believed to act as prodrugs that require enzyme-mediated reduction by nitroreductases to generate cytotoxic species that cause DNA, lipid and protein damage [24]. As the genomes of *leishmania* parasites contain a homologous nitroreductase

gene a study to assess the leishmanicidal activity and preclinical profile of fexinidazole was done[25].

**2.1.1.2 *In vitro* and *In vivo* sensitivity of *L. donovani* to fexinidazole and its metabolites:** The leishmanicidal activity of fexinidazole and its two predominant metabolites (fexinidazole sulfoxide and sulfone) was observed *in vitro* against promastigotes and axenic amastigotes of *L. donovani* (strain LdBOB).The $EC_{50}$ values of fexinidazole against promastigotes and amastigotes was 5.6 ± 0.2 and 2.8 ± 0.1 μM while that of miltefosine was 6.1 ± 0.3 and 4.4± 0.2 μM respectively. The sulfoxide and sulfone metabolites of fexinidazole was as sensitive as the unmetabolized form of the drug. However, in intracellular *L. donovani* (LV9) amastigotes in peritoneal mouse macrophages fexinidazole itself had little effect while fexinidazole sulfoxide and sulfone had $EC_{50s}$ of 5.3 ± 0.1 and 5.3 ± 0.2 μM, respectively which was comparable to miltefosine ($EC_{50}$ - 3.3 ± 0.3 μM).This suggests that the sulfoxide and sulfone and not the parent compound, were likely to be therapeutically important. *In vivo* sensitivity of *L. donovani* to the drug was excellent with five single daily doses of 200 mg/ kg in BALB/c mice suppressing infection by 98.4%.Lower doses of drug were also effective in treating the murine model of infection, with the $ED_{50}$ and $ED_{90}$ estimated at 12 and 57 mg/ kg, respectively[25]. In similar *in vivo* studies miltefosine had $ED_{50}$, 4 mg/ kg and $ED_{90}$, 27 mg/ kg and pentostam had $ED_{50}$, 20 mg/ kg and $ED_{90}$, 57 mg/ kg which shows that the results of fexinidazole were comparable to currently used antileishmanial[26]. To determine whether the drug was cytocidal or cytostatic axenic amastigotes were incubated with fexinidazole sulfone at a concentration equivalent to 10 times its $EC_{50}$ value. Growth of drug-treated cultures ceased almost immediately with cell numbers declining after 10h with no intact or viable cells visible by 30h. Fexinidazole sulfoxide also had a similar cytotoxic profile suggesting that both fexinidazole sulfoxide and sulfone were leishmanicidal[25].

**2.1.1.3 *In vivo* pharmacokinetic properties:** The oral absorption potential of fexinidazole was assessed in Caco-2 cell model for intestinal epithelial permeability which showed high absorption potential. The absolute bioavailability of oral fexinidazole was 41% in mice, 30% in rats and 10% in dogs. In all species tested, fexinidazole was rapidly and extensively metabolised to the sulfoxide and subsequently sulfone derivatives. Whole-body autoradiography in rats using [14C]-radiolabelled fexinidazole showed that the parent drug and/or its metabolites were broadly distributed to all organs and tissues, with peak concentrations in most tissues 2 h after oral dosing. After 48 h, most radioactivity was eliminated from the body and no tissue specific accumulation was noted[20]. In an *in vivo* sensitivity study of *L.donovani* to the drug in BALB/c mice the total blood concentrations of both the sulfoxide and sulfone comfortably exceeded their respective $EC_{99}$ levels shortly after oral dosing, The sulfoxide accumulated rapidly in the blood then its concentration dropped below the $EC_{99}$ after 8h.While blood concentrations of the sulfone were slower to accumulate, it remained above therapeutic levels for more than 24h.Thus their cumulative blood concentrations exceeded the $EC_{99}$ for ~30h, underlining the potential of fexinidazole as a once daily[25]. It was metabolised extensively by multiple CYP450 isoforms but none of the enzymes tested metabolised either the sulfoxide or the sulfone to any significant degree[20].

Overexpression of the leishmanial homologue of this nitroreductase in *L. donovani* increased sensitivity to fexinidazole sulfone by 19-fold indicating that nitroreductase played a crucial role in activation of fexinidazole and its metabolites in *L. donovani* [25].

**2.1.1.4 Safety:** Safety pharmacology and 4-weeks repeated-dose toxicokinetics in rat and dog showed that fexinidazole was well tolerated. The No Observed Adverse Event Levels in the 4-weeks repeated dose toxicity studies in rats and dogs was 200 mg/kg/day in both species, with no issues of concern identified for doses up to 800 mg/kg/day[20]. While fexinidazole and its metabolites was mutagenic in the Ames test due to bacterial specific metabolism however, mutagenicity was either attenuated or lost in Ames Salmonella strains that lacked one or more nitroreductase(s).It was not genotoxic to mammalian cells in an *in vitro* micronucleus test on human lymphocytes, an *in vivo* mouse bone marrow micronucleus test, and an *ex vivo* unscheduled DNA synthesis test in rats[20, 27].

Redox measurements showed that fexinidazole and its sulfoxide and sulfone metabolites possessed highly negative single-electron redox potentials of - 511, - 493 and - 488 mV, respectively. This was consistent with the absence of mutagenic activity in mammalian cells as mammalian nitroreductases are expected to be incapable of nitro-reducing compounds with such negative single-electron redox potentials[27].

**2.1.1.5 Human Studies:** Phase 1study of fexinidazole in healthy male volunteers has been completed[28]. Another phase 1study in which 3 or 4 tablets of fexinidazole 600mg per day were administered during 4 days (loading dose) then 2 tablets of fexinidazole 600mg for 6 days to 36 healthy male subsaharan volunteers was terminated due to poor tolerability at the higher dose.[29] A phase 1 study to assess the bioavailability of fexinidazole tablets after single oral dose of 1200mg under different food intake conditions has been completed.[30]

In the ongoing phase II/III study the efficacy and safety of fexinidazole 1800 mg once a day for 4 days, followed by 1200 mg once a day for 6 days is being compared to Nifurtimox-Eflornithine combination therapy in patients with late-stage Human African Trypanosomiasis (HAT) due to *T.b. Gambiense*[31]. Curently, a phase II proof of concept trial to determine efficacy of fexinidazole at the daily dose of 1800 mg (3 tablets) once a day for 4 days continued by 1200mg (2 tablets)once a day for 6 days in VL patients in Sudan is recruiting patients.[32]

**2.1.2 PA-824—**Another nitroimidazole compound PA-824, (*S*)-2-nitro-6-[4-(trifluoromethoxy)benzyloxy]-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine), is a 4-nitroimidazo-oxazine. It exhibits potent bactericidal activity against both replicating and nonreplicating *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), and is currently being tested in phase II clinical trials[33-35].

**2.1.2.1 *In vitro* sensitivity of *L. donovani* to (*S*)- and (*R*)-PA-824 ( Fig:2):** The potency of (*S*)-PA-824 was determined *in vitro* against *L. donovani* (LdBOB) promastigotes and against intracellular amastigotes in peritoneal mouse macrophages. (*S*)-PA- 824 showed antileishmanial activity against both developmental stages of the parasite, with $EC_{50}$ of 0.9± 0.1 and 4.9± 0.3μM against promastigotes and amastigotes, respectively[36]. On testing the

antileishmanial activity of *R* enantiomer of PA-284 which had minimal activity against *M. tuberculosis* (MIC, >100μM)[37] it was observed to be 5-fold-more potent inhibitor of *L. donovani* growth *in vitro* than the *S*-enantiomer candidate, with $EC_{50}$ of 0.16±0.03 and 0.9± 0.1 μM against promastigotes and intracellular amastigotes, respectively[36].

The efficacy of both (*R*)- and (*S*)-PA-824 was assessed in a mouse model of VL. Seven days following infection with *L. donovani* LV9 *ex vivo* amastigotes, groups of BALB/c mice were dosed orally with (*R*)- or (*S*)-PA-824 (30 or 100 mg/ kg) twice daily for five days. Fourteen days following inoculation, parasite burdens in the livers of infected mice were determined. Mice dosed twice daily at 30 mg /kg with (*R*)- or (*S*)-PA-824 suppressed infection in the murine model by approximately 35% compared to untreated controls. Increased dose of 100 mg/ kg did not improve the efficacy of (*S*)-PA-824 *in vivo*, while treatment with (*R*)-PA-824 at 100 mg/ kg effectively cured the murine model of infection, suppressing infection by 99.9%. Dosing with (*R*)-PA-824 at this level proved to be superior to treatment with sodium stibogluconate (41.9% suppression) and miltefosine (68.7% suppression)[36].

**2.1.2.2 *In vivo* pharmacokinetic properties of (*S*)- and (*R*)-PA-824:** Mice dosed with (*S*)- or (*R*)-PA-824 at 50 mg/ kg showed a maximum concentration in blood after 4 h of 11,600 or 10,500 ng/ ml, respectively Thereafter, blood concentrations decreased [elimination half-life (*t*1/2) ~5 h and 2 h for (*S*)- or (*R*)-PA-824, respectively], reaching undetectable levels at a time point between 8 and 24 h for (*R*)-PA-824. At 24 h, (*S*)-PA-824 still had a blood concentration of 900 ng/ ml.(*R*)-PA-824 had an $EC_{50}$ of 0.93μM for *L. donovani* cultured in macrophages and $EC_{90}$ value of 3.4μM(equivalent to 1,200 ng/ ml). (*R*)-PA-824 blood levels exceeded the $EC_{90}$ 1 h after dosing and remained above the $EC_{90}$ for at least 7 h. Thus, it was predicted that 100 mg/ kg twice daily of (*R*)-PA-824 would provide adequate exposure. Although (*S*)- and (*R*)-PA-824 reached comparable blood levels, the lower potency of (*S*)-PA-824 ($EC_{90}$= 22μM) resulted in a maximal free concentration below $EC_{90}$ (37,000 ng $ml^{-1}$) after oral dosing at 50 mg/ kg[36].

(*S*)-PA-824 is believed to function as a prodrug which requires bioreductive activation prior to exhibiting antitubercular activity[38, 39]. In *M. tuberculosis*, this reduction is catalyzed by an unusual deazaflavin (F420)-dependent nitroreductase[39, 40]. However, overexpression of nitroreductase in promastigotes did not significantly alter the sensitivity to either (*S*)-PA-824 or (*R*)-PA-824 suggesting that this nitroreductase did not play a role in the activation of PA-824 in *L. donovani*. Drug combination studies *in vitro* indicated that fexinidazole and (*R*)-PA-824 are additive whereas (*S*)-PA-824 and (*R*)-PA-824 showed mild antagonistic behaviour[36].

**2.1.2.3 Human studies:** Phase 1 study was done in 58 healthy male volunteers using single oral doses (50, 250, 500, 750, 1,000, 1,250, or 1,500 mg) or multiple doses of 200, 600 and 1,000 mg of (S)- PA-824 each day for 7 days. PA-824 reached maximal plasma levels in 4 to 5 h independently of the dose. Maximal blood levels averaged 3 μg/ml (1,500-mg dose) in the single-dose study and 3.8 μg/ml (600-mg dose) in the multiple-dose study. Steady state was achieved after 5 to 6 days of daily dosing, with an accumulation ratio of approximately 2. The elimination half-life averaged 16 to 20 h[34]. There was an increase in exposure to (S)-PA-824 when administered with high-calorie, high-fat meal compared to the fasted state[41].

It did not inhibit or induce CYP3A4 and is not likely to markedly affect the pharmacokinetics of CYP3A4 metabolized drugs[42].

**2.1.2.4 Safety:** (S)-PA-824 was well tolerated at all doses in the phase 1 study, with no serious adverse events. Headache was the most common adverse event, followed by elevated serum creatinine levels, stomach discomfort (nausea, vomiting, flatulence, and/or diarrhea), and back pain. In the multiple-dose study, maximum elevation of serum creatinine was 1.3 (200-mg-dose group) and 1.4 mg/dl (600-mg-dose group). In the 1,000-mg-dose group by day 5 of dosing serum creatinine levels had risen in five of six subjects by an average of 0.28 mg/dl relative to the baseline level; the highest recorded absolute value was 1.6 mg/dl. Consequently, dosing was stopped on day 5, however, serum creatinine levels returned to clinically normal levels during the ensuing 7-day[34]. Another study was conducted to assess the effect of the drug in renal functions of healthy volunteers. In this study other than creatinine elevations, multiple doses of 800- and 1,000-mg (S)-PA-824 were not associated with clinically meaningful alterations in any of the other renal function measured by glomerular filtration rate (GFR), change in effective renal plasma flow (ERPF), filtration fraction (FF), blood urea nitrogen (BUN), and uric acid (UA)[43]. Currently (S)-PA-824 has completed a phase II trial in which the combination of (S)-PA-824-moxifloxacinpyrazinamide was found suitable for treating drug-sensitive and multidrug-resistant tuberculosis [44].

## 2.2 New Amphotericin B formulations

AmphotericinB( AmB)(Fig:3) is one of the most potent antileishmanial agent however its major drawbacks are need for prolonged injections, toxicity and cost. Recently, novel drug delivery systems including liposomes, niosomes, microspheres, nanoparticles, carbon nanotubes have been used to provide targeted delivery of the drug to macrophages [45]. The lipid formulations have excellent efficacy and are less toxic but their cost are prohibitive. Thus efforts are ongoing to make cheaper and less toxic formulations of this drug and to make it orally bioavailable.

**2.2.1 Nano-amphotericin B—**The rationale behind testing nanoparticles of amphotericin B deoxycholate in leishmaniasis is that nanoparticles are recognized as foreign bodies and phagocytosed by the macrophages which also harbours the parasite leading to target specific delivery of the drug [46]. *In vitro* efficacy of nano-amphotericin B was done in intracellular amastigotes of *L. donovani* parasite (MZP 301strain). The $ED_{50}$ of nano-amphotericin B was 3.69-fold lower than that of amphotericin B. Cytotoxicity study of this formulation and amphotericin B in the J774A cell line revealed that $CC_{50}$ values were far higher (12.67 and 10.61 mg/L) than the $ED_{50}$ doses (0.004 and 0.012 mg/L, respectively) for intracellular amastigotes. *In vivo* studies in hamster with nano-amphotericin B and conventional amphotericin B injected intraperitoneally at 5 mg/kg per day for 5 days revealed inhibition of amastigotes in the splenic tissue with nanoamphotericin B was significantly more than with conventional amphotericin B (92.18% versus 74.57%, P= 0.005). Similarly, the suppression of parasite replication in the spleen was also found to be significant (99.18% versus 97.17%, P= 0.05)[47]. Amphotericin B (AmB)-encapsulated chitosan nanocapsules (CNC-AmB) showed 86% parasite inhibition in *Leishmania donovani*-infected hamsters

along with the upregulation of tumor necrosis factor alpha (TNF-α), interleukin-12 (IL-12), and inducible nitric oxide synthase and with the downregulation of transforming growth factor β (TGF-β), IL-10, and IL-4[48].

**2.2.2 Carbon Nanotubes—**In the family of nanomaterials, carbon nanotubes (CNTs) have emerged as a efficient tool for transporting drugs [49]. Functionalized carbon nanotubes (f-CNTs) prepared by carboxylation and amidation with amphotericin B attached to it was tested for antileishmanial activity. In intracellular amastigotes the efficacy of f-CNT–AmB was significantly higher than that of AmB ($IC_{50}$ 0.00234+0.00075 mg/mL versus 0.03263+0.00123 mg/mL; P≤0.0001). *In vivo* toxicity assessment in BALB/c mice revealed no hepatic or renal toxicity. The percentage inhibition of amastigote replication in hamsters treated with f-CNT–AmB injected intraperitoneally (i.p.) at 5 mg/kg body weight per day for 5 days, was significantly more than that with AmB at the same dose (89.85%+2.93% versus 68.97%+1.84%; P=0.0004)[50]. As the chemical synthesis of f-CNT–AmB involves covalent coupling rather than biological molecules,its production could be cheaper than the existing liposomal AmB. Covalent modification by the organic functionalization of end groups and side walls of f-CNTs increases the solubility of functionalized carbon nanotubes in a range of solvents, including water[51].With this idea the leishmanicidal efficacy of oral f-CNT-AmB was tested in hamsters. This experiment showed this formulation could be administered orally and resulted in 99% inhibition of parasite growth following a 5-day course at 15 mg/kg body weight[52].

**2.2.3 Oral lipid-based formulation of amphotericin B—**An oral lipid-based formulation of amphotericin B (iCo-009) composed of monoglycerides, diglycerides and distearoylphosphatidylethanolamine polyethylene glycol 2000 significantly reduced liver parasitemia in a murine model of VL at 10 and 20 mg/kg twice daily for 5 days and was also effective in systemic fungal infections [53, 54] . However, instability of the product at tropical temperature limited the potential of iCo-009 and a new oral lipid-based formulation of AmB (iCo-010) composed of monoglycerides, diglycerides, polyethylene glycol glycerides and D-alpha-tocopheryl polyethylene glycol succinate was developed and tested against VL in a murine model[55]. The formulation showed good antileishmanial activity at both 10 mg/kg po bid for 5 days (<99% reduction in parasitic infection) and 20 mg/kg po qd for 5 days (95% inhibition when compared to control) and very good stability at tropical temperatures. Histopathological analysis detected no gastrointestinal, liver or kidney toxicity following multiple dose oral administration of iCo-010 in BALB/c mice [56]. Multiple dosing of oral iCo-010 led to higher steady state concentrations in the tissues of rats , which could lead to enhanced eradication of tissue borne fungal and parasitic infections[57].

**2.2.4 Other Amphotericin B preparations—**AmB has been formulated in lipo-polymerosome (L-Psome) by spontaneous self-assembly of synthesized glycol chitosan stearic acid copolymer as a low cost, stable and safe alternative. *In vitro* and *in vivo* toxicity studies revealed high plasma stability and less toxicity of AmBL-Psome compared to commercialized Fungizone (amphotericin deoxycholate) and Ambisome (L-AmB). The rank order of antileishmanial efficacy was AmB-L-Psome > Ambisome > Fungizone. AmB-L-Psome also upregulated Th-1 cytokines (TNF-α, IL-12 and IFN-γ) and inducible nitric oxide

synthase, and downregulated of Th-2 cytokines (TGF-β, IL-10 and IL-4) in *in vitro* (macrophage amastigote system) and *in vivo* (hamsters) study[58].

It was hypothesized that direct non-covalent association of AmB with poly(methacrylic acid) (PMAA) would replicate many of the properties of liposomal AmB (L-AmB) thus water-soluble AmB–PMAA complexes with AmB loadings ranging from 20 to 45% were reproducibly prepared. The AmB–PMAA complex had *in vitro* activity against *Leishmania donovani* amastigotes with no macrophage toxicity observed at an $IC_{50}$ of 0.043 (±0.003) μM. It was well tolerated *in vivo* and a total dose of 6 mg/ kg achieved greater than 90% parasite inhibition *in vivo* after a single dose against *L. donovani* in HU3 infected BALB/c mice [59].

N-(2-hydroxypropyl)-methacrylamide-GFLG-amphotericin B copolymer conjugates decreased parasites by up to 94% in the liver of *L. donovani* infected BALB/c mice after intravenous administration of 1mg/kg amphotericin B equivalent on 3 alternate days and by up to 99.6% at a dose of 3mg/kg amphotericin B equivalent [60].

Besides these a number of novel drug delivery system have been used to carry AmB to the site of infection [61-66].

### 2.3 Quinoline derivatives

Quinoline scaffold-based derivatives, such as indolyl quinoline analogues , 4-substituted quinoline, 2-substituted quinoline, 8-amino quinoline derivatives (sitamaquine) , and 2 propylene quinoline derivatives , quinoline derivative (S-4), 2-(2-methylquinolin-4-ylamino)-*N*-phenylacetamide display antiparasitic activities [67-72].

**2.3.1 2-substituted quinoline—**2-substituted quinoline alkaloids were originally isolated from a Bolivian medicinal plant (*Galipea longiflora* ) and shown to have an effect in the treatment of experimental New World CL[73]. Activity of 2-substituted quinoline alkaloids was subsequently reported in the *L.donovani* – BALB/c mouse model with 2-n-propylquinoline showing significant activity after oral administration and chimanine D after subcutaneous administration[74]. Among more than one hundred 2-substituted quinolones, 2-n-propylquinoline was found to be the most stable and safe compound in various conditions[75]. As this compound was in oily state, a 2-npropylquinoline formulation as camphorsulfonic salt ( Fig:4) was prepared and characterised. *In vivo* studies in Balb/c mice model, oral treatment at 60 mmoles/kg/day for 10 days with this formulation was compared to 2-n-propylquinoline alone and to miltefosine. The salt formulation did not alter the activity of the 2-n-propylquinoline and reduced the parasite burden 76% compared to 89% for miltefosine [76].

Recently 26g a new 2-substituted quinoline compound with morpholine at C4 position, chloro at C6 position and fluoro at C7 position (Fig:5) , was found to have *in vitro* and *in vivo* antileishmanial activity; it exhibited an $IC_{50}$ value of 0.2 mM and >180 fold selectivity. The hydrochloride salt of 26g showed 84.26± 4.44 percent inhibition at 50 mg/kg for 5 days (twice daily, oral route) dose in *L. donovani*/hamster model[77]. Studies have shown the efficacy of 2-substituted quinolines against both HIV and *Leishmania*, suggesting that oral

therapy with these compounds could be an effective therapy for *Leishmania*-HIV co infection [69, 78].

**2.3.2 4-Aminoquinaldine compounds—**PP-9 and PP-10 are analogues of 4-aminoquinaldine with chlorine substituents( Fig:6). They showed antiparasitic activity against both SAG-sensitive and -resistant strains of *Leishmania* in *in vitro* and as well as *in vivo studies*. There was 95 to 98% reductions in the parasite burden of spleen and liver with only 4 doses, administered orally to BALB/c mice and they were more active than miltefosine at similar doses. Both the compounds did not cause any changes in haematological or cardiac functions in BALB/c mice and were not hepato or nephrotoxic. Investigation of their mode of action revealed that killing by PP-10 involved moderate inhibition of dihydrofolate reductase and elicitation of the apoptotic cascade[79].

**2.3.3 8 aminoquinoline—**Sitamaquine (WR- 6026) is a 8-aminoquinoline analogue (Fig: 7) discovered by the Walter Reed Army Institute of Research (WRAIR, USA) being developed as a oral treatment for VL.

**2.3.3.1 In vivo efficacy:** In 1950's 8-aminoquinoline had shown to have antileishmanial activity and oral availability against *L. donovani* in the hamster model[80]. Later, 8-[[6-(diethylamino) hexyl]amino]-6-methoxy-4-methylquinoline or WR6026, now called sitamaquine was shown to be 708 times more active than meglumine antimoniate (Glucantime®) against *L. donovani* in hamsters[81].

Pharmacokinetics data in humans showed that sitamaquine has a short elimination half-life (about 26 hr) in contrast to miltefosine half-life (150-200 hr)[82]. The metabolism of sitamaquine was studied in a rat hepatic microsomal system[83]. Two metabolites were found: 8(-6-ethylaminohexylamino)-6-methoxy-lepidine and 8(-6-iethylaminohexylamino)-6-methoxy-4-hydroxy-methyl-quinoline[84]. The formation of both metabolites was catalyzed by different cytochrome P450 isozymes. Multiple-dose pharmacokinetic profile of sitamaquine and its metabolite desethyl-sitamaquine after administration of 2 mg/kg/day of sitamaquine with or without food for 21 days are as follows. Area under curve $(AUC)_{(0-\tau)}$ = 6,627−8,903 ng.hr/mL, $AUC_{(0-16)}$ = 4,859−6,633 ng.hr/mL, maximum plasma concentration ($C_{max}$) = 401−570 ng/mL, apparent terminal half-life ($t_{1/2}$) = 18.3−22.8 hr, time to reach $C_{max}$ ($t_{max}$) = 3.5−6 hr; and desethyl-sitamaquine, $AUC_{(0-\tau)}$ = 2,307−3,163 ng.hr/mL, $C_{max}$ = 109−154 ng/mL, $t_{1/2}$ = 23.0−27.9 hr, $t_{max}$ = 2−10 hr, with no significant food effect [85].

**2.3.3.2 Human studies:** The first phase 2 study was done in Kenya in 16 patients at a dose of 0.75-1mg/kg for 2-4 weeks with 50% cure rate for 28day treatment with 1mg/kg[70]. This was followed by a study in Brazil. Cure rates for Brazilian patients treated for 28 days were as follows: 1 mg/kg/day:0 of 4 (0%); 1.5 mg/kg/day: 1 of 6 (17%); 2.0 mg/kg/day: 4 of 6 (67%); 2.5 mg/kg/day: 1 of 5 (20%); and 3.25 mg/kg/day: 0 of 1 (0%)[86]. A randomized, open label, multicenter study was conducted in India to study the dose response and safety profile one of four sitamaquine doses (1.5, 1.75, 2.0, or 2.5 mg/ kg/ day) daily for 28 days. Final cure (primary efficacy outcome) was achieved in 92 of 106 (87%) patients overall and 25 of 31 (81%), 24 of 27 (89%), 23 of 23 (100%), and 20 of 25 (80%) patients at doses of

1.5, 1.75, 2.0, or 2.5 mg/ kg/day sitamaquine, respectively [87]. In Africa, cure was achieved in 79 (83%) of 95 patients overall, and in 11 (92%) of 12, 49 (80%) of 61, 9 (82%) of 11, and 10 (91%) of 11 patients at sitamaquine doses of 1.75, 2.0, 2.5, or 3.0 mg/kg/day, respectively[88]. In another recent study with sitamaquin at 2mg/kg for 28 days in India the final clinical cure (day 180) was 85% (95% confidence interval = 70.8–94.4%)[85].

**2.3.3.3 Safety:** Methhemoglobinemia and nephrotoxicity are the two main toxicity of the drug. Increased methemoglobin levels are a known class effect of 8-aminoquinolines; all patients are at risk regardless of glucose-6-phosphate dehydrogenase status. In the first phase2 study in India laboratory reports showed 40 (33%) of 120 patients had methemoglobin level increases ≥ 10% while six patients were symptomatic[87]. In another study only 5 patients had methemoglobin levels > 5%, the increase was reversible after the end of therapy and patients were asymptomatic[85]. Methemoglobinemia was not reported in the Kenyan study[88].

The study from India reported nephritic syndrome in 3% and glomerulonephritis in 2% patients with doses ≥ 2.5 mg /mg/day[87]. Nephropathy was also reported in the study from Brazil in 3 patients[86]. In the recent study from India, proteinuria was the most significant renal safety finding observed in 7 of 41 patients, 6 with a protein:creatinine ratio increase of > 3.5 from baseline. These changes were reversible after drug discontinuation. Two patients had a transient decrease in creatinine clearance which reversed within two weeks of completion of therapy. Two patients had asymptomatic hematuria (normalized by day 90 and day 180)[85].

**2.3.3.4. Drug resistance:** To study experimental resistance to the drug a *L. donovani* promastigote line resistant to 160 μM sitamaquine was selected by *in vitro* drug pressure. The resistant line was infective for murine peritoneal macrophages *in vitro* as its parent wild-type line but less infective for Balb/c mice, suggesting that a low transmission of resistant parasites could occur in the field. The sitamaquine $IC_{50}$ of the resistant line was about five and three times higher than those of the wild-type line on promastigote and intramacrophage amastigote forms, respectively. No cross-resistance with other antileishmanial agents was observed. However, this resistance was stable when parasites were subcultured in drug-free medium for a long time or after *in vivo* passage[89, 90].

## 2.4. Naphthoquinones

Plumbagin, a naphthoquinones derived from the stem barks of *Pera benensis* a medicinal plant was used by the Chimane Indians in the Bolivian Amazonia as treatment of cutaneous leishmaniasis[91].

**2.4.1. Buparvaquone and Derivatives—**Buparvaquone a hydroxynaphtoquinone, was shown to be highly active *in vitro* against intracellular *L. donovani* amastigotes in macrophages, but less active *in vivo* in the BALB/c mouse [92]. Buparvaquone-oxime demonstrated moderate *in vitro* activity against amastigotes of *L. Donovani*[93]. *In vivo* study of naphthoquinone buparvaquone and two phosphate prodrugs showed that hydrous gel and water-in-oil emulsion of buparvaquone significantly reduced cutaneous parasite burden. In

the visceral model, both prodrugs were more effective at reducing liver parasite burden than the parent drug, buparvaquone,however the liver parasite burden was reduced only by 34%[94]. *In vivo* effectiveness of buparvaquone entrapped in phosphatidylserine liposomes (BPQ-PS-LP) was studied in *L.infantum chagasi*-infected hamsters. BPQ-PS-LP at 0.33 mg/kg/day for eight consecutive days reduced the number of amastigotes by 89.4% ($P<0.05$) in the spleen and by 67.2% ($P>0.05$) in the liver, compared to 84.3% ($P<0.05$) and 99.7% ($P<0.05$), respectively, following Glucantime® treatment at 50 mg/kg/day[95]. *In vitro* antileishmanial activity of 2-methyl-5-(3'-methyl-but-2'-enyloxy)- [1,4]naphthoquinone , a prenyloxy-naphthoquinone isolated and characterised from roots of the plant *Plumbago zeylanica* (family-Plumbaginaceae) was evaluated. The observed $EC_{50}$ of this compound against promastigote and amastigote forms of *L. donovani* was significantly ($p<0.001$) lower than miltefosine[96]. In another *in vitro* study both naphthoquinones 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (TR 001) and 2,3-dibromo-1,4-naphthoquinone (TR 002) also showed antileismanial activity[97]. Treatment of infected BALB/c mice with D17 a di-epoxide derivative of diospyrin (bis- naphthoquinone ) at 2mg/kg/day reduced the hepatic parasite load only by 38% [98].

### 2.5 Doxorubicin

Doxorubicin, a well characterized anticancer drug, showed strong antileishmanial activity. It achieved up to 95% reduction of parasite in spleen of infected mouse model at a dose of 625 μg doxorubicin/kg body weight/day in 4 consecutive doses, which is far less than the toxic dose [99].

To provide improved delivery of the drug into macrophage, doxorubicin conjugated to mannose-human serum albumin (man-HSA) was tested in experimental VL to give excellent results[100]. However, the limitation was that the drug was being directed not only to the infected macrophages but also to the normal macrophages, thereby causing some toxicity. A 51-kDa *Leishmania* species-specific protein on the infected macrophage surface was characterized[101] and active targeting of doxorubicin to infected macrophages was studied by incorporating it in immunoliposomes prepared by grafting $F(ab)'_2$ of anti-51- kDa antibody onto the liposomal surface. In a 45-day mouse model of VL, complete elimination of splenic parasite burden was achieved by doxorubicin incorporated in immunoliposome (immunodoxosome) at a dose of 250 μg/kg/day for 4 consecutive days[102]. To improve the delivery of this toxic drug into the macrophages a number of drug delivery systems have been tried like gel-assisted layer-by-layer nanomatrix with high payload of doxorubicin, chitosan microparticles as a carrier of doxorubicin, phosphatidylserine specific ligand-anchored nanocapsules bearing doxorubicin and doxorubicin loaded nanocapsules [103-106].

## 3. Other investigational agents

Strategies to improve the antileishmanial activity and to decrease the side effects of currently available drugs by the use of different drug delivery system have also been undertaken[45]. Liposome encapsulated antimonials and pentamidine, niosomal formulation of SSG, pentamidine with polymethacrylate nanoparticles have shown superior activity as compared to the free drug[107-110]. Micellar systems composed of AMB molecules incorporated into miltefosine to provide both the drugs orally were evaluated. However,

permeability studies showed that transport of both drugs across the intestinal barrier was reduced when they were present together[111-113]. With the large number of compounds being evaluated daily Nwaka et al[114] proposed a set of criteria based on biological activity, physico-chemical characteristics and pharmacokinetics to facilitate the identification of effective antileishmanial drug candidates. According to the authors, a leishmanicidal hit should fit the following criteria: In *in vitro* studies, $IC_{50}$ for the amastigote (in macrophages) ~1–2μg/mL, selectivity over parasites >20 (selectivity index, SI), *in vivo* activity in *Leishmania infantum* hamster model or *L donovani* mouse model with > 80% reduction in amastigote burden. Besides this the compound should have confirmed and elucidate structure, established synthetic route, good drug-likeness scores (DLS), no violation of Lipinski's Rule of Five and chemically exploitable [114]. Many natural products have shown antileishmanial effect however, most of them are preliminary *in vitro* studies. To develop these natural products as antileishmanial drug candidates *in vivo*, toxicity and other studies to fulfil Nwaka's criteria needs to be done[115-117]. Some natural and synthetic products that have shown *in vitro* and *in vivo* antileishmanial activity have been enumerated in table 1.

## 4. Expert opinion

At present only a small number of antileishmanial drugs are available for clinical use, three injectables and the sole orally adminstrable miltefosine. There is a pressing need to develop more antileishmanial drugs as there are several handicaps with each of the available drug. Among all the investigational drugs fexinidazole, a nitroimidazole, has reached the stage of phase 2 clinical trial for VL. Fexinidazole has excellent *in vitro* and *in vivo* antileishmanial activity. The phase II trial in Sudanese VL patients which will be completed in 2015, will give us important efficacy and safety data. The oral advantage,comparable leishmanicidal activity to miltefosine, and safety reiterates the potential of fexinidazole as a much needed additional oral therapy for VL. As fexinidazole acts as a prodrug that must be activated by nitroreduction, this reliance on a single enzyme for activation makes it vulnerable to the emergence of drug resistance. Presently, monotherapy is not the preferred way of treating VL, and combination therapy with another antileishmanial agent would prevent the emergence of drug resistance. Work to identify an appropriate partner drug for fexinidazole is currently underway.

(*R*)-PA-824, another nitroimidazole compound with its *in-vitro* and *in vivo* leishmanicidal activity, oral bioavailability, safety, has the potential to be used as an antileishmanial drug. The fact that it shows additive effect in combination with fexinidazole is an added advantage. At present the (S) enantiomer has reached phase II trials for the treatment of tuberculosis therefore studies of the efficacy of the (*R*)-enantiomer in the treatment of VL are needed.

A number of novel delivery system of amphotericin B and oral formulations of Amphotericin B are in investigational stages for several years. Unfortunately, their development has been slow with none of them reaching the stage of a clinical trial.

Sitamaquine is the second orally active antileishmanial drug after miltefosine which has reached phase 2 trials. Unfortunately due to its low efficacy, development of this drug has been stopped for VL.

Naphthoquinone, buparvaquone except for the liposomal formulation is more active for CL than VL, but its other derivatives could be explored for activity against VL.

Doxorubicin an anticancer drug has shown strong antileishmanial activity at low doses. Active targeting of this drug to infected macrophages are being experimented, however at present its toxicity is its major drawback.

Leishmaniasis is a neglected tropical disease and pharmaceutical industries have very little interest in the development of drugs for such diseases due to lack of financial incentives. Thus, there is a huge gap between screening for antileishmanial hits and identification and optimization of leads, preclinical and clinical studies. As majority of these screening is being done by the academia they are not being further evaluated due to lack of resources. Recently, support from public-private partnerships (PPP) such as the Drugs for Neglected Diseases Initiative (DNDi) has helped in identifying new drug targets for neglected tropical diseases . Such PPPs could play an important role in ensuring that these drug candidates are promptly taken forward into development.

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## Article Highlight

Nitroimidazole compound fexinidazole has reached phase II trials as oral therapy for VL.

Sitamaquin has completed phase II trials but has shown inadequate efficacy for monotherapy.

The R enantiomer of PA-824 has shown excellent antileishmanial activity and an additive effect with fexinidazole.

Novel delivery system and oral formulations of Amphotericin B are still in investigational stages.

Active targeting of doxorubicin to infected macrophages are being done to increase efficacy and decrease toxicity of this anticancer drug.

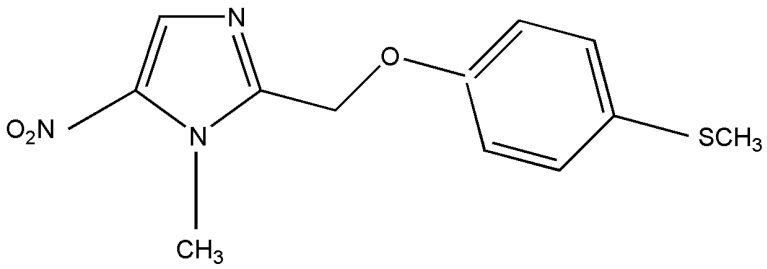



Figure 1. Chemical Structure of Fexinidazole

**Figure 1.**
Chemical structure of fexinidazole

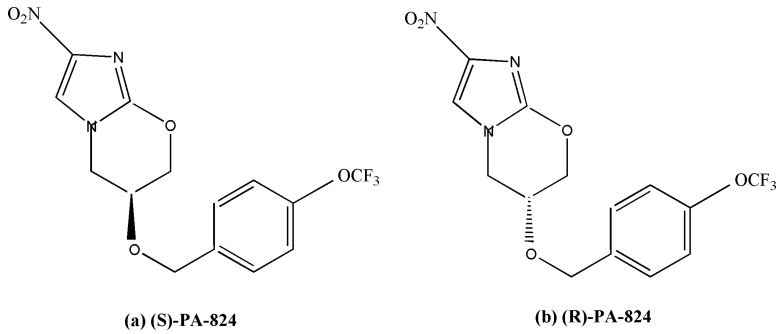



**Figure 2.**
Chemical structure of (S)- and (R)-PA-824

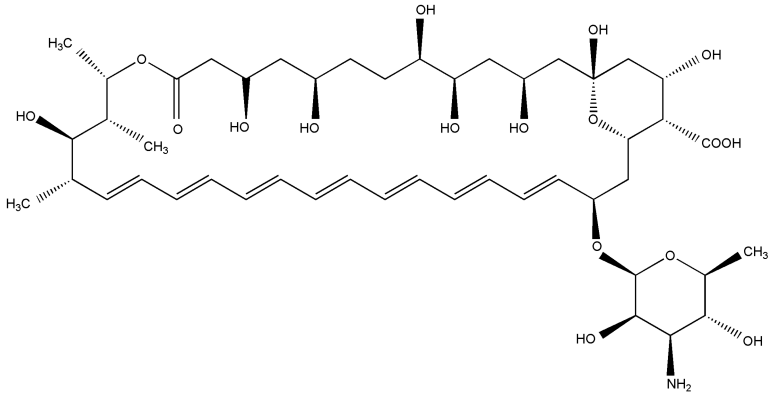
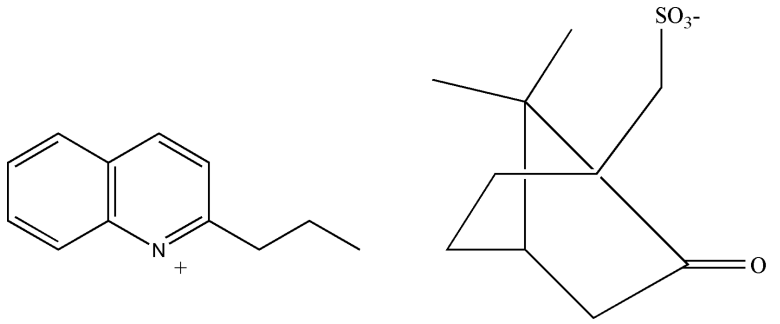
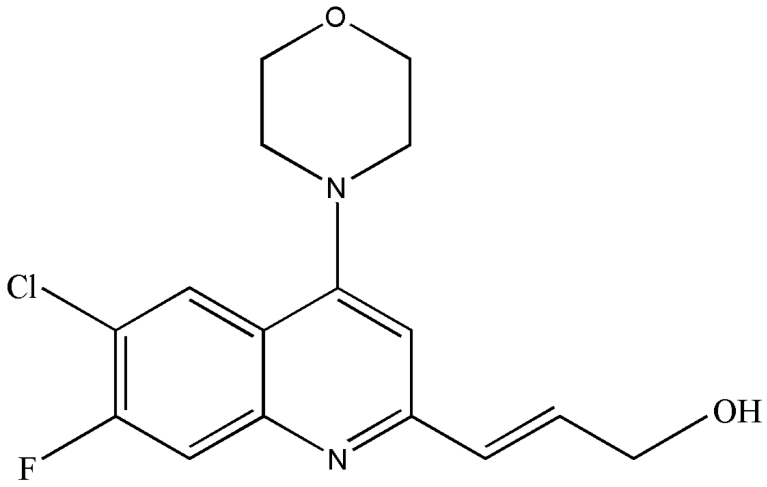



**Figure 3.**
Chemical structure of amphotericin B



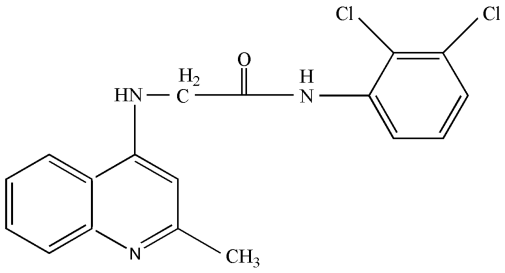

**Chemical Structure of 2-n-propylquinoline camphorsulfonic salt**

**Figure 4.**
Chemical structure of 2-npropylquinoline camphorsulfonic salt

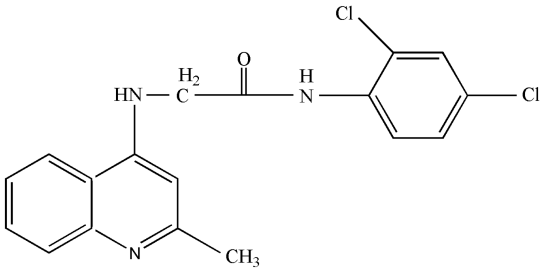



**Chemical Structure of Compound 26g**

**Figure 5.**
Chemical structure of Compound 26G




**(a) PP-9**




**(a) PP-10**

**Figure 6.**
Chemical structures of PP-9 and PP-10

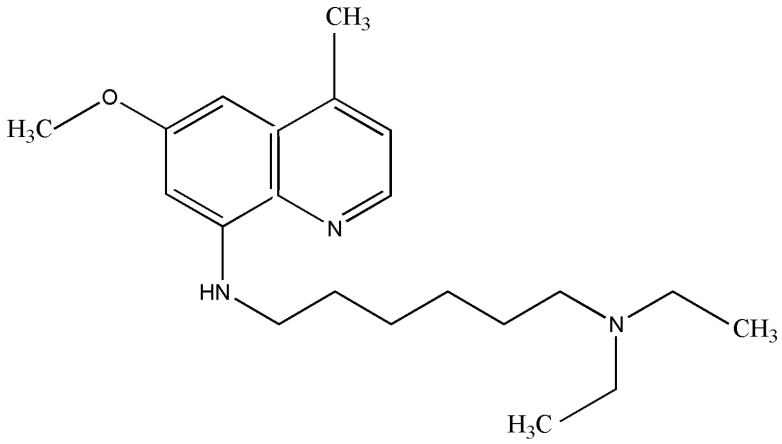



**Figure 7.**
Chemical structure of sitamaquine

## Table 1

Investigational agents in visceral leishmaniasis

| Reference | Active compound | *In vitro* efficacy | *In vitro* or *Ex vivo* toxicity | *In vivo* efficacy | Other characteristics |
|---|---|---|---|---|---|
| Mukherjee et al[118] | Imipramine, N-(c-dimethylaminopropyl)-iminodibenzyl HCl, a tricyclic antidepressant | $IC_{50}$ and $EC_{50}$ same in SSG(S) or SSG (R) *L. donovani* strains | 60μM cleared 100% of intracellular parasites while 100% MOs remained viable upto 90 μM of imipramine | 5 mg/kg/day for 4 weeks, cleared 99.5% parasites in SSG-R or SSG-S hamster | FDA approved oral drug ↓ IL 10 and TGFβ level ↑ TNF α, IFN γ and iNOS in imipramine treated infected hamsters |
| Palit et al[119] | Sertraline a selective serotonin reuptake inhibitor | $IC_{50}$ against *L. donovani* promastigotes & amastigotes of 2.2 and 2.3 mg/L | | 10 mg/kg body weight, two times per week for 4 weeks orally ↓ed parasite load in spleen by 72%& in liver by 70% in BALB/c mice | Induces ↓ in cytoplasmic ATP levels & oxygen consumption rate in promastigotes suggesting the involvement of an apoptosis mode of cell death. |
| Suryawanshi et al[120] | Triazole integrated phenyl heteroterpenoids compound 3a | $IC_{50}$-6.4 ± 1.2 μM in amastigotes of *L. donovani* 100% Inhibition at 40 μM | $CC_{50}$ - 112.4 ± 10.9 μM SI - 18 | 79 ± 11% inhibition of parasite multiplication at 50 mg/ kg × 5 days I.P. in hamster model. | Inhibits growth of *Leishmania* amastigotes by preventing the 14 a-demethylation of lanosterol and effectively blocking synthesis of ergosterol. |
| Sharma et al[121] | Quinazolinone-Triazine. 8a 8g | $IC_{50}$–against promastigote ± amastigotes - 7.05 ± 2.3 μM ± 3.95 ± 2.3 μM $IC_{50}$ against promastigotes ± amastigotes - ±40 μM ± 4.39 ±1.4 μM | $CC_{50}$ > 400 SI >101.26 $CC_{50}$ > 400 SI >91.1 | 73.15 ± 12.69% inhibition of parasite 80.93 ± 10.50%. against *L.donovani in* hamster model | |
| Kyriazis et al[122] | Olive tree extracts Oleuropein Hydroxytyrosol | Oleuropein – IC50-110±32 in amastigotes Hydroxytyrosol-IC50-38.7±3 in amastigotes | Oleuropein $CC_{50}$ -356±23 SI 3.24 Hydroxytyrosol - $CC_{50}$ 180±16 SI 4.65 | 14 alternate day IP oleuropein reduced spleen parasitic burden >80% at 3 days & >95% in liver and spleen at 6weeks post treatment in BALB/c mice | |
| Bhattacharya et al[123] | Bungarus caeruleus snake venom (BCV) | $IC_{50}$ - *L. donovani* promastigote - 14.5 μg/ml Amastigote - 11.2 μg/ml. | | 20μg/kg and 40μg/kg body ↓parasite count by 54.9% andj 74.2% in spleen and 41.4% and 60.4% in liver of infected BALB/c mice. | Increased production of TNF-α, IFN- γ, ROS, NO in infected mice. |
| Khaliq et al[124] | peganine hydrochloride. *Peganum harmala* seeds | *L. donovani* promastigotes $IC_{50}$ 38 (±1.23) μg/ ml. In intracellular Amastigotes $IC_{50}$ μg/ml | Devoid of cytotoxic effect in J774A.1 macrophages | *In-vivo* activity, 79.6 (+/−8.07)% ± 87.5 ±9.10)% f inhibition of parasites in hamsters at a oral dose of 100mg/kg & 200mg/kg for 5 days respectively. | Causes apoptosis-like cell death due to *L. donovani's* topoisomerase I inhibition |

| **Reference** | **Active compound** | ***In vitro* efficacy** | ***In vitro* or *Ex vivo* toxicity** | ***In vivo* efficacy** | **Other characteristics** |
|---|---|---|---|---|---|
| Maes et al[125] Germonprez et al[126] | Maesabalide III triterpenoid saponins, from plant *Maesa balansae* | $IC_{50}$ value 7 ng/ml in *L. infantum* amastigotes | $CC_{50}$ > 32μg/mL in human fibroblast (MRC-5) cell line | Single dose of MB-III at 0.8 mg/kg causes 94.2% reduction of the liver amastigote burden in hamsters | Efficacy comparable to that of a single dose of liposomal amphotericin B at 5 mg/kg |
| Mittra et al[127] | luteolin flavonoids | IC50 value 12.5μM in *L.donovani* promastigotes 12.5 μM reduced the intracellular parasite load by 70% in *L. Donovani*-infected BALB/c mice peritoneal macrophages | No effect on normal human T-cell blasts. | A dose of 3.5-mg/kg twice a week for 1month reduced the splenic parasite load by over 80% in hamsters | Inhibited DNA synthesis in promastigotes, and promoted topoisomerase-II mediated linearization of kDNA minicircles. |
| Zhai et al[128] | oxygenated chalcones 35m4ac 24m4ac | $IC_{50}$ value −0.81 ± 0.3 $IC_{50}$ value −0.65 ± 0.35μM in *L.donovani* amastigotes | | 20mg/kg for 6 days ↓ in parasite load in liver 97% 88% | Interferes with the function of the parasite mitochondria |
| Chen et al[129] | licochalcone A | | | 20 mg/kg per day i.p. for 6 days resulted in a > 96% ↓ in parasite load in the liver and the spleen 5to 150 mg/kg oral for 6days resulted in >65 and 85% ↓ in the parasite load in liver and spleen | |
| Medda et al[130] | Amarogentin, a secoiridoid glycoside from Indian medicinal plant *Swertia chirata* | | blood pathology, histological staining of tissues and liver enzyme levels showed no toxicity. | 2.5 mg/kg of amarogentin, in 0.5 mL niosomal suspension s.c. every 3 days for a total of 6 doses ↓ 9o% parasite load in spleen in hamsters | Inhibit the activity of DNA-topoisomerase I of *L. donovani*. |

SSG-S -antimony sensitive SSG-R- antimony resistant

MO‑ peritoneal exudate cells of BALB/c mice

IC 50- half maximum inhibitory concentration

CC 50, half maximum cytotoxic con- centration

SI -Selectivity index -The selectivity index (SI) is defined as the ratio of CC 50 on Vero cells to IC 50 on *L. donovani* intramacrophagic amastigotes.

I.P.- intraperitoneal

ROS, reactive oxygen species; NO, nitric oxide;