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Antileishmanial chemotherapy: present status and future perspectives

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Abstract: Parasitic diseases affect about 30% of the world's population. Leishmania organisms are endemic in more than 80 countries and 350 million people are measured to be at risk worldwide. A huge number of compounds are filed for antileishmanial activity annually, but only a few are more potent than reference drugs such as miltefosine, pentamidine and metronidazole. In addition, most of the compounds are not as efficient as amphotericin B. Therefore, there is a need for novel compounds that are not only potent than the FDA-approved AmBisome and miltefosine, but are also less toxic and more cost effective in humans. Filling the antileishmanial drug discovery pipeline has never been as challenging as it is nowadays. The discovery and development of novel antileishmanial in the last decade has continued to be an area of significant effort, both in academia and industry. However, the challenges of leveraging this information into cost-effective drugs against leishmaniasis remain significant. Nevertheless, research in this area must continue if we are to be able to address the real needs that we will face over the next several years. It is hoped that the present review will have vital implications in this noteworthy area of antileishmanial drug discovery. Therefore, herein, we recapitulated the present status and future perspectives in developing novel lead for antileishmanial chemotherapy.

Keywords: Promastigotes, Amastigote, Pentamidine, Dihydrofolate reductase (DHFR), Drugs Resistant, Antileishmanial activity.

1.1. Introduction

Leishmaniasis is a devastating disease caused by parasites of the genus *Leishmania* in the family *Trypanosomatidae*. The disease observable as three types: Cutaneous, Mucocutaneous, and

Visceral Leishmaniasis, which is also known as kala-azar.^{1,2} Cutaneous Leishmaniasis, the most general forms a group of diseases with a varied spectrum of clinical manifestations, which range from small cutaneous nodules to gross mucosal tissue destruction.³ It is the most severe

form, in which the parasites have migrated to vital organs. It is a severe, debilitating disease characterized by prolonged fever, splenomegaly, hypergammaglobulinemia, and pancytopenia. Patients gradually become ill over a period of a few months, and nearly always die if untreated.⁴ Leishmaniasis is transmitted through the bite of female phlebotomine sandflies infected with the protozoan.^{5, 6} The parasite is then internalized via macrophages in the liver, spleen, and bone marrow. *Leishmania* parasites are dimorphic organisms, i.e., with two morphological forms in their life cycle: amastigotes in the mononuclear phagocytic system of the mammalian host, and promastigotes in the digestive organs of the vector.^{7, 8}

It is one of the most neglected tropical diseases in the world,^{9, 10} about 1.5 million new cases of Cutaneous Leishmaniasis and 500000 new cases of visceral disease occur each year. Cutaneous Leishmaniasis is endemic in more than 70 countries worldwide. Visceral Leishmaniasis occurs in 65 countries; the majority (90%) of cases occur in agricultural areas and among the suburban poor countries. The number of cases is increasing globally at an alarming rate. Ecological chaos caused by humans has enabled the Leishmaniasis to expand beyond their natural ecotopes, and this in turn affects the level of human exposure to the sandfly vectors. Cases of *Leishmania* and human immunodeficiency virus (HIV) co-infection have also recently increased.¹¹

The classical treatment of Leishmaniasis requires the administration of toxic and poorly tolerated drugs.¹² The pentavalent antimonials-meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam), are the first-line compounds used to treat Leishmaniasis. Other drugs that may be used include pentamidine, miltefosine,¹³ paromomycin,¹⁴ amphotericin B and its lipid formulations.¹⁶ However, parasite resistance greatly reduces the efficacy of conventional medications.¹⁷

In the last 15 years, clinical misapplication of medications has enabled the development of generalized resistance to these agents in Bihar, India, where half of the global Visceral Leishmaniasis cases occur. High toxicity, emergence of resistance and lack of cost effectiveness are the main drawbacks of the present drugs.¹⁸ Moreover, there are no effective vaccines to prevent Leishmaniasis.¹⁹ Therefore it is utmost importance to look for effective new drugs to treat Leishmaniasis.

In this review, we have summarized the current treatment options for Leishmaniasis, promising lead molecules, drug targets and recent advances in the development of novel chemotherapies and future prospective of leishmanial chemotherapy.

1.2. Morphology of *Leishmania* parasite

Leishmania is dimorphic parasites. It exists in two entirely different morphological structures, parasitizing in two different, hosts, promastigotes stage in sandfly and amastigotes stage in vertebrate host.²⁰

1.2.1. Promastigotes stage

Promastigotes stage of *Leishmania* exists in sandfly.²¹ They are in flagellated and spindle like shape and measure 15-20 μm in length and 1.5-3.5 μm in width. The amastigotes transform to promastigotes in posterior midgut of sandfly within hours of bite. On other side, promastigotes transform to amastigotes inside macrophages. In this form, nucleus is situated at the center and kinetoplast transversely towards the anterior end the single and delicate flagellum 15-28 μm in length. These promastigotes are morphologically similar to those grown in culture.

1.2.2. Amastigote stage

This form exists in macrophages of reticuloendothelial system of vertebrates such

as spleen, liver, bone-marrow and lymph node etc. There are ovoid and non-flagellated forms of *Leishmania* which are 3-8 μm length. The centrally located round/oval nucleus and adjacent but smaller round/road shaped kinetoplast are distinguished structure of it. The flagellum is not functional in amastigotes and does not extend beyond the cell body, there is 'Flageller pocket' which serves as a site of endocytosis and exocytose. The cytoplasm contains mitochondria, neutral red vacuoles and basophilic, and volutin granules containing RNA. The organism is surrounded by a double membrane below which is a row of 130-200 hollow fibrils. Closely situated nucleus and kinetoplast which are known as "*Torpedo forms*", frequently present in human beings.

1.3. Life cycle of Leishmania

The life cycle begins as an infected female sandfly inoculates a vertebrate host²² with flagellated promastigotes during a blood meal. Macrophages are the host's first line of defense and promptly phagocytose the invading organisms. Unfortunately, *Leishmania* organisms are capable of survival within the macrophage where they undergo a transformational change from flagellated promastigotes to non-motile amastigotes. In the vertebrate host, the amastigotes (contained within macrophages) are capable of binary fission. Division continues until the macrophage lyses and amastigotes are released to infect neighbouring phagocytic cells.

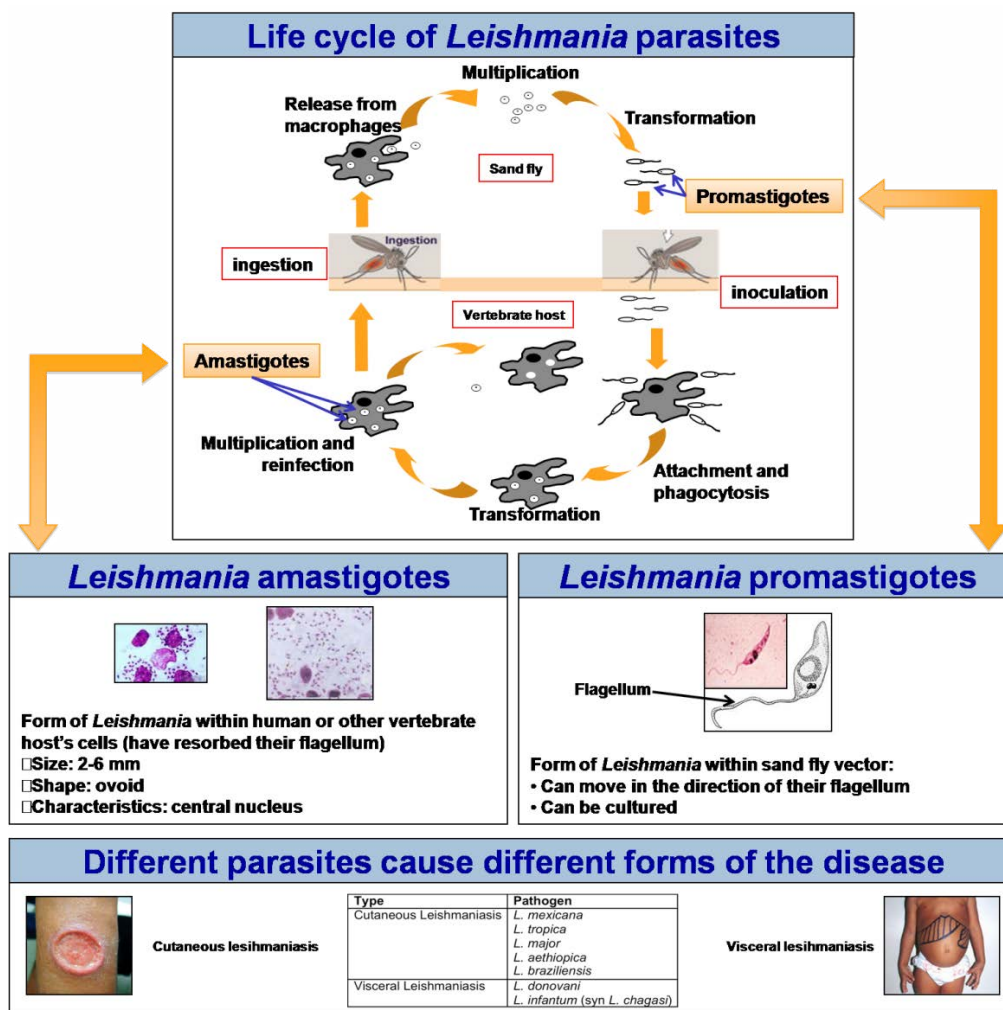


Figure 1.1. Life cycle of *Leishmania*

Infected macrophages or individual amastigotes enter the systemic circulation and subsequently disseminate to visceral organs leading to internal disease. Once the organism has entered the systemic circulation, it can once again be taken up during a blood meal by the female sandfly. The ingested amastigotes travel to the gut of the sandfly and are once again transformed into promastigotes. In the vector, it is the promastigote stage within the gut of the sand fly that is capable of binary fission. These flagellated organisms subsequently migrate to the hypostome of the sandfly and are inoculated into another vertebrate host completing the life cycle.²³

1.4. Clinical Signs

Mainly two forms of Leishmaniasis, Cutaneous and Visceral, are seen in humans. Some texts also distinguish a mucocutaneous form, while others consider it to be a subset of Cutaneous Leishmaniasis. The form of the disease and the usual clinical signs vary with the species of *Leishmania*. Some infections remain asymptomatic.

1.4.1. Cutaneous Leishmaniasis

Cutaneous Leishmaniasis often involves only the skin, and may be characterized by one to dozens of lesions. Depending on the species of *Leishmania*, ulcers, smooth nodules, flat plaques or hyperkeratotic wart-like lesions may be seen. The initial lesions, which occur on skin that was exposed to sandflies, are usually papules.²⁴ Many lesions remain localized, but in some cases, the parasites may spread via the lymphatics and produce secondary lesions on the skin, or occasionally the mucosa, of other parts of the body. Regional lymphadenopathy sometimes occurs. Cutaneous Leishmaniasis is usually painless unless the lesions become secondarily infected, and except in the ear, the ulcers tend to remain confined to the skin and do not affect the subcutaneous tissues. Most

skin lesions heal spontaneously. However, the speed of healing varies with the species of *Leishmania*. In some cases, it may take several months to a year or longer. Some forms leave permanent scars. HIV-infected individuals can have unusually severe cases, and the disease is more difficult to cure.²⁵ Steroid treatment or other forms of immune suppression can also result in unusually severe disease.

Disseminated Leishmaniasis is a rare form of cutaneous disease.²⁶ It is seen especially with *L. amazonensis* in the Western Hemisphere, although other organisms can also be involved. It also occurs in the Eastern Hemisphere, often in people who have concurrent HIV infections. In diffuse Cutaneous Leishmaniasis, the nodules do not ulcerate but they spread widely on the skin. They may cause damage to deep tissues, and can persist indefinitely. The diffuse form can be incurable in some cases. Leishmaniasis recidivans (lupoid leishmaniasis), another rare form, is characterized by the development of new lesions around the edges of a healed skin lesion. It is most often caused by *L. tropica* or *L. braziliensis*, and it does not heal without treatment. Mucocutaneous Leishmaniasis (espundia) usually occurs in Latin America, where it is caused by *L. braziliensis* and, less often, by *L. Panamensis*/*L. guyanensis*. Mucocutaneous Leishmaniasis tends to occur 1 to 5 years after Cutaneous Leishmaniasis caused by these organisms has healed, but it can also be seen while skin lesions are still present. The initial signs are erythema and ulcerations at the nares, followed by destructive inflammation that can spread to involve the nasal septum, and in some cases, the pharynx or larynx. Frequent nosebleeds can be an early sign. The inflammation may perforate the nasal septum, cause severe disfigurement of the face, or block the pharynx or larynx. In some cases, the genitalia may also be involved. Mucocutaneous Leishmaniasis does not heal spontaneously.

1.4.2. Visceral Leishmaniasis

Visceral Leishmaniasis is usually an insidious, chronic disease among the inhabitants of endemic areas; however, the onset may be acute in travelers from *Leishmania*-free areas. In some cases (especially in Africa), a primary granuloma appears on the skin before the systemic signs. The most common symptoms of Visceral Leishmaniasis are a prolonged undulant fever, weight loss, decreased appetite, signs of anemia, and abdominal distension with splenomegaly and hepatomegaly. Thrombocytopenia may cause bleeding tendencies, including petechiae or hemorrhages on the mucous membranes, and leukopenia can result in increased susceptibility to other infections. Other symptoms may include coughing, chronic diarrhea, darkening of the skin, lymphadenopathy, and in many cases, signs of chronic kidney disease. Mild cases with only a few symptoms may resolve spontaneously. Unless they are treated, most other cases are eventually fatal, often from secondary infections and other complications. Fulminant disease or atypical cases can also occur, especially in patients co-infected with HIV. People with successfully treated infections continue to carry the parasite, and the disease may recur if they become immune suppressed. Similarly, asymptotically infected individuals may later develop clinical signs.

Post-kala azar dermal Leishmaniasis (PKDL) occurs after recovery in some cases of Visceral Leishmaniasis caused by *L. donovani*. This syndrome is characterized by a maculopapular, macular or-nodular rash around the mouth, which spreads. In Africa, PKDL is common, usually occurs within six months of Visceral Leishmaniasis, and typically disappears within a year without treatment. In South Asia, this syndrome is relatively rare, occurs several years after Visceral Leishmaniasis has been cured, and required prolonged treatment. In India, PKDL is seen in 1-3% of successfully treated cases of Visceral Leishmaniasis.

1.5. Diagnosis

Cutaneous Leishmaniasis can be diagnosed by direct observation of the parasites in skin scrapings, impression smears or skin biopsies stained with Giemsa, Leishman's, Wright's or other stains. Amastigotes are easiest to find in recent or active lesions. Polymerase chain reaction assays (PCR)²⁷ are often used for diagnosis in areas where they are available. *Leishmania spp.* can also be cultured. However, each species will grow only in certain media, and some species can be difficult to isolate. Novy-MacNeil-Nicole (NMN) medium, brain-heart infusion (BHI) medium, Evan's modified Tobie's medium (EMTM), Grace's medium and Schneider's Drosophila medium might be used initially. Animal inoculation into hamsters may also be valuable, especially with contaminated material. Diagnosing Leishmaniasis by *in vitro* culture requires 5 to 30 days, while animal inoculation can take weeks or months. The species, subspecies and/or strain can be identified by PCR,²⁷ DNA hybridization, kinetoplast DNA restriction endonuclease analysis,²⁸ isoenzyme analysis, or immunological techniques that use monoclonal antibodies. A delayed hypersensitivity test, the leishmanin skin test (Montenegro skin test), is useful in the diagnosis of Cutaneous and Mucocutaneous Leishmaniasis, but it is usually negative in the diffuse cutaneous form. Antibodies are often slow to develop and of low titer.

Visceral Leishmaniasis can be diagnosed using some of the same techniques, including direct observation of the parasites. Amastigotes may be found in peripheral blood, or more often, in aspirates or biopsy smears from the spleen, bone marrow or lymph nodes. PCR, culture or animal (hamster) inoculation may be particularly useful early, when parasite numbers are low. Serology can also be helpful in this form of Leishmaniasis. Common serological tests used in humans include the immune fluorescent antibody test (IFA), direct agglutination, enzyme-linked immune

sorbent assay (ELISA),²⁹ fast agglutination-screening test (FAST), and a rapid immune chromatographic assay (K39 dipstick or strip-test).³⁰ Other assays including gel diffusion, complement fixation, indirect hemagglutination and counter current electrophoresis have also been used. Cross-reactions can occur in some serological tests with leprosy, Chagas disease, malaria and schistosomiasis. The leishmanin skin test/ Montenegro skin test is usually negative in cases of Visceral Leishmaniasis, but reactions can be seen once the disease is cured.

1.6. Controlling strategies of Leishmaniasis

The current control strategies for Leishmaniasis³¹ rely on reservoir and vector control, the use of insecticide-impregnated materials and active case detection and treatment, anti-leishmanial vaccines are still being developed.³²

1.6.1. Reservoir control

Dogs are the main reservoir of *L. infantum* in zoonotic VL.³³ Despite evidence from experimental studies showing a decreased incidence of VL in both dogs and children following serological screening of dogs and killing of sero positive animals,³⁴ the efficiency and acceptability of this control strategy is increasingly being debated. Treating infected dogs is not an effective control strategy as relapses are frequent and dogs can regain infectivity weeks after treatment, despite being clinically cured. Moreover, the widespread veterinary use of VL drugs might lead to resistance in parasites. A new control approach is the use of deltamethrine-treated collars, which reduced the risk of infection in dogs (by 54%) and children (by 43%) in a study conducted in Iran.³⁵ Vaccination of dogs would nevertheless be the best strategy if an efficacious vaccine can be developed.

1.6.2. Vector control

Sandflies are susceptible to the same insecticides as Anopheles mosquitoes, the malaria vector. Residual insecticide spraying of houses and animal shelters was shown to be efficacious in India,³⁶ where the vector (*Phlebotomus argentipes*) is restricted to areas in and around the home. Following the large scale antimalarial insecticide (dichloro-diphenyl-trichloroethane (DDT)) spraying campaigns that was implemented in the 1950s, VL almost completely disappeared from the Indian subcontinent. Unfortunately, the disease quickly re-emerged when these spraying campaigns were discontinued. Resistance of *P. argentipes* to DDT remains limited, but has been reported in Bihar. In Sudan and other endemic countries in East Africa, transmission occurs mainly, but not exclusively, outside villages, during shepherding for example. Indoor residual spraying for disease control is therefore unlikely to be as efficient in this region.³⁷

1.7. Vaccines against Leishmaniasis

There is currently no vaccine available for any form of Leishmaniasis, including Visceral Leishmaniasis (VL), which if left untreated is almost always fatal. Preventive vaccines are recognized as the best and most cost effective protection measure against pathogens. *Leishmania* vaccine development has proven to be a difficult and challenging task and is hampered by an inadequate knowledge of disease pathogenesis, the complexity of immune responses needed for protection, and the cost of vaccine development. The burden of VL is concentrated in resource poor nations, and a lack of political will and philanthropic investment further aggravates the situation.³⁸ However, the rise of biotechnology industries in endemic countries, such as India, may provide an impetus for VL vaccine development and investment.³⁹ A recent clinical trial in India assessed the safety and immunogenicity⁴⁰ of the LEISH-F1+MPL-SE vaccine,⁴¹ which is the only Second-generation vaccine currently

in clinical development for human VL. There are currently several new European-based VL vaccine efforts including a synthetic vaccine RAPSODI, DNA-based LEISHDNAVAX and an adenovirus vectored therapeutic vaccine.⁴² New adjuvants are also being developed, and there are several clinical vaccine trials in progress and in planning.⁴³ Given the rapid progress in the fields of parasite immunology and genomics, a successful anti-*Leishmania* vaccine should be achievable sooner rather than later. There is a clear need for greater investment in research and development to move promising vaccine leads along the development pathway toward an effective and affordable VL vaccine.

1.8. Present Status of Antileishmanial Chemotherapy

Over the past decades, few alternative drugs or new formulations of old ones have become available.^{18,44} But, as yet, none of them are ideal

for treatment due to high toxicity, resistance issues, prohibitive prices, long treatment length or inadequate mode of administration not adapted to the field. In addition, many patients are unable to complete the whole treatment, increasing the risk of drug resistance development. Drug combinations have demonstrated positive results and may be a short term solution to delay or prevent the emergence of resistance, increasing efficacy, or shortening the course of treatment.⁴⁵

Chemotherapy is currently the only way to treat the various forms of Leishmaniasis, since no vaccine is yet available. However, the arsenal of drugs against the disease is still limited. Today, first line antileishmanial drugs are pentavalent antimonials (sodium stibogluconate and meglumine antimonate), which are slowly being replaced by liposomal amphotericin B, pentamidine or the first oral drug against the visceral form of the disease, miltefosine.⁴⁶

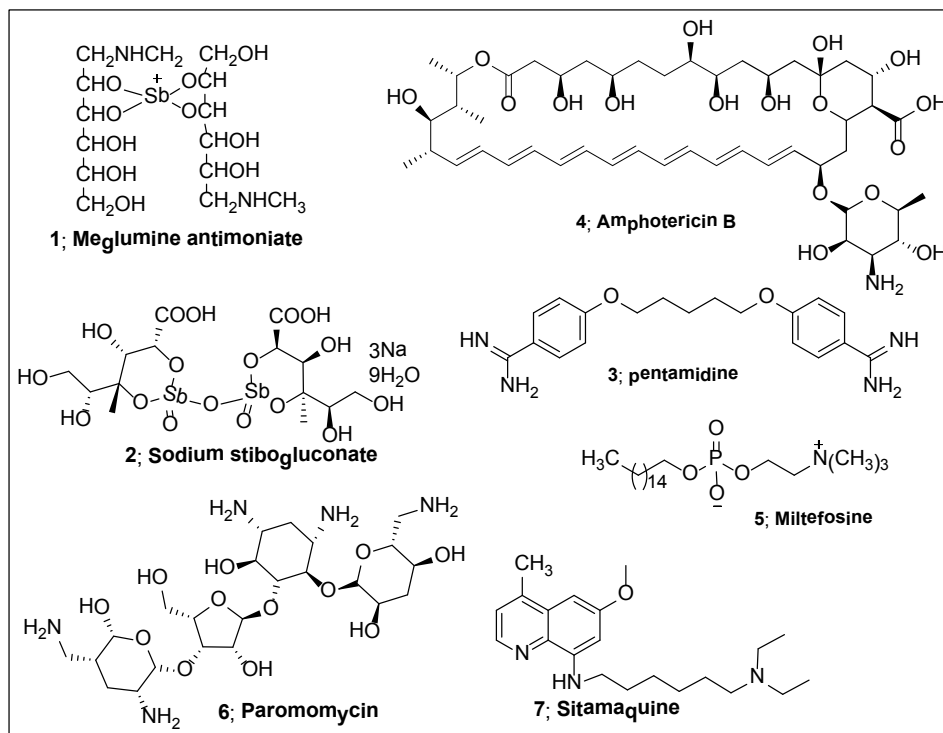


Figure1.2. Antileishmanial drugs

1.8.1. Pentavalent antimonials (1 & 2)

Pentavalent antimonial compounds sodium stibogluconate (Sb^{v}) or meglumine antimoniate has been the mainstay in the treatment of kala-azar being used as a first-line drug.⁴⁷ Sodium stibogluconate. Globally, including India, the treatment of VL has centred around pentavalent antimony compounds (Sb^{v}) for more than seven decades. Initially Sb^{v} was used in a dose of 10 mg/kg for 6-10 days, but increasing unresponsiveness in India led to successive upward revisions and currently the amount of drug being used is 10 times more than in earlier years. The last few years have seen the emergence of large scale Sb^{v} resistance in north Bihar, India, where over 60 per cent of previously untreated patients are unresponsive to Sb^{v} rendering the drug useless for routine use. Resistance seems to be a feature of intensive transmission of anthroponotic *L. donovani* as epidemic turns to endemic in foci where Sb^{v} has been used as a solo drug, often with poor supervision and compliance. However, there is a regional variation in the response to Sb^{v} as patients in other States like Uttar Pradesh continue to be responsive. Current recommendations are replacement of Sb^{v} by amphotericin B in these Sb^{v} refractory zones.⁴⁸ However, outside Bihar, Sb^{v} remains the drug of choice to be used parenterally in a dose of 20 mg/kg daily for 30 days without any upper limit. Till very recently, an unanswered question was whether Sb^{v} unresponsiveness was linked to the host or parasite. It has now been firmly established that antimonial resistance⁴⁹ is an inherent feature of the *Leishmania* parasite.⁵⁰

To date, the precise mechanism of action of sodium antimony gluconate (SAG) remains an enigma; a general consensus is that Sb^{v} acts upon several targets that include influencing the bioenergetics of *Leishmania* parasites by inhibiting parasite glycolysis, fatty acid beta-oxidation and inhibition of ADP phosphorylation. It has also been reported to

cause non specific blocking of SH groups of amastigote proteins and cause inhibition of DNA topoisomerase. More recently, it has been demonstrated that antimony can alter the thiol-redox potential⁵¹ in both forms of the parasite by actively promoting efflux of thiols, glutathione and trypanothione, thus rendering the parasite more susceptible to oxidative stress.

1.8.2. Pentamidine isethionate (3)

Pentamidine, an aromatic diamidine and was highly effective as a second line antileishmanial drug in antimony unresponsive patients initially.^{52, 53} But its precise mode of action has yet to be elucidated. Since it is a competitive inhibitor of arginine transport and noncompetitively inhibits putrescine and spermidine, its leishmanicidal activity is possibly mediated via its influence on polyamine biosynthesis and the mitochondrial membrane potential. Pentamidine was initially proven to be useful in Sb^{v} resistant kala-azar, but the limiting factors were the expense and unacceptable toxicity as it causes irreversible insulin dependent diabetes mellitus and death.⁵⁴ Further, its declining responses, efficacy (as only about 70% patients could be cured) has led to its use being totally abandoned in India.

Pentamidine was used as second-line drug in antimony-resistant VL treatment. High toxicity combined with decreased efficacy in treatment of patients suggesting resistance drove to a complete abandonment of this drug to treat VL in India. However, this compound is still valuable for combined therapies. The cellular target of pentamidine is unknown, but it seems to play a role in the mitochondria, as it accumulates in this organelle.

1.8.3. Amphotericin B and its lipid formulations (4)

Amphotericin B is a polyamine antibiotic. It inhibits the biosynthesis of ergosterol like sterols, which membrane sterol for both

fungi and *Leishmania*, leading to increase membrane permeability and ultimate killing of *Leishmania* parasite. Amphotericin B has excellent leishmanicidal activity. Faced with increasing Sb^V unresponsiveness of VL over the last decade,⁵⁵ amphotericin in a dose of 0.75-1 mg/kg for 15 to 20 infusions either daily or on alternate days has consistently produced cure rates of about 97 per cent and is now the drug of choice.⁵⁶ Major limiting factors include an almost universal occurrence of infusion based reactions like high fever with rigor and chills, thrombophlebitis and occasional serious toxicities like myocarditis, severe hypokalaemia, renal dysfunction and even death. Thus, its use at peripheral health posts was prevented by frequent adverse events, the need for prolonged hospitalization and close monitoring.

Toxic effects of amphotericin B deoxycholate have been largely ameliorated with the advent of lipid formulations of amphotericin B.⁵⁷ In these formulations, deoxycholate has been replaced by other lipids that mask amphotericin B from susceptible tissues, thus reducing toxicity, and facilitate its preferential uptake by reticuloendothelial cells, thus achieving targeted drug delivery to the parasite resulting in increasing efficacy and reduced toxicity. Three such lipid associated formulations of amphotericin⁵⁸ are commercially available: (i) lipids that mask amphotericin B (ii) amphotericin B lipid complex (iii) amphotericin B colloidal dispersion.

1.8.4. Miltefosine (5)

Miltefosine (Impavido), also known as hexadecylphosphocholine, was simultaneously discovered as an anticancer and antileishmanial drug. It is the most recent antileishmanial drug on the market and the first effective oral treatment against VL,⁵⁹ being recommended as first line drug for childhood VL. Also use of miltefosine in cutaneous leishmaniasis has been addressed in a few clinical trials.⁶⁰ An important advantage

of this drug is its oral administration when compared with the standard parenteral drugs in the context of a large-scale use in the inner regions of the endemic countries. Miltefosine also shows activity in severe or refractory cases. Although its toxicity is not very high, its teratogenicity is a problem. The mechanism of action of miltefosine can be a direct action against the parasite by impairing the lipid metabolism and causing parasite apoptosis.⁶¹ A combination therapy of miltefosine with amphotericin B or paromomycin is very efficient and could be helpful to treat antimony-resistant VL infections in India.⁶²

1.8.5. Paromomycin (6)

Paromomycin is an aminoglycoside with clinically important antileishmanial activity. Both visceral and cutaneous forms can be treated with this antibiotic, but poor oral absorption has led to the development of parenteral and topical formulations for the visceral and cutaneous forms, respectively.⁶³ However, new topical formulations of paromomycin have given good results. A randomized, controlled study was undertaken to compare the therapeutic efficacy⁶⁴ of two paromomycin topical preparations with meglumine antimoniate. The results showed that topical paromomycin can be a therapeutic alternative for Cutaneous Leishmaniasis, although a longer period is required for clinical healing. A hydrophilic gel containing 10% paromomycin was evaluated in Balb/c mice infected with *L. Amazonensis* and hamsters infected with *L. braziliensis*. Compared to the antimony treatment, the activity of the paromomycin gel was significantly higher against *L. amazonensis*, whereas these two medications were equally effective against *L. braziliensis*. The gel formulation may represent an alternative topical treatment for Cutaneous Leishmaniasis. Paromomycin impairs the mitochondrial membrane potential, inhibits protein synthesis, and leads to respiratory dysfunction. It also alters membrane fluidity

and lipid metabolism. Paromomycin, with its excellent efficacy, low cost, shorter duration of administration and good safety profile, has the potential to be used as a first-line drug.

1.8.6. Sitamaquine (7)

A new primaquine analogue, sitamaquine (WR 6026), was developed by Walter Reed Army Institute of Research (United States) originally for malaria and has no biochemical rationale. Sitamaquine is the second oral drug⁶⁵ in development for Leishmaniasis treatment, after miltefosine, and was recently in clinical trials. Preliminary clinical studies in Kenya and Brazil showed satisfactory efficacy against different species of *Leishmania*.⁶⁶ A recent study showed that it targets succinate dehydrogenase causing oxidative stress in *L. donovani* promastigotes.⁶⁷ The higher doses showed the major side effects of sitamaquine were nephrotoxicity, gastrointestinal, vomiting and diarrhea. However, very recently unresponsive strains of *Leishmania spp.* have been reported against this drug too.⁶⁸

1.9. Other important lead molecules at various stages of development

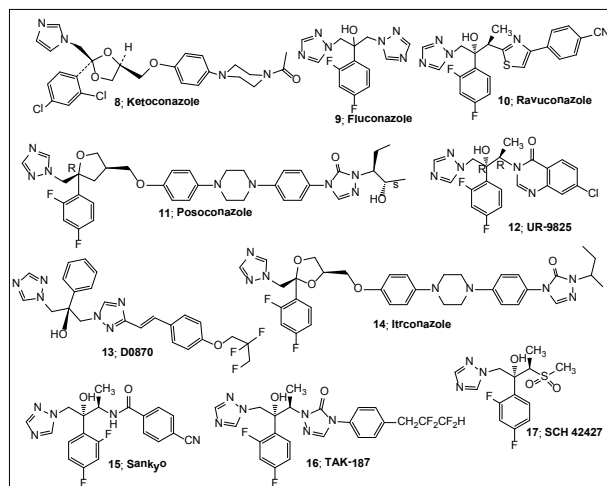


Figure 1.3: Antileishmanial azoles

Leishmania parasite, resembles fungi in

synthesizing 24-substituted sterols such as ergosterol, whereas mammals have just cholesterol. That's why ergosterol is a useful target for design the antileishmanial drugs.⁹ The antimycotic azoles, ketoconazole (**8**), fluconazole (**9**) and itraconazole (**14**), show activity against *Leishmania spp* by inhibiting the cytochrome P450-mediated 14 α -demethylation of lanosterol, resulting in ergosterol depletion and accumulation of 14 α -methyl sterols.⁶⁹⁻⁷¹ There have been several clinical trials of the azoles (**8-17**) in the treatment of Leishmaniasis, but their effectiveness has been varied. Itraconazole has also been tested in the treatment of Mucocutaneous Leishmaniasis, but was found to be effective only in mild to moderate forms of the disease, limiting its usefulness. Posaconazole (**11**), a triazole that is being developed as an antifungal agent, has also been studied in experimental models of Cutaneous and Visceral Leishmaniasis.⁷² When used at a higher dose (60 mg/kg/day), this drug was found to be superior to amphotericin B in reducing the size of cutaneous lesions, but was inferior to amphotericin B in treating Visceral Leishmaniasis in a mouse model of disease .

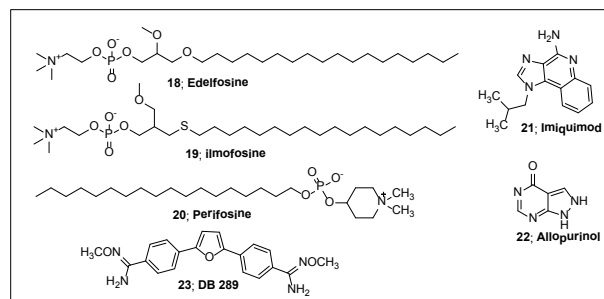


Figure 1.4. Potent Antileishmanial molecules

Alkylphospholipids such as edelfosine (**18**) and ilmofofosine (**19**), as well as perifosine (**20**), and allopurinol⁷³(**22**) have proved to possess potent *in vitro* antileishmanial activity.^{74, 75} Cabrera-Serra *et al.* tested edelfosine and perifosine in Balb/c infected with *L. amazonensis*.⁷⁶ This preclinical showed that perifosine had higher activity in the *in vivo* assay and may be a possible alternative

treatment against Cutaneous Leishmaniasis.

The imidazoquinolone compound Imiquimod (**21**) is an immune-response modifying agent,⁷⁷ first approved by FDA for the topical treatment of external genital and perianal warts in 1997. It induces, through stimulation of Toll-like receptors (TLRs) localized on the surface of antigen-presenting cells, synthesis and release of several endogenous pro-inflammatory cytokines such as interferon- α (IFN- α), tumor necrosis factor- α (TNF- α) and interleukins (IL) 6 and 12, which in turn stimulate both the innate and acquired immune pathways. It has been used for the treatment of a wide variety of dermatologic conditions in which the immune system is thought to play a role in regression of the disease. When tested in macrophages and in a mouse model of infection, Imiquimod (**21**) demonstrated macrophage-mediating antileishmanial activity both *in vitro* and *in vivo*. This was probably due to the ability of the drug to stimulate the release of nitric oxide from macrophages. A study that tested the efficacy of imiquimod treatment combined⁷⁸ with meglumine antimoniate (MA) in patients with Cutaneous Leishmaniasis not responsive to treatment with MA alone demonstrated a 90% cure rate.⁷⁴ However, when tested alone, imiquimod demonstrated only a short lived benefit in the treatment of Cutaneous Leishmaniasis, which was followed by clinical deterioration. Where the lesions of most patients had regressed in the first 3 weeks of treatment, by 8 weeks, all lesions had progressed.

1.10. Mechanism of Actions of antileishmanial drugs

Generic name of drug (Chemical type)	Mechanism of action
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Pentavalent antimonials: Meglumine antimoniate (Glucantime) Sodium stibogluconate (Pentostame)	Exact mechanism of action is still not known despite its use for over 50 years. May be activated within the amastigote, but not in the promastigote, by conversion to a lethal trivalent form. Activation mechanism not known. Antileishmanial activity might be due to action on host macrophage. Complexes with 24-substituted sterols, such as ergosterol in cell membrane, thus causing pores which alter ion balance and result in cell death.
Amphotericin B (polyene antibiotic)	Accumulated by the parasite; effects include binding to kinetoplast DNA. Primary mode of action uncertain
Pentamidine (diamidine)	In bacteria, paromomycin inhibits protein synthesis by binding to 30S subunit ribosomes, causing misreading and premature termination of mRNA translation. In <i>Leishmania</i> , paromomycin also affects mitochondrion.
Paromomycin (aminoglycoside antibiotic)	Primary effect uncertain, possible inhibition of ether remodelling, phosphatidylcholine biosynthesis, signal transduction and calcium homeostasis.
Miltefosine (hexadecylphosphocholine)	Unknown, might affect mitochondrial electron transport chain.
Sitamaquine	Stimulates nitric oxide production from macrophages.
Imiquimod (imidazoquinoline)	

1.11. Potential drug targets and experimental agents with leishmanicidal activity

The first step in drug discovery is to identify a suitable drug target, it plays a crucial role in any rational drug designing program.⁷⁹ The genome sequences of *L. major* and *L. infantum* has revealed immense amount of information and has given the opportunity to identify novel drug targets that are unique to these parasites.^{80, 81} Utilization of this information in order to come up with a candidate drug molecule requires combining all the technology and using a multi-

disciplinary approach, right from characterizing the target protein to high throughput screening of compounds. *Leishmania* belonging to the order kinetoplastidae emerges from the ancient eukaryotic lineages. They are quite diverse from their mammalian hosts and there are several cellular processes that we are getting to know of, which exist distinctly in these parasites. We are going to discuss some of the metabolic pathways that are essential and could be used as potential drug targets in *Leishmania*.

1.11.1. Dihydrofolate reductase (DHFR) inhibitors

Leishmania contain a bifunctional protein possessing the enzyme activity of both thymidylate synthetase and dihydrofolate reductase (DHFR) in contrast to mammalian cells, in which the enzyme activities reside on separate proteins.^{82, 83} The crystal structure of DHFR from *Leishmania major* has been reported, *Leishmania* and other *trypanosomatid* protozoa require reduced pteridines (pterins and folates) for growth, suggesting that inhibition of these pathways could be targeted for effective chemotherapy.⁸⁴ Most of the clinically used DHFR inhibitors show less selectivity for leishmanial enzymes. This is due to the fact that the gene for pteridine reductase (PTR1) is amplified in some leishmanial mutants. PTR1 can reduce pterins and folates and therefore act as a bypass for DHFR inhibition.⁸⁵ This implies that antifolate drugs must simultaneously target both DHFR and PTR1 to be successful antileishmanials. Aminopyrimidines are reported to be selective inhibitors of *trypanosomal* and leishmanial dihydrofolate reductase.

Several compounds were found to be inhibiting both DHFR-TS and PTR1.⁸³ Sirawaraporn *et al.* studied a series of substituted 5-benzyl-2,4-diaminopyrimidines, some of which showed selectivity for the *L. major* DHFR in enzyme assays and moderate activity against *L. major* promastigotes and *L. donovani* amastigotes.⁸⁶

The most active and selective compound in the enzyme assay experiments was the 8-octyloxy derivative (**24**) with a selectivity of 130-fold for the *L. major* DHFR and EC_{50} against *L. major* promastigotes of 5.6 μ M and *L. donovani* amastigotes of 4.6 μ M. Some compounds (**24-34**) with good selectivity for the leishmanial enzyme and *in vitro* activity against both *L. major* promastigotes (form found in the insect vector) and *L. donovani* amastigotes (form found in the human host) are shown in the Figure 1.5.

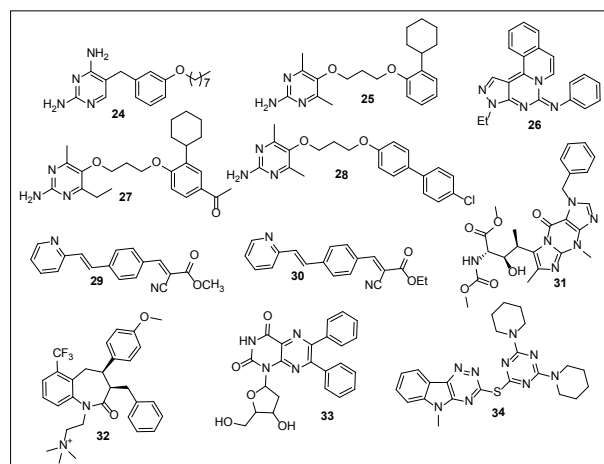


Figure 1.5: Structures of some potent DHFR inhibitors

1.11.2. Deoxyuridine Triphosphate Nucleotidohydrolase (dUTP) as drug target

dUTPase is widespread in nature and has been found in a variety of prokaryotic and eukaryotic organisms as well as in many viruses.⁸⁷ It was shown to be essential for viability in *L. major* and is essential for all cellular systems.⁸⁸ The structures of the dUTPases from *trypanosomes* are vastly different from the human trimeric enzymes makes them an attractive drug target for the development of new drugs against the diseases caused by these organisms.⁸⁹ Persson *et al.* had synthesized dUTP analogue, 2'-deoxyuridine 5'-(α , β -imido)diphosphate (dUPNP, **36**) and its corresponding triphosphate analogue

(dUPNPP, **37**), are most potent nonnucleotide inhibitors of human dUTPase.⁹⁰ Nguyen *et al.* extensively studied the Deoxyuridine Triphosphate Nucleotidohydrolase analogues, two compounds shown good inhibition against *L. donovani*, best IC₅₀ values at 13 μ M (**38**) and 17 μ M (**39**).⁸⁹

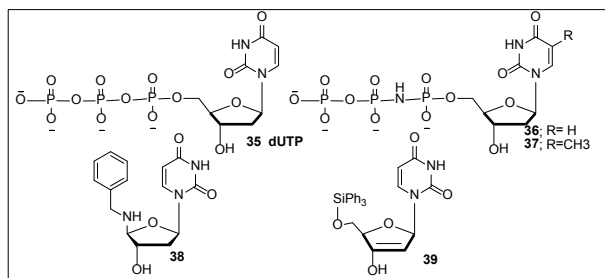


Figure 1.6: dUTPase inhibitors

1.11.3. DNA Topoisomerases as a drug target

DNA topoisomerases are ubiquitous enzymes needed to overcome topological problems encountered during DNA replication, transcription, recombination and maintenance of genomic stability.

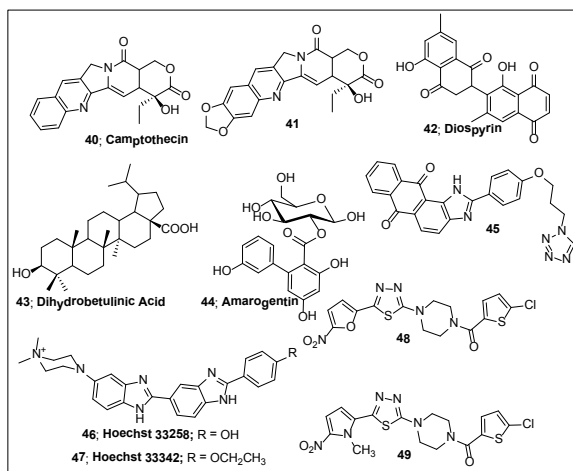


Figure 1.7: DNA topoisomerases inhibitors

They have proved to be valuable targets for therapy.⁹¹ There are three categories of such enzymes: DNA topoisomerases types IA, IB and II. Type I topoisomerases are monomeric ATP-independent enzymes with relaxation activity

for both positively and negatively supercoiled DNA.⁹² They introduce single-stranded breaks in DNA followed by passage and rejoining, thereby allowing single step changes in the linking number of circular DNA. They are subdivided into two distinct classes: type IA enzymes that bind covalently to the 5' end, and type IB enzymes that form covalent bonds with the 3' end of the broken DNA strand. Type II DNA topoisomerases are homodimeric ATP-dependent enzymes that introduce transient double stranded breaks in the double helix, followed by passage and rejoining.⁹³ These enzymes can relax, catenate/decatenate, knot/un knot or introduce supercoils in the DNA molecule. Type II topoisomerases have been cloned and functionally expressed from several parasitic sources, including *Leishmania spp.*, *African* and *American trypanosomes*, and *P. Falciparum*. These new developments of DNA topoisomerases as targets of novel therapeutic agents being excellent opportunities for drug discovery in the treatment of infectious and parasitic diseases.⁹⁴ Few DNA topoisomerases inhibitors^{95, 96} with promising antileishmanial activity were shown in the Figure 1.7.

1.11.4. Protein kinases as drug target

Cyclin dependent kinases (CDKs) are the family of Protein kinases, are known to play a crucial role in cell division. They have been found to be abnormally regulated in cancer cells and have therefore drawn attention as drug targets.⁹⁷

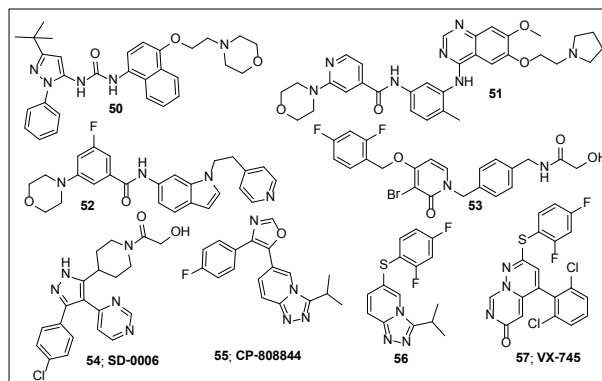


Figure 1.8: Kinases inhibitors

In *Leishmania*, the cdc-2 related kinase (CRK) family has attracted attention as potential drug targets.⁹⁸ CRK3 is found to be active throughout the life cycle in *L. mexicana*.⁹⁹ CRK3 from *L. major* was able to complement a temperature sensitive cdc-2 mutant in *S. pombe*. Inhibitors of CRK3, inhibited the growth and replication of *L. donovani* amastigotes in peritoneal macrophages. These kinases inhibitory compounds caused growth arrest of the parasite in culture, and cells exposed to indirubins had aberrant morphology and an altered DNA content, consistent with disruption of the cell cycle through inhibition of a CDK. Several interesting group of compounds (**50-57**) such as azoles,^{100, 101} aryl pyridinones¹⁰² and fused heterocyclic systems were discovered, some of those shown in the Figure.1.8.

1.11.5. Polyamines Transportase

The Polyamines putrescine, spermidine and spermine are polycationic molecules, which are essential for eukaryotic growth. Intracellular polyamine levels are maintained by both de novo synthesis and uptake of extracellular polyamines. Promastigotes of *Leishmania species* have high levels of putrescine and spermidine and their growth can be inhibited by polyamine biosynthesis antagonists.¹⁰³ Polyamine uptake has been described for many mammalian cells. Polyamine metabolism by parasitic protozoa has attracted considerable attention and it appears to be an important target for drug action. *Leishmania* have the capacity to transport polyamines,¹⁰⁴ although few detailed data have been reported,¹⁰⁵ Polyamine transport was shown to be saturable and temperature-sensitive for both developmental stages of *Leishmania*. Transport was shown to be pH-dependent, The uptake process was independent of extracellular Na⁺, but inhibited by protonophores and H⁺-ATPase inhibitors. Targeting polyamine transporters could be a viable approach for obtaining anti-trypanosomatid drugs. The aromatic diamidine

pentamidine, the drug of choice for treatment of antimonial-resistant cases of Leishmaniasis, inhibited both putrescine and spermidine transport non-competitively.¹⁰⁶ Considerable number of reports where different sets of substituted polyamine analogs have been prepared,¹⁰⁷ few systematic studies involving the synthesis of a number of aliphatic diamines and amino-alcohols and several of their alkyl, acyl and carbamoyl derivatives, have been synthesised and evaluated *in vitro* on cultures of *Leishmania spp.* Souza *et al.* studied the effect of *N*-dodecyl-1,2-ethylenediamine (NDDE, **59**) on the morphology and replication of *Leishmania* using macrophages cultured from the peritoneal exudate of mice infected *in vitro* with three species of *Leishmania*. NDDE inhibited *Leishmania* amastigotes multiplication into inflammatory peritoneal cells in concentrations which were not toxic to mammalian cells (0.5-1 µg/mL). More recently, Caminos *et al.*¹⁰⁸ prepared a library of *N*, *N*-disubstituted diamines, and screened *in vitro* for antiparasitic activity on the causative agents Visceral Leishmaniasis. The most potent compounds were derived from a subset of diamines that contained a 4-OBn substitution (**60**) had shown the IC₅₀ of 31nM, and a selective index of 137 which is several folds more potent than standard drugs. Many synthetic analogues^{107, 109} were reported as polyamine transport inhibitor, some of the potent most potent molecules (**61-66**) are shown in the Figure. 1.9.

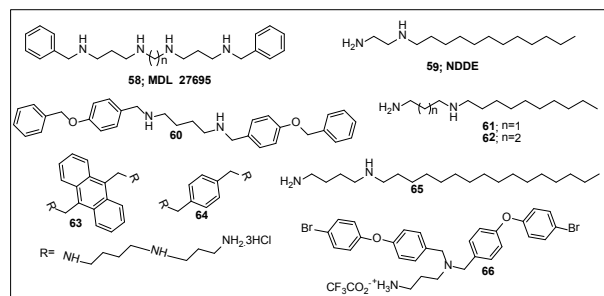


Figure 1.9: Polyamines Transport inhibitors

1.11.6. Squalene synthase

The ergosterol biosynthesis pathway is a promising target in the development of a rational chemotherapeutic strategy against *Leishmania* and other *trypanosomatids*, since ergosterol is essential for the parasite's viability and is absent in mammalian cells. Different classes of ergosterol biosynthesis inhibitors have been shown to be active against *trypanosomatid* parasites. One important enzyme of the sterol biosynthesis pathway is squalene synthase¹¹⁰ (SQS), which catalyzes the head-to-head condensation of two molecules of farnesyl pyrophosphate (FPP) to produce squalene. This is the first committed step in the sterol pathway, and its inhibition does not affect the biosynthesis of other essential isoprenoids. SQS has been under intense scrutiny with the aim of developing new cholesterol-lowering agents for humans. Several reports have described the potent and selective activity of the same class of compounds¹¹¹ against parasites such

as *Leishmania*, *Trypanosoma cruzi*, and *Toxoplasma gondii*. Two novel quinuclidine-based SQS inhibitors (**69**, **70**) developed by Eisai Co. (Tokyo, Japan) as cholesterol- and triglyceride-lowering agents in humans, have recently been shown to be potent anti-*Trypanosoma cruzi* and antileishmanial agents *in vitro* and *in vivo*, and their activities have been shown to be associated with a dramatic depletion of the parasite's endogenous sterols. Souza *et al.*¹¹² found that the compounds E5700 (**69**) and ER-119884 (**70**) were potent inhibitors of the growth of *L. amazonensis* that induce dramatic effects on the morphology and ultra structure of these unicellular organisms, most probably resulting from inhibition of endogenous sterol biosynthesis at the level of SQS and the resulting depletion of essential endogenous sterols, many synthetic compounds^{113, 114} were reported as potent squalene synthase inhibitors in *Leishmania*.

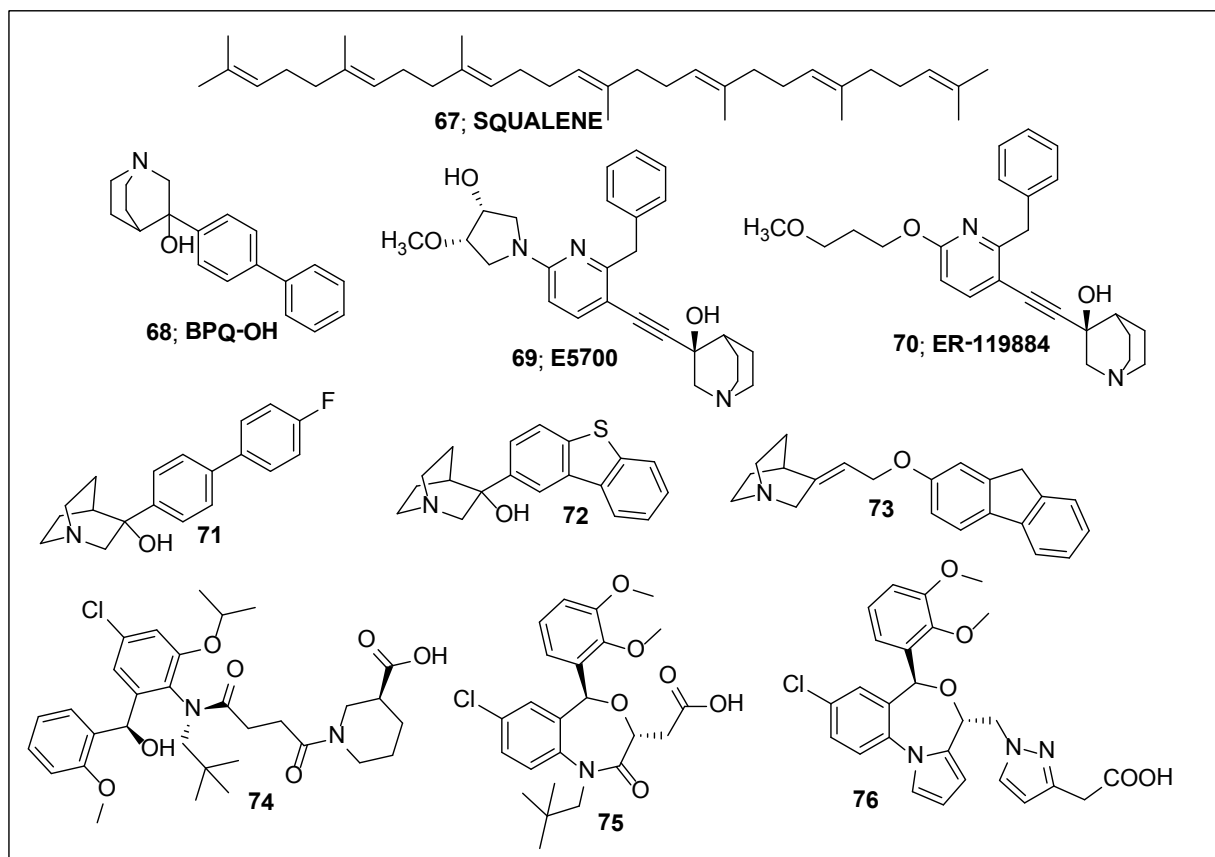


Figure 1.10: Structures of squalene inhibitors

1.11.7. Cysteine proteases

Proteases are ubiquitous enzymes that are involved in various important metabolic functions. These are of major two types in eukaryotes-cysteine proteases (papain like)¹¹⁵ and serine proteases (trypsin like), while in case of *Leishmania* cysteine protease is the major protease involved in survival of parasite within the host cell and adaptation to the changing environment. These proteases cause nucleophilic attack cleaving the peptide bond. Several studies confirmed the efficacy of cysteine protease inhibitors in arresting and killing parasites in tissue culture models of parasite replication or cell invasion. The relative lack of redundancy of cysteine proteases in parasites compared to their mammalian hosts makes them attractive targets for the development of new antiparasitic chemotherapy. K. A. Scheidt *et al.*¹¹⁶ synthesized the conformationally constrained cysteine inhibitors and tested against *L. major*; three compounds (**77-79**) were very effective inhibitors of the *L. major* cathepsin B-like cysteine protease.

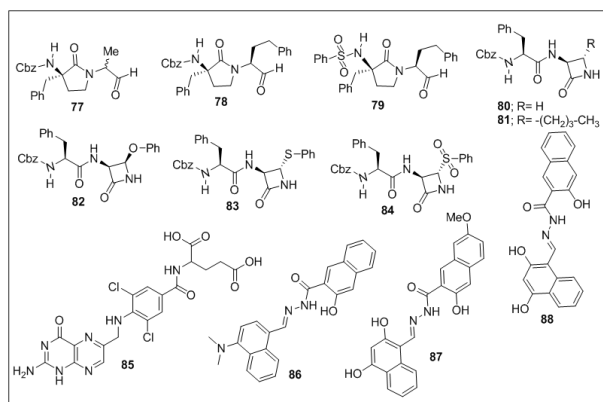


Figure 1.11: Cysteine proteases inhibitors

Compound (**79**) displayed an IC_{50} of 20 nM was most potent compound against the *L. major* protease. Zhou *et al.*¹¹⁷ identified the β -lactam moiety (**80-84**) as a new class of pharmacophore, Most of these β -lactam compounds selectively inhibit the papain type of cysteine proteases (cathepsins) with no inhibition or non-significant

inhibition of serine protease, human leukocyte elastase. Selzer *et al.*¹¹⁸ reported a new class of nonpeptide cysteine proteases Inhibitors, Some of these compounds (**85-88**) proved to be potent inhibitors of both the protease and parasite growth in culture and IC_{50} 's in the nanomolar range against *L. major*.

1.11.8. Glyoxalase a potential target

The glyoxalase system is a ubiquitous detoxification pathway that protects against cellular damage caused by methylglyoxal, a mutagenic and cytotoxic compound that is mainly formed as a by-product of glycolysis.¹¹⁹ It is also formed during catabolism of amino acids via aminoacetone and hydroxyacetone. The glyoxalase system comprises of two enzymes, glyoxalase I (GLOI) (lactoylglutathione lyase, EC 4.4.1.5) and glyoxalase II (GLOII) (hydroxyacylglutathione hydrolase, EC 3.1.2.6). Glyoxalase I catalyses the formation of S-D-lactoyl glutathione from the hemithioacetal formed nonenzymatically from methylglyoxal and glutathione. Glyoxalase II converts S-D-lactoyl glutathione to lactate and free glutathione. Thus, glutathione acts as a cofactor in the overall reaction pathway. The glyoxalase system is present in the cytosol of cells and cellular organelles particularly mitochondria. It is found throughout biological life and is thought to be ubiquitous. The widespread distribution suggests it fulfils a function of fundamental importance to biological life. Glyoxalase has a distinct role in cell proliferation and maturation. The glyoxalase I activity has been reported in *Leishmania*,¹²⁰ but very low levels of GLOI and GLOII activities were detected in lysates using glutathione as the substrate. Glyoxalase system of the pathogenic kinetoplastids has been recently reported to be unique, as a consequence of these protozoa possessing an unusual thiol metabolism. In these organisms, instead of glutathione, the major low molecular mass thiol is trypanothione [N^1 , N^8 -bis(glutathionyl) spermidine].

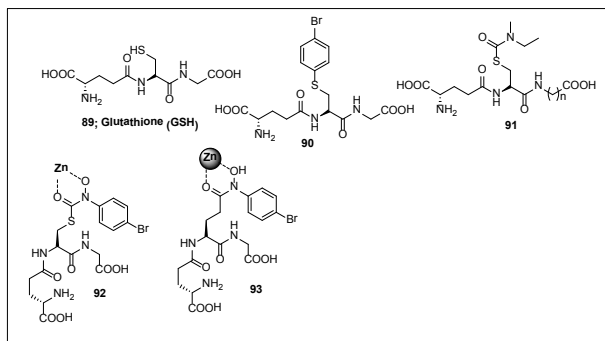


Figure 1.12: Glyoxalase inhibitors

It has been recently reported that the GLOI system in *L. major* uses trypanothione as the substitute for glutathione.¹²¹ The metal cofactor is zinc in eukaryotes and nickel in *Escherichia coli* and *L. major*. Thus, both the substrate and cofactor of *Leishmania* glyoxalase are different from those of mammalian glyoxalases.¹²¹ The deference in cofactor dependence is reflected in deferences between the active sites of the human and *Leishmania* enzymes, suggested that glyoxalase be a target. Numerous inhibitors

have been developed, most of which share the glutathione moiety. Among the most tightly binding family of inhibitors to the human enzyme are derivatives of *S*-(*N*-aryl-*N*-hydroxycarbamoyl)glutathione,¹²² most notably the *p*-bromophenyl derivative, More and Vince¹²³ were studied A series of rational modifications to the structure of known *S*-(*N*-aryl-*N*-hydroxycarbamoyl)glutathione based glyoxalase I inhibitors (**92-93**) culminated in the discovery of the first single-digit nanomolar inhibitor. This study makes available key information about possible means to address the issues of metabolic instability, low potency, and synthetic complexity that have plagued the area of glyoxalase I inhibition.

1.11.9. Sterol biosynthetic pathway

Sterols are important components of the cell membrane¹²⁴ that are vital to cellular function and maintenance of cell structure. Unlike mammalian cells, which have cholesterol as

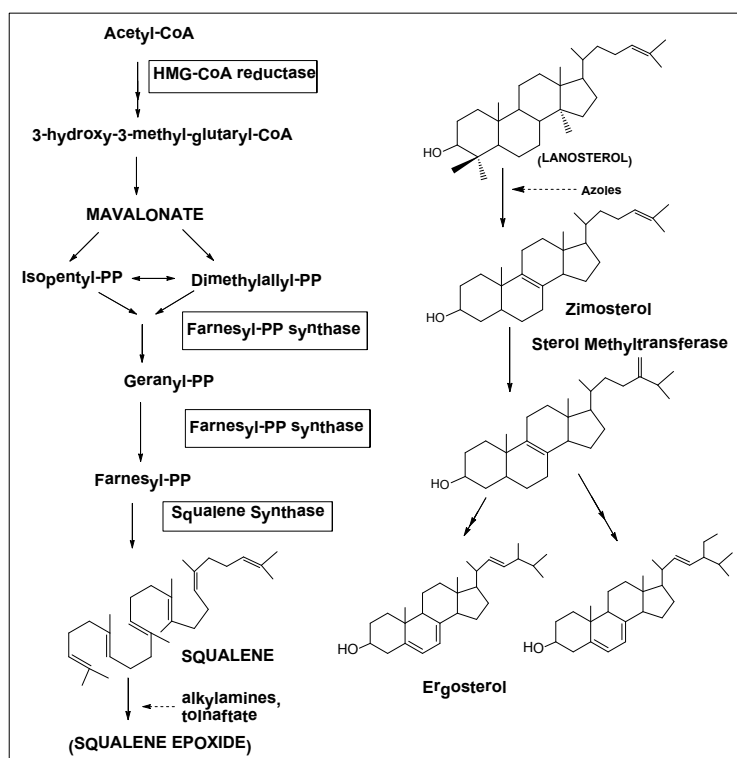


Fig. 1.13. Sterol biosynthetic pathway in *Leishmania*.

the major membrane sterol, *trypanosomatids* synthesize ergosterol and other 24-methyl sterols that are required for their growth and viability. These sterols are absent from the mammalian cells. Therefore, the sterol biosynthetic pathway from *Leishmania* is considered to be an important drug target.¹²⁵ One of the enzymes that is being studied deeply is squalene synthase (SQS), which was described above. Another class of inhibitors, bisphosphonates, inhibits the isoprenoid pathway,¹²⁶ that is catalyzed by the enzyme farnesyl diphosphate synthase (FPPS). Another potent inhibition of the cell growth and suppression of the activity of isolated enzymes (from *L. major*) was observed thus validating the isoprenoid pathway as a drug target.¹²⁷ Another important putative target in ergosterol biosynthesis is the enzyme 24,25-sterol methyltransferase (SMT). This enzyme is only present in *trypanosomatids* and absent from the human host. Therefore, this enzyme could be exploited as a potential drug target. Azasterols are known to inhibit SMT in case of *Candida spp.*. The effect of azasterols has been studied on *Leishmania* and *Trypanosoma* species among the protozoan parasites. Several azasterols^{128, 129} have been shown to have anti-proliferative effect with their IC_{50} s in submicromolar to nanomolar range against *Leishmania spp.* signifying that this step could be a potential chemotherapeutic target. In, *L. amazonensis*, they also led to disorganization of the mitochondrial membrane followed by intense swelling and loss of matrix contents.

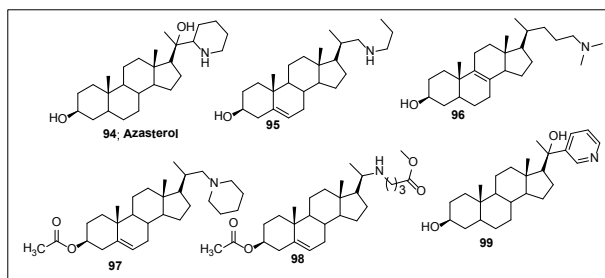


Figure1.14. Azasterol and its analogues

Lorente *et al*¹³⁰ and Gigante *et al*¹³¹ have synthesized some novel azasterol compounds (**95-99**), showed activity at micromolar and nanomolar concentrations when tested against *Leishmania spp.* and *Trypanosoma spp.* These compounds were shown to have ultrastructural effects on *L. amazonensis* promastigote membranes, including the plasma membrane, the mitochondrial membrane, and the endoplasmic reticulum. Compounds **98** and **99** show good antiparasitic activity, with inhibition of IC_{50} in the range of 12-80 nM better than that of the reference compound azasterol (**94**). Interestingly, azasterols when used in combination with azoles act synergistically and are even more effective suggesting that inhibiting multiple steps of this pathway, that is, combination therapy may be used against the parasitic protozoa.

1.12. Azole based lead molecules

Azoles act on ergosterol biosynthesis at the C-14 demethylation stage, a three-step, oxidative reaction catalyzed by the cytochrome P-450 enzyme 14 α -sterol demethylase (P-450_{DM}) (Figure 1.13). The resulting ergosterol depletion and accumulation of lanosterol and other 14-methylated sterols interferes with the 'bulk' functions of ergosterol as a membrane component. It disrupts the structure of the plasma membrane, making it more vulnerable to further damage, and alters the activity of several membrane-bound enzymes, such as those associated with nutrient transport and chitin synthesis. Severe ergosterol depletion (>99%) may additionally interfere with the hormone-like ('sparking') functions of ergosterol, affecting cell growth and proliferation. By inhibiting P-450_{DM}, azoles may, in addition, sensitize parasite cells to oxidative metabolites produced by phagocytes.

Azoles were originally developed as antifungal. But have also been used in the treatment of Leishmaniasis. Poorrajab *et al*¹³² and Tahghighi

*et al*¹³³ studied a series of 5-(5-nitrofuran-2-yl)-1,3,4-thiadiazol-2-amines, which were synthesized by introducing *N*-[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl] moiety as a new functionality on the C-2 amine of thiadiazole ring. Most of the compounds exhibited good antileishmanial activity against the promastigote form of *L. major*. 4-methylbenzyl analogue (**100**) was found to be the most active compound with an IC_{50} of 12 μ M.

Bernardino *et al*¹³⁴ synthesized and evaluated the *in vitro* antileishmanial activity of new series of pyrazole-4-carbohydrazides for their inhibitory effects against the extracellular

promastigote stage of *L. amazonensis*, *L. chagasi* and *L. braziliensis* parasites, as its direct toxic effect on macro phage host cells. Among all two compounds (**101** & **102**) showed to be most effective on promastigotes forms of *Leishmania spe.* and also reasonably less toxic than pentamidine. A variety of new imidazole and triazole derivatives¹³⁵ carrying either a carbaldehyde or a difluoromethylene functionality were synthesized and evaluated for their antileishmanial activity. Two Compounds (**103** & **104**) inhibited the growth of promastigote forms of *L. amazonensis* significantly.¹³⁵ In addition, no apparent hepatic

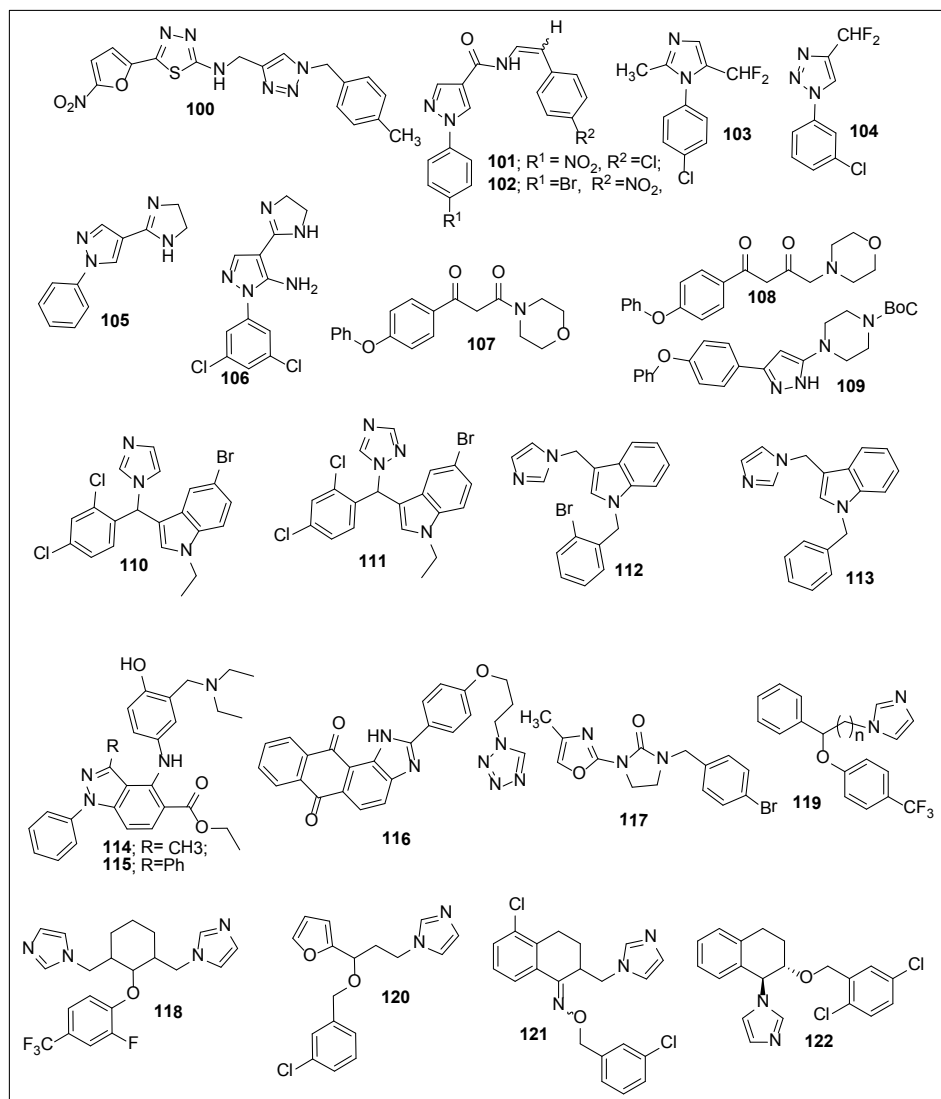


Figure 1.15. Antileishmanial azoles

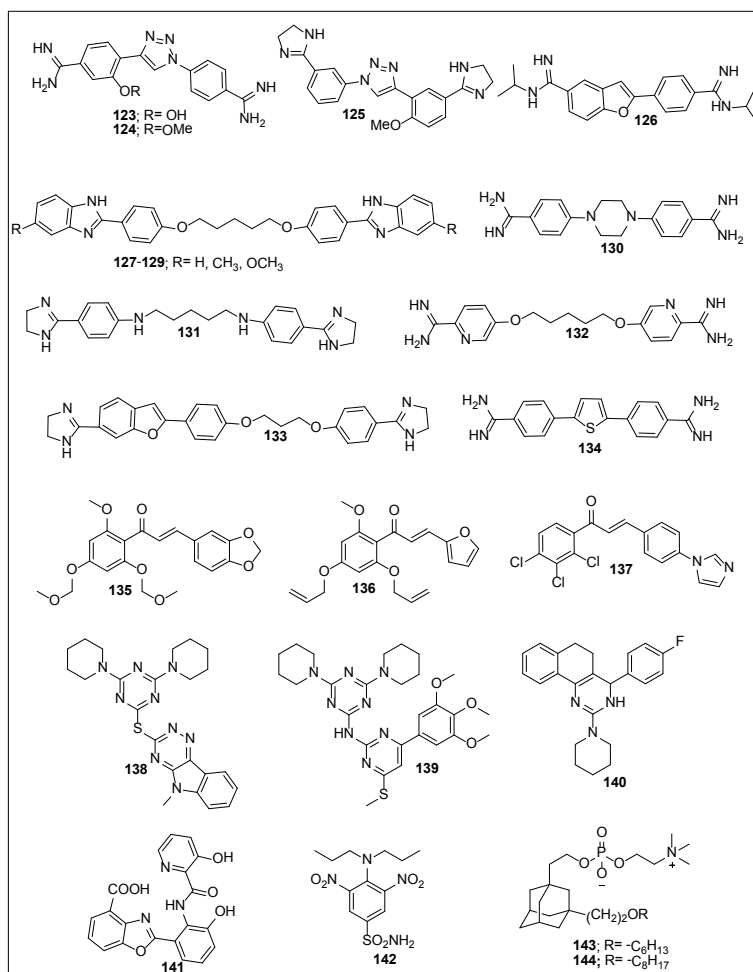


Figure 1.16: Some potent antileishmanial agents

or renal toxicity due to these compounds were found. Santos *et al*¹³⁶ synthesized a series of 1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles and 5-amino-1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles were and evaluated *in vitro* against three *Leishmania* species: *L. amazonensis*, *L. braziliensis* and *L. Infantum*. Among the derivatives examined, two compounds (**105** & **106**) emerged as the most active on promastigotes forms of *L. amazonensis* with IC_{50} values ranging from 15 to 40 μ M. These compounds also exhibited significant *in vivo* inhibition relative to an untreated control.

Kuettel *et al*¹³⁷ tested 4-[5-(4-phenoxyphenyl)-2*H*-pyrazol-3-yl]morpholine derivatives against *L. donovani*, three compounds were found active with, (**107-109**) IC_{50} values of 2.3-

5.2 μ M. Azolylbenzyl indoles¹³⁸⁻¹⁴⁰ derivatives (**110-113**), pyrazolopyridines¹⁴¹ derivatives (**114** & **115**), Anthra[1,2-*d*]imidazole-6,11-dione¹⁴² (**116**) and imidazolidin-2-ones¹⁴³ (**117**) have been discovered as potent antileishmanial agents with IC_{50} less than 1 μ M. Bhandari *et al* Carried out the synthesis, *in vitro* and *in vivo* biological evaluation of arylxoyazole¹⁴⁴⁻¹⁴⁷ based antileishmanial agents against *L. donovani*. Several compounds (**118-122**) exhibited promising antileishmanial activity both *in vitro* and *in vivo*.

A large number of cationically substituted pentamidine congeners (**123-134**),¹⁴⁸⁻¹⁵¹ chalcones (**135-137**),¹⁵²⁻¹⁵⁴ triazines (**138** & **139**),^{155, 156} quinazoline derivatives (**140**),¹⁵⁷ benzoxazoles (**141**),¹⁵⁸ sulfonamides (**142**),¹⁵⁹

ether phospholipids (**143** & **144**)¹⁶⁰ have been synthesized, and there *in vitro* and *in vivo* antiprotozoal properties against *Trypanosoma*, *Leishmania* species, as well as cytotoxicity against mammalian cells, have been evaluated. Some of the most potent molecules are shown in the Figure. 1.16.

1.13. Our group contribution in antileishmanial chemotherapy –

We are mounting new molecules as potent anti-parasitic agents from past 30 years. We were prepared various libraries for antileishmanial chemotherapy which were based on diverse medicinal chemistry approaches like molecular hybridization, natural product inspired, diversity oriented synthesis, drug inspire synthesis, biology-oriented synthesis, target based drug design and fragment based drug design etc (**Figure. 1.17**). In this section, we are going to

take a snapshot of our recent efforts.

1.13.1. A natural product inspired hybrid approach towards the synthesis of novel pentamidine based scaffolds as potential antileishmanial agents –

A natural product inspired molecular hybridization approach led us to a series of novel pentamidine based pyrimidine and chalcone scaffolds. All the hybrids were evaluated for their anti-leishmanial potential. Most of the screened compounds have showed significant *in vitro* anti-leishmanial activity with less cytotoxicity in comparison to the standard drugs (pentamidine, sodium stibogluconate, and miltefosine). Additionally, anti-malarial screening of these compounds was also done and four compounds have shown superior activity against chloroquine resistance strain (K1) of *Plasmodium falciparum*¹⁶¹. (Figure. 1.18)

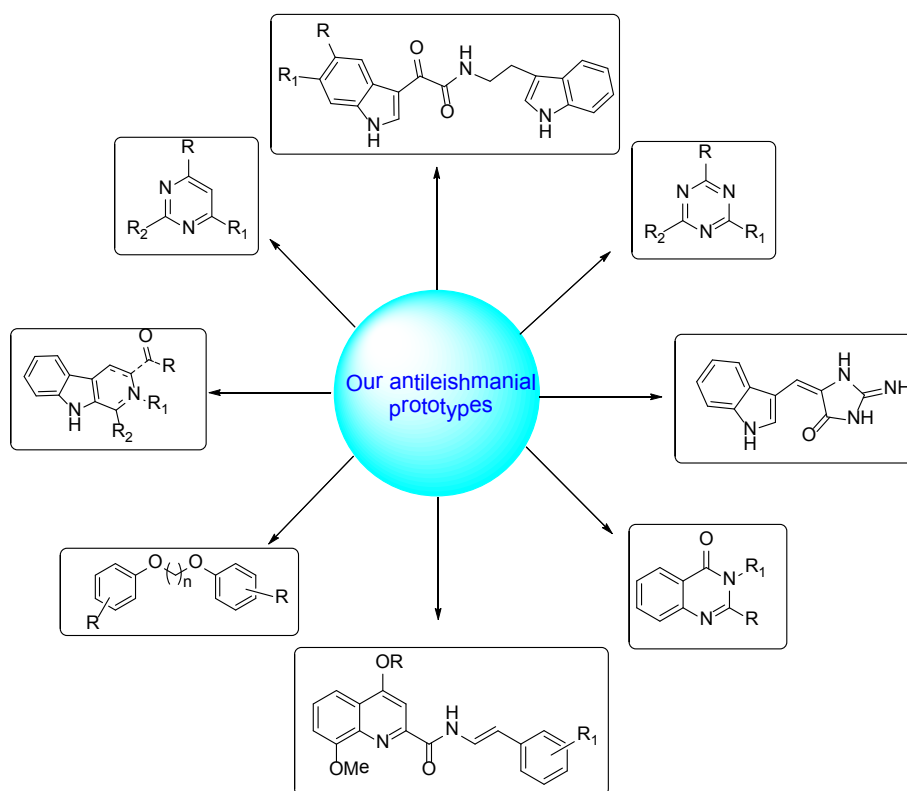


Figure 1.17: Some potent antileishmanial prototypes of our lab.

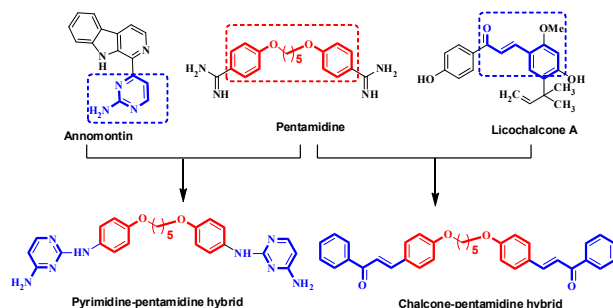


Figure 1.18: A natural product inspired hybrid approach to the synthesis of anti-parasitic agent

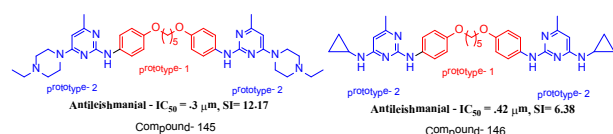


Figure 1.19: most active compounds of this sequence

All the synthesized compounds were evaluated *in vitro* against transgenic *L. donovani* amastigotes. All the pyrimidine-pentamidine hybrids showed very good inhibitory activity with the IC_{50} values in the range of 0.30-1.72 μM and SI values in the range of 1.11- >232, when compared to the standard drugs like Pentamidine (IC_{50} = 20.43 μM , SI = 2.58), SSG (IC_{50} = 71.90 $\mu g/mL$, SI = 5.53) and miltefosine (IC_{50} = 12.5 μM , SI = 0.25). Moreover, in case compounds 145 and 146 having *N*-ethyl piperazine and cyclopropyl amine functionalities showed IC_{50} = .3Mm, 0.42 μM and SI = 12.17, 6.38, respectively.

1.13.2. New Class of Natural Product-Inspired Quinazolinone Hybrid as Potent Antileishmanial agents-

Four new series of Natural Product-Inspired quinazolinone hybrid bearing interesting bioactive scaffolds (pyrimidine, triazine, tetrazole and peptide) were synthesized (Figure 1.20). A number of such compounds were found to be more potent against the intracellular amastigote of *L. donovani* than the standard drugs and were not found to be cytotoxic.

Compound 149 found to be the most potent *in vitro*, which is 13-fold better active with IC_{50} values of 0.73 μM and 129 fold more selective (SI >547.94) than that of miltefosine. Compound 147, 148, and 149 showed very consistent and promising leishmanicidal activity against intracellular amastigotes and *in vivo*. Moreover, it displayed no toxicity for macrophages, and its high value of selective index was better to other current antileishmanials like miltefosine and SSG. Therefore, these are good candidates for a lead optimization and could contribute greatly to new drug discovery for this serious and neglected disease. Interaction of most potent compounds 147, 148, and 149 with blood proteins showed static and dynamic quenching of intrinsic fluorescence. The data obtained for absorption and distribution profiles provide valuable information on the pharmacokinetic properties of the quinazolinone derivatives relevant for the therapeutic treatment of leishmaniasis. Therefore, these new compounds represent excellent leads for identifying new analogues to curb the unnecessary loss of life globally¹⁶².

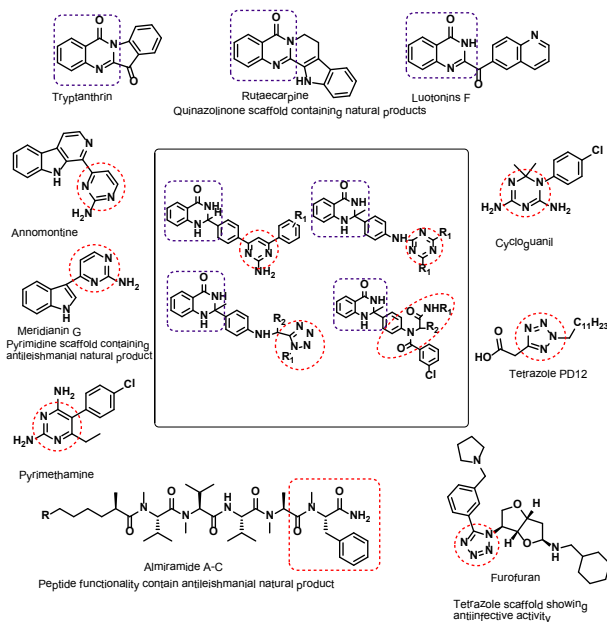


Figure 1.20. Designing of quinazolinone-based hybrids showing antileishmanial activity

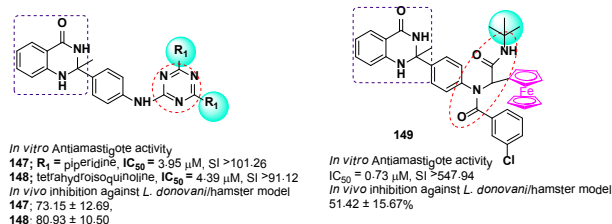


Figure 1.21: most active compounds of this sequence

1.13.3. Synthesis of Antileishmanial Perspicamide A and Related Diverse Analogues –

The quinoline nucleus enclosed metabolite perspicamide A (150) and B (151) (**Figure 1.22**), first isolated from the Australian ascidian *Botrylloides perspicuum* in 2005 by Quinn et al.,¹⁶³ has attracted much attention as it is found in many drug-like compounds. We have documented the first synthesis of perspicamide A and a wide range of structurally related analogues and their biological evaluation as potential antileishmanial agent employing an efficient and flexible route via Cu-mediated cross-coupling reaction under mild condition.

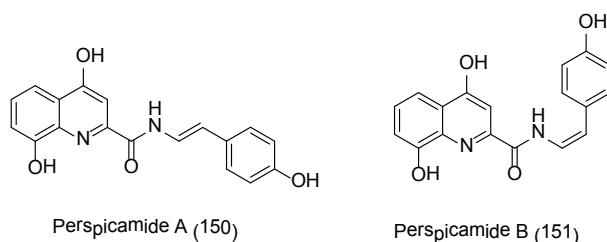


Figure 1.22: Structure of Perspicamide A and B.

All of the synthesized compounds were evaluated for their antileishmanial activity where analogues 152, 153, 154, 155, and 156 were found to be potentially active with IC_{50} values or 4.16, 4.55, 3.75, 10.37, and 6.98 μ M and SI values or 9.58, 53.12, 25.27, 38.57, and 22.91, respectively, which seems more promising than values for the standard drug Miltefosine (IC_{50} 12.4 μ M and SI 4.41) (**Figure**

1.23).

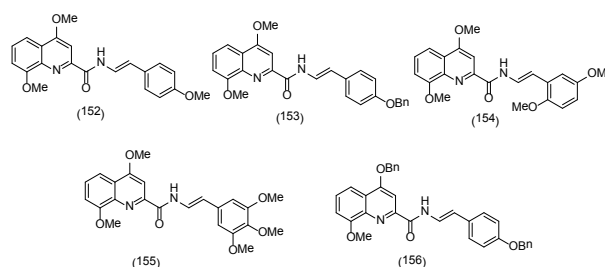


Figure 1.23: most active compounds of perspicamide sequence.

1.14. Conclusion

The control of Leishmaniasis is globally challenged by the widespread emergence of resistance for present drugs. In the last decade new formulations of conventional antileishmanial drugs as well as new agents became available; several mechanisms have been identified for antileishmanial resistance, with little success. However, understanding the molecular and biochemical characteristics as well as identification of proper drug targets will lead to the design of new drugs and also the molecular surveillance of resistance. Since Leishmaniasis is strongly linked with poverty, new cost effective drugs are needed. It is believed that this review will be instrumental in stressing the need for an urgent convergence of resources and academic interest and is sure to inform strategy and programming in this important area.

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1.15. References

1. P. Desjeux, *Comp. Immunol. Microbiol. Infect. Dis.* **2004**, 27, (5), 305-318.
2. P. Mitropoulos, P. Konidas, M. Durkin-Konidas, *J. Am. Acad. Dermatol.* **2010**, 63, (2), 309-322.
3. M. Mokni, A. Mebazaa, S. Boubaker, *Ann. Dermatol. Venereol.* **2011**, 138, (4), 354-356.
4. R. E. Unlu, S. Altun, O. Ssenoz, *J. Craniofac. Surg.* **2007**, 18, (3), 708-710.
5. E. M. Moore, D. N. Lockwood, *Clin. Med.* **2011**, 11, (5), 492-497.
6. J. C. Lemos, C. Lima Sdo, *Rev. Soc. Bras. Med. Trop.* **2005**, 38, (1), 22-26.
7. T. C. Hammarton, J. C. Mottram, C. Doerig, *Prog. Cell. Cycle. Res.* **2003**, 5, 91-101.
8. R. J. Wheeler, E. Gluenz, K. Gull, *Mol. Microbiol.* **2011**, 79, (3), 647-662.
9. A. Boutayeb, *Int. J. Equity Health*, **2007**, 6, 20.
10. A. Fenwick, *Public Health*, **2012**, 126, (3), 233-236.
11. O. B. Ngouateu, P. Kollo, C. Ravel, J. Dereure, P. Kamtchouing, A. Same-Ekobo, E. von Stebut, M. Maurer, B. Dondji, *Trans. R. Soc. Trop. Med. Hyg.* **2012**, 106, (3), 137-142.
12. L. F. Oliveira, A. O. Schubach, M. M. Martins, S. L. Passos, R. V. Oliveira, M. C. Marzochi, C. A. Andrade, *Acta. Trop.* **2011**, 118, (2), 87-96.
13. K. Griewank, C. Gazeau, A. Eichhorn, E. von Stebut, *Antimicrob. Agents. Chemother.* **2010**, 54, (2), 652-659.
14. M. R. Jaafari, N. Bavarsad, B. S. Bazzaz, A. Samiei, D. Soroush, S. Ghorbani, M. M. Heravi, A. Khamesipour, *Antimicrob. Agents. Chemother.* **2009**, 53, (6), 2259-2265.
15. S. Sundar, J. Chakravarty, *Expert Opin. Investig. Drugs.* **2008**, 17, (5), 787-794.
16. M. Solomon, F. Pavlotsky, E. Leshem, M. Ephros, H. Trau, E. Schwartz, *J. Eur. Acad. Dermatol. Venereol.* **2011**, 25, (8), 973-977.
17. S. L. Croft, S. Sundar, A. H. Fairlamb, *Clin. Microbiol. Rev.* **2006**, 19, (1), 111-126.
18. S. L. Croft, P. Olliaro, *Clin. Microbiol. Infect.* **2011**, 17, (10), 1478-1483.
19. R. Ravindran, N. Ali, *Curr. Mol. Med.* **2004**, 4, (6), 697-709.
20. P. Volf, J. Hostomska, I. Rohousova, *Parasite.* **2008**, 15, (3), 237-243.
21. P. F. Pimenta, S. J. Turco, M. J. McConville, P. G. Lawyer, P. V. Perkins, D. L. Sacks, *Science* **1992**, 256, (5065), 1812-1815.
22. R. Killick-Kendrick, *Ann. Parasitol. Hum. Comp.* **1990**, 65 Suppl 1, 37-42.
23. A. A. Pan, S. M. Duboise, S. Eperon, L. Rivas, V. Hodgkinson, Y. Traub-Cseko, D. McMahon-Pratt, *J. Eukaryot. Microbiol.* **1993**, 40, (2), 213-223.
24. R. Reithinger, J. C. Dujardin, H. Louzir, C. Pirmez, B. Alexander, S. Brooker, *Lancet Infect. Dis.* **2007**, 7, (9), 581-596.
25. P. Soni, N. Prasad, K. Khandelwal, B. C. Ghiya, R. D. Mehta, R. A. Bumb, P. Salotra, *Indian J. Dermatol. Venereol. Leprol.* **2011**, 77, (2), 251.
26. M. Mohebbali, A. Malmasi, H. Hajjaran, S. Jamshidi, B. Akhoundi, M. Rezaei, S. Janitabar, H. Zarei, S. Charehdar, *Central Iran. Iran. J. Parasitol.* **2011**, 6, (2), 69-73.
27. S. Khademvatan, N. Neisi, S. Maraghi, J. Saki, *Ann. Trop. Med. Parasitol.* **2011**, 105, (8), 559-565.
28. M. Berzunza-Cruz, G. Bricaire, S. Z. Romero, R. Perez-Becker, E. Saavedra-Lira, R. Perez-Montfort, M. Crippa-Rossi, O. Velasco-Castrejon, I. Becker, *Exp. Parasitol.* **2000**, 95, (4), 277-284.
29. I. Mauricio, L. Campino, P. Abranches, *Acta. Med. Port.* **1995**, 8, (11), 607-611.
30. N. Sakru, S. O. Toz, M. Korkmaz, Y. Ozbel, *Turkiye Parazit. Derg.* **2011**, 35, (3), 125-128.
31. P. Desjeux, *Bmj* **2010**, 341, c6751.
32. K. R. Matthews, *Science* **2011**, 331, (6021), 1149-1153.
33. R. J. Quinnell, O. Courtenay, *Parasitology* **2009**, 136, (14), 1915-1934.
34. M. Podaliri Vulpiani, L. Iannetti, D. Paganico, F. Iannino, N. Ferri, *Vet. Med. Int.* **2011**, 215964.
35. E. Ferroglio, M. Poggi, A. Trisciuglio, *Zoonoses Public Health* **2008**, 55, (3), 145-148.
36. M. Maroli, C. Khoury, *Parassitologia* **2004**, 46, (1-2), 211-215.
37. B. Ostyn, V. Vanlerberghe, A. Picado, D. S. Dinesh, S. Sundar, F. Chappuis, S. Rijal, J. C. Dujardin, M. Coosemans, M. Boelaert, C. Davies, *Trop. Med. Int. Health* **2008**, 13, (8), 1073-1085.
38. L. Kedzierski, Y. Zhu, E. Handman, *Parasitology* **2006**, 133 Suppl, S87-112.
39. J. M. Requena, S. Iborra, J. Carrion, C. Alonso, M. Soto, *Expert. Opin. Biol. Ther.* **2004**, 4, (9), 1505-1517.
40. I. D. Velez, K. Gilchrist, S. Martinez, J. R. Ramirez-Pineda, J. A. Ashman, F. P. Alves, R. N. Coler, L. Y. Bogatzki, S. J. Kahn, A. M. Beckmann, K. D. Cowgill, S. G. Reed, F. M. Piazza, *Vaccine* **2009**, 28, (2), 329-337.
41. E. Nascimento, D. F. Fernandes, E. P. Vieira, A. Campos-Neto, J. A. Ashman, F. P. Alves, R. N. Coler, L. Y. Bogatzki, S. J. Kahn, A. M. Beckmann, S. O. Pine, K. D. Cowgill, S. G. Reed, F. M. Piazza, *Vaccine* **2010**, 28, (40), 6581-6587.
42. <http://www.leishnavax.org/>.
43. R. Nagill, S. Kaur, *Int. Immunopharmacol.* **2011**, 11, (10), 1464-1488.
44. S. L. Croft, V. Yardley, *Curr. Pharm. Des.* **2002**, 8, (4), 319-342.
45. H. C. Maltezou, *J. Biomed. Biotechnol.* **2010**, 617521.
46. J. Mishra, A. Saxena, S. Singh, *Curr. Med. Chem.* **2007**,

- 14, (10), 1153-1169.
47. A. K. Haldar, P. Sen, S. Roy, *Mol. Biol. Int.* **2011**, 571242.
48. F. Chappuis, E. Alirol, D. T. Worku, Y. Mueller, K. Ritmeijer, *Antimicrob. Agents Chemother.* **2011**, 55, (1), 455-456.
49. M. K. Mittal, S. Rai, Ashutosh, Ravinder, S. Gupta, S. Sundar, N. Goyal, *Am. J. Trop. Med. Hyg.* **2007**, 76, (4), 681-688.
50. S. Sundar, D. K. More, M. K. Singh, V. P. Singh, S. Sharma, A. Makharia, P. C. Kumar, H. W. Murray, *Clin. Infect. Dis.* **2000**, 31, (4), 1104-1107.
51. S. Wyllie, M. L. Cunningham, A. H. Fairlamb, *J. Biol. Chem.* **2004**, 279, (38), 39925-39932.
52. I. Hellier, O. Dereure, I. Tournillac, F. Pratlong, B. Guillot, J. P. Dedet, J. J. Guilhou, *Dermatology* **2000**, 200, (2), 120-123.
53. A. F. E. J. Lai, M. A. Vrede, R. M. Soetosenojo, A. F. R. F. Lai, *Int. J. Dermatol.* **2002**, 41, (11), 796-800.
54. A. Feddersen, K. Sack, *J. Antimicrob. Chemother.* **1991**, 28, (3), 437-446.
55. P. Minodier, S. Robert, G. Noel, P. Blanc, K. Retornaz, J. M. Garnier, *Arch. Pediatr.* **2005**, 12, (7), 1102-1108.
56. S. Sundar, H. W. Murray, *J. Infect. Dis.* **1996**, 173, (3), 762-765.
57. V. S. Amato, A. Rabello, A. Rotondo-Silva, A. Kono, T. P. Maldonado, I. C. Alves, L. M. Floeter-Winter, V. A. Neto, M. A. Shikanai-Yasuda, *Acta. Trop.* **2004**, 92, (2), 127-132.
58. R. Herbrecht, S. Natarajan-Ame, Y. Nivoix, V. Letscher-Bru, *Expert Opin. Pharmacother.* **2003**, 4, (8), 1277-1287.
59. C. Fischer, A. Voss, J. Engel, *Med. Microbiol. Immunol.* **2001**, 190, (1-2), 85-87.
60. I. Velez, L. Lopez, X. Sanchez, L. Mestra, C. Rojas, E. Rodriguez, *Am. J. Trop. Med. Hyg.* **2010**, 83, (2), 351-356.
61. N. K. Verma, C. S. Dey, *Agents Chemother.* **2004**, 48, (8), 3010-3015.
62. S. Sundar, M. Rai, J. Chakravarty, D. Agarwal, N. Agrawal, M. Vaillant, P. Oliario, H. W. Murray, *Clin. Infect. Dis.* **2008**, 47, (8), 1000-1006.
63. S. L. Croft, V. Yardley, H. Kendrick, *Trans. R. Soc. Trop. Med. Hyg.* **2002**, 96 Suppl 1, S127- S129.
64. R. X. Armijos, M. M. Weigel, M. Calvopina, M. Mancheno, R. Rodriguez, *Acta. Trop.* **2004**, 91, (2), 153-160.
65. H. Sangraula, K. K. Sharma, S. Rijal, S. Dwivedi, S. Koirala, *J. Assoc. Physicians India* **2003**, 51, 686-690.
66. M. K. Wasunna, J. R. Rashid, J. Mbui, G. Kirigi, D. Kinoti, H. Lodenyo, J. M. Felton, A. J. Sabin, M. J. Albert, J. Horton, *Am. J. Trop. Med. Hyg.* **2005**, 73, (5), 871-876.
67. L. Carvalho, J. R. Luque-Ortega, C. Lopez-Martin, S. Castanys, L. Rivas, F. Gamarro, *Antimicrob. Agents Chemother.* **2011**, 55, (9), 4204-4210.
68. P. M. Loiseau, S. Cojean, J. Schrevel, *Parasite* **2011**, 18, (2), 115-119.
69. A. Z. Momeni, M. Aminjavaheri, M. R. Omidghaemi, *J. Dermatol. Treat.* **2003**, 14, (1), 26-29.
70. A. Q. Sousa, M. S. Frutuoso, E. A. Moraes, R. D. Pearson, M. M. Pompeu, *Clin. Infect. Dis.* **2011**, 53, (7), 693-695.
71. A. Baroni, F. S. Aiello, A. Vozza, G. Vozza, F. Faccenda, M. Brasiello, E. Ruocco, *Dermatol. Ther.* **2009**, 22 Suppl 1, S27- S29.
72. A. E. Paniz Mondolfi, C. Stavropoulos, T. Gelanew, E. Loucas, A. M. Perez Alvarez, G. Benaim, B. Polsky, G. Schoenian, E. M. Sordillo, *Antimicrob. Agents Chemother.* **2011**, 55, (4), 1774-1776.
73. S. M. da Silva, I. F. Amorim, R. R. Ribeiro, E. G. Azevedo, C. Demicheli, M. N. Melo, W. L. Tafuri, N. F. Gontijo, M. S. Michalick, F. Frezard, *Antimicrob. Agents Chemother.* **2012**.
74. S. Shamsi Meymandi, A. Javadi, S. Dabiri, Shamsi M. Meymandi, M. Nadji, *CArch. Iran. Med.* **2011**, 14, (4), 238-243.
75. S. Azzouz, M. Maache, R. G. Garcia, A. Osuna, *Basic Clin. Pharmacol. Toxicol.* **2005**, 96, (1), 60-65.
76. M. G. Cabrera-Serra, B. Valladares, J. E. Pinero, *Acta. Trop.* **2008**, 108, (1), 20-25.
77. F. Lacarrubba, M. R. Nasca, G. Micali, *Ther. Clin. Risk Manag.* **2008**, 4, (1), 87-97.
78. I. Arevalo, G. Tulliano, A. Quispe, G. Spaeth, G. Matlashewski, A. Llanos-Cuentas, H. Pollack, *Clin. Infect. Dis.* **2007**, 44, (12), 1549-1554.
79. G. J. Crowther, D. Shanmugam, S. J. Carmona, M. A. Doyle, C. Hertz-Fowler, M. Berriman, S. Nwaka, S. A. Ralph, D. S. Roos, W. C. Van Voorhis, F. Agüero, *PLoS Negl. Trop. Dis.* **2010**, 4, (8), e804.
80. M. Smith, F. Bringaud, B. Papadopoulou, *BMC Genomics* **2009**, 10, 240.
81. P. J. Myler, S. M. Beverley, A. K. Cruz, D. E. Dobson, A. C. Ivens, P. D. McDonagh, R. Madhubala, S. Martinez-Calvillo, J. C. Ruiz, A. Saxena, E. Sisk, S. M. Sunkin, E. Worthey, S. Yan, K. D. Stuart, *Med. Microbiol. Immunol.* **2001**, 190, (1-2), 9-12.
82. R. Grumont, W. Sirawaraporn, D. V. Santi, *Biochemistry* **1988**, 27, (10), 3776-3784.
83. I. H. Gilbert, *Biochim Biophys. Acta.* **2002**, 1587, (2-3), 249-257.
84. B. Nare, J. Luba, L. W. Hardy, S. Beverley, *Parasitology* **1997**, 114 Suppl, S101-S110.
85. B. Nare, L. W. Hardy, S. M. Beverley, *J. Biol. Chem.* **1997**, 272, (21), 13883-13891.
86. W. Sirawaraporn, R. Sertsriwanich, R. G. Booth, C. Hansch, R. A. Neal, D. V. Santi, *Mol. Biochem. Parasitol.* **1988**, 31, (1), 79-85.
87. V. Bernier-Villamor, A. Camacho, D. Gonzalez-Pacanowska, E. Cedergren-Zeppeauer, A. Antson, K. S. Wilson, *Acta. Crystallogr. D. Biol. Crystallogr.* **1999**, 55, (Pt 2), 528-530.
88. A. Camacho, F. Hidalgo-Zarco, V. Bernier-Villamor, L. M. Ruiz-Perez, D. Gonzalez-Pacanowska, *Biochem. J.* **2000**, 346 Pt 1, 163-168.

89. C. Nguyen, G. Kasinathan, I. Leal-Cortijo, A. Musso-Buendia, M. Kaiser, R. Brun, L. M. Ruiz-Perez, N. G. Johansson, D. Gonzalez-Pacanowska, I. H. Gilbert, J. Med. Chem. **2005**, 48, (19), 5942-5954.
90. T. Persson, G. Larsson, P. O. Nyman, Bioorg. Med. Chem. **1996**, 4, (4), 553-556.
91. Y. C. Tse-Dinh, Infect. Disord. Drug. Targets **2007**, 7, (1), 3-9.
92. K. D. Corbett, J. M. Berger, Annu. Rev. Biophys. Biomol. Struct. **2004**, 33, 95-118.
93. J. M. Berger, Curr. Opin. Struct. Biol. **1998**, 8, (1), 26-32.
94. B. B. Das, A. Ganguly, H. K. Majumder, Adv. Exp. Med. Biol. **2008**, 625, 103-115.
95. F. Poorrajab, S. K. Ardestani A., Foroumadi, S. Emami, A. Kariminia, M. Behrouzi-Fardmoghdam, A. Shafiee, Exp. Parasitol. **2009**, 121, (4), 323-330.
96. J. Walker, N. G. Saravia, J. Parasitol. **2004**, 90, (5), 1155-1162.
97. A. Stone, R. L. Sutherland, E. A. Musgrove, Crit. Rev. Oncog. **2012**, 17, (2), 175-98.
98. C. Naula, M. Parsons, J. C. Mottram, Biochim. Biophys. Acta. **2005**, 1754, (1-2), 151-159.
99. P. Hassan, D. Fergusson, K. M. Grant, J. C. Mottram, Mol. Biochem. Parasitol. **2001**, 113, (2), 189-198.
100. B. L. Burnette, S. Selness, R. Devraj, G. Jungbluth, R. Kurumbail, L. Stillwell, G. Anderson, S. Mnich, J. Hirsch, R. Compton, P. De Ciechi, H. Hope, M. Hepperle, R. H. Keith, W. Naing, H. Shieh, J. Portanova, Y. Zhang, J. Zhang, R. M. Leimgruber, J. Monahan, Pharmacology **2009**, 84, (1), 42-60.
101. K. F. McClure, M. A. Letavic, A. S. Kalgutkar, C. A. Gabel, L. Audoly, J. T. Barberia, J. F. Braganza, D. Carter, T. J. Carty, S. R. Cortina, M. A. Dombroski, K. M. Donahue, N. C. Elliott, C. P. Gibbons, C. K. Jordan, A. V. Kuperman, J. M. Labasi, R. E. Laliberte, J. M. McCoy, B. M. Naiman, K. L. Nelson, H. T. Nguyen, K. M. Peese, F. J. Sweeney, T. J. Taylor, C. E. Trebino, Y. A. Abramov, E. R. Laird, W. A. Volberg, J. Zhou, J. Bach, F. Lombardo, S. Bioorg. Med. Chem. Lett. **2006**, 16, (16), 4339-4344.
102. S. R. Selness, T. L. Boehm, J. K. Walker, B. Devadas, R. C. Durley, R. Kurumbail, H. Shieh, L. Xing, M. Hepperle, P. V. Rucker, K. D. Jerome, A. G. Benson, L. D. Marrufo, H. M. Madsen, J. Hitchcock, T. J. Owen, L. Christie, M. A. Promo, B. S. Hickory, E. Alvira, W. Naing, R. Blevis-Bal, R. V. Devraj, D. Messing, J. F. Schindler, J. Hirsch, M. Saabye, S. Bonar, E. Webb, G. Anderson, J. B. Monahan, Bioorg. Med. Chem. Lett. **2011**, 21, (13), 4059-4065.
103. M. Basselin, G. H. Coombs, M. P. Barrett, Mol. Biochem. Parasitol. **2000**, 109, (1), 37-46.
104. M. Kandpal, B. L. Tekwani, Life Sci. **1997**, 60, (20), 1793-1801.
105. M. P. Hasne, B. Ullman, J. Biol. Chem. **2005**, 280, (15), 15188-15194.
106. U. Bachrach, L. Abu-Elheiga, L. F. Schnur, Exp. Parasitol. **1983**, 55, (3), 280-288.
107. N. Kaur, J. G. Delcros, J. Imran, A. Khaled, M. Chehtane, N. Tschammer, B. Martin, O. T. Phanstiel, J. Med. Chem. **2008**, 51, (5), 1393-1401.
108. A. P. Caminos, E. A. Panozzo-Zenere, S. R. Wilkinson, B. L. Tekwani, G. R. Labadie, Bioorg. Med. Chem. Lett. **2012**, 22, (4), 1712-1715.
109. G. R. Labadie, S. R. Choi, M. A. Avery, Bioorg. Med. Chem. Lett. **2004**, 14, (3), 615-619.
110. J. A. Urbina, J. L. Concepcion, S. Rangel, G. Visbal, R. Lira, Mol. Biochem. Parasitol. **2002**, 125, (1-2), 35-45.
111. S. Orenes Lorente, R. Gomez, C. Jimenez, S. Cammerer, V. Yardley, K. de Luca-Fradley, S. L. Croft, L. M. Ruiz Perez, J. Urbina, D. Gonzalez Pacanowska, I. H. Gilbert, Bioorg. Med. Chem. **2005**, 13, (10), 3519-3529.
112. J. C. Fernandes Rodrigues, J. L. Concepcion, C. Rodrigues, A. Caldera, J. A. Urbina, W. de Souza, Antimicrob. Agents Chemother. **2008**, 52, (11), 4098-4114.
113. T. Ishihara, H. Kakuta, H. Moritani, T. Ugawa, I. Yanagisawa, Bioorg. Med. Chem. **2004**, 12, (22), 5899-5908.
114. M. Ichikawa, A. Yokomizo, M. Itoh, K. Sugita, H. Usui, H. Shimizu, M. Suzuki, K. Terayama, A. Kanda, Bioorg. Med. Chem. **2011**, 19, (6), 1930-1949.
115. D. Turk, B. Turk, V. Turk, Biochem. Soc. Symp. **2003**, (70), 15-30.
116. K. A. Scheidt, W. R. Roush, J. H. McKerrow, P. M. Selzer, E. Hansell, P. J. Rosenthal, Bioorg. Med. Chem. **1998**, 6, (12), 2477-2494.
117. N. E. Zhou, D. Guo, G. Thomas, A. V. Reddy, J. Kaleta, E. Purisima, R. Menard, R. G. Micetich, R. Singh, Bioorg. Med. Chem. Lett **2003**, 13, (1), 139-141.
118. P. M. Selzer, X. Chen, V. J. Chan, M. Cheng, G. L. Kenyon, I. D. Kuntz, J. A. Sakanari, F. E. Cohen, J. H. McKerrow, Exp. Parasitol. **1997**, 87, (3), 212-221.
119. S. Wyllie, A. H. Fairlamb, Semin. Cell. Dev. Biol. **2011**, 22, (3), 271-277.
120. P. K. Padmanabhan, A. Mukherjee, S. Singh, S. Chattopadhyaya, V. S. Gowri, P. J. Myler, N. Srinivasan, R. Madhubala, Biochem. Biophys. Res. Commun. **2005**, 337, (4), 1237-1248.
121. A. Ariza, T. J. Vickers, N. Greig, K. A. Armour, M. J. Dixon, I. M. Eggleston, A. H. Fairlamb, C. S. Bond, Mol. Microbiol. **2006**, 59, (4), 1239-1248.
122. N. S. Murthy, T. Bakeris, M. J. Kavarana, D. S. Hamilton, Y. Lan, D. J. Creighton, J. Med. Chem. **1994**, 37, (14), 2161-2166.
123. S. S. More, R. Vince, J. Med. Chem. **2009**, 52, (15), 4650-4656.
124. L. F. Bonewald, G. R. Mundy, Connect. Tissue. Res. **1989**, 23, (2-3), 201-208.
125. W. de Souza, J. C. Rodrigues, Interdiscip. Perspect. Infect. Dis. **2009**, 642502.
126. M. B. Martin, J. S. Grimley, J. C. Lewis, H. T. Heath, B.

- N. Bailey, H. Kendrick, V. Yardley, A. Caldera, R. Lira, J. A. Urbina, S. N. Moreno, R. Docampo, S. L. Croft, E. Oldfield, *J. Med. Chem.* **2001**, 44, (6), 909-916.
127. M. Rohmer, C. Grosdemange-Billiard, M. Seemann, D. Tritsch, *Curr. Opin. Investig. Drugs* **2004**, 5, (2), 154-162.
128. F. Magaraci, C. J. Jimenez, C. Rodrigues, J. C. Rodrigues, M. V. Braga, V. Yardley, K. de Luca-Fradley, S. L. Croft, W. de Souza, L. M. Ruiz-Perez, J. Urbina, D. Gonzalez Pacanowska, I. H. Gilbert, *J. Med. Chem.* **2003**, 46, (22), 4714-4727.
129. L. Gros, V. M. Castillo-Acosta, Jimenez C. Jimenez, M. Sealey-Cardona, S. Vargas, A. Manuel Estevez, V. Yardley, L. Rattray, S. L. Croft, L. M. Ruiz-Perez, J. A. Urbina, I. H. Gilbert, D. Gonzalez-Pacanowska, *Antimicrob. Agents Chemother.* **2006**, 50, (8), 2595-2601.
130. S. O. Lorente, J. C. Rodrigues, C. Jimenez Jimenez, M. Joyce-Menekse, C. Rodrigues, S. L. Croft, V. Yardley, K. de Luca-Fradley, L. M. Ruiz-Perez, J. Urbina, W. de Souza, D. Gonzalez Pacanowska, I. H. Gilbert, *Antimicrob. Agents Chemother.* **2004**, 48, (8), 2937-2950.
131. F. Gigante, M. Kaiser, R. Brun, I. H. Gilbert, *Bioorg. Med. Chem.* **2009**, 17, (16), 5950-5961.
132. F. Poorrajab, S. K. Ardestani, S. Emami, M. Behrouzi-Fardmoghdam, A. Shafiee, A. Foroumadi, *Eur. J. Med. Chem.* **2009**, 44, (4), 1758-1762.
133. A. Tahghighi, S. Razmi, M. Mahdavi, P. Foroumadi, S. K. Ardestani, S. Emami, F. Kobarfard, S. Dastmalchi, A. Shafiee, A. Foroumadi, *Eur. J. Med. Chem.* **2012**, 50, 124-128.
134. A. M. Bernardino, A. O. Gomes, K. S. Charret, A. C. Freitas, G. M. Machado, M. M. Canto-Cavalheiro, L. L. Leon, V. F. Amaral, *Eur. J. Med. Chem.* **2006**, 41, (1), 80-87.
135. S. B. Ferreira, M. S. Costa, N. Boechat, R. J. Bezerra, M. S. Genestra, M. M. Canto-Cavalheiro, W. B. Kover, V. F. Ferreira, *Eur. J. Med. Chem.* **2007**, 42, (11-12), 1388-1395.
136. M. S. dos Santos, M. L. Oliveira, A. M. Bernardino, R. M. de Leo, V. F. Amaral, F. T. de Carvalho, L. L. Leon, M. M. Canto-Cavalheiro, *Bioorg. Med. Chem. Lett.* **2011**, 21, (24), 7451-7454.
137. S. Kuettel, A. Zambon, M. Kaiser, R. Brun, L. Scapozza, R. Perozzo, *J. Med. Chem.* **2007**, 50, (23), 5833-5839.
138. P. Marchand, M. Le Borgne, Y. M. Na, F. Pagniez, H. Abdala, G. Le Baut, P. Le Pape, *J. Enzyme. Inhib. Med. Chem.* **2002**, 17, (6), 353-358.
139. Y. M. Na, N. Lebouvier, M. Le Borgne, F. Pagniez, N. Alvarez, P. Le Pape, G. Le Baut, *Inhib. Med. Chem.* **2004**, 19, (6), 451-457.
140. F. Pagniez, H. Abdala-Valencia, P. Marchand, M. Le Borgne, G. Le Baut, S. Robert-Piessard, P. Le Pape, *J. Enzyme. Inhib. Med. Chem.* **2006**, 21, (3), 277-283.
141. H. de Mello, A. Echevarria, A. M. Bernardino, M. Canto-Cavalheiro, L. L. Leon, *J. Med. Chem.* **2004**, 47, (22), 5427-5432.
142. P. Chaudhuri, H. K. Majumder, S. Bhattacharya, *J. Med. Chem.* **2007**, 50, (10), 2536-2540.
143. J. M. Robert, C. Sabourin, N. Alvarez, S. Robert-Piessard, G. Le Baut, P. Le Pape, *Eur. J. Med. Chem.* **2003**, 38, (7-8), 711-718.
144. K. Bhandari, N. Srinivas, V. K. Marrapu, A. Verma, S. Srivastava, S. Gupta, *Bioorg. Med. Chem. Lett.* **2010**, 20, (1), 291-293.
145. N. Srinivas, S. Palne, S. Nishi Gupta, K. Bhandari, *Bioorg. Med. Chem. Lett.* **2009**, 19, (2), 324-327.
146. V. K. Marrapu, N. Srinivas, M. Mittal, N. Shakya, S. Gupta, K. Bhandari, *Bioorg. Med. Chem. Lett.* **2011**, 21, (5), 1407-1410.
147. V. K. Marrapu, M. Mittal, R. Shivahare, S. Gupta, K. Bhandari, *Eur. J. Med. Chem.* **2011**, 46, (5), 1694-1700.
148. S. M. Bakunova, S. A. Bakunov, D. A. Patrick, E. V. Kumar, K. A. Ohemeng, A. S. Bridges, T. Wenzler, T. Barszcz, S. K. Jones, K. A. Werbovetz, R. Brun, R. R. Tidwell, *J. Med. Chem.* **2009**, 52, (7), 2016-2035.
149. J. J. Brendle, A. Outlaw, A. Kumar, D. W. Boykin, D. A. Patrick, R. R. Tidwell, K. A. Werbovetz, *Antimicrob. Agents Chemother.* **2002**, 46, (3), 797-807.
150. S. M. Bakunova, S. A. Bakunov, T. Wenzler, T. Barszcz, K. A. Werbovetz, R. Brun, R. R. Tidwell, *J. Med. Chem.* **2009**, 52, (15), 4657-4667.
151. S. A. Bakunov, S. M. Bakunova, A. S. Bridges, T. Wenzler, T. Barszcz, K. A. Werbovetz, R. Brun, R. R. Tidwell, *J. Med. Chem.* **2009**, 52, (18), 5763-5767.
152. J. C. Aponte, D. Castillo, Y. Estevez, G. Gonzalez, J. Arevalo, G. B. Hammond, M. Sauvain, *Bioorg. Med. Chem. Lett.* **2010**, 20, (1), 100-103.
153. J. C. Aponte, M. Verastegui, E. Malaga, M. Zimic, M. Quiliano, A. J. Vaisberg, R. H. Gilman, G. B. Hammond, *J. Med. Chem.* **2008**, 51, (19), 6230-6234.
154. T. Hussain, H. L. Siddiqui, M. Zia-ur-Rehman, M. Masoom Yasinza, M. Parvez, *Eur. J. Med. Chem.* **2009**, 44, (11), 4654-4660.
155. N. Sunduru, S. Nishi Palne, P. M. Chauhan, S. Gupta, *Eur. J. Med. Chem.* **2009**, 44, (6), 2473-2481.
156. L. Gupta, N. Sunduru, A. Verma, S. Srivastava, S. Gupta, N. Goyal, P. M. Chauhan, *Eur. J. Med. Chem.* **2010**, 45, (6), 2359-2365.
157. K. C. Agarwal, V. Sharma, N. Shakya, S. Gupta, *Bioorg. Med. Chem. Lett.* **2009**, 19, (18), 5474-5477.
158. S. K. Tipparaju, S. Joyasawal, M. Pieroni, M. Kaiser, R. Brun, A. P. Kozikowski, *J. Med. Chem.* **2008**, 51, (23), 7344-7347.
159. G. Bhattacharya, M. M. Salem, K. A. Werbovetz, *Bioorg. Med. Chem. Lett.* **2002**, 12, (17), 2395-2398.
160. I. Papanastasiou, K. C. Prousis, K. Georgikopoulou, T. Pavlidis, E. Scoulica, N. Kolocouris, T. Calogeropoulou, *Bioorg. Med. Chem. Lett.* **2010**, 20, (18), 5484-5487.
161. V. Tyagi, S. Khan, R. Shivahare, K. Srivastava, S. Gupta, S. Kidwai, K. Srivastava, S. K. Puri, P. M. S. Chauhan,

- Bioorg. Med. Chem. Lett. 2013, 23, 291–296.
162. M. Sharma, K. Chauhan, R. Shivahare, P. Vishwakarma, M. K. Suthar, A. Sharma, Gupta, S. Saxena, J. K. Lal, J. Chandra, P. Kumar, B., P. M. S. Chauhan, J. Med. Chem. **2013**, 56, 4374–4392.
163. M. J. McKay, A. R. Carroll, R. J. Quinn, J. Nat. Prod **2005**, 68, 1776.
164. A. K. Pandey, R. Sharma, R. Shivahare, A. Arora, N. Rastogi, S. Gupta, P. M. S. Chauhan, J. Org. Chem. **2013**, 78, 1534–1546.