Antileishmanial drug discovery: comprehensive review of the last 10 years

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Leishmaniasis, a group of diseases caused by hemoflagellate obligate intracellular protozoa (trypanosomatids) from the genus Leishmania, has not received the attention it deserves and has developed into a major health problem in developing countries. No effective vaccine is available against leishmaniasis, so chemotherapy is the only effective way to treat all forms of the disease. However, the drugs currently used for treatment of human cutaneous and visceral leishmaniasis are toxic, having severe adverse reactions which limit their use. Therefore, development of novel, effective, and safe antileishmanial agents, with reduced side effects, is a major priority for health researchers, and large numbers of research reports have been published on antileishmanial agents in the last 10 years. Herein, we comprehensively review the developments of the last decade, covering all aspects of leishmaniasis including clinically used drugs, various new classes of antileishmanial agents (synthetic as well as natural), patented antileishmanial agents, and possible drug targets.

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1. Introduction

Leishmaniasis is a vector-borne, poverty-associated disease developing in the mammalian host by protozoan parasites (obligate, hemoflagellate, intracellular in nature) belonging to the order Kinetoplastidae, family Trypanosomatidae from the genus Leishmania. These parasites reside in and are transmitted through the bites of more than 30 species of female sand flies. The World Health Organization (WHO) classified leishmaniasis as a major tropical disease, ranking second only after malaria. Ever-increasing cases worldwide are resulting in high morbidity and mortality levels with a wide spectrum of clinical syndromes. It has become a major focus of concern and a serious world problem that affects the poorer sections of the society. It is estimated that 12 million people worldwide are infected by over 20 different species of Leishmania, and about 350 million people living in the endemic areas are at risk of infection. This parasite exists in many tropical and temperate countries. Researchers have reported that more than 90% of visceral cases of leishmaniasis (visceral leishmaniasis, VL) occur in India, Nepal, South Sudan, Sudan, Bangladesh, Brazil, and Ethiopia, while about 70–75% of cutaneous cases (cutaneous leishmaniasis, CL) occur mainly in Afghanistan, Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Iran, Syria, North Sudan, and Peru.

When sand flies (vectors) feed on an infected host, Leishmania parasites enter their digestive tracts and multiply there as promastigotes, which can then be passed to a mammalian host when the sand flies bite healthy humans for blood meal. In this vertebrate host, the parasite multiplies inside the macrophages (where they survive and multiply within phagolysosomal compartment) in an amastigote form.

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Rohidas Arote did his PhD in gene delivery from Seoul National University, Seoul, Republic of Korea in 2008. He continued as a postdoctoral fellow in the same University for the period of 2009–2010 and is currently working as an Assistant Professor with School of Dentistry, Seoul National University, Republic of Korea. His Ph.D. research involved development of a biodegradable polymeric gene carrier system for the treatment of various types of diseases, mainly cancer. His postdoctoral research was developing efficient gene carriers for the treatment of lung and liver cancer. Presently, he is focusing on targeted drug and gene delivery by using multifunctional nanocarriers in the field of nanomedicine.

Rajendra H. Patil completed his master from Swami Ramanand Teerth Marathwada University, Nanded, India, in 2002. He submitted doctorate studies to the Department of Biotechnology, Savitribai Phule Pune University, Pune, India, in 2014, and later joined as an assistant professor in the same department. He is a recipient of UGC-CSIR fellowship for completing his doctorate studies. His interest has been in the area of bioorganic chemistry wherein the microbial diversity is explored for synthesis of bioactive molecules and nanoparticles. He also has published many research articles wherein the chemically synthesized nanoparticles were used to eradicate the biofilm in Pseudomonas aeruginosa.

Fig. 1 Life cycle of Leishmania.
Leishmania parasites have the capability to survive in stress conditions, lyse macrophages, and are phagocytosed by new host cells.11–13 Depending on the tropism, the disease is characterized as one of four major syndromes: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), visceral leishmaniasis (VL), and post-kala-azar dermal leishmaniasis (PKDL). Other cutaneous manifestations such as diffuse cutaneous leishmaniasis (DCL) and recidivans leishmaniasis (RL) may also occur.6,14

Among the various causative organisms, viz. L. donovani, L. major, L. tropica, L. aethiopica, L. mexicana, L. venezuelensis, L. amazonensis, L. braziliensis, L. panamensis, L. guyanensis, L. peruviana, L. infantum (L. chagasi) etc., that infect a variety of the population, some are associated significantly with morbidity.6 If untreated, most VL caused by L. donovani is fatal. CL caused by L. major, L. mexicana, L. braziliensis, and L. panamensis is associated with morbidity, but can self-cure within 3–18 months leaving behind disfiguring scars.10,13,14 Leishmaniasis is said to be recidivans leishmaniasis, if caused by L. tropica which is difficult to treat and leaves behind extensive scars. MCL is characterized by the destruction of mucosa and cartilage of mouth and pharynx, followed by the involvement of facial tissue (several species are able to cause MCL, the most important being L. braziliensis).17

There are very few reports published regarding leishmanial disease history and parasite biology. Also, to the best of our knowledge, there are very few comprehensive reviews covering all current aspects of leishmanial disease.17 So the focus of this article is to review comprehensively developments in antileishmanial agents and their progress during the past decade. In this review, we cover disease history and parasite biology, followed by a summary of currently available treatments, and, finally, review reports of novel small molecules (synthetic as well as natural) with antileishmanial activity. We also discuss all possible drug targets reported for anti-leishmanial agents.

2. Current antileishmanial therapy

As there is no vaccine currently available against leishmaniasis, drugs are the only available tool for treatment and control of both VL and CL.47 Severity of disease is dependent on the infecting Leishmania species and the associated host-immune response. Visceral disease (may result in PKDL) caused by visceral Leishmania species requires systemic treatment, whereas cutaneous disease (may further evolve into recidivans, diffuse or mucosal complication) caused by dermotropic Leishmania species is treated either systemically or locally (Fig. 2).17

The following details the different drugs used in current antileishmanial therapy.

2.1. Pentavalent antimonials

Prof. Brahmy-Chari from India was nominated for a Nobel Prize (in 1929) for the first effective drug against L. donovani, the urea stibamine (discovered by him in 1912). Although it saved the lives of many poor Indians, it had some side effects. Pentavalent antimonials were developed subsequently, which showed promise in reduction of these side effects.18 The antimonials were first introduced in 1945 and remained the drug standard for about six decades. The activation mechanism of pentavalent antimonials is still not clearly known, but it has been reported that pentavalent antimonite (Sb, v) is a prodrug requiring biological reduction to its trivalent form (Sb, III) for antileishmanial activity. However, site (amastigote or macrophage) and mechanism of reduction (enzymatic or non-enzymatic) remain controversial. Studies indicate that axenic amastigotes are susceptible to Sb(v) but that promastigotes are not, suggesting that some stage-specific reduction occurs during the life cycle, but the mechanism by which amastigotes reduce Sb(v) is not clear. Both glutathione and trypanothione can non-enzymatically reduce Sb(v) to Sb(III), particularly under acidic conditions. However, promastigotes contain higher intracellular concentration of trypanothione and glutathione than amastigotes, and both stages maintain intracellular pH values close to neutral, independent of external pH. Thus, it is difficult to account for the selective action of Sb(v) against amastigotes by a non-enzymatic mechanism.19 As both stages can take up Sb(III) and Sb(v), the insensitivity of promastigotes to Sb(v) cannot be attributed to drug exclusion. Two possible candidates for the enzymatic reduction of Sb(v) to Sb(III) in amastigotes, a thiol-dependent reductase related to glutathione-S-transferase highly expressed in amastigotes and a homologue of a glutaredoxin-dependent yeast arsenate reductase, have been identified recently. However, the level of expression of arsenate reductase has not been reported and the low specific activity of the recombinant enzyme with glutaredoxin raises questions as to the physiological nature of the electron donor in Leishmania species.13,20–23

These drugs are used with variable efficacy against both major types of leishmaniasis (VL and CL) and were recommended frontline treatment until observation of development of drug-resistance in the Indian state, Bihar.17 Pentavalent antimonials can be administered intramuscularly, intravenously, or even by an intralymphatic route.24 The recommended dose is 20 mg per kg of body weight for about 20–30 days, achieving more than 95% cure. In Bihar, however, 60% or more of patients did not respond, suggesting that there was development of resistance to the drugs by the parasite. However, they are still used as frontline drugs in areas in which resistance has not developed.25–27

Since the mid-1940s, in English-speaking East Africa, stibogluconate (I) has been used (manufactured by GlaxoWellcome, London, UK, under the brand name Pentostam T, PSM, containing sodium stibogluconate), whereas in the former French and Italian colonies of Africa, meglumine antimoniate (2) (prepared by Rhone-Poulenc-Rohrer, Paris, under the brand name Glucantime T) has been the only drug used for treatment of kala-azar (VL) and post-kala-azar dermal leishmaniasis (PKDL). Frezard et al. proposed the structures of stibogluconate (1) and meglumine antimoniate (2), identified by ESI(−)-MS in aqueous solutions.28 However, because of
increasing demand not being met by the manufacturer, the urgency of treatment, and the exceptionally high cost (approximately 200 US dollars per patient), Albert David Ltd. (Calcutta, India) began to manufacture generic sodium stibogluconate B.P. (SSG), which costs approximately 13 US dollars per patient. Since 1997, the International Dispensary Association (IDA, Amsterdam, Netherlands) also has supplied generic SSG.

Several limitations have decreased the use of antimonials. They routinely cause pancreatitis during treatment, with other side effects including pancytopenia, reversible peripheral neuropathy, elevations in serum aminotransferases, pain at the site of injection, stiff joints, gastrointestinal problems, hepatic and renal insufficiency (nephrotoxicity). Cardiotoxicity may occur and this may cause sudden death. In addition, the long duration of treatment can result in accumulation of drug in the tissues in the liver and spleen.\(^\text{30-33}\)

Parenteral administration, long-term treatment (up to 4 weeks), and variation in efficacy against VL and CL, along with development of resistance, are some of the factors that have led to decreased use of antimonials. Recently, Fernandes \textit{et al.} reported a novel oral delivery strategy for pentavalent antimonials in treatment of visceral leishmaniasis. This was based on formation of an amphiphilic antimony(\(V\)) complex on reaction of antimony(\(V\)) with nonionic surfactants from the \(N\)-alkyl-\(N\)-...
methylglucamide series. Improved oral bioavailability and pharmacokinetics of Sb in mice were achieved with this strategy, compared with use of meglumine antimoniate. The resulting amphiphilic complexes were found to be active by the oral route in a murine model of VL.\(^8\)

### 2.2. Amphotericin B

In the areas where resistance is commonly seen to antimonial antileishmanial treatment is, amphotericin B (3), a macrolide polyene antifungal antibiotic, is recommended as drug of choice.\(^ {34,35} \) In India, amphotericin B is recommended by the National Expert Committee.\(^ {36} \) It was discovered in 1956 in actinomycetes: Streptomyces nodosus, a bacterium collected from the soil of Orinoco River in Venezuela.\(^ {14} \) Amphotericin B has high affinity for 24-substituted sterol, ergosterol (a major component of leishmanial cell membrane), forming a complex with it and thus interfering with the ergosterol pathway, which ultimately results in formation of aqueous pores leading to increased membrane permeability to monovalent cations, anions, and small metabolites, causing cell death (leishmanicidal action).\(^ {35,37,38} \)

Currently used preparations of amphotericin B are amphotericin B desoxycholate (Fungizone®) and various liposomal formulations (e.g. AmBisome® involving unilamellar liposomes).\(^ {39} \) Lipid formulations (liposomal) of amphotericin B are prepared to improve its bioavailability and pharmacokinetic properties, which helps in masking amphotericin B from susceptible tissues, facilitating its preferential uptake by reticuloendothelial cells and thereby reducing side effects and increasing its efficacy.\(^ {40-45} \) Smaller liposomes reside in the bloodstream for longer duration, whereas larger lipid particles are rapidly engulfed by mononuclear phagocytes, for example hepatic macrophages (Kupffer cells, the site at which VL parasites such as L. donovani attack and accumulate, and VL develops). Thus, the mechanism of AmBisome is such that it accumulates rapidly in the liver and reaches its therapeutic concentration at a faster rate than antimonials, and with increased effect because of its longer half-life.\(^ {39,41,44,45} \)

AmBisome is registered and approved for treatment of VL in various countries such as USA and Europe, its use is recommended by a WHO working group. It can also be used for CL and complex forms of CL (such as MCL), as well as for PKDL.\(^ {34,46,4} \) Recently, after a single infusion therapy analysis on a particular group of patients in India, it was reported that AmBisome cures 95% of patients with minimal adverse effects.\(^ {48} \)

Despite having higher toxicity profiles and lesser efficacy than AmBisome, other formulations such as an amphotericin B colloidal dispersion (Amphocil\(^ {74} \)), an amphiphilic liposomal suspension (Amphotec®),\(^ \), and a multilamellar liposomal formulation, are also in use, but these are not as common.\(^ {49,50} \) Besides the main drawbacks of high cost, administration route, and lack of stability at high temperature (manufacturer guarantee 25 °C) which limits usefulness, liposomal amphotericin B has been proven to be an efficient drug with more than 95% efficacy over Amphocil and Abelcet.\(^ {8,51,52} \) It can be administered intravenously in therapeutic doses of about 0.75–1.0 mg per kg body weight as 15–20 infusions either daily for about 20–30 days or 1.5–2.0 g on alternate days, or as 5–20 mg kg\(^{-1}\) total dose in 4–10 doses over 10–20 days.\(^ {24,25,43} \)

Although minimal, amphotericin B does bind to the cholesterol present in human cell walls and thus exhibits some toxic effects. Infusion-related adverse effects such as “nephrotoxicity, fever with rigor and chills, bone pain, hypotension, anorexia, dyspnea, thrombophlebitis, cardiac arrest (rarely), myocarditis and delayed side effects such as hypokalemia” explain fluctuations in use of amphotericin B before the 1990s, when there were no available lipid formulations.\(^ {53-55} \) The use of amphotericin B also requires prolonged hospitalization and close monitoring.\(^ {41} \) Although no resistance has been reported as yet, there is evidence of development of resistance in laboratory Leishmania strains.\(^ {38} \)

### 2.3. Miltefosine

Miltefosine (4), chemically known as hexadecylphosphocholine, was originally developed as an antineoplastic agent used in topical treatment (Miltex) of skin metastases of breast cancer. Its use as an antileishmanial agent was initiated in the mid-1980s. It is the first orally administered drug effective in treatment of leishmaniasis, and the most recent antileishmanial drug to enter in the market. In the mid-to-late 1990s, collaborative development of miltefosine by Asta Medica (now Zentaris) and a WHO/TDR partnership showed that it has oral activity in VL patients, including antimonial-unresponsive patients. On administering a dose of 2.5 mg kg\(^{-1}\) of miltefosine daily for 28 days in phase III clinical trials, it was observed that there was about 94% cure in VL patients. The drug was then registered in 2002 and entered the Indian market. Thereafter, a phase IV study was performed. Two years later, the drug was approved in Germany, showing usefulness in treatment of immunocompromised patients. More recently, study of miltefosine in treatment of CL was carried out in regions of Colombia, where L. panamensis is a commonly infecting parasite. A 91% cure rate was observed with the same oral dose as described above. However, in regions of Guatemala (Central America) where L. braziliensis and L. mexicana are common, only 53% cure rates were observed, much lower than the cure rate of antimonials (more than 90%).\(^ {17,56} \) Therefore, considering these studies, miltefosine was registered in India, Germany, and Columbia (Impavid®).\(^ 8 \)
In a recent study, children suffering from cutaneous leishmaniasis were given miltefosine, with results being similar to those after administration of meglumine antimoniate, and the added advantage of an oral delivery route that is more easily tolerated by pediatric patients in comparison with other routes.\textsuperscript{37} The primary effect of miltefosine is uncertain, but possible mechanisms are action by blocking proliferation of \textit{Leishmania}, inhibition of phosphatidylinositol biosynthesis, alteration of phospholipid and sterol composition, activation of cellular immunity, or inhibition of signal transduction and calcium homeostasis.\textsuperscript{58,59} Also, it has been reported that the activity of miltefosine may be enhanced by intracellular accumulation, which is regulated by drug transporters. Researchers also have found that it causes apoptosis-like processes in \textit{L. donovani}, but the exact mechanism is not known.\textsuperscript{60,61} Miltefosine has been shown to stimulate production of inducible nitric oxide synthetase 2 (iNOS2), causing generation of nitric oxide (NO) which further helps to kill the parasite within the macrophage.\textsuperscript{62}

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In a phase IV clinical trial of miltefosine in India, preliminary results on treatment with weekly supervision suggested that there was doubling of its relapse rate.\textsuperscript{63} It has less severe toxicity but long terminal residence time (approx. 152 h), and can cause teratogenicity, therefore its use should be avoided during pregnancy. Other adverse reactions of this drug include gastrointestinal disturbances, hepatotoxicity, and renal toxicity, but these can be reversed. Resistance for the drug was found to be established in laboratory strains.\textsuperscript{24,64} Also, some studies suggest that a few \textit{Leishmania} species, such as \textit{L. braziliensis}, \textit{L. guyanensis}, and \textit{L. mexicana}, are insensitive to miltefosine.\textsuperscript{55} There is potential for use of combination therapy of miltefosine with either paromomycin or amphotericin B, and this may even prove useful in treatment of antimonial-resistant VL patients in India.\textsuperscript{66}

### 2.4. Paromomycin

Paromomycin (5), an aminoglycoside antibiotic, is a relatively new broad-spectrum antibiotic drug that has been used for treatment of leishmaniasis. It can be used for treatment of both types of leishmaniasis (VL as well as CL), although paromomycin is more effective for CL. Limited availability restricts its use in endemic regions.\textsuperscript{67,68} Paromomycin is available as an intramuscular injection for parenteral administration to treat systemic infections \textit{i.e.} VL, and as an ointment formulation to treat local skin infections \textit{i.e.} CL.\textsuperscript{69,70} It has been found to be effective in India, Kenya, and more recently in Tunisia, but was found to be less effective in Sudan and Colombia.\textsuperscript{71,72} It has also undergone phase IV clinical trials.\textsuperscript{73} A bacterial pathogen \textit{Streptomyces rimosus var. paromomycus} is the organism responsible for origination of paromomycin by a fermentation mechanism.\textsuperscript{74} Paromomycin is now an off-patent drug and is recognized as an Orphan Drug by the US FDA and EU EMEA.\textsuperscript{75} The drug is recommended by the WHO and was approved by the Indian government in August 2006 to treat VL-infected patients.\textsuperscript{76} It is inexpensive, but a dose of 16 mg kg\textsuperscript{-1} is required daily for about 21 days \textit{via} an intramuscular route.\textsuperscript{77}

Although paromomycin acts specifically to treat other diseases, the mechanism specific to \textit{Leishmania} requires further study and elucidation. However, it has been observed that in the cytoplasm as well as in the mitochondria of \textit{L. donovani}, following low Mg\textsuperscript{2+} concentration-induced dissociation, ribosomal subunit association was promoted by paromomycin, resulting in inhibition of subunit recycling and, ultimately, inhibition of leishmanial protein synthesis.\textsuperscript{78} Other mechanisms may include alteration of membrane fluidity and lipid metabolism.\textsuperscript{79} Paromomycin can induce a local conformational change in the A-site of 16s rRNA and also respiratory dysfunction in \textit{L. donovani} promastigotes.\textsuperscript{73,80} Some adverse reactions or side effects of paromomycin have been observed, including elevated hepatic transaminases, ototoxicity, pain at injection site, nausea, abdominal cramps, and diarrhea.\textsuperscript{76,81} Experimental evidence has shown that a laboratory strain of \textit{L. donovani} promastigotes developed resistance to paromomycin.\textsuperscript{79}

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### 2.5. Pentamidine

Pentamidine (6) is an aromatic diamidine. This drug is used in antimonial resistance cases. It was originally used in treatment of VL \textit{via} an intramuscular route, but because of increasing resistance and toxicity, its use was forfeited. It also has potential as a useful drug for maintenance treatment in immunocompromised hosts.\textsuperscript{82} Although its primary mechanism of action is unknown and yet to be explored, it is thought that the drug is accumulated in the parasite, with effects including binding to kinetoplast DNA. It also has been reported that the drug enters promastigotes through arginine and polyamine transporters and is accumulated in mitochondria, acting to inhibit mitochondrial topoisomerase II.\textsuperscript{1,83-86}
Treatment with pentamidine may cause myalgia, pain at the injection site, nausea, headache, and, less commonly, results in a metallic taste, a burning sensation, numbness and hypotension, irreversible insulin-dependent diabetes mellitus, and death. \textsuperscript{87} Increasing unresponsiveness in India, emergence of drug resistance, especially in HIV co-infections, and toxicity (reversible hypoglycemia and nephrotoxicity) are some other limitations to its usefulness. \textsuperscript{88}

3. Drawbacks of current antileishmanial therapy

Although the range of antileishmanial drugs has expanded somewhat, currently available antileishmanial drugs do not meet the increasing requirements of managing infection in different patient populations, and drug resistance and toxicity have been reported with all the drugs (Table 1). \textsuperscript{75,89}

4. Recent progress in antileishmanial agents

In the last 10 years, various cores and their derivatives have been reported to possess antileishmanial activity (Fig. 3).

4.1. Acridines

Acridine and its derivatives have been found to have ability for intercalation in DNA and to interfere with various metabolic processes of both eukaryotic and prokaryotic cells. In recent times, various acridine derivatives have been investigated for potential activity as antileishmanial agents. \textsuperscript{90,91} Giorgio et al. reported the synthesis and antileishmanial activity of 6-monosubstituted and 3,6-disubstituted acridines. Among the array of synthesized compounds, the most active compound, 3,6-disubstituted acridine (7), with benzoylamino groups at the third and sixth positions, demonstrated a strong affinity for both parasite forms. Its IC\textsubscript{50} values were found to be about 1.1 ± 0.2 μM for amastigotes and 4.3 ± 1.2 μM for promastigotes, while it exhibited a lower antiproliferative activity against human monocytes (IC\textsubscript{50} = 110.3 ± 15.2 μM) with a SI (selectivity/speciﬁcity index) value of 100.2, suggesting that acridine compounds could interact with protozoan and mammalian cells in different ways. \textsuperscript{92}

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<tr>
<th>Sr. no.</th>
<th>Current drugs</th>
<th>Toxicity</th>
<th>Resistance</th>
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<tbody>
<tr>
<td>1</td>
<td>Pentavalent antimonials</td>
<td>Pancreatitis, pancytopenia, reversible peripheral neuropathy, elevations in serumaminotransferases, pain at the site of injection, stiff joints, gastrointestinal problems, hepaticrenal insufficiency (nephrotoxicity), cardiotoxicity, accumulate inside the tissues, such as particularly in liver and spleen, etc.</td>
<td>Resistance developed in Bihar state, India</td>
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<tr>
<td>2</td>
<td>Amphotericin B</td>
<td>Nephrotoxicity, fever with rigor and chills, bone pain, hypotension, anorexia, dyspnoea, thrombophlebitis, rarely cardiac arrest, myocarditis, and delayed side effects such as hypokalemia, etc.</td>
<td>Resistance in laboratory strains</td>
</tr>
<tr>
<td>3</td>
<td>Miltefosine</td>
<td>Gastrointestinal disturbances, hepatotoxicity, renal toxicity, etc.</td>
<td>Resistance in laboratory strains, L. braziliensis, L. guyanensis, and L. mexicana are insensitive towards miltefosine</td>
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<tr>
<td>4</td>
<td>Paromomycin</td>
<td>Elevated hepatic transaminases, ototoxicity, pain at injection site, nausea, abdominal cramps, diarrhoea, etc.</td>
<td>Laboratory strain of L. donovani promastigote developed resistance</td>
</tr>
<tr>
<td>5</td>
<td>Pentamidine</td>
<td>Myalgia, pain at the injection site, nausea, headache, and less commonly results in a metallic taste, a burning sensation, numbness and hypotension, irreversible insulin-dependent diabetes mellitus, and death</td>
<td>Unresponsiveness in India, emergence of drug resistance especially in HIV co-infections</td>
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The same study group also reported synthesis of (1,3-benzothiazol-2-yl)amino-9-(10H)-acridinone derivatives as potent antileishmanial agents via a procedure based on the Ullman reaction. After evaluation for antileishmanial activity against *L. infantum*, compound (8) with 6-amino benzothiazole substitution (on the amino group of 1-(1,3-benzothiazol-2-yl)amino-9-(10H)-acridinone) revealed selective antileishmanial activity. It showed IC\textsubscript{50} values of 20.1 \( \mu \)M against promastigote form, 4.3 \( \mu \)M against amastigote form, and 223.1 \( \mu \)M against human monocytes (lesser antiproliferative action) with a promising SI (selectivity index) of 51.9. While explaining its SAR, they reported that a benzothiazole group on a parent ‘amino-9-(10H)-acridinone’ ring could enhance antileishmanial abilities and the presence of a 6-amino-benzothiazole group at the second position of the amino chain was essential for specific anti-amastigote properties.

Ralph *et al.* synthesized several 9-anilinoacridine derivatives as antileishmanial agents. The compounds possessing 1’-NH-alkyl substituents have shown more than 80% growth inhibition of (macrophage infected) *L. major* amastigotes at or below a concentration of 1 \( \mu \)M. These compounds were also evaluated for their activities against promastigote and amastigote forms of *L. major*, and also for their toxicities to human Jurkat leukemia cells. Among the synthesized compounds, compound (9) was found to be one of the most active against intracellular parasites (>80% killing at 1 \( \mu \)M concentration). It had strong antileishmanial activity and also was found to be the least toxic compound to human Jurkat cells (IC\textsubscript{50} = 17.0 \( \mu \)M). After evaluating the activity of these compounds, Ralph *et al.* suggested that it might be possible to modify existing anticancer drugs targeted at DNA topoisomerase II to improve their activities and specificities against other organisms, such as those causing leishmaniasis.

4.2. Chromenes and coumarins

The 2\( H \)-chromene-2-ones, oxygenated heterocycles, are also known as natural coumarins. Naturally available derivatives of coumarins have been found to be effective against the promastigote form of the *Leishmania* parasite within a range of 17–50 \( \mu \)g mL\(^{-1}\) for IC\textsubscript{50} determination. Therefore, coumarins have potential as antileishmanial agents. Dubey *et al.* synthesized a series of chromene-2-thione derivatives by molecular docking (into the active site of the trypanothione reductase (TryR) enzyme, which is required for redox balance of the parasite and the inhibition of which leads to parasite death) and evaluated their antileishmanial activity *in vitro* on promastigote, axenic amastigote, and intracellular amastigote forms of *L. donovani*. The derivatives showed high levels of antileishmanial activity together with minimal toxicity to human peripheral blood mononuclear cells compound (10) was one of the most active of the tested compounds, showing IC\textsubscript{50} values of 36, 97, and 22 \( \mu \)M against axenic amastigote, promastigote, and intracellular amastigote forms, respectively, with % cytotoxicity to the human peripheral blood mononuclear cells observed at IC\textsubscript{50} as 28.84 \( \mu \)M. *In silico* evaluation revealed that none of the synthesized ligands violated any criterion of the Lipinski rule of five, suggesting that these ligands have good potential for development as oral agents and as potentially active drug candidates.
Combes et al. reported synthesis of a series of 4-arylcoumarins by a Suzuki–Miyaura cross-coupling reaction. On performing evaluation against *L. donovani*, 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2H-chromene-2-one (11) exhibited potent activity against amastigote form ($IC_{50} = 1.1 \mu M$ $IC_{90}$ against human monocytes THP1: $>292 \mu M$) with a SI of 265, twice that of amphotericin B (SI = 140).  

4.3. Benzodiazepines

Mackay et al. prepared a series of synthetically amenable benzodiazepines and pyrrolobenzodiazepines structurally related to the paullone nucleus to probe for activity using a macrophage amastigote infection model. Paullones (12) can inhibit cyclin-dependent kinases, which completely inhibits growth of *L. mexicana* promastigotes *in vitro*.

Mackay et al. reported that the tricyclic pyrrolobenzodiazepine-2,5-diones were more effective antileishmanial agents than sodium stibogluconate at the concentrations tested, with no evidence of toxicity against the host macrophage cells. Also the activity was found to be independent of chirality in the tricyclic 2,5-diones. The authors concluded that 7-chloro substituted 1,4-benzodiazepine-2,5-dione (13) had an amastigote suppression efficacy comparable with that of the clinically used sodium stibogluconate, and was non-toxic in the test model. It demonstrated efficacy at a concentration of 11.5 μM, whereas sodium stibogluconate had a plasma concentration of 1.47–2.95 μM after clinical dosing.

4.4. Chalcones

Chalcones are open chain flavonoids with two aromatic rings linked by a carbonyl group and two α,β-unsaturated carbon atoms. Kharazmi et al. reported that licochalcone A (14), an oxygenated chalcone, inhibits *in vitro* growth of both *L. major* and *L. donovani* promastigote form. Their preliminary studies showed that it destroyed the ultrastructure of the parasite’s (promastigote form) mitochondria.

Furthermore, while expanding their studies on the function of parasitochondria, they noted that licochalcone A inhibited respiration of the parasite in a concentration-dependent manner, as illustrated by inhibition of O$_2$ consumption and CO$_2$ production by the parasites. Moreover, licochalcone A inhibited the activity of the parasite mitochondrial dehydrogenase. These findings demonstrate that licochalcone A alters the ultrastructure and function of the mitochondria of *Leishmania* parasites. Again, further investigation on the mechanism of action of chalcones, focusing on the parasite’s respiratory chain, showed that licochalcone A inhibited the activity of fumarate reductase (FRD) in a permeabilized *L. major* promastigote form as well as in the parasite mitochondria, and also inhibited solubilized FRD and purified FRD from *L. donovani*. This indicates that FRD, one of the enzymes of the parasite respiratory chain, might be the specific target for antiprotozoal chalcones. As FRD exists in the *Leishmania* parasite and does not exist in mammalian cells, it has potential as an excellent target for antiprotozoal drugs.

Foroumadi et al. prepared a series of novel chalconoids containing 6-chloro-2H-chromen-3-yl groups, and evaluated these against the promastigote form of *L. major* using MTT assay. All the compounds showed high antileishmanial activity.
in vitro at concentrations less than 3.0 µM. Cytotoxicity assessment against mouse peritoneal macrophage cells showed that the antileishmanial activity of these compounds was achieved at non-cytotoxic concentrations. The most potent compounds statistically were the compound containing a 2-chlorophenyl group (15) with IC\textsubscript{50} value 1.22 ± 0.31 µM, and the compound containing a 2-bromophenyl group (16) with IC\textsubscript{50} value 1.33 ± 0.52 µM.\textsuperscript{110}

Tadigopulla et al. reported antileishmanial activities of chalcone derivatives. Compounds (17) and (18), with IC\textsubscript{50} values of 2 and 2.5 µM, and CC\textsubscript{50} values of 325.4 µM (SI = 162.5) and 258.1 µM (SI = 103.2), respectively, were found to be most potent against L. donovani amastigote form. These compounds also showed almost 100% inhibition of promastigote form of the same species at a concentration of 25 µM. Introduction of alkylated amino substituents on rings A and B increased the activity profile significantly from that of the parent compound (IC\textsubscript{50} > 20 µM against amastigote form). Remarkably, these di-alkylated amino substituted analogues were also found to be more effective than standard drugs miltefosine (IC\textsubscript{50} = 8.40 µM) and SSG (IC\textsubscript{50} = 49.7 µM).\textsuperscript{111}

Vasconcellos et al. synthesized a new chalcone-like series using the Morita-Baylis-Hillman reaction, and reported that compound (20) bearing an o-nitro group had the most potent leishmanicidal activity, with IC\textsubscript{50} values of 7.65 and 10.14 µM on L. amazonensis and L. chagasi, respectively.\textsuperscript{111}

Nunes et al. reported the effects of a new set of sulfonamide 4-methoxychalcones against the promastigote form of L. braziliensis, with compound (21) presenting the best antileishmanial profile (IC\textsubscript{50} = 3.5 ± 0.6 µM).\textsuperscript{114} The same study group also reported the synthesis, antileishmanial activity, molecular modeling, and structure–activity relationship (SAR) evaluation of a series of novel chalcone derivatives based on a 1,3-diacetyl biphenyl nucleus without sulfonamide group. After evaluation, the most active compound (22) showed reduced toxicity level in a Vero cell assay (CC\textsubscript{50} = 216 µM) with low IC\textsubscript{50} (3.9 µM), and the best SI of 55.4 against the promastigote form of L. braziliensis. The position of the methoxy group in phenyl ring A (especially di-ortho-substituents) and the chlorine atom in phenyl ring B (compound 22) seem to be important for

Nielsen et al. reported synthesis and evaluation of a large number of substituted chalcones with antileishmanial activities against L. donovani promastigote. Among the synthesized compounds, the IC\textsubscript{50} value of the most potent chalcone (19) was 3.7 µM.\textsuperscript{112}
antileishmanial activity. This compound fulfilled the Lipinski rule of five, identifying it as a hit compound for further exploration with potential in design of new candidates for leishmaniasis treatment.\textsuperscript{115}

Shaﬁee \textit{et al.} prepared two regioisomeric chromene-based chalcones and investigated these for antileishmanial activity against the promastigote form of \textit{L. major}. The chloro-substituted 1-(6-methoxy-2\textsuperscript{H}-chromen-3-yl)-3-phenylpropen-1-one showed excellent activity at non-cytotoxic concentrations. The compound \textsuperscript{(23)} with chloro substitution at the para position of the phenyl ring was found to be most potent, having an IC\textsubscript{50} of 0.7 ± 0.3 \textmu M.\textsuperscript{116}

Gupta \textit{et al.} reported synthesis, structure–activity relationships, and biological studies of chromeno-chalcones as potential antileishmanial agents. From the compounds that exhibited better activity than the marketed drug miltefosine against the intracellular amastigote form of \textit{L. donovani}, a potent compound \textsuperscript{(24)} showed IC\textsubscript{50} = 0.78 and 5.4 \textmu M against promastigote and amastigote forms, respectively, with a CC\textsubscript{50} value of 40.07 \textmu M on mammalian kidney fibroblast cells (Vero cell lines) and SI value of 7.5 compared with miltefosine (IC\textsubscript{50} = 8.4 \textmu M, CC\textsubscript{50} = 52.5 \textmu M with SI of 6.2). Oral administration of compound \textsuperscript{(24)} in a hamster model, at a concentration of 100 mg per kg of body weight per day for 5 consecutive days, resulted in >84\% parasite inhibition at day 7 post treatment and activity was retained until day 28. Molecular and immunological studies revealed that compound \textsuperscript{(24)} has a dual nature to act as a direct parasite killing agent and as a host immune stimulant. Pharmacokinetics and serum albumin binding studies suggested that compound \textsuperscript{(24)} has potential as a candidate for treatment of the non-healing form of leishmaniasis.\textsuperscript{117}

Vishwakarma \textit{et al.} developed an efficient protocol for synthesis of 3,3\textsuperscript{-}diindolylmethane. They observed that all synthesized 3,3\textsuperscript{-}diindolylmethanes had promising antileishmanial activity against \textit{L. donovani} promastigotes as well as axenic amastigotes. Of the synthesized compounds, the nitroaryl substituted diindolylmethanes showed potent antileishmanial activity, with the most potent being compound \textsuperscript{(26)}. Compound \textsuperscript{(26)}, which contains a 4-nitrophenyl moiety linked to 3,3\textsuperscript{-}diindolylmethane, showed IC\textsubscript{50} values of 7.88 and 8.37 \textmu M against \textit{L. donovani} promastigote and amastigote forms, respectively.\textsuperscript{119}

Sarragiotto \textit{et al.} synthesized and evaluated a series of 1-phenylsubstituted \textit{\beta}-carbolines containing an N-butylcarboxamide group at C-3 of the \textit{\beta}-carboline nucleus. After \textit{in vitro} evaluation of this series of compounds against the promastigote by intraperitoneal route, at day 7 post treatment when tested in a hamster model.\textsuperscript{118}
form of *L. amazonensis*, compound (27) with 4-methoxy phenyl as a substituent was found to be most active drug candidate with an IC$_{50}$ of 0.25 ± 0.07 μM, and to have the lowest cytotoxicity to macrophages (CC$_{50}$ = 521.0 ± 6.36 μM). The selectivity index ratio (SI) was 2084.$^{120}$

Singh et al. reported synthesis of *N*(1-methyl-1H-indol-3-yl)methylenamines and 3,3-diaryl-4-(1-methyl-1H-indol-3-yl)azetidin-2-ones as potential antileishmanial agents. After screening for antileishmanial activity against *L. major*, the most potent compound (28) was shown to have an IC$_{50}$ value of 0.122 μM, similar to that of the standard drug amphotericin B (IC$_{50}$ = 0.06 μM).$^{121}$

![Figure 28](image)

Chauhan et al. synthesized a series of indolyl glyoxylamides and evaluated their *in vitro* activity against the amastigote form of *L. donovani*. Compound (29) with a para-ethylphenyl ring on tetrahydro-β-carboline was identified as the most active analog of the series, with IC$_{50}$ and CC$_{50}$ values of 5.17 μM and 162.76 μM, respectively (SI = 31.48). This lead molecule was also found to be 12- and 5-fold more selective than the standard drugs pentamidine (IC$_{50}$ = 20.43 μM, SI = 2.58) and sodium stibogluconate (IC$_{50}$ = 71.90 μM, SI = 5.53), respectively.$^{122}$

![Figure 29](image)

4.6. Furan

A systematic lead discovery program was employed and evaluated for *in vitro* and *in vivo* antileishmanial activities, mutagenicities, and toxicities of two novel AIAs (arylimidamides/bis-arylimidamides), DB745 and DB766 (30a). In intracellular *Leishmania* assays, compound (30a), which has unsymmetrical substitutions on the diphenylfuran linker, was found to be substantially more potent than miltefosine, paromomycin, and pentamidine, and similar in potency to amphotericin B (Table 2). After assessment for activity using J774 macrophages infected with clinical isolates of antimony-resistant *L. donovani*, IC$_{50}$ values were found to range from 0.064 to 0.090 μM. An Ames screening assay was performed and it was found that compound (30a) did not exhibit any mutagenicity. Furthermore, *in vivo* analysis showed that compound (30a) when given orally, produced dose-dependent inhibition of liver parasitemia in two efficacy models, *L. donovani*-infected mice.
and hamsters. Most notably, compound (30a) (100 mg per kg of body weight per day for 5 days) reduced liver parasitemia in mice and hamsters by 71% and 89%, respectively. Werbovetz et al. have synthesized analogs of this antileishmanial lead (30a), along with an additional compound containing isopropoxy groups to the central furan. After in vitro evaluation of all the prepared compounds against intracellular amastigote forms of L. donovani and L. amazonensis, the target compounds displayed IC$_{50}$ values in the nanomolar range with selectivity indices >100 compared with J774 macrophages. Compound (30b) bearing a meta-isopropoxy group was found to be the most potent (Table 2), with an IC$_{50}$ of 11 000 nM against J774 macrophages and an approximate SI value of 2075. This compound was well tolerated by mice and showed activity in a murine model of visceral leishmaniasis; however, the unsymmetrical analogues were found to be toxic in nature.\(^{133}\)

A series of amidoxime derivatives was synthesized by Vanelle et al. Among the tested compounds, compound (31), a mono-amidoxime derivative with the lowest IC$_{50}$ of 8.3 μM, showed better antileishmanial activity than that of pentamidine (IC$_{50}$ = 11.2 μM), and had the highest SI of 6.6 (SI of pentamidine = 2.8) against L. donovani promastigote form. It was suggested that a single amidoxime group appears to be sufficient for antileishmanial activity.\(^{134}\)

### 4.8. Thienopyridine

We recently reported synthesis, antileishmanial activity, and docking study of N'-substituted benzylidene-2-(6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl)acetohydrazides. The synthesized series was evaluated for antileishmanial activity against L. donovani promastigotes. Among all tested compounds, 4-N,N-dimethylamino substituted phenyl ring compound (32) was found to be the most promising, with an IC$_{50}$ value of 27.41 μM when compared with sodium stibogluconate (IC$_{50}$ = 537.92 μM) as standard.\(^{125}\)

### Table 2  Intracellular Leishmania assays: comparison of DB766 (30a) and compound (30b) with miltefosine, paromomycin, pentamidine, and amphotericin B

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Compounds</th>
<th>L. donovani axenic amastigotes [IC$_{50}$ in μM]</th>
<th>L. donovani (LV82) intracellular amastigotes [IC$_{50}$ in μM]</th>
<th>L. amazonensis intracellular amastigotes [IC$_{50}$ in μM]</th>
<th>L. major intracellular amastigotes [IC$_{50}$ in μM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DB766 (30a)</td>
<td>0.50 ± 0.10</td>
<td>0.036 ± 0.005</td>
<td>0.087 ± 0.015</td>
<td>0.014 ± 0.004</td>
</tr>
<tr>
<td>2</td>
<td>Compound (30b)</td>
<td>5.3 ± 1.2</td>
<td>—</td>
<td>93 ± 28</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>Miltefosine</td>
<td>8.5 ± 1.2</td>
<td>2.7 ± 0.3</td>
<td>15 ± 3</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>4</td>
<td>Pentamidine</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>19 ± 3</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>5</td>
<td>Amphotericin B</td>
<td>0.098 ± 0.013</td>
<td>0.066 ± 0.012</td>
<td>0.14 ± 0.01</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

\(\text{--}\) indicates not done.
4.9. Quinoline

Quinoline based chemotherapeutic agents have attracted considerable interest as antileishmanial agents. The Walter Reed Army Institute (USA) discovered an 8-aminoquinoline (primaquine) analogue WR6026 (sitamaquine) (34). This was in development with GlaxoSmithKline (UK) for oral treatment against VL (caused by L. chagasi). Studies revealed that it cured 50% of patients with kala-azar in Kenya at a dose of 1 mg per kg per day for 28 days. After phase II clinical trials, WR6026 demonstrated the unusual clinical features of lack of increased efficacy against Brazilian kala-azar with increased dosing above 2 mg per kg per day and toxicity that was not present in previous investigations.

Quinolines also have been found to inhibit leishmanial GDP-mannose-pyrophosphorylase, an enzyme system producing a range of mannose-rich glycoconjugates that are essential for parasite survival and its virulence. Chauhan et al. synthesized a novel series of 1,2,4-triazino[5,6b]indole-3-thiones covalently linked to 7-chloro-4-aminoquinoline. After evaluation for their in vitro activity, compound (33) was found to be the most potent, with an IC\textsubscript{50} value of 0.36 \( \mu \text{M} \) and CC\textsubscript{50} > 400 \( \mu \text{M} \) in Vero cell lines (SI of >1111) against amastigote form of L. donovani, which is several times more potent than the standard drugs, miltefosine (IC\textsubscript{50} = 8.10 \( \mu \text{M} \), SI = 7) and sodium stibogluconate (IC\textsubscript{50} = 54.60 \( \mu \text{M} \), SI = 7).

Cryptolepine (5-methyl-10H-indolo[3,2-b]quinoline) is an indoloquinoline alkaloid isolated from a medicinal plant Cryptolepis sanguinolenta. The antileishmanial properties of synthetic derivatives of cryptolepine against L. donovani parasites were evaluated for the first time by Hazara et al. From the series, compound (36), a 2,7-dibromocryptolepine, was the only drug to exhibit selective toxicity against the promastigote form of a classical L. donovani strain (AG83), with an IC\textsubscript{50} value of 0.5 \( \pm \) 0.1 \( \mu \text{M} \) and IC\textsubscript{50} against mouse peritoneal macrophage cells of 9.0 \( \pm \) 1.2 \( \mu \text{M} \) (SI ~ 18) in comparison with cryptolepine (IC\textsubscript{50} = 1.1 \( \pm \) 0.3 \( \mu \text{M} \); SI ~ 0.7). Furthermore, compound (36) was found to inhibit substantially the intracellular amastigote forms of two clinical isolates, one of them being a Sb\textsuperscript{V}-resistant strain of L. donovani. Compound (36) was reported to be a prospective “lead” to novel antileishmanial therapy, supported by studies on the mechanism of cytotoxicity induced by (36) in L. donovani promastigotes (AG83). This revealed a mode of cell death in L. donovani promastigotes characterized by disruption of mitochondrial membrane integrity in terms of depolarization of membrane potential, and degradation of chromosomal DNA into oligonucleosomal fragments—the characteristic event of apoptosis.

Bertinaria et al. synthesized new amodiaquine derivatives bearing modified lateral basic chains, and the compounds were tested in vitro against L. donovani (MHOM/ET/67/HU3). The authors were the first to report the antileishmanial action of amodiaquine (37) and some newly synthesized analogs against the intracellular amastigote form of L. donovani. Amodiaquine showed potent activity against leishmaniasis, with an IC\textsubscript{50} value of 1.4 \( \mu \text{M} \), but an IC\textsubscript{50} value of 90 \( \mu \text{M} \) on KB cells, therefore it was found to be non-cytotoxic in nature. Derivatives of amodiaquine showed good antileishmanial activity, but were found to be cytotoxic and to have a narrow therapeutic index.

Azas et al. prepared a series of 2-substituted nitroquinolines and evaluated these for in vitro antileishmanial properties. From the series, they identified 2-hydroxy-8-nitroquinoline (38)
as a hit molecule displaying IC$_{50}$ values of 6.6, 6.5, and 7.6 $\mu$M against *L. donovani* promastigote form, amastigote form, and *L. infantum* promastigote form, respectively. Compound (38) was also found to possess low cytotoxicity (CC$_{50}$) on human HepG2 cell lines and murine J774 cell lines, 126.3 $\mu$M and 105 $\mu$M, respectively. To explain the SAR, the authors reported that the presence of a hydroxyl group at position 2 (involved in a prototropic tautomeric equilibrium between quinoline-2-ol and its [1H]-quinolin-2-one counterpart) and a nitro group at position 8 of the quinoline ring result in reasonable activity against both promastigote (*L. donovani* and *L. infantum*) and amastigote (*L. donovani*) parasite stages.\footnote{134}

\[ \text{(38)} \]

Silva *et al.* reported synthesis of 4-amino-7-chlороquinoline derivatives and evaluated these for antileishmanial activity against promastigotes of different *Leishmania* species. Among the tested compounds, compound (39) containing an amino group was found to be almost 50 times (IC$_{50} = 0.01\ \mu$M) more active than the reference drug amphotericin B (0.004 $\mu$M) against *L. chagasi*. To predict the SAR, from the obtained results they stated that the presence of the amino group is essential for good activity of such compounds against *Leishmania*, as addition of alkyl groups, either mono or di-alkyne substituents, results in loss of antileishmanial activity.\footnote{135}

\[ \text{(39)} \]

Loiseau *et al.* evaluated a 2-substituted series of quinolines, styrylquinolines, and 7-arylstyrylquinolines for in vitro antileishmanial activities and cytotoxicities. Among the quinolines and the styrylquinoline derivatives, the most interesting compound was compound (40), with an IC$_{50}$ of 4.1 $\mu$M for *L. donovani* intramacrophage amastigotes and a SI of 8.3, whereas from the 7-arylstyrylquinolines, compound (41) exhibited an IC$_{50}$ of 1.2 $\mu$M and a SI of 121.5, which is 10-fold and 8-fold more active than miltefosine (IC$_{50} = 13.4\ \mu$M; SI = 0.2) and sitamaquine (IC$_{50} = 9.7\ \mu$M; SI = 2) with SI values 607-fold and 60-fold higher, respectively. The authors concluded that because of its high in vitro antileishmanial activity and low toxicity, compound (41) is the most interesting compound to emerge from more than 150 derivatives of 2-substituted quinolines that were synthesized and evaluated. Compound (41) has now been selected as a candidate for evaluation in vivo with *L. donovani* mouse or hamster models via the Drugs for Neglected Diseases initiative (DNDi) pipeline.\footnote{136}

\[ \text{(40)} \]

Gupta *et al.*, researchers from Advinus Therapeutics Ltd., Bangalore, India, Division of Parasitology, CSIR-Central Drug Research Institute, Lucknow India, and Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland, together reported that substituted quinoline, compound (42), was found to have in vitro activity (IC$_{50} = 0.22 \pm 0.06\ \mu$M) against *L. donovani* amastigotes. Its SI was 187.5. The compound was found to have good in vivo efficacy (84.26 $\pm$ 4.44\% inhibition) and also promising ADME properties.\footnote{137} The same group, in
expansion of their study, reported synthesis of analogs of (42) and their subsequent characterization for in vitro activity against the intracellular form of \textit{L. donovani}. The resulting quinolines were found to have similar efficacy against the parasite to that of (42). From these tested compounds, compound (43) was found to be the most active, with an IC$_{50}$ value of 0.17 μM.$^{138}$

4.10. Quinazoline

Sahu et al. synthesized a series of a new class of \{hetero\}aryl-2-piperazino quinazolines and assessed these for in vitro activity against extracellular promastigotes and intracellular amastigotes of \textit{L. donovani}. Among the compounds evaluated, compound (44) (a 4-methyl piperazinyl and 2,4,6-trimethoxyphenyl substituted quinoline derivative) showed the lowest toxicity, having a CC$_{50}$ value above 25.38 μM. The authors reported its SI value above 8.03, which is comparable with that of sodium stibogluconate and pentamidine (SI = 6.38 and 2.07, respectively). It was also observed that compound (44) exhibited higher anti-amastigote activity against \textit{L. donovani} with an IC$_{50}$ value of 3.16 μM when compared with standard drugs sodium stibogluconate (IC$_{50}$ = 7.92 μM) and pentamidine (IC$_{50}$ = 3.56 μM). 2,3,5-Trimethoxy benzene together with an N-methyl group (44) enhanced the antileishmanial activity remarkably, and thus represent an interesting lead as antileishmanial agents.$^{139}$

After synthesizing a series of novel substituted quinazoline derivatives and evaluating these for antileishmanial activity, Agarwal et al. reported that all the compounds exhibited higher activities against \textit{L. donovani} compared with reference drugs sodium stibogluconate and pentamidine. The most active compound (45), having a 3,4,5,6-tetrahydrobenzoquinazoline ring with a pyridyl piperazinyl group and 4-chlorophenyl ring substituted at the second and fourth positions, respectively, showed 99.9 ± 0.07% inhibition against \textit{L. donovani} promastigote at a concentration of 2.19 μM, whereas its antileishmanial activity in vitro (IC$_{50}$) against a luciferase-amastigote system of \textit{L. donovani} was found to be at its lowest at 0.58 μM when compared with the reference drugs.$^{140}$

Bhattacharjee et al. reported that when tested against \textit{L. donovani} amastigotes, several tryptanthrin (indolo[2,1-b]quinazoline-6,12-dione) derivatives exhibited remarkable in vitro activity at concentrations below 0.0004 μM. The parent compound can be produced by \textit{Candida lipolytica} (when grown in media containing an excess of tryptophan, hence the name tryptanthrin). When tested for toxicity against murine J774 macrophages and rat neuronal NG-108-15 cells, the best selectivity was obtained with compound (47), which was found to be 69-fold more toxic to the parasites than to both mammalian cell lines (toxic at 0.00088 μM). Compound (47) was one of the most active, having an IC$_{50}$ value of 0.000013 μM when compared with amphotericin B (IC$_{50}$ = 0.0045 μM).$^{141}$ After performing 3D-QSAR analysis, the researchers concluded that the presence of a five-membered carbonyl moiety in the molecule appears to be a structural requirement for potent activity. Stereoelectronic factors of the substituents at the third position of the D ring in

Berman et al. (Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, D.C.) assessed 2,4-diaminoquinazoline analogs of folate against \textit{L. major} in human macrophages. After in vitro testing of these analogs, compound (46) containing a side chain with an aromatic tertiary amine, was found to be the most active, possessing an ED$_{50}$ value of 0.00365 μM with a 50% macrophage toxic dose of 1.52 μM, which indicates an in vitro therapeutic index of approximately 10$^5$. It was reported that the activity of compound (46) depends on the aromatic tertiary amine being attached directly to the benzyl group of the quinazolines nucleus. Restricted amine (even if tertiary, aromatic, or bound directly to the ring) was found to be without activity. In their conclusion, they stated that “The remarkable activity of 2,4-diaminoquinazolines in vitro suggests that these or other folate analogs have strong potential to be investigated as novel antileishmanial agents”.$^{142}$
indolo[2,1-b]quinazoline-6,12-dione skeleton appear to have a significant effect on potent activity. The carbonyl groups of the five- and six-membered rings in the tryptanthrin moiety, and electron transfer ability from a receptor are likely to be crucial in the mechanism of action of the compounds.\textsuperscript{143}

Manetsch \textit{et al.} recently reported the antileishmanial activity of a series of N\textsuperscript{2},N\textsuperscript{4}-disubstituted quinazoline-2,4-diamines, which were tested \textit{in vitro} against intracellular amastigotes of \textit{L. donovani} and \textit{L. amazonensis}. Compound (48), a benzyl-substituted quinazoline, was the most potent compound (50% effective dose, EC\textsubscript{50} = 0.15 ± 0.02 µM and 0.90 ± 0.27 µM against \textit{L. donovani} and \textit{L. amazonensis}, respectively), but it failed to exhibit the same actions in an \textit{in vivo} murine visceral leishmaniasis model. Quinazoline (49) (EC\textsubscript{50} of 0.83 ± 0.32 µM against \textit{L. donovani} and 4.1 ± 1.2 µM against \textit{L. amazonensis}) with EC\textsubscript{50} value of >33 against J774A.1 cell line and SI value of >40, was found to reduce parasitemia by 37% when given at 15 mg per kg per day via the intraperitoneal route for 5 consecutive days. Pharmacokinetic studies of compound (49) revealed a maximum plasma concentration that was threefold higher than the EC\textsubscript{50}, and it has a terminal half-life of 5 hours after i.p. administration. Although a clear correlation among \textit{in vitro} activity, \textit{in vitro} physicochemical properties, and \textit{in vivo} activity was not clearly observed, the potencies of front runner compounds such as (48) and (49) in conjunction with favorable physicochemical properties make N\textsuperscript{2},N\textsuperscript{4}-disubstituted quinazoline-2,4-diamines a suitable platform for future development of antileishmanial agents.\textsuperscript{144}

Chauhan \textit{et al.} discovered four novel series of quinazolinone hybrids \textit{via} introducing heterocyclic systems with different possible functionalities based on the concept of molecular hybridization: (i) among the substituted quinazolinone-triazines, compound (50), having IC\textsubscript{50} of 7.05 ± 2.3 and 3.95 ± 0.8 µM against promastigote and amastigote forms of \textit{L. donovani}, respectively (CC\textsubscript{50} as >400 µM against both J774A.1 and Vero cell lines), was the most potent compound with SI of about 101.26; (ii) from the quinazolinone–peptide hybrids, compound (51) demonstrated potent activity, possessing IC\textsubscript{50} of 0.73 ± 0.2 µM against amastigote form of \textit{L. donovani} (CC\textsubscript{50} as >400 µM against both J774A.1 and Vero cell lines) with the best SI value of >547.94. Both compounds were found to have higher potency compared with reference drug miltefosine (IC\textsubscript{50} = 8.4 ± 2.1 µM and SI = 1.48). When administered \textit{in vivo} in a hamster model, % inhibition of \textit{L. donovani} parasite was found to be 73.15 ± 12.69 for compound (50) and 51.42 ± 15.67 for compound (51). Furthermore, it was reported that activation of T helper type 1 (Th1 type) and suppression of T helper type 2 (Th2 type) immune responses and induction in nitric oxide (NO) generation proved that compound (50) induces murine macrophages to prevent survival of parasites.\textsuperscript{145}
4.11. Quinone
Costa et al. synthesized pterocarpanquinones, aza-pterocarpanquinone derivatives. Compound (52) showed the best activity against amastigote form of *L. amazonensis*. It was found to have IC<sub>50</sub> values of 1.27 and 1.25 μM against promastigote and amastigote forms, respectively, with a SI (M J774 cell lines/IC<sub>50</sub> amastigote) of 14.4.  

4.12. Thiophene
Robinson et al. reported potent antileishmanial activity of thiophene derivatives against *L. infantum* LV9. More than a decade later, Ram et al. reported synthesis of thiophenes and thienc[3,2-c]pyran-4-ones. On evaluating all the synthesized compounds in vitro against *L. donovani* promastigotes, the researchers noted that compounds (53) and (54) were the most potent, displaying 100% growth inhibition against promastigotes at a concentration of 25 μM. After performing SAR analysis, they explained that most of the highly active compounds possessed a –COOEt group except for compound (53) which has a –COOH substituent at the second position. The high order of activity may be a result of increased lipophilicity as ester groups are present. The nature of the aryl substituent also potentiates the antileishmanial activity.

4.13. Triazine
Chauhan et al. synthesized compounds of triazine dimers. They reported that most of the synthesized derivatives exhibited better activity against intracellular amastigotes (IC<sub>50</sub> ranging from 0.77 to 10.32 μM) compared with standard pentamidine (IC<sub>50</sub> = 13.68 μM), and the derivatives also were found to be non-toxic to Vero cells. Compound (56) with IC<sub>50</sub> of 1.99 ± 0.31 μM against *L. donovani* intracellular amastigote form and CC<sub>50</sub> of 216.08 ± 5.89 μM on Vero cells, possessing a SI value of 108.58, showed 74.41% inhibition in vivo in a *L. donovani* hamster model. Investigations of the immunostimulatory properties clearly indicated that compound (56) treated cells in *Leishmania* infected mouse macrophages (J-774A.1) had induced Th1 (T helper type 1) type immune responses by (i) remarkable production of interleukin (IL)-12, tumor necrosis factor (TNF)-α, and nitric oxide (NO), and (ii) effective suppression of Th2 (T helper type 2) type cytokines, IL-10, and transforming growth factor (TGF)-β. Furthermore, molecular docking studies of compound (56) revealed that it shared the same binding residues as shared by pentamidine. The docking studies also indicated that compound (56) showed H-bonding and pi-stacking with Tyr191 residue, whereas of the interaction of pentamidine with Tyr191 was limited to pi-stacking only.

Chauhan et al. synthesized a series of 2,4,6-trisubstituted pyrimidines and triazines, and screened these for in vitro antileishmanial activity in a promastigote model of *L. donovani*. A 2,4,6-trisubstituted triazine compound (57) having piperidine substituted at the fourth and sixth positions showed inhibition of 98%, 94%, 78%, and 73% against the promastigote form of...
L. donovani at concentrations of 2.14, 1.07, 0.43, and 0.21 μM, respectively.\textsuperscript{151}

Berman et al. noted that formycin B (59), formycin A (60), formycin B and A monophosphate (61 and 62), and formycin A triphosphate (63) all had 50% effective doses of 0.02 to 0.04 μM and eliminated 90% of organisms at ≤0.5 μM, and therefore were the most active agents with favorable therapeutic-toxic ratios when tested \textit{in vitro} against \textit{L. tropica} infected human macrophages. They reported that the activity of 3-deazaguanosine (64) (EC\textsubscript{50} = 3.6 μM) in the same model suggested that guanosine derivatives may have potential as antileishmanial agents. They concluded that the apparent mechanism of action of formycin B is that it gets metabolized to formycin B monophosphate, formycin A monophosphate, then formycin A triphosphate by the organisms, which then incorporate the triphosphate form into RNA.\textsuperscript{153-155}
4.15. **Pyrimidine**

Perez-Perez *et al.* synthesized two series of 5′-triphenylmethyl-(trityl)-substituted thymidine derivatives and tested them against *L. infantum* axenic promastigotes and amastigotes. Compound (65), having dipeptides coupled at the third position showed good leishmanicidal activity against intracellular parasites, similar to that observed for the control drug edelfosine (87% decrease in the number of infected macrophages), with an estimated IC~50~ value of 8.0 ± 0.15 μM. After performing an assay of this compound, they concluded that mitochondrial nuclease LiEndoG (*L. infantum* endonuclease G) was a target for the action of this family of compounds.156

In early 2000, it was reported that methionine aminopeptidase 2 (MetAP2) inhibitors such as fumagillin and TNP-470, arrest parasite growth in *L. donovani* parasites.157 As a part of a collaboration between Pfizer and WHO-TDR to discover new hits and leads to treat neglected tropical diseases,158 Chen *et al.* reported synthesis and SAR study of the 2-(2-pyridinyl)-pyrimidine scaffold as an antileishmanial agent.159 Whitlock *et al.*, researchers from Pfizer Global R&D, Swiss Tropical Institute, and University of Cape Town, reported that analogs (66) and (67) had the best combination of c log P (2.7 and 2.9, respectively) and *L. donovani* activity (IC~50~ values as 1.1 and 0.53 μM, whereas cytotoxic IC~50~ of 115 and 80 μM, respectively), therefore these could form the basis for a hit-to-lead program to identify additional compounds with increased *L. donovani* potency.159

Chauhan *et al.* synthesized a series of dihydropyrido[2,3-]*d* pyrimidines and screened them for in vitro antileishmanial activity in *L. donovani* promastigote and amastigote models. At a concentration of 13.62 μM, compound (68), with substitution of the phenyl ring with an isopropyl group at the para position of dihydropyrido[2,3-]*d*pyrimidine, exhibited 100% activity in both forms of the parasite. At a concentration of 2.72 μM, it also showed 84.2% and 94.2% inhibition of promastigote and amastigote forms of *L. donovani*, respectively.161

Suryawanshi *et al.* reported a series of substituted aryl pyrimidine derivatives evaluated in vitro for their anti-leishmanial potential against intracellular amastigote form of *L. donovani* using reporter gene luciferase assay. Among the 4-S-
substituted pyrimidine derivatives and 4-N-substituted pyrimidine derivatives, they found that compound (69), a 4-S-substituted pyrimidine derivative having benzoxyl aryl substitution, was the most promising, with IC\textsubscript{50} and CC\textsubscript{50} (on Vero cell line) values of 2.0 ± 0.1 and 375.9 ± 5.1 μM, respectively, whereas its SI was found to be 188. On the basis of the SI, some compounds from both series were further evaluated for \textit{in vivo} antileishmanial activity using a \textit{L. donovani} hamster model. Again, compound (69) when administered intraperitoneally, had shown significant inhibition of parasitic multiplication (88.4%) at a daily dose of 50 mg kg\textsuperscript{-1} × 5 days. Therefore, the respective researchers concluded that compound (69) was the most promising, and may provide a new lead as an antileishmanial agent.\textsuperscript{162}

4.16. \textbf{Hydrazone/Schiff base}

Taha \textit{et al.} synthesized a library of Schiff bases of 2-methoxybenzoyl hydrazide. After evaluation of the compounds for \textit{in vitro} antileishmanial activity, they found that compound (70), having 2-hydroxy-4-methoxy substituents on ring A, showed excellent activity (IC\textsubscript{50} = 1.95 ± 0.04 μM). They suggested that 2-hydroxy substitution on ring A along with a methoxy group is vital for antileishmanial activity of this type of compound.\textsuperscript{163}

Yasinzai \textit{et al.} reported that synthesized azomethines inhibited parasite growth and most showed highly potent action towards \textit{L. major} promastigotes. Of these, the most potent compound (72) had an IC\textsubscript{50} of 0.23 μM.\textsuperscript{165}

4.17. \textbf{Steroids}

Silva \textit{et al.} reported synthesis of steroids and \textit{in vitro} antileishmanial screening against promastigote forms of \textit{L. amazonensis}, \textit{L. braziliensis}, and \textit{L. major}. Compound (73), a 6-thiopurine/steroid conjugate was found to be active with IC\textsubscript{50} values of 22.8, 13.9, and 17.3 μM for \textit{L. amazonensis}, \textit{L. braziliensis}, and \textit{L. major}, respectively. The compound showed no toxicity on mouse peritoneal macrophages at the maximum concentration tested (100 μM).\textsuperscript{166}

Rando \textit{et al.} synthesized a series of nitro derivatives and screened these against \textit{L. donovani} promastigote forms. They reported that nitrothiophene analogs were more potent than nitrofuran ones. Among the nitrothiophene analogs, compound (71) containing chloro substitutions at the \textit{meta} and \textit{para} positions of the phenyl ring, showed an IC\textsubscript{50} value of 0.41 μM (with IC\textsubscript{90} as 0.87 μM), which was lower than that of standard drugs pentamidine (IC\textsubscript{50} = 1.06 μM) and amphotericin B (IC\textsubscript{50} = 1.19 μM), and thus compound (71) was identified as the most potent in the series. They also mentioned that substitution was important for the activity. Further, they explained that the potency of nitrothiophene analogs was attributed to the ability of sulfur atoms to accommodate electrons from nitro groups, facilitating reduction and therefore formation of free radicals that are lethal to the parasites.\textsuperscript{164}
4.18. Azoles

4.18.1. Imidazole. Marchand et al. reported synthesis and biological evaluation of 2,3-diarylimidazo[1,2-a]pyridines as antileishmanial agents. They found that compound (74) exhibited very good antileishmanial activity and therapeutic index against amastigote and promastigote forms of *L. major*, with 95% inhibition at 10 μM concentration for amastigote form, whereas the IC₅₀ value for anti-promastigote form was found to be 7.0 ± 1.0 μM. It was also observed that compound (74) had low cytotoxicity against the human HeLa cell line (38 ± 7 μM), and also a good selectivity index (SI = 5.43). ¹⁶⁷

In a study on synthesis and evaluation of new furanyl and thiophenyl coupled imidazoles as antileishmanial agents, Bhandari et al. synthesized a series of benzyloxy furanyl and benzyloxy thiophenyl imidazoles and performed screening for their *in vitro* antileishmanial activity against both forms of *L. donovani*. Among the tested compounds that were found to be several times less toxic (against J774A.1 cell line) than reference drugs miltefosine (CC₅₀ = 3.23 μM) and miconazole (CC₅₀ = 9.93 μM), compound (75), a 3-chlorobenzyloxy furanyl imidazole, emerged as the most active, with an IC₅₀ value of 3.04 μM (CC₅₀ being 60.21 μM) and SI of 19.80, which was a better SI than those of miltefosine (SI = 0.24) and miconazole (SI = 1.66). ¹⁶⁸

Gupta et al. reported antileishmanial activity of synthetic oximino benzocycloalkyl imidazoles evaluated *in vitro* against extracellular promastigote and intracellular amastigote forms of *L. donovani*. Compound (76), with 5-chlorotetrahydronaphthyl and 3-chlorobenzyl moieties, showed 93.41% inhibition during anti-promastigote activity testing at a concentration of 2.5 μM. It also showed anti-amastigote activity, with an IC₅₀ value observed at 0.23 μM. With CC₅₀ of 5.95 μM (on mouse macrophage cell line: J-774A.1), its SI was found to be 25.59. Also, in *in vivo* testing in a hamster model, compound (76) showed 70.13 ± 5.23% inhibition of parasite. ¹⁶⁹

Bhandari et al. reported preparation of aryloxy cyclohexane-based mono and bis imidazoles and their *in vitro* antileishmanial activities against *L. donovani*, along with a cytotoxicity study using a mouse macrophage cell line (J-774-A.1). Their *in vitro* studies revealed that compound (77) was the most...
potent among the series, having IC$_{50}$ anti-promastigote, IC$_{50}$ anti-amastigote, and SI values of 0.34 μM, 0.22 μM, and 140.84, respectively. The authors reported that compound (77) was better than existing drugs sodium stibogluconate (SI = 6.38) and pentamidine (SI = 2.58). After in vitro assay along with other promising compounds in a L. donovani/hamster model, compound (77) showed 55.35% inhibition. Bis methylimidazoles (78) containing a 2-fluoro, 4-nitro arlyloxy group (in vitro IC$_{50}$ anti-promastigote, IC$_{50}$ anti-amastigote, and SI of 0.89 μM, 0.29 μM, and 33.64 respectively), exhibited significant inhibition of 77.9%. In terms of SAR of the synthesized compounds, the researchers explained that the highest activity (in vitro as well as in vivo) was shown by compounds containing 2-fluoro and 4-NO$_2$ arlyloxy moieties. They suggested that arlyloxy moiety with 2-fluoro and 4-NO$_2$ substituents should be investigated for development of highly selective antileishmanial compounds.

4.18.2. Triazole. A novel series of triazole integrated phenyl hetero terpenoids was synthesized by Suryawanshi et al. After in vitro activity screening against intracellular amastigote form of L. donovani, compound (79), a β ionone based triazole integrated with phenyl pyrazoline, was found to be the most active, having IC$_{50}$ of 6.4 ± 1.2 μM and CC$_{50}$ of 112.4 ± 10.9 μM (on Vero cells) with better selectivity index of 18 compared with reference drugs miltefosine (IC$_{50}$ = 8.6 μM and SI = 6) and miconazole (IC$_{50}$ = 5.4 μM and SI = 7). The authors reported that compound (79) exhibited 79 ± 11% inhibition of parasite multiplication at 50 mg kg$^{-1}$ × 5 days on day 7 post treatment in vivo in a L. donovani/hamster model.

4.18.3. Pyrazole. Santos et al. reported novel pyrazole derivatives as antileishmanial agents. Compound (80) with a chloro substituent attachment at two meta-positions on the aryl nucleus of the aminopyrazole derivatives was the most active having an IC$_{50}$ of 15.5 ± 6.8 μM against the extracellular promastigote stage of L. amazonensis. After in vivo evaluation of the same compound, it was seen that there was inhibition of the progression of cutaneous lesions in CBA mice infected with L. amazonensis relative to an untreated control.

4.18.4. Benzimidazole. Nema et al. synthesized 1,5-bis(5-substituted benzimidazole) alkanes from substituted benzimidazoles. In vitro screening of these compounds found compound (81) to have the most potent antileishmanial activity, with IC$_{50}$ values of 0.43 μM against promastigote form and 1.53 μM against amastigote form.

Eynde et al. prepared a small library of 2,2'-[(p,p-alkanediylbis(oxyphenylene))bis-1H-benzimidazoles. After in vitro screening, the synthesized derivatives emerged as promising hits characterized by IC$_{50}$ values lower than that determined for pentamidine against L. donovani. Compound (82) had the lowest IC$_{50}$ of 1.4 μM and IC$_{50}$ of 3.1 μM, with an IC$_{50}$ of 28.7 μM against Vero cells.

4.18.5. Benzoxazole. Kozikowski et al. reported benzoxazole analogs as potential antileishmanial agents. Compounds (83) and (84), antibiotics isolated a couple of decades ago from a culture broth of Streptomyces sp. NRRL 12068, displayed the most notable activities. Compound (83) was found to have lower toxicity (IC$_{50}$ = 203.7) toward L6 cells than that of miltefosine (IC$_{50}$ = 147.0 μM), with IC$_{50}$ against L. donovani (axenic amastigote form) of 0.52 μM and SI as 392 being comparable to those of miltefosine (IC$_{50}$ = 0.26; SI = 565). Compound (84) had threefold more activity against the axenic amastigote form of L. donovani than that of miltefosine (IC$_{50}$ = 0.08 μM vs. 0.26 μM).

4.18.6. Thiadiazoles. Foroumadi et al. reported synthesis and antileishmanial activity of novel 5-(5-nitrofurany-2-yl)-1,3,4-thiadiazoles with piperazinyl-linked benzamide substituents. On in vitro screening, the most active compound (85) demonstrated an IC$_{50}$ value of 0.08 μM against an L. major
promastigote model. This compound showed a very low level of toxicity to mouse peritoneal macrophages \( \text{CC}_{50} = 785 \mu M \), and the highest selectivity index \( \text{SI} = 78.5 \) of the tested compounds. The authors discussed the potential of propyl, butyl, and benzyl substitutions on the amidine residue to improve activity against promastigotes.\(^{176}\)

The same researchers also reported synthesis and antileishmanial activity of 5-(5-nitroaryl)-2-substituted-thio-1,3,4-thiadiazoles against the promastigote form of \( L. \) \textit{major} using a tetrazolium bromide salt (MTT) colorimetric assay. Compound (86) appeared to be most potent with a lowest \( \text{IC}_{50} \) of 1.11 \( \mu M \). A structure–activity relationship study indicated that the S-pendant group attached to the 2-position of the thiadiazole ring has high flexibility for structural alteration, therefore retaining good antileishmanial activity.\(^{177}\)

5. Brief summary of promising scaffolds

From the above reported synthetic derivatives, we selected the five most promising scaffolds (32, 46, 47, 84, and 85) for antileishmanial activities (Fig. 4). Herein, we discuss these compounds in detail.

Potent antileishmanial activities have been reported for a dihydropyridine class of compounds bearing phenyl and other sugar residues at the fourth position of the dihydropyridine ring. To optimize these dihydripyridine derivatives, Tripathi \textit{et al.} extended the work and synthesized a series of 1-phenyl-4-glycosyl-dihydropyridines, evaluating the \textit{in vitro} and \textit{in vivo} activities of these against \( L. \) \textit{donovani}. Compound (32) emerged as a potent antileishmanial agent. It had promising...
antileishmanial activities against \textit{L. donovani} with IC$_{50}$ values of 0.04 μM (anti-promastigote) and 1.16 μM (anti-amastigote) when compared with standard drugs like pentamidine and miltefosine. Compound (32) was also evaluated against \textit{J-774A.1} growing cells for cytotoxic activity, and showed good selective index (SI = 8.04). The compound was screened for \textit{in vivo} activity against \textit{L. donovani} in a hamster model, showing 49.73% inhibition. A molecular docking study revealed that such compounds inhibit PTR1 (Pteridine reductase 1) enzyme of leishmanial parasites. Thus, compound (32) has potential for further exploration in development of safe and effective antileishmanial drugs.\textsuperscript{125}

Another compound (46) that showed potent antileishmanial activity is from the 2,4-diamoquinazoline class. The ED$_{50}$ value of compound (46) was found to be 0.00365 μM against \textit{L. major} amastigotes. The activity was structurally specific because it depended on an unconstrained tertiary aromatic amine attached directly to the benzyl group of the quinazoline. Compound (46) has been suggested to be primarily leishmanialstatic in nature. It showed inhibition of 88% of \textit{L. mexicana} promastigote DHFR at a concentration of 7.5 μg mL$^{-1}$. The remarkable activity of compound (46) suggests that such analogs have potential for investigation as novel antileishmanial agents.\textsuperscript{126} The compound (47) tryptanthrin derivative has shown promising antileishmanial activity against \textit{L. donovani} amastigotes (IC$_{50}$ = 0.000013 μM). \textit{In vitro} toxicity studies indicate that compound (47) is fairly well tolerated in both murine \textit{J774} macrophages and rat neuronal NG-108-15 cell lines. The carbonyl groups of the five- and six-membered rings in the indolo[2,1-b]quinazoline-6,12-dione skeleton and the electron transfer ability to the carbonyl atom appear to be crucial for activity. Compound (47) is found to be less toxic to mammalian cell lines than to \textit{Leishmania} \textit{in vitro}. Thus, this compound shows remarkable promise for further study as a potential antileishmanial candidate.\textsuperscript{127,128}

Compound (84), a benzoxazole derivative, has shown potent activity against \textit{L. donovani} amastigote (IC$_{50}$ = 0.08 μM) with low cytotoxicity against L6 cells (CC$_{50}$ = 14.2 μM). This compound is threefold more active than miltefosine (IC$_{50}$ = 0.26 μM). Thus, discovery of compound (84) underscores the importance of the \textit{N}-2-benzoxazole-2-ylphenyl benzamides as an important lead scaffold in design and synthesis of antileishmanial agents.\textsuperscript{129} Compound (85) has shown promising antileishmanial activity against \textit{L. major} promastigotes (IC$_{50}$ = 0.08 μM) with a very low level of toxicity against macrophages (CC$_{50}$ = 785 μM and SI = 78.5). The potent activity of compound (85) indicates that propyl substitution on the amidine residue improve antileishmanial activity. Thus, compound (85) is a promising new hit for development of antileishmanial chemotherapy.\textsuperscript{130}

6. Antileishmanial natural products

6.1. Plant origin

6.1.1. Xanthone. 1,3,6,7-Tetrahydroxy-2-(3-methylbut-2-enyl)xanthone (87a) and a new xanthone derivative allanxanthone D (87b) were isolated from the stem bark of \textit{Allanblackia gabonensis} (Guttiferae). After assaying them, the IC$_{50}$ values were found to be 4.05 μM for compound (87a) and 4.24 μM for allanxanthone D (87b) against axenic amastigote form of \textit{L. amazonensis}.\textsuperscript{178-180}

6.1.2. Flavonoids. Quercetin (88) was obtained from \textit{Cecropia pachystachya}. Trecul had shown antileishmanial activity with an IC$_{50}$ value of 3.8 μM against \textit{L. amazonensis} promastigote form.\textsuperscript{181} A flavonoid glycoside named quercetin-3-O-β-D-galactopyranoside (89) was isolated from the extracts of \textit{Corymbia maculate}. Hook exhibited an IC$_{50}$ value of 6.9 ± 0.3 μM against \textit{L. donovani} promastigotes.\textsuperscript{182} A flavono-18-prenylmucronulatol (90) was extracted from the plant \textit{Smirnowia airanica}, with IC$_{50}$ value was found to be 6.9 μM against \textit{L. donovani} promastigotes.\textsuperscript{183} Another flavonol glycoside luteolin (91) was isolated from \textit{Vitex negundo} (Verbenaceae) and \textit{Fagopyrum esculentum} (Polygonaceae). Its IC$_{50}$ was found to be 12.5 μM when tested against intracellular amastigote form of \textit{L. donovani}.\textsuperscript{184}

6.1.3. Alkaloids. Among the tested compounds that were isolated from \textit{Holarrhena curtisii} (Apocynaceae), the most potent compound was found to be holamine (92), a steroidal compound having IC$_{50}$ values in the range of 1.23–4.94 μM against promastigote form of \textit{L. donovani}.\textsuperscript{185}

An indole alkaloid corynantheine (93), present in the bark of \textit{Corynanthe pachyceras} (Rubiaceae), exhibited antileishmanial activity against \textit{L. major} promastigotes with an IC$_{50}$ value of
Another alkaloid named rhodesiacridone (95), which contains an acridone ring, was obtained from *Thamnosma rhodesica* (Rutaceae). After performing antileishmanial assay against *L. major*, it was found that at 10 μM concentration rhodesiacridone inhibited 69% of promastigote forms, whereas against amastigote form of the same species, over 90% and 50% inhibition were observed at concentrations of 10 μM and 1 μM, respectively. Compound (95) was found to be non-toxic to murine macrophages at the same concentrations.188

Among the compounds that were tested against *L. donovani* promastigotes, anistrotanzanine B (96), an isoquinoline alkaloid isolated from the plant *Ancistrocladus tanzaniensis* (Ancistrocladaceae), was found to be most potent with an IC₅₀ value of 3.81 μM.189

Dihydrochelerythrine (97), having a phenanthridine ring coupled with a benzene ring, isolated from the stem bark of *Garcinia lucida* (Clusiaceae), was found to be the most active from compounds tested against *L. donovani* axenic amastigotes, showing an IC₅₀ value of 2.0 μM.190

About 3 μM.186 A macroline-derived indole alkaloid (94) obtained from stem bark of *Alstonia angustifolia* (Apocynaceae) was found to be potent against *L. mexicana* promastigotes, with an IC₅₀ value of 57.8 μM.187
From the seeds of plant known as *Peganum harmala*, peganine hydrochloride dihydrate (98), a quinazoline alkaloid (identified as an orally active antileishmanial lead molecule), showed *in vitro* anti-promastigote and anti-amastigote activity against *L. donovani*, with IC\(_{50}\) values, respectively, of 16.99 \(\mu\)M and 18.30 \(\mu\)M. On testing its *in vivo* activity, peganine hydrochloride dihydrate (98) showed 79.6 ± 8.07% inhibition against the same species of established VL in hamster models at a dose of 100 mg per kg b.wt.\(^{191}\)

6.1.4. Terpenoids. Linalool (99), a monoterpenoid that is present in oil of the plant *Croton cajucara* (Euphorbiaceae), exhibited anti-promastigote activity with an IC\(_{50}\) value of 0.028 \(\mu\)M, whereas its anti-amastigote activity (IC\(_{50}\)) was found to be 0.143 \(\mu\)M. It presented no cytotoxic effects against mammalian cells. Treatment of pre-infected mouse peritoneal macrophages with 0.015 \(\mu\)g mL\(^{-1}\) of essential oil containing linalool (99) reduced the interaction between these macrophages and *L. amazonensis* with an increase in the level of nitric oxide production by the infected macrophages.\(^{192}\)

Agarofuran derivative (1S,4R,5R,6R,7R,8S,9R,10R)-8-acetoxy-1,9-dibenzoyloxy-4-hydroxy-6-nicotynoyloxy-dihydro-b-agarofuran (100a), a sesquiterpene obtained from root and barks of *Maytenus apurimacensis*, showed antileishmanial activity against *L. tropica* amastigotes with an IC\(_{90}\) value of 7 \(\mu\)M.\(^{193}\) One sesquiterpene furanoeremophil-1-on-13-oic acid (100b), obtained from the woody shrub *Drypetes chevalieri* Beille (Euphorbiaceae), was screened against the *L. major* promastigotes and showed significant antileishmanial activity (IC\(_{50}\) = 15.27 \(\mu\)M) compared with control drug pentamidine (IC\(_{50}\) = 11.18 \(\mu\)M).\(^{193}\) Another sesquiterpene known as nerolidol (a mixture of cis- and trans-nerolidol) (101), present in essential oil of several plants,
showed antileishmanial activity when tested against promastigote forms of various Leishmania species such as L. amazonensis (IC\textsubscript{50} = 85 μM), L. braziliensis (IC\textsubscript{50} = 74 μM), and L. chagasi (IC\textsubscript{50} = 75 μM). It also exhibited antileishmanial activity against L. amazonensis amastigote form, with IC\textsubscript{50} value of 67 μM.

194 10-Deacetylbaccatin III (a precursor of the well-known drug taxol), a diterpenoid (102) isolated from Taxus baccata, showed an IC\textsubscript{50} value of 0.07 μM against L. donovani intracellular amastigotes.

6.1.5. Coumarins. A sesquiterpene coumarin derivative conferol (106a), isolated from the plant Ferula narthex Boiss, showed an IC\textsubscript{50} value of 3.99 μM against L. major promastigote form. Umbelliprenin (106b), another prenylated sesquiterpene coumarin present as one of the components in the extract of Ferula szowitsiana (Apiaceae) roots, showed significant activity with an IC\textsubscript{50} value of 13.3 μM against promastigotes of L. major.

6.2. Marine origin
Almiramide C (107), a peptide present in the crude extract of marine cyanobacterium Lyngbya majuscula (isolated from mangrove roots), exhibited a strong antileishmanial property with an EC\textsubscript{50} of 1.9 μM against L. donovani amastigotes. Dragonamide E (108), another marine peptide obtained from the same source, showed an EC\textsubscript{50} of 5.1 μM when tested in vitro against L. donovani axenic amastigotes. Viridamide A (109), a marine peptide isolated from Oscillatoria nigro-viridis, displayed potent in vitro antileishmanial activity (EC\textsubscript{50} = 1.5 μM) against amastigote form of L. mexicana.

Isoaapentine (110), a marine alkaloid obtained from marine sponge, that is Aaptes sponge, was found to have good antileishmanial activity with an EC\textsubscript{50} of 0.31 μM when assayed in vitro against L. donovani promastigote form. Plakortide P (111), a polyketide isolated from marine sponge Plakortis angulospiculatus, exhibited in vitro antileishmanial activity against L. chagasi promastigotes with an EC\textsubscript{50} value of 0.52 μM.

7. Recent patents on antileishmanial drug moieties
In a recent patent, Satoskar et al. discussed sterol compounds (isolated from P. andrieuxii and/or obtained hemi-synthetically using appropriate sterol precursor) as useful therapeutic agents for leishmaniasis. Among the compounds isolated from the roots of P. andrieuxii, the IC\textsubscript{50} values of compounds (112) and (113) against amastigote form of L. mexicana were observed to be 0.03 and 1.4 μM, respectively, with compound (113) found to
be most active against promastigote form of the same species with IC\textsubscript{50} value of 9.2 m\textmu M.

Out of the nine stem fractions that were isolated from \textit{P. andrieuxii}, the fraction PASD3F2 showed potent activity against promastigote form (IC\textsubscript{50} = 21.5 \mu g mL\textsuperscript{-1}). None of the compounds were cytotoxic to the non-infected bone marrow-derived macrophages (IC\textsubscript{50} > 100 \mu g mL\textsuperscript{-1}), suggesting that such compounds are selective for protozoal cells.\textsuperscript{204} In the invention by Vasquez \textit{et al.}, use of quaternary ammonium salts is described for treatment of \textit{Leishmania} infections. A screening the compounds against axenic amastigotes of \textit{L. panamensis}, the authors commented that compound (114) was the most effective of the tested compounds (EC\textsubscript{50} = 14.0 \pm 0.9 \mu M).\textsuperscript{205}

The patent by Boechat \textit{et al.} refers to new 1,2,3-triazoles and imidazoles included in families of compounds represented by general formula (115), and also to a pharmaceutical composition comprising at least one of theazole compounds represented by the same general formula (115), to the use of such compositions, and to the method of treatment or inhibition of leishmaniasis.

where, X = “N” and the radicals of the triazole ring are represented by R\textsubscript{1} = CF\textsubscript{3}R\textsubscript{2}; R\textsubscript{2} = R\textsubscript{3} = alkyl group and the radical R\textsubscript{n} can be located in any one or in more than one of the aromatic rings, and is represented by a halogen.

On \textit{in vitro} analysis against promastigote form of \textit{L. amazonensis}, the difluoromethyl derivative (116) showed potent activity (inhibition of parasite = 93%) at 10 \mu g mL\textsuperscript{-1} concentration when compared with the standard drug pentamidine (at 160 \mu g mL\textsuperscript{-1} concentration inhibits 53\% of parasites).\textsuperscript{206}

Kimura \textit{et al.} found that an extract from \textit{Sargassum yamade}, a brown alga (family: Sargassaceae; order: Fucales) had high antileishmanial activity. From the compounds that were subjected to \textit{in vitro} analysis against promastigote form of \textit{L. major}, compound (117) showed almost equal growth inhibition rate to that of amphotericin B, which was used as a positive control. The authors also evaluated \textit{in vivo} analysis of the same compound on a leishmaniasis mouse model by administering 200 \mu g via a peritoneal route once a day for 3 weeks. It was found that compound (117) again exhibited activity equal to that of amphotericin B.\textsuperscript{207}
Sevilla et al. claimed that N<sup>6</sup>-(ferrocenmethyl)quinazoline-2,4,6-triamine compound (118) presents leishmanicidal activity at a concentration starting from 0.1 μg mL<sup>-1</sup>. On in vitro analysis against L. mexicana, they reported that compound (118) is lethal (in less than 5 h) at concentrations greater than 5 μg mL<sup>-1</sup>. They observed that the parasite structure was modified such that it lost its characteristic form, lost refringence, became spherical, and was incapable of multiplying. Although the mechanism could not be identified, the authors suggested that a necrosis process was likely to be involved. The same compound was found to be up to 10-fold faster at killing the total number of parasites when compared with other compounds such as metronidazole and hydroxyurea (having leishmanicidal activity). There was also no occurrence of cytotoxicity with murine cells in in vitro analysis, nor in mice (the in vivo analysis model) when administered orally, parenterally, or dermally.<sup>208</sup></p>

In their patent, Shairah et al. commented that certain metronidazole derivatives (2-methyl-5-nitro-imidazolyl compounds) are useful against L. donovani and L. tropica promastigotes. On their in vitro assay, compound (119) showed IC<sub>50</sub> values of 109 μM and 54.54 μM against L. donovani and L. tropica, respectively.<sup>209</sup>

Curtis et al. reported that compounds, for example (120), that contain a substituted five- or six-membered ring core containing one or two oxygen, nitrogen, or sulfur atoms as constituent atoms of the ring, can be used to treat leishmaniasis or its symptoms by inhibiting sirtuin (e.g. SIRT1) present in the parasite. They further reported that the described SIRT1 inhibitor (e.g. 120) decreases the ability of parasite to develop resistance to conventional treatments, and/or decreases the viability and/or infectivity of the parasite. They tested various possible compounds for activity against SIRT1. Different compounds were found to have different activities (IC<sub>50</sub>), even lower than 1 μM.<sup>210</sup>

Cohen et al. reported their invention represented by the general formula (121), related to thiosemicarbazone and semicarbazone inhibitors of cysteine proteases, and methods of using such compounds to prevent and treat protozoan infections such as leishmaniasis.<sup>211</sup>

Searle et al. from GlaxoSmithKline, USA, reported sitamaquine tosylate (122) for treatment of leishmaniasis.<sup>212</sup>

Werbovetz et al., in their patent “Antileishmanial dinitroaniline sulfanoamides with activity against parasite tubulin,” highlighted usefulness of these compounds particularly in the treatment of leishmaniasis. Compound (123) showed good
activity against *L. donovani*. Its IC\(_{50}\) values were found to be 8.02 ± 0.42 and 9.0 ± 0.7 μM against promastigote and amastigote forms, respectively.\(^{213}\)

From the invention by Masataka et al. (Japan Science and Technology Corporation, Japan), compound (124) was found to be most potent, having an IC\(_{50}\) value of 0.0018 μM, more than that of standard drug amphotericin B (IC\(_{50}\) = 0.015 μM).\(^{214}\)

Rios et al. in their patent had claimed that none of the current treatments for leishmaniasis use compounds with chemical structure comparable with the compounds of their present invention, which belong to the class of substances known as aporphine alkaloids. Compound (125) was found to be most potent of the tested compounds, with IC\(_{50}\) values against *L. mexicana* and *L. panamensis* of 3 ± 0.27 and 6 ± 0.07 μM, respectively. The compound was also found to have 37-fold higher toxicity towards *L. mexicana* than macrophages (IC\(_{50}\) = 112 ± 0.2 μM).\(^{215}\)

8. **Possible antileishmanial drug targets**

8.1. Sterol pathway (enzymes of sterol biosynthesis)

Unlike cholesterol which is present in mammals, the *Leishmania* parasite has endogeneous ergosterol (counterpart of cholesterol) and stigmasterol in its cell membrane. This feature may be useful in drug targeting of antileishmanial agents. For example, azasterols inhibit 24-methyltransferase, an enzyme vital for ergosterol biosynthesis.\(^{216}\) Inhibitors of 14-α-methylsterol-14-demethyase, such as some azoles and triazoles, are effective against *Leishmania*.\(^{217}\)

8.2. Thiol pathway (enzymes of thiol metabolism)

Some reports indicated that a characteristic thiol metabolic defense mechanism developed by the parasite was involved in neutralization of host oxidative outcome (harmful to the parasite), explaining why the *Leishmania* parasite can withstand and proliferate in a toxic environment developed by macrophages of mammalian host. For example, Gradoni et al. reported that trypanothion [T(SH)\(_2\)], a dithiol found in *L. infantum*, is capable of reducing nitric oxide (generated in mammals) and iron into a harmless stable dinitrosyl iron complex with 600 time more affinity than mammalian GSH (glutathione) reductase system. This is the mechanism by which the parasite protects itself from such lethal environments. In homology modelling of *L. infantum*, TR (trypanthione reductase, one of the antioxidant enzymes present in *Leishmania*) and mammalian glutathione reductase (GR) have shown remarkable differences in their three-dimensional and catalytic active sites.\(^{218-220}\)

8.3. Hypusine pathway

Hypusine (derived from the polyamine spermidine) is synthesized in two enzymatic steps as a result of post-translational modification in all eukaryotes. The first step is catalysed by the enzyme deoxyhypusine synthase (DHS). Recently, Chawala et al. showed that hypusine biosynthesis occurs in *L. donovani* and they identified two genes from this containing DHS domains. They further concluded that the gene DHS34 (DHS-like gene from chromosome 34) is essential for functional activity in vitro in *L. donovani*.\(^{218-223}\)

8.4. GPI pathway

A major component of the *Leishmania* surface coat is the glycosylphosphatidylinositol (GPI)-anchored polysaccharide called lipophosphoglycan (LPG), having some free GPIs which protect the parasite from the alternate complement pathway and external hydrolases. Sacks et al. reported that LPG is essential for infectivity of *L. major* promastigotes in both mammalian and insect hosts. They further concluded that LPG is required to maintain infection in the fly during excretion of the digested blood meal.\(^{224-225}\)
8.5. Glycolytic pathway
The unique compartmentalization of glycolytic enzymes (in glycosomes of Leishmania) and their large phylogenetic distance from the mammalian hosts provides them with unique features that can be targeted. Specific inhibitors have been designed for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which is an essential enzyme for conversion of glucose to pyruvate in glycolysis occurring in Leishmania. For example, N\textsuperscript{6}-[1-naphthalenemethyl]-2\textsuperscript{'}-(3-methoxybenzamido) adenosine inhibited growth of L. mexicana with an IC\textsubscript{50} of 0.28 µM. In another study, it was reported that two different types of iron superoxide dismutases FeSOD (absent in mammalian counterpart), LcFeSOD1 and LcFeSOD2, were characterized in L. chagasi (within glycosomes), and these were found to be responsible for survival and protection from lethal superoxide radicals.

8.6. Purine salvage pathway
Like other hemoflagellates, Leishmania parasites are incapable of synthesizing the purine nucleus. Therefore to utilize purine bases from their mammalian hosts, they depend solely on an exogenous supply of preformed purines by means of a purine “salvage” pathway. The enzyme phosphoribosyl transferase (PRT) is a mediator in salvage of purines. Adenine phosphoribosyl transferase (APRT), hypoxanthine guanine phosphoribosyl transferase (HGPRT), and xanthine phosphoribosyl transferase (XPRT) are the three PRTs identified as present in Leishmania species. Because of differences in substrate specificity of parasitic purine salvage enzymes from host enzymes, various inhibitors could be designed or developed to target them (e.g. allopurinol that targets HGPRT, gets phosphorylated therein and thus incorporated into parasitical nucleic acid leading to its leishmanicidal action).

8.7. Nucleoside transporters
LdNT1 (present in promastigotes as well as amastigotes responsible for transportation of adenosine and pyrimidine nucleosides) and LdNT2 (present in amastigotes that transport purine nucleosides such as inosine, guanosine, etc.) are the two transporters documented from L. donovani. The parasitic transporters are different from mammalian transporters in terms of their higher specificity towards the substrate, making them vital targets as these transporters also uptake toxic nucleosides, which are inhibitory in action to the cell growth.

8.8. Cyclin dependent kinase
Cyclin dependent kinases (CDKs) are important in cell division, transcription, etc. In Leishmania, the cdc-2 related kinase (CRK) family have attracted attention as potential drug targets which are homologous to CDKs and are thought to be vital for cell cycle progression. For example, CRK3 was found to be active throughout the life cycle of L. mexicana, and it inhibitors of CRK3 inhibited the growth and replication of L. donovani amastigotes in peritoneal macrophages. Most potent inhibitors of CRK3 belongs to the indirubin class.

8.9. Mitogen activated protein kinase (MAPK)
In L. mexicana, MAPK was found to be important for transformation and cellular growth. MAPKs are not only important to amastigotes but also promastigotes. Therefore, they have potential as antileishmanial agents.

8.10. Enzymes of polyamine biosynthesis
The putrescine, spermidine, and spermine-like polyamines and their metabolic pathways have important roles in growth and differentiation of parasites from promastigote to amastigote stages, and also downregulate lipid peroxidation generated by oxidant compounds and make the environment compatible for parasite survival. Arginase and ornithine decarboxylase are the two enzymes present in Leishmania involved in synthesis of putrescine and thereby spermine and spermidine, offering potential as targets.

8.11. Dihydrofolate reductase (DHFR)
Thymidylate synthase (TS) and dihydrofolate reductase (DHFR) are enzymes involved in the folate pathway during DNA biosynthesis. Classic inhibitors of DHFR were found to be ineffective against Leishmania. Another enzyme, pteridine reductase (PTR1) was viewed in some Leishmania mutants resistant to methotrexate, an inhibitor of DHFR-TS.

8.12. Peptidase
A total of 154 peptidases were found to be present in the L. major genome. Secretary endosomal system consists of subtilisin like serine peptidase, which participate in processing of secreted proteins and may be useful as a drug target. It was reported that TPCK (N-tosyl-l-lysyl-chloromethylketone) and benzamidine, the serine peptidase inhibitors, reduce the viability and induce morphological changes in L. amazonensis promastigotes, suggesting that serine peptidases could be useful potential drug targets. In vitro study revealed that proteasome was essential for growth of both parasitic forms, that is promastigotes and amastigotes. Hence, the proteasome of Leishmania is a potential therapeutic target.

8.13. Topoisomerase
DNA topoisomerases are important enzymes required in many essential processes like DNA replication, transcription, recombination, and repair. Both types of enzymes viz. type I
topoisomerase and type II topoisomerase, have been characterized from *L. donovani*. Anti-leishmanial compounds such as sodium stibogluconate and urea stibamine are inhibitors of type I topoisomerase. Camptothecin, a plant alkaloid, was also found to be an inhibitor of *L. donovani*. Antibacterial and anticancerous drugs like novobiocin, etoposide, and fluorouracil can be used to target topoisomerase II to inactivate genetic integrity and cell survival. A derivative of betulinic acid, a pentacyclic triterpenoid *i.e.* dihydrobeticulic acid (DHBA), was found to be active against both topoisomerase I and topoisomerase II of *L. donovani*.

8.14. Metacaspase
Two metacaspases (MCAs), LdMCA1 and LdMCA2, are reported in *L. donovani* promastigotes and amastigotes. It has been reported that parasites which overexpress metacaspases are more sensitive to 

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<th>8.15. Glyoxalase system</th>
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<td>The function of the glyoxalase system is to detoxify cells by eliminating toxic and mutagenic methylglyoxal, which is mainly formed in glycolysis as a byproduct. Glyoxalase I (characterized from <em>L. donovani</em> and <em>L. major</em>) and glyoxalase II (characterized from <em>L. donovani</em>) are the two enzymes involved in glyoxalase system. A derivative of betulinic acid, a pentacyclic triterpenoid <em>i.e.</em> dihydrobeticulic acid (DHBA), was found to be active against both topoisomerase I and topoisomerase II of <em>L. donovani</em>.</td>
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9. Conclusions
Leishmaniasis is a life-threatening disease that mainly affects people in developing countries. There has been significant progress in the treatment of leishmaniasis during recent decades. Various drugs like miltefosine, paromomycin, pentamidine, and liposomal amphotericin B have substantially improved the options for treatment. However, growing incidences of resistance and toxicities with available drugs warrant precise use of antileishmanial drugs as well as necessitating the search for and development of newer effective drugs and vaccine candidates.

Several new synthetic molecules with interesting anti-leishmanial activity have been proposed. Various cores and derivatives like indole, coumarin, quinoline, azoles, triazine, thiophosphorylpyridine, pyrimidine, etc., have been reported to possess potent antileishmanial activity with good selectivity indices. Screening of natural compounds seems to be an attractive approach for development of effective new lead compounds or drugs. Importantly, natural products, *viz.*, quercetin (flavonoid), luteolin (flavonoid), holamine (steroid), corynantheine (indole), rhodesiacridone (acridone), dihydrochelerythrine (phenanthridine), pegamine (quinazoline), linalool, agarofuran and nerolidol (terpenoid), conferol (coumarin), and isoapantamina (alkaloid) demonstrate interesting in vitro antileishmanial activity. Also, a number of products with antileishmanial activity have been patented following different strategies old and new. Several interesting drug targets also have been proposed including many proteins and enzymes namely, sterol pathway, thiol pathway, hpyusine pathway, glycolytic pathway, purine salvage pathway, polyamine pathway, protein kinase, dihydrofolate reductase, topoisomerase, *etc.*, that differ from their mammalian counterparts.

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