LEISHMANIASIS: Current Treatment Strategies and Future Opportunities

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ABSTRACT
Leishmaniasis, in its variety of visceral (VL), cutaneous (CL) and mucocutaneous (MCL) forms, a vector-borne parasitic disease, is caused by the infection with the obligate intracellular protozoan parasite, Leishmania, transmitted by about 30 species of Phlebotomine sandflies. The control of leishmaniasis remains a problem—principally a zoonotic infection, except in epidemics where it is anthropontic, interruption of transmission is difficult, though not impossible. No vaccines exist for VL, CL or MCL and chemotherapy is inadequate and expensive. Current regimes use pentavalent antimony as primary therapy, which must be administered parenterally. Should this fail, a number of other drugs may be employed, depending upon the species of Leishmania concerned and the resources available to the health professionals involved. The most widely used of these is amphotericin B, which is highly active but has extensive toxicity complications. Pentamidine and Paromomycin are used in some instances, and a new anti-leishmanial, Miltefosine, may be used in the future. In short, there remains a pressing need for new anti-leishmanials and this chapter reviews the current status of chemotherapy, the various Novel Targets and some important lead compounds in antileishmanial chemotherapy.

Key Words: Leishmaniasis, Deoxyuridine Triphosphate, Miltefosine, J-Binding Protein
INTRODUCTION

Leishmaniasis, a vector-borne parasitic disease, is caused by the infection with the obligate intracellular protozoan parasite, *Leishmania*, transmitted by about 30 species of Phlebotomine sandflies[1] which is the commonest mode of transmission. It is presumed that skin lesions or peripheral parasitaemia act as reservoirs, from where the female sandfly takes up the infective form of the parasite (amastigotes) during the blood meals and transmits to new human host through another bite. Other than the insect route, transmission through placental[2], semen[3], injection needles[4] and laboratory acquired infections have also been reported[5], though rarely. The infection presents with a wide range of clinical forms in the human host.[6] are cutaneous leishmaniasis (CL)[7] produces skin ulcers on the exposed parts of the body, such as the face, arms and legs. The number of ulcers may vary from one to as many as 200, causing serious disability and leaving the patient permanently scarred. Visceral leishmaniasis (VL) [8], also known as kala-azar, is the most severe form of the disease, which, if untreated, has a mortality rate of almost 100%[9]. The third form is mucocutaneous leishmaniasis (MCL) or espundia[10]. It can lead to extensive and disfiguring destruction of mucous membranes of the nose, mouth and throat cavities and can involve even the cartilages. Sometimes the cutaneous form may lead to disseminated form, known as diffuse cutaneous leishmaniasis (DCL).[11] Leishmaniasis is regarded as a major public health problem (WHO, 2002), causing significant morbidity and mortality in Africa, Asia and Latin America. The disease currently threatens about 350 million people in 88 countries around the world[12], with about 2 million affected annually. An increase in the incidence of leishmaniasis can be associated with urban development, forest devastation, environmental changes and migrations of people to areas where the disease is endemic.

There have been several comprehensive reviews with respect to chemotherapy along with new developments, new targets, cause of resistance and methods to tackle them for the current threatening disease.[13-17] Nevertheless in order to initiate an exploratory research exercise targeted toward discovery of new molecular framework for the bioactivity against the leishmaniasis, we have overview the chemotherapy, new promising leads and the important novel metabolic and enzymatic pathways in leishmanial parasite which have been reported in recent past for new drug discovery endeavors.

Modes of transmission

1. Vector-borne transmission
   It is the most common mode of transmission, throughout the world[14]. When sandflies bite an infected host, they swallow *Leishmania* amastigotes, which circulate freely in the host’s blood or inside peripheral blood mononuclear cells. These amastigotes migrate to the sandfly’s proboscis where they develop into stationary, infective-stage organisms that could be qualified as “metacyclic” promastigotes. When this infected sandfly bites a second host, *e.g.*, a human being, these promastigotes are released and deposited on the site of bite or injected along with potent vasodilators (*i.e.*, maxadilan) that produce long-lasting erythema.[15]

2. Congenital transmission
   The first case of congenital leishmaniasis was reported in 1926 by Low & Cooke.[16] *L. donovani* has been found to pass through the placenta of the Syrian hamster and mice but no
parasite could be demonstrated in the organs of an aborted 5 months foetus while the placenta had numerous amastigotes.[17] This indicated that the infection might have occurred in most of these cases during the exchange of mother’s blood at the time of passage of foetus through birth canal. Congenital VL manifests within three months of life and manifestations are by and large similar to that *Leishmania* acquired through sandfly bite, but the course is usually rapid.[17,18] Pregnant women become more susceptible to leishmaniasis due to shift of cell mediated immunity to humoral immunity.[19]

3. Sexual transmission
Urine and prostatic fluid cultures from patients with VL have yielded promastigotes. Reports of sexual transmission[20] include transmission from a man to his wife, as well as probable transmission in a homosexual man with AIDS who had rectal lesion and to have admitted frequent receptive anal intercourse while vacationing in endemic areas.

4. Occupational (needle stick) exposures
In Spain number of cases of AIDS and about 200 cases of HIV-associated leishmaniasis were detected earlier, of which more than 85 per cent occurred among intravenous drug users (IVDUs). The infection is so common that 17 % of 111 bone marrow aspirates (BMAs) in HIV-positive subjects with fever had amastigote. Alvar et. al.[21] described a high variability of *L. infantum* zymodemes circulate among drug users who share syringes and, therefore, act as reservoirs to a degree that is as yet unknown. In another study Molina et al[22] tested the indirect xenodiagnosis of VL in 10 HIV-infected patients, of whom nine were IVDUs; they found that minute volumes of blood (0.3-0.5 µl) proved infective to Phlebotomus perniciosus, thereby concluding that the possibility of needle-mediated transmission cannot be ruled out. Other studies have been conducted with similar findings.[21a,b,22,23]

Morphology of the parasite
*Leishmania* parasite exists basically in two forms i.e. (1) Amastigote (2) promastigote

1. Amastigote form
This stage exist as ovoid and nonflagellated form of leishmania, measuring 3-5 µm in length.[24] On simple light microscopy, a central round or oval nucleus and adjacent but smaller round or rod shaped kinetoplast can be discovered.

2. Promastigote form
In the sandfly host the parasite is found in the promastigote form. The transformation of amastigotes to promastigotes starts within hours of ingestion of the amastigotes (either free or intracellular) and occurs exclusively in the gut. The amastigotes are completely transformed into motile promastigotes within 24-48 h and keep on dividing by binary division.[25-33]

Present Status of Antileishmanial Chemotherapy
The control of *Leishmania* infections rely primarily on the chemotherapeutic treatments. Despite the continuous ongoing efforts in antileishmanial drug discovery and development, the arsenal of current chemotherapeutic drugs available for the treatment of *Leishmania* infection is not satisfactory and reflects the need for discovery of more effective antileishmanial agents.[34]
1. Amphotericin B (2)
This polyene antibiotic has selective activity against fungi as well as Leishmania and T. cruzi, due to its higher affinity for ergosterol, the predominant sterol in these microorganisms, than to host cell cholesterol [35-37]. There has been no study in vitro comparing the sensitivity of different species of Leishmania to amphotericin B, although differences might be expected due to variation in the type and quantity of sterols in the membranes of different species. Clinical comparisons are also lacking.[38-39]

2. Pentavalent antimonials (1 & 3)
The variation in clinical response to the pentavalent antimonials, sodium stibogluconate 3 (Pentostam) and meglumine antimoniate 1 (Glucantime), has been a persistent problem in treatment over the past 50 years. Wide variations in sensitivity of isolates to pentavalent antimonials have been demonstrated. Clinical response was correlated with a decrease in the sensitivity in vitro of L. donovani amastigotes in macrophages but not of the promastigote stage.[40]

3. Pentamidine (7)
Pentamidine was used in visceral leishmaniasis treatment for years[39, 41]. In late 1980s, pentamidine mesylate was replaced by pentamidine isethionate (Pentacarinats) in order to reduce side-effects. Pentamidine isethionate is also used in South American cutaneous leishmaniasis, especially when caused by L. guyanensis which seem to be less sensitive to antimony. The failure rate depends on the timeliness of the treatment, elevated muscle enzymes are noted frequently with pentamidine. In an area where L. braziliensis, L. amazonensis, L. shawi and L. guyanensis coexist in Brazil,[42] a regimen of three intramuscular injections (days 1, 3 and 5 with 4 mg/kg pentamidine-base; maximum dosage: 300 mg/d) has been compared to 20 days of antimony. Most of the observed failures in the pentamidine group were attributed to the withdrawal of patients with persistent presence of parasites.[43]

4. Miltefosine (4)
An alkylphosphocholine originally developed as an anticancer agent, which interferes with cell signal-transduction pathways and inhibits phospholipids and sterol biosynthesis. It has been developed for treatment of both VL and CL by Zentaris and the WHO TDR. It is orally active drug,[44] showed sensitivity against both the promastigotes and amastigotes stages of L. donovani, L. major, L. tropica, L. aethiopica, L. mexicana and L. panamensis and has emerged as a powerful drug among the other active members of the series such as ilmofosine (6)[45] and edelfosine (8). Eflornithine (5) is an irreversible inhibitor of ornithine decarboxylase and polyamine biosynthesis that was also originally developed as an anticancer agent but is now used for the treatment of stage 2 Trypanosoma brucei gambiense infection.

5. The diamidine DB 289 (9). This is a prodrug of 2,5-bis (4-amidinophenyl) furan (DB75 or furamidine) and is currently in Phase III clinical trials for treatment of stage–1 Trypanosoma brucei gambiense infection in central Africa (in development with the North Carolina University and Gates Consortium).

6. Azoles (10–16). As for amphotericin B, certain azole antifungal drugs inhibit the 14 α-demethylation of lanosterol, mediated by cytochrome 450. They cause an accumulation of 14 α–
methyl sterols and block ergosterol synthesis of Leishmania parasites. The azoles can be given orally. Ketoconazole 10 (600 mg/d in adults and 10 mg/kg/d in children, for a month) was tried during the 1980s.[46] It’s efficacy varies with the species and this drug is not used commonly. Fluconazole 12 has a long half-life, high solubility in water and a concentration in skin that is ten times that in plasma. A randomized, double-blind, placebo-controlled trial was conducted in Saudi Arabia, in patients with 2 years of infection with L. major,[47-50] and its limitations have been emphasized.[51] The time to healing was also significantly shorter in the fluconazole group (median, 8.5 weeks). Itraconazole (100–400 mg/d) has been used with certain efficacy in small series, in India, Brazil, Argentina, Italy and United Kingdom. However, larger series in Iran (L. major cutaneous leishmaniasis) have found low response rates. Ravuconazole 13 and posaconazoles 16 are inhibitors of Trypanosoma cruzi sterol C14α sterol demethylase that have potent and selective activity and pharmacokinetic properties.

Figure 1.2: Antileishmanial Drugs
Figure 1.3. New drugs in clinical or preclinical development for the trypanosomiases and leishmaniasis

7. Novel Targets and some important lead compounds in antileishmanial chemotherapy
In order to fight leishmaniasis newer options are required to be investigated. These include development of new chemotypes, re-evaluation and modification of existing drugs and identification and validation of novel enzymatic and metabolic pathways for Leishmania.[52] The advancement in unraveling the newer targets for development of new antileishmanials have been limited In the following text some of the putative biochemical targets which have been investigated for design and discovery of new drugs against Leishmania parasites are being described. Additionally we have made an effort to compile various chemotypes which have been reported recently to display antileishmanial activity.

A. Folate Metabolism
Leishmania is a folate and pterin auxotroph and both the folate and pterin metabolisms are interconnected.[53] In Leishmania, the enzyme DHFR exist as dimer of DHFR and TS and both are fused resulting in a bifunctional DHFR-TS protein.[54] TS participate in the folate metabolic pathway by catalyzing the conversion of methylene THF to DHF Pteridine reductase (PTR1) is an NADPH-dependent short-chain reductase which participates in the salvage of pterins. PTR1 has been reported to be involved in the reduction of biopterin to dihydrobiopterin and terahydrobiopterin and is capable of converting DHF to THF. Several Leishmania DHFR
inhibitors are reported in literature amongst which compounds 17, 18 and 22 have been described to be novel through docking studies (Fig. 1.4.).[55] Compounds 16, 19–21, 24, 25–27 were identified to be potent DHFR inhibitors derived from derivatives of 2, 4-diaminopyrimidine. Several quinazolines 28–32 and pteridine analogues were also demonstrated to be potent antileishmanials by inhibiting the DHFR enzyme.[56]
vitro activity sufficient to show efficacy in the mouse model. In addition to that, the enzyme deoxyuridine 5-triphosphate nucleotidohydrolase (dUTPase, E.C. 3.6.1.23) is involved in nucleotide metabolism,[57] it catalyses the hydrolysis of deoxyuridine triphosphate (dUTP) to deoxyuridine monophosphate (dUMP) and inorganic pyrophosphate in the presence of magnesium ions (Figure 1.5.). dUTPase is widespread in nature and has been found in a variety of prokaryotic and eukaryotic organisms as well as in many viruses.[58] It was shown to be essential for viability in Leishmania major [59] and is essential for all cellular systems. The enzyme is thought to be crucial to DNA integrity in two ways. It prevents the build up of dUTP and ensures the provision of dUMP, the substrate for thymidylate synthase in the biosynthesis of deoxythymidine triphosphate (dTTP). As a result, a low dUTP/dTTP ratio is maintained, which greatly reduces uracil incorporation into DNA because DNA polymerases do not discriminate between dUTP and dTTP. Under normal circumstances, following dUTP misincorporation into DNA, uracil is replaced by thymine through a repair process catalyzed by uracil−DNA glycosylase. However, when dUTP levels are abnormally high, repetitive cycles of introduction and excision of uracil take place, giving rise to DNA fragmentation and ultimately cell death.

![Figure 1.5](image)

The pyrimidine anologues dUPNPP 33 and dTPNPP 34,[60] compounds 35 and 36 are most potent nonnucleotide inhibitors of human dUTPase. Essentially, two of the compounds were notably active against the L. donovani parasite, with the best IC50 values are for 37 (13 µM) and 38 (17 µM). The amino acid uracil acetamide compounds 39 and 40 were evaluated for dUTPase enzyme inhibition in anti-leishmanial parasite successfully.

C. Glutathione S-transferase

Recent studies on GST have shown their involvement in cell proliferation[61] and regulation of lipid peroxides responsible for the cell apoptosis[62] and have been documented to be a promising target for antileishmanial chemotherapy.[63] Several functions of the enzyme, including catalytic glutathione conjugation, passive ligand binding and modulation of signal transduction, may be selectively targeted for inhibition.
Figure 1.6 Structure of dUTPase inhibitors

Figure 1.7 GST Inhibitors

Recently bivalent analogues of the non-selective GST inhibitor of ethacrynic acid were prepared and compounds 41–49 (Figure 1.7.) were found to possess promising inhibitory activity.[64]
D. J-Binding Protein of Leishmania
The DNA of Leishmania organisms contains a modified base, J (b-D-glucosyl-hydroxymethyluracil), which does not occur in higher eukaryotes. Leishmania species contain a protein, J-binding protein (JBP), that binds J-containing DNA sequences and is involved in the conversion of thymine to J. Deletion of JBP is lethal for the organisms. Therefore, most likely compounds that interfere with the binding of JBP to J are detrimental to growth or survival of Leishmania organisms. Because J and J do not occur in the host, such a compound might lead to a therapeutic drug for treatment of leishmaniasis. The compound 51 also recently found J-B Protein inhibitor.[65]

E. DNA Topoisomerases.
Sodium stibogluconate and Ureastibamine, two potent antileishmanial drugs specifically inhibit the relaxation of supercoiled plasmid pBR322 catalyzed by DNA topoisomerase I of Leishmania donovani. DNA topoisomerases[66] are ubiquitous enzymes, catalyzing changes in the topological state of duplex DNA during replication,[67] transcription, recombination and DNA-repair processes. [68] Topoisomerases are classified as type-I or type-II enzymes according to their specific mode of action. Type I DNA topoisomerases are monomeric ATP independent enzymes with relaxation activity for positively and negatively supercoiled DNA. They introduce single-stranded breaks in DNA followed by passage and rejoining, thereby allowing single step changes in the linking number of circular DNA. They have been subdivided into two distinct classes: type IA enzymes that bind covalently to the 5 end and type IB enzymes that form covalent bonds with the 3 end of the broken DNA strand. Type II DNA topoisomerases are homodimeric ATP dependent enzymes, which introduce transient double-stranded breaks in the double helix, followed by passage and rejoining. These enzymes can relax, catenate–decatenate, knot–unknot or introduce supercoils in the DNA molecule.[69-71]Indeed out of several topoisomerase inhibitors showing antileishmanial activity which has been recently reported a few are presented in Figure 1.9.

F. Polyamine Transport
The transport of putrescine and spermidine into Leishmania donovani promastigotes and Leishmania mexicana promastigotes and amastigotes has been characterized by Basselin et. al.[72] Polyamine transport was shown to be saturable and temperature-sensitive for both developmental stages of Leishmania. Transport was pH-dependent with pH optima of 7.4 and 5.5 for promastigotes and amastigotes, respectively. The uptake process was independent of extracellular Na+, but inhibited by protonophores and H+ ATPase inhibitors
According to inhibition data suggested that putrescine and spermidine use different transporters, the aromatic diamidine pentamidine, the drug of choice for treatment of antimonial-resistant cases of leishmaniasis, inhibited both putrescine and spermidine transport non-competitively. Herein the polyamines have an additional role participating in the endogenous redox equilibrium through the compound (N1, N8-bis (glutathionyl) spermidine), named trypanothione T(S)2, which is maintained in its reduced dithiol form, T(SH)2, by trypanothione reductase (TR).[73] T(S)2 is the major redox reactive metabolite in trypanosomatids[74] because of which this molecule and the enzymes involved in its metabolism are good drug targets.[75] This role of spermidine as an essential polyamine required for the maintenance of normal proliferation is supported by the sensitivity of L. donovani cell lines deficient in the spermidine synthase gene.[76] It has been earlier proposed that the complete arrest of polyamine dependent cellular proliferation in Leishmania could be achieved by blocking the de novo biosynthesis as well as uptake of polyamines from the surroundings.[77-79] It was also demonstrated that the promastigote and amastigote stages express different polyamine transporters and have separate transporters for both putrescine and spermidine.
Recently Avery et al. have reported the synthesis of substituted ethylenediamines possessing micromolar activities against Leishmania promastigotes.[80] 63-65 [81] and the EC50 of the active compounds of the series 66, 67 and 68 (Fig. 1.10.) were 0.88, 0.67 and 0.37 µM respectively.

G. Squalene synthase

Squalene synthase (SQS, E.C. 2.5.1.21) catalyzes the first committed step in sterol biosynthesis and is currently under intense study as a possible target for cholesterol-lowering agents in humans, but it has not been investigated as a target for anti-parasitic chemotherapy. SQS is a membrane-bound enzyme in both T. cruzi epimastigotes and Leishmania mexicana promastigotes with a dual subcellular localization, being almost evenly distributed between glycosomes and mitochondrial/microsomal vesicles. Kinetic studies showed that the parasite enzymes display normal Michaelis-Menten kinetics and the values of the kinetic constants are comparable to those of the mammalian enzyme. The synthesized compound of 3-(biphenyl-4-yl)-3-hydroxyquinuclidine 69 (BPQ-OH),[82] a potent and specific inhibitor of mammalian SQS and found that it is also a powerful non-competitive inhibitor of T. cruzi and L. mexicana SQS. BPQ-OH induced a dose-dependent reduction of proliferation the extracellular stages of these parasites with minimal growth inhibitory concentrations (MIC) of 10-30 µM. From these results support the notion that SQS inhibitors could be developed as selective anti-leishmanial agents. The Glaxo[83] and Merck[84] groups discovered that Squalestatin 71 (Zaragozic Acid A) is a potent, selective inhibitor of squalene synthase both in vitro and in vivo.[85] Many synthetic analogues 72–78 are reported as potent squalene synthase inhibitors in leishmania (Figure 1.11.) and the piperidine-4-acetic acid derivative 79 and 80 was also effective inhibitors.[86]

H. Lanosterol 14a-demethylase inhibitors

Inhibitors of sterol and phospholipid biosynthesis in kinetoplastid parasites such as different species of Leishmania have potent and selective activity as chemotherapeutic agents in vitro and in vivo. The biosynthetic pathway of ergosterol, the major sterol in fungi as well as Leishmania
spp. and *Trypanosoma cruzi*, is a target for some of the most important antifungal drugs. Two classes of these drugs, the allylamines (for example, terbinafine) that inhibit squalene epoxidase and the azoles (for example, ketoconazole and itraconazole) that inhibit C14α-demethylase, have generated the most interest as antileishmanials. In a comparative study on the sensitivity of promastigotes to ketoconazole, *L. donovani*, *L. braziliensis*, and *L. amazonensis* were found to be more sensitive than *L. aethiopica*, *L. major*, *L. tropica*, and *L. mexicana*. However, in contrast, Rangel et al. observed that *L. braziliensis* was relatively insensitive to ketoconazole and the bistriazole D0870 (fig. 1.13), whereas *L. mexicana* was sensitive to ketoconazole. Both sets of results differ from those of an earlier study using an amastigote-macrophage model, which showed that *L. donovani* was more sensitive to ketoconazole than *L. mexicana* or *L. major*. The lack of concordance is probably due to different assay conditions.

![Figure 1.11. Squalene Synthase Inhibitors](image-url)

Schematic diagram of the sterol biosynthesis pathway in protozoan parasites such as *Trypanosoma cruzi* and fungi is shown in Figure 1.12. Each arrow corresponds to a distinct metabolic step. The sites of action of sterol biosynthesis inhibitors (SBI) are currently used or still under development are indicated with dashed arrows (Figure 1.12.).
The results of the previous study indicate the potential of lipid biosynthesis inhibitors as useful therapeutic agents in the treatment of leishmaniasis. Some of the potent azole containing “Lanosterol 14a-demethylase inhibitors” are shown in (Figure 1.14.)

I. Azole based lead compounds

The imidazole and triazole derivatives carrying either the carbaldehyde or the difluoromethylene functionalities were reported to be active against the *L. amazonensis* promastigotes. Among the compounds tested 82 and 83 inhibited the parasite growth significantly at the IC50 of 1.5 and 2.6 µM respectively.[91] The substituted indole-alkyl azole compounds 84 and 85 were discovered to be highly active against the *Leishmania* amastigotes, therefore investigation towards the oral efficacy of these compounds was being taken up.[92] One of the compound exhibited good anti-leishmanial activity against the promastigote form of *L. major* at non-cytotoxic concentrations that is, 1-[(5-chloro-2-thienyl) carbonyl]-4-[5-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4 thia-diazol-2-yl] piperazine 86 which also showed good activity against intracellular form of *L.*
major [93] and it was found that among all the 1H-pyrazole-4-carbohydrazides derivatives examined, the most active compounds 88 and 87 derivatives which showed to be most effective on promastigotes forms of L. amazonensis than on L. chagasi and L. braziliensis species. When tested against murine peritoneal macrophages as mammalian host cell controls of toxicity, 1-(4-Br-phenyl)-N’-[(4-NO2-phenyl)methylene]-1H-pyrazole-4-carbohydrazides 88 (EC50 = 50 µMl–1) and 1-(4-NO2-phenyl)-N’-[(4-Cl-phenyl)methylene]-1H-pyrazole-4-carbohydrazides 87 EC50 = 80 µM.

was reasonably toxic. However, both compounds were less toxic than pentamidine and ketoconazole.[94] Compounds 89–91 and 103–105 (MIC ≤ 5 µg/mL and ≤ 10 µg/mL) were equipotent to ketoconazole, econazole, and miconazole.[95] The O-alkyl substituted compounds (92–95) were found to be more active in both screening paradigms anti-microbial and convulsant. Compound 98 (piperazine analog) was found to be most active compound in invitro.
Imiquimod stimulates nitric oxide production from macrophages. The promising results showed for the 3'-diethylaminomethyl-substituted compounds as the most active (IC50 = 0.39 [101] and 0.12 µM [102]). Compound 106 was tested in L. donovani infected macrophages and resulted (IC50 0.78 µM), appreciably more active than the reference drug miltefosine (IC50 1.74 µM). The obtained results from the compound 107 and its analogues showed activity comparable with the current used antiprotozoal drugs metronidazole and pentamidine, however, exhibited even higher bioactivity especially toward L. mexicana. A small library of 2,2’-[(α,ω-alkanediylbis(oxyphenylene)] bis-IH-benzimidazoles has been prepared and screened in vitro against Leishmania donovani. Among the six tested compounds one derivative emerged as promising hit characterized by IC50 values lower than that determined for pentamidine against L. donovani. A series of 52 pentamidine congeners was reported in which the flexible pentyldioxy linker in pentamidine was replaced with several restricted linkers. The most active antileishmanial agents obtained from the series 109 and 110 were reported to be 10 and 7-fold more potent than pentamidine, respectively. Three series of benzimidazole N-oxide derivatives were developed by Boiani, M. et al. using simple chemical methodologies. Among them, benzimidazole 1,3-dioxide derivatives displayed remarkable activities against T. cruzi epimastigotes and Leishmania promastigotes. Due to their therapeutic index, compounds 111 and 112 were evaluated in vivo in an acute model of Chagas disease, where both derivatives were able to rescue mice from death and lowered anti-T. cruzi antibodies with only 10 doses in a short-term scheme. Structureactivity relationship studies pointed to the relevance of the reduction potential and electrophilicity to anti-T. cruzi activity. Chudhari et al. has successfully developed a series of imidazole-linked, anthraquinone-based topoisomerase inhibitors 113-116 by systematically varying the side chain appended to the central aromatic moiety. Five of the nine drugs.
studied have emerged as very potent and selective inhibitors of topo I of *L. donovani*. As the compounds are also synthetically facile and chemically stable, the observations made in the present study provide useful insights toward developing potent inhibitors of the parasitic enzyme. Indeed, the nature of the amine-based side chain and its pKa would hold the key in such design. A series of 4-anilino-1H-pyrazolo [3,4-b] pyridine-5-carboxylic esters were showed promising anti-leishmanial results with [IC50 0.39 (117) and 0.12 µM (118)]. Molecular modeling, using semi empirical AM1 method, predicted the most active compounds through the low-energy conformers superimposition on amodiaquine structure.[105]

**CONCLUSION**

Thus from the preceding text it is clear that in the recent past several efforts have been made to investigate the bioactivity in new molecular frameworks with little success. However better understanding of the enzyme structures and molecular targets have led medicinal chemists not to deter from the path of exploration. It is obvious that a few targets are similar to this disease and new compounds may be developed which display activity for this disease. This exercise is only an attempt in this direction.

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