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Review

Natural product based leads to fight against leishmaniasis

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ABSTRACT

The growing incidence of parasitic resistance against generic pentavalent antimonials, specifically for visceral disease in Indian subcontinent, is a serious issue in *Leishmania* control. Notwithstanding the two treatment alternatives, that is amphotericin B and miltefosine are being effectively used but their high cost and therapeutic complications limit their use in endemic areas. In the absence of a vaccine candidate, identification, and characterization of novel drugs and targets is a major requirement of leishmanial research. This review describes current drug regimens, putative drug targets, numerous natural products that have shown promising antileishmanial activity alongwith some key issues and strategies for future research to control leishmaniasis worldwide.

Contents

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1. Introduction

Leishmaniasis is a poverty-associated disease caused by more than 20 species of protozoan parasites that belong to family kinetoplastida and genus *Leishmania*. It is a wide spectrum of vector born disease with great epidemiological and clinical diversity. The disease is spreaded by more than 30 species of sand fly of the genus *Phlebotomus* in the old world and *Leutzomia* in the new world.[1] The *Leishmania* species are generally zoonotic in nature and carried by rodents and canids that are main reservoir hosts. Only two *Leishmania* species can maintain anthroponotic human–human cycle. They are *Leishmania donovani*, responsible for visceral leishmaniasis (VL) in Indian subcontinent & East Africa, and *Leishmania tropica*, responsible for cutaneous leishmaniasis (CL) in the old world.[2,3] Three major clinical forms of the disease are visceral (VL), cutaneous (CL), and mucocutaneous leishmaniasis (MCL), which differ in immunopathologies and degree of morbidity and mortality. Most VL caused by *L. donovani* is fatal if untreated, whereas CL caused by *Leishmania major, Leishmania mexicana, Leishmania braziliensis,* and *Leishmania panamensis* is significantly associated with morbidity.[4,5]

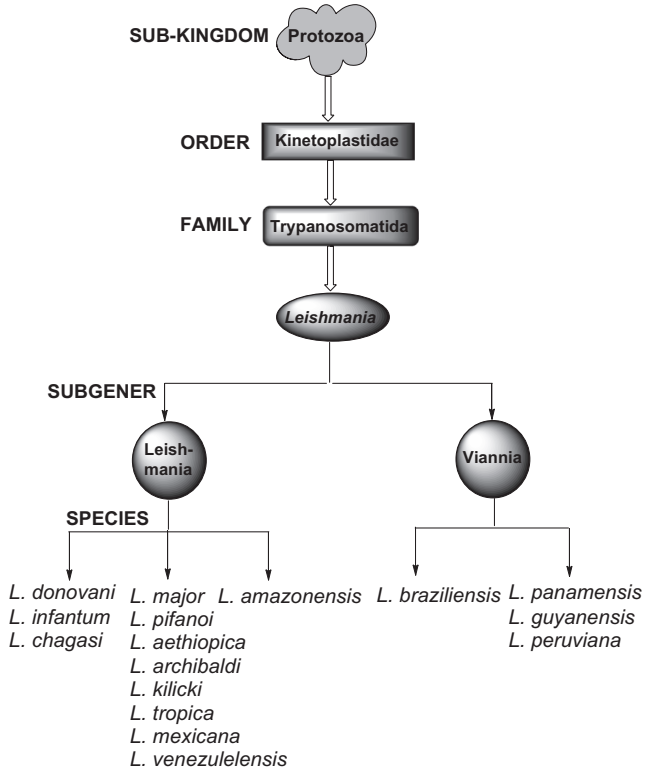


Figure 1. Taxonomic classification of *Leishmania* spp.

Leishmanial infections are prevalent in more than 98 countries most of which are either poorly developed or developing. The global annual burden of all forms of leishmaniasis is approximately 12 million per year in which about 350 million people are at risk however, exact statistical data are lacking.[6] In a recent report, it has been observed that approximately 0.2 to 0.4 VL cases and 0.7 to 1.2 million CL cases occur each year however, there is gross under reporting of cases in endemic areas.[7] More than 90% cases of VL ensue in five countries: India, Bangladesh, Nepal, Sudan, Brazil, and 90% of CL cases occur in seven countries: Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia, and Syria.[8] Although, spread of disease in endemic and non-endemic regions is multi-factorial but lack of effective control measures for both, parasite and its vector are main factors.[9]

The poor knowledge about the disease and lack of effective health policies are the primary hurdles in the elimination of leishmaniasis from every corner of the world is far from reality. Sodium stibogluconate, a drug belongs to class of pentavalent antimonials, is the cornerstone of leishmanial chemotherapy in disease endemic countries especially in Indian subcontinent.[10] However, the growing incidence of resistance has raised serious concern for its use in disease endemic area. The other second line drugs like amphotericin B, its liposomal formulations, and miltefosine have become prevalent as first line treatments. These drugs are being used in the treatment with more efficacies and dramatic potential for

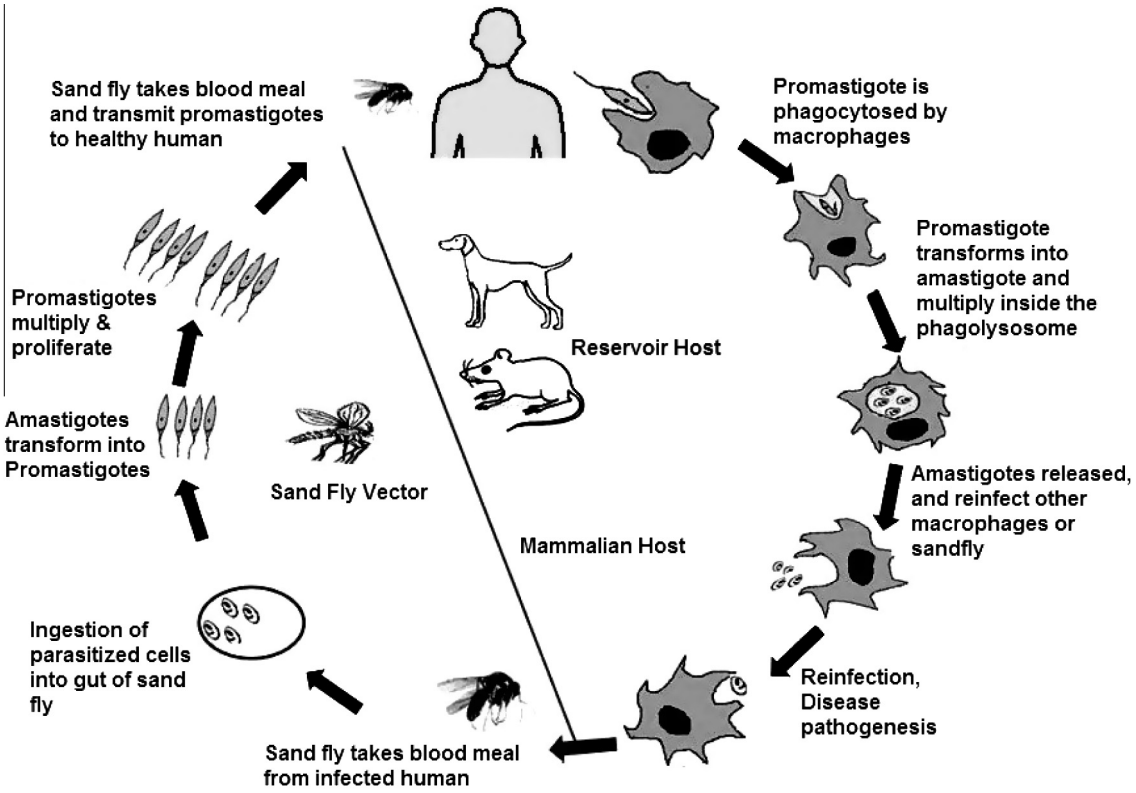


Figure 2. Life cycle of *Leishmania.*

curing leishmaniasis; however, their high cost and therapeutic complications limit their use.[11] Other drugs such as paromomycin and pentamidine have shown some usefulness and would be a potential supplement in the drugs regimen but their use and availability in disease endemic regions is limited.[12] The efficacy of different drugs seems to vary according to the *Leishmania* species involved. Although the drugs recommended for the treatment of leishmaniasis have been used for decades and some adverse effects are known, there are no systematic reviews about their safety.

The main thrust of protozoan research worldwide is focused on the identification and characterization of cellular targets and answering the problem of drug resistance that occur in leishmaniasis. However, the increasing incidences of multi drug resistance (MDR) in leishmaniasis and absence of vaccine necessitates a focused attention on identification of novel targets and development of new drugs.[13,14] This review hereafter analyses the limitation of current drug regimen, presents new drug targets, and broadly discuss numerous antileishmanial natural products reported from mid-1984 to early 2013.

2. Leishmania taxonomy

Taxonomic classification of genera *Leishmania*[4] is summarized in Figure 1.

3. Morphology and life cycle

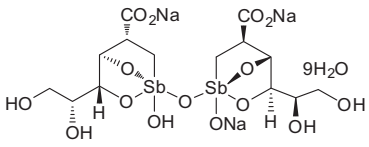
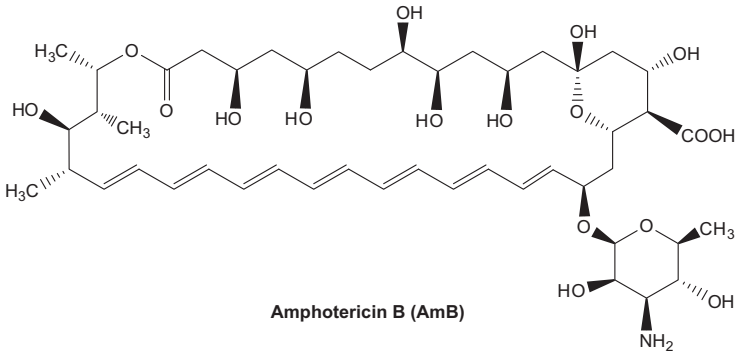
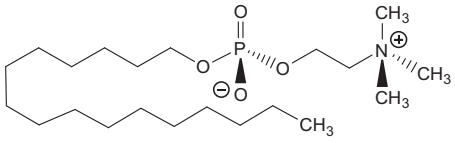
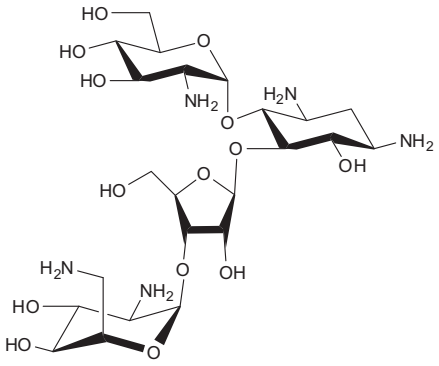
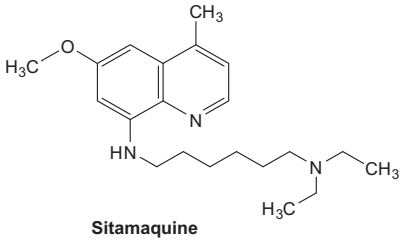
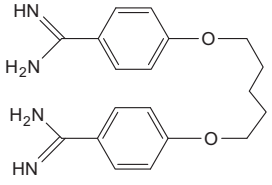
The digenetic life cycle (Fig. 2) of *Leishmania* consists of motile, flagellated, extracellular promastigotes (10–15 μm in length, measuring 1.5–3.5 μm at their widest part) form in the gut of sand fly vector that infects mammalian host. The promastigotes are phagocytosed by host macrophages where they transform into nonmotile, spherical, nonflagellated amastigotes (approximately 2–3 μm in diameter), which survive and multiply within phagolysosomal compartment of macrophages.[15]

Infection starts after a sand fly takes a blood meal from an infected host (e.g., canines, marsupials, edentates, and rodents). Small amounts of blood, lymph, and macrophages infected with *Leishmania* amastigotes are ingested. Once ingested, the amastigotes migrate to the midgut of the sand fly, where they transform into the promastigotes by binary fission and move forward (after four or five days) to the esophagus, and the salivary glands. Infected sand fly during the second blood meal regurgitates the infectious promastigotes into the bloodstream of the host vertebrates. Once inside the bloodstream of reservoirs, phagocytosis of promastigotes by the host mononuclear phagocytic cells takes place. The *Leishmania* are able to resist the microbiocidal action of the acid hydrolases release from the lysozymes, thus easily survive, and multiply inside the macrophages. Eventually, the host cells lyse, releasing the free parasites which spread to new cells and tissues of different organs (especially the spleen, liver and bone marrow) causing lesions and tissue destruction.

4. Current drug regimen for leishmaniasis

Over more than five decades, pentavalent antimonials, the generic sodium stibogluconate (pentosam) and branded meglumine antimoniate etc., are being used in the treatment for all forms of leishmaniasis worldwide (Table 1), and still they are the first line drugs of choice where resistance is not reported.[16,17] The recommended therapeutic dose of 20 mg per kg of body weight for 20–30 days achieves more that 95% cure rate but in the state of Bihar, India, more than 60% patients were reported to develop resistance for pentamonials.[18] **Sodium stibogluconate,** marketed under the name Pentostam to treat leishmaniasis, is only available for administration by injection. It belongs to the class of drugs known as the

Table 1
Current drugs, structures, doses, drug targets and their mechanism of action

Drug	Doses	Target	Mechanism of action
 **Sodium stibogluconate**	20 mg/kg body weight, daily for 30 days, intra-muscular	Trypanothione reductase (TR)	Active trivalent SbIII form inhibits trypanothione reductase and exposes parasite to oxidative stress of the host.
 **Amphotericin B (AmB)**	0.75–1.0 mg/kg body weight for 15–20 infusions either daily or on alternate days	Sterol metabolism	Forms complex with 24 substituted sterol, ergesterol in the cell membrane that create pores and subsequent ion imbalance, which ultimately lead to cell death.
 **Miltefosine**	100 – 150 mg for four weeks, oral	Ionositol metabolism	Interacts with the cell memebrane of parasite by cell surface modulation, and phospholipase activation
 **Paromomycin**	16 mg/kg for 21 days or 20 mg/kg for 17 days, intramuscular	Protein synthesis	Inhibit protein synthesis by binding to 30S subunit of ribosome and affects mitochondria
 **Sitamaquine**	1.72–2 mg/kg/ day for 28 days	Mitochondrial respiratory chain	Mechanism still uncertain, might effect through mitochondrial respiratory chain.
 **Pentamidine**	2 mg/kg every other day for a total of seven injections, intramuscular	Kinetoplast DNA	Primary effect uncertain, accumulated by parasite and bind to kinetoplastid DNA

pentavalent antimonials since it contain antimony in its +5 oxidation state. The **pentavalent antimony** (Sb^V) considered as a pro-drug, which is further converted to trivalent antimonite (Sb^{III}) that eventually kills parasite. Both forms of antimonials, Sb^V and

Sb^{III}, have been reported to kill *Leishmania* species by DNA fragmentation, suggesting its role in apoptosis however, the exact mechanisms of action are still unexplored.[19–21] In addition, pentamonials are also known to inhibit trypanothion reductases, glycolysis, and metabolic pathways. They increase efflux of intracellular thiols by an ATP binding cassette (ABC) transporter, multi drug resistant protein A (MRPA).[22–24] Further, various genes identified in antimonial unresponsive clinical isolates suggests the multifactorial mechanism of resistance and hence this disease seriously require therapeutic alternatives.[25,26]

Fortunately, few treatment alternates are available albeit these are not true antileishmanials in nature. Among these, the first drug of choice is **amphotericin B** (AmB), a polyene antifungal, is widely being used in endemic regions where antimonials resistance is common.[27] AmB shows high affinity for ergosterol, the major sterol of leishmanial cell membrane forming aqueous pores leading to increase membrane permeability and killing of parasite. Despite its high efficiency, AmB is toxic and may cause numerous side effects.[28] Adverse effects of plain AmB have been circumvented with its three clinical formulations in which deoxycholate have been replaced by other lipids are liposomal AmB (L-AmB: Ambiosome), AmB colloidal dispersion (ABCD: Amphocil) and AmB lipid complex (ABL: Abelcit). These lipid formulations retain their antifungal activity, show very high efficacy, and are less toxic. In VL, liposomal AmB has been proved as an efficient drug with more than 95% efficacy over amphocil and abelcit but high cost limits its use to a common person suffering from this deadly disease in the diseases endemic areas of Indian subcontinent and Sudan.[29,30] In addition, resistance has been reported in clinical isolates that warrants a possibility of resistance.

Miltefosine, an alkylphosphocholine (hexadecylphosphocholine), is the first oral drug originally developed as anti cancer agent.[31] It is used for the treatment of VL at doses 50 mg/day, 100 mg/day and 2.5 mg/kg/body weight/day for adults >25 kg, adult (>50 kg) and children, respectively for 28 days but unfortunately side effects raise questions against extreme efficacy of this drug.[32] The main observance of this drug is its long terminal residence time and teratogenicity. Miltefosine has a median long half-life of approximately 152 h, which could encourage development of clinical resistance. The activity of miltefosine is due to intracellular accumulation of drug, which is regulated by drug transporters.[33] Although, the exact mode of antileishmanial action is still unclear but it has been found that it causes apoptosis like processes in *L. donovani* but how does it occur is still unknown.[34]

Paromomycin is chemically an aminoglycosidic antibiotic and has both antileishmanial and antibacterial activity. It cures both,

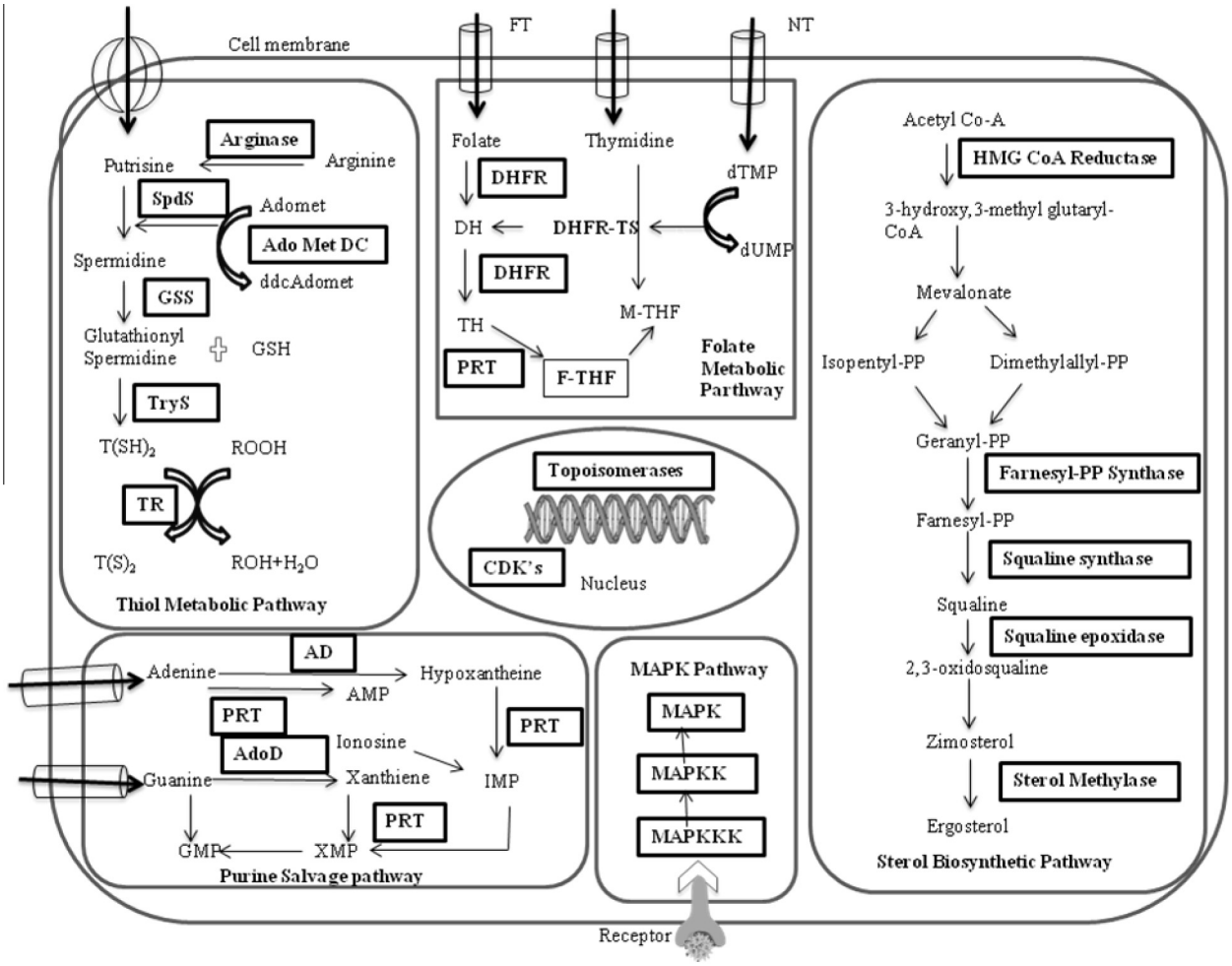


Figure 3. An overview of potential drug targets (in bold letters, inside box) of various biochemical and metabolic pathways in *Leishmania* species. PRT: Phosphoribosyl transferase, AD: Adenine deaminase, AdoD: Adenosine deaminase, SpdS: Sprermidine Synthase, TR: Trypanothione Reductase, TDPx: Tryparedoxin Peroxidase, DHFR: Dihydrofolate Reductase, DHFR-TS: Dihydrofolate ReductaseThymidylate Synthase, MAPKKK: Mitogen Activated Protein Kinase Kinase Kinase, MAPKK: mitogen Activated Protein kinase Kinase, MAPK: Mitogen Activated Protein Kinase, POT1: Putrisine transporter1, FT: Folate transporter, NT: Nucleotide Transporter, CDKs: Cyclin dependent kinases; ADP: Adenosine Diphosphate, AMP: Adenosine Monophosphate, IMP: Ionosine Monophosphate, XMP: Xantheine Monophosphate, GMP: Guanosine Monophosphate, GDP: Guanosine Diphosphate, DH: Dihydrofolate, TH: Tetrahydrofolate, M-THF: Methylene Tetrahydrofolate.

VL and CL (more effectively) but limited availability restricts its use in endemic regions.[35,36] This drug has recently undergone phase 4 trial and achieved 99% and 94% initial and final cure rate, respectively after 6 month at a dose of 11 mg/kg/day. The mechanism of action is largely unclear. Recently, it has been shown that cationic paromomycin binds to the negatively charged leishmanial glycocalyx suggesting mitochondria as a primary target.[37] In addition, paromomycin in *L. donovani* promotes association of 50S and 30S subunits of both, cytoplasmic and mitochondrial ribosomes, and stops their recycling that eventually inhibits protein synthesis.[38] Due to its limited use, resistance is not yet reported in outpatient treatment but resistance has been reported in vitro in *L. donovani* and *L. tropica.*[39–41]

Sitamaquine, chemically 8-aminoquinoline (an analog of antimalarial primaquine), is the only drug developed originally for treatment of VL by Walter Reed Army Institute, USA. The advantages associated with this drug are its oral administration, and short half-life of 26 h. At high concentration, it affects parasite motility, morphology, and growth.[42] It accumulates in *Leishmania* cytosolic acidic compartments, acidocalcisome however, correlation between its action and accumulation is not clear.[43] In clinical trials, a cure rate ranging from 87% to 100% has been achieved at doses between 1.5 and 3.0 mg/kg/day for 28 days.[44] However, with available status of knowledge, more studies are required to understand its efficacy, mode of action as well as toxicity.

Pentamidine, a second line drug whose isothionate and methansulfonate salts are mainly used for the treatment of VL. Currently this drug is mainly used for antimony resistance and immuncompromised patients as well as in combination with other drugs but with limited success.[45] Although, its precise mode of action is not known but it is reported that the drug enters inside promastigote through arginine and polyamine transporters and gets accumulated in mitochondria and inhibit mitochondrial topoisomerase II.[46–49] Pentamidine is highly toxic and causes hypoglycemia, nephrotoxicity, hypotension, etc.

Despite the significant progress during last few years in leishmanial chemotherapy, an ideal drug is still awaited. The current drug regimen is highly compromised because of price, feasibility, safety, efficacy, toxicity, side effects, and probability of growing resistance. The most important consideration of present therapeutics is the gradual increase in resistance to antimonials in particular and others in general, predominantly in Indian subcontinents. In recent years, clinical trials of combination therapies are operational. Combination of two or more drugs could reduce treatment duration and drug doses and consequently drug toxicity but probabilities of resistance development against currently available drug regimen could not be overruled. Hence, identification of novel drug targets and development of a true antileishmanial drug is priority of research to curb leishmanial infections in endemic areas of disease-affected countries.

Possible drug targets: In the past several decades, very little emphasis has been made on novel control strategies to find new drug targets for leishmaniasis. The search of novel parasitic drug targets mainly focus on biochemical and metabolic pathways.[50] The target enzymes of these pathways that have significant structural and functional differences from its mammalian counterparts are the most reasonable candidates for the selective inhibition using chemical compounds. A desirable target should involve vital aspects of parasitic growth and metabolic pathways, whose inactivation lead to parasite death and inability to persist in the host. Moreover, the realistic targets that are involved in the pathogenesis of disease should be given more consideration in drug designing and development. Further strategies may be devised to target more than one of the metabolic pathways to shorten the lengthy invasive leishmanial treatment, which will eventually eliminate the chances of drug resistance. The various possible targets of *Leishmania* species are summarized in Figure 3.

5. Enzymes of metabolic pathways

5.1. Polyamine pathway

The putrescine, spermidine, and spermine like polyamines and their metabolic pathways play very important role in the growth and differentiation of parasite from promastigote to amastigote stages.[51] The polyamines are significantly involved in parasitic survival as they play important role in the down regulation of lipid peroxidation, and make the environment compatible for survival of parasite in the host cells.

In *Leishmania,* the first target enzyme of polyamine biosynthesis is arginase that converts arginine to ornithine,[52] which is further converted to putrescine through decarboxylation by enzyme ornithine decarboxylase, a next target enzyme. Putrescine is converted ahead to spermidine and spermine by the enzymes complex, s-adenosylmethionine decarboxylase (Adomet DC) and spermidine synthase.[53] The parasites over expresses these enzymes however, polyamine pool remains unchanged or marginally affected during their growth that implies existence of some regulatory mechanisms, which in turns offer a greater possibility for a future drug target.[54] Moreover, these substances are also responsible for Th_2 type immune response that protects parasite from the host defense mechanisms, which further prove their importance to curb the leishmanial infections. In addition, the polyamine transporters (LmPOT1) that transport both, putrescine and spermidine should also be explored as drug target since they essentially regulate intracellular poyamines level.[55,56] Hence, development of inhibitors to stop polyamine biosynthesis and transportation may be quite useful and may result into a novel antileishmanial therapeutic approach.

5.2. Purine pathway

Leishmania lacks enzymes required for *de novo* biosynthesis of purine nucleotides and depend on purine salvage pathway to utilize purine bases from the mammalian host. The enzyme phosphoribosyl transferase (PRT) play a key mediator role in the salvage of purines as it converts de-phosphorylated purines into nucleoside mono-phosphate. In *Leishmania* three PRT's have been identified that is adenine phosphoribosyl transferase (APRT), hypoxanthine guanine phosphoribosyl transferse (HGPRT), and xanthine phosphoribosyl transferse (XPRT).[57] Nucleotide transporter transports adenine from host to parasite where it gets converted to hypoxanthin by the activity enzyme adenine deaminase. The HGPRT converts hypoxanthin to ionsine mono-phosphate and guanine-to-guanine monphosphate. Due to subtle differences in substrate specificity of parasitic purine salvage enzymes from host enzymes, various inhibitors can be either designed or developed to target them for for example allopurinol that targets HGPRT.[58]

5.3. Glycolytic pathway

In trypanosomatids a peroxisomes like organelle, glycosome, play important role in many metabolic activities like glycolysis, oxidation of fatty acid, lipid biosynthesis, and purine salvage pathways, etc. Like other trypanosomatids, *Leishmania* depends only on host for source of carbon to meet nutritional and energy requirements. Enzymes like hexokinase (HKK), phospho-fructokinases (PFK), etc. are autocatalytic, and accumulation of their hexose phosphate intermediates may lethal to parasite. To escape from this lethality, a unique mechanism evolved in trypanosomes

wherein first seven steps of glycolysis occur inside glycosomes while rest three occurs in cytosol. This unique compartmentation regulates HXK and PFK autocatalysis, which depend on ATP.[59] Hence, unique organization of glycolytic pathway and evolutionary distance from mammalian host offer great possibility for potential drug targets.

5.4. Thiol pathways

A characteristic thiol metabolic defense system is present in protozoan parasite, which is essentially involved in neutralization of host oxidative response. The *Leishmania* parasite makes silent entry into the host macrophages, and proliferates inside the hazardous environment of phagolysosomes. It remains a puzzle that how parasite protects itself from reactive oxygen and reactive nitrogen species produced by host immune effectors mechanisms. The thiol metabolic defense mechanism of parasite includes a cascade of three antioxidant enzymes of trypanothion metabolism that are required to counteract host oxidative stress. Trypanosomatids contain trypanothion [$T(SH)_2$], a dithiol, instead of a glutathione as a main reductant and possess redox couple [$T(SH)_2$]/trypanothione reductase (TR).[60,61] Why [$T(SH)_2$] is evolved inside parasitic system remains an enigma. *Leishmania* lacks H_2O_2 detoxifying catalase, a glutathione dependent antioxidant enzyme. However, it expresses antioxidant enzyme like TR, thioredoxin (Txn) and triparedoxin peroxidase (TxnP)/peroxidoxine. These enzymes are mainly responsible to protect parasitic proteins, lipids and nucleic acid from host oxidative damage.

TR reduces thioredoxin, tryparedoxin and some short chain protein like dithiols. These reactions delivers reducing equivalent to peroxidase for detoxification of toxic fee radicals. Trypanothione maintains low molecular mass thiols trypanothione and monoglutathionyl spermidine, glutathiol and ovathiol in their reduced state, which is essentially required for uninterrupted progression of metabolic pathways of the parasite.[62] In *Trypanosoma brucie* and *L. infantum,* it is reported that [$T(SH)_2$] is capable of reducing NO and Fe into a harmless stable dinitrosyl iron complex with 600 time more affinity than mammalian GSH reductase system that protects parasites from potentially lethal nitric oxide (NO).[63,64] The absence of this pathway in mammalian host and trypansomatids sensitivity towards oxidative stress, trypanothione reductase, and enzymes of trypanothione metabolism offers an attractive drug targets to design novel antileishmanial compounds.[65] Homology modeling of *L. infantum* TR and mammalian glutathione reductase (GR) has shown remarkable difference in their three dimensional and catalytic active sites. Hence, specific inhibitors can be designed against TR to get an ideal compound that will stop parasite growth without altering host GR activity.

5.5. Sterol pathway

In *Leishmania* species, the main endogenous sterols are ergosterol and stigmasterol. These are an important component of cell membrane and responsible for various cellular functions as well as maintain integrity of cell membrane. Ergosterol, which completely differs from mammalian counterpart cholesterol, has two important functions; first, it is a structural component of cell membrane and second, it might play hormonal role. Various inhibitor of sterol biosynthesis have been found to be effective and potent antileishmanial agent. Azasterols, a known class of s-adenosyl-L-methionine, showed antileishmanial activity and inhibits 24-methyltransferase, which is a vital enzyme in ergosterol biosynthesis.[66] Other sterols inhibitors like azol and triazole, which inhibit 14α-methylsterol 14-demethylase, have also been found effective against *Leishmania* species.[67] Although, *Leishmania* has potential to survive in altered sterol profile hence the most appropriate way to use these targets is combinational approach by the use of inhibitors of sterol biosynthesis along with inhibitors of other metabolic pathway.[68]

5.6. Dihydrofolate reductase, metacaspase, and topoisomerase: key enzymes of cellular machinery

A key enzyme in folate metabolism, dihydrofolate reductase (DHFR) is linked to the production of thymidine.[69,70] DHFR reduces dihydrofolate to tetrahydrofolate using NADPH as cofactor. Therefore, inhibition of DHFR prevents biosynthesis of thymidine and as a consequence, DNA biosynthesis. Fortunately, this enzyme from *L. major* and *Trypanosoma cruzi* has been crystallized and the structural data may be exploited to observe structural difference between parasite and human enzymes that may help to design selective DHFR inhibitors.[71,72] An approach to discover novel parasite DHFR inhibitors using database mining has also been made to search the Cambridge structural database but DHFR as drug target but requires further attention.[73,74] In addition, the enzyme dihydrofolate reductase-thymidylate (DHFR-TS) that catalyzes conversion of dihydrofolate from methyhylene tetrahydrofolate (M-THF) and thymidine is related with parasite survival.[75] This enzyme offers an appropriate drug target and require further exploration In spite of these advantages, few potential resistance mechanisms to DHFR have been reported including over-expression of the enzyme DHFR-TS and enzyme pteridine reductase 1 (ptr I).[76,77] The enzyme ptr1 is predominantly involved in reduction of biopterin to dihydrobiopterin and tetrahydrobiopterin but it is also capable to reduce dihydrofolate to tetrahydrofolate. Hence, a combined strategy to target both DHFR and ptrI will be more effective to stop parasitic growth and survival.

Metacaspases (MCA) are orthologous to caspases and play crucial role in cellular apoptosis however; they are not well understood in pathogenic protozoa. It has been found that metacaspases may be possible candidates to induce programmed cell death in trypasomatids.[78] In *L. donovani* two metacaspases, LdMCA1 and LdMCA2 are reported.[79] A metacaspase from *L. major* (LmjMCA) has found to be essential for the proper segregation of the nucleus and kinetoplast.[80] In addition, it is well documented that metacaspase gene is actively expressed in amastigotes and procyclic promastigotes, which is essentially good. It has been observed that metacaspases on treatment with H_2O_2 trigger process of programmed cell death of parasites. It has also been found that parasites, which over express metacaspases are more sensitive to H_2O_2 induced programmed cell death.[81] Molecules that can target to metacaspase biosynthetic machinery and induces their early expression might prove as efficient antileishmanial agents. In addition, since they are required for chromosomal segregation and parasite survival, they can also be directly targeted.[82] However, more studies are required to understand the complete function of leishmanial metacaspases.

DNA topoisomerases are ubiquitous enzymes needed to remove torsional stress in DNA by introducing transient protein-bridge DNA breaks either on one (type I) or both (type II) DNA strands. Topoisomerases are major targets in cancer and bacterial chemotherapy.[83,84] In parallel protozoan parasites are not distinct and they also require topoisomerase specially topoisomerase II due to the presence of complex intercatenated network of thousands of minicircles and maxicircles in kinetoplastids. Topoisomerase II has been reported over expressed and shows increased activity in arsenite resistance *L. donovani* that shows their importance.[85] Antibacterial and anticancerous drugs like novobiocin, etoposide, and fluoroquinolones can be used to target topoisomerase II in order to inactivation of genetic integrity and cell survival.[86] More efforts are required towards topoisomerase targeted drug interaction and

development of anti-topoisomerase chemicals against drug resistant parasites.

5.7. Proteinases (peptidases)

Proteinases are divided into four types based on residues present on the active site of the enzymes that is cysteine, serine, aspartate, and metalloenzymes. In the recent years, these enzymes are increasingly being seen as potential drug targets and their inhibitors have been successfully introduced to treat diseases like HIV, hypertension, pancreatitis, multiple myeloma, etc. Cysteine proteinase (CP) is the most identified and characterized proteinase and has been found to play crucial role in infection, replication, and development of parasite. It also modulates host immune response in its favor. Hence, CP has become potential candidate for chemotherapy development.[87]

In *Leishmania*, two aspartic peptidases has been identified; one with sequence similarity to presenlin1 (PS1), which is a multipass membrane peptidase, and other with intra membrane signal peptide peptidase (SPP).[88] PS1 is potentially involved in autophagy while SPP cleaves the transmembrane domain of signal peptidase that may be vital drug target.[89] Approximately twenty-one threonine peptidases have been identified and classified as proteasome subunit in *Leishmania.*[90] The proteasome is a multisubunit, multicatalytic peptidase responsible for degradation of ubiquitinated proteins in the cytosol.[91] Similar to plasmodial and trypanosomatids proteasome, the proteasome of *Leishmania* is a potential therapeutic target, as the use of specific inhibitors in in vitro study has shown the proteasome was essential for growth of promastigotes and amastigotes.[92] Among several serine peptidases of protozoan, subtilisin-like serine peptidases, which participate in processing of secreted proteins, may be very useful as drug target.[93] Serine peptidase inhibitors *N*-tosyl-L-lysyl-chloromethylketone (TPCK) and benzamidine reduce viability and induce morphological changes in promastigotes, suggesting there usefulness as potential drug targets.[94]

5.8. Cyclin dependent and mitogen activated protein (MAP) kinases

Cellular processes like cell division cycle, transcription, apoptosis, and differentiation are regulated by various cyclin dependent kinases (cdk). Genomic analysis of *Leishmania* species has reported 10 orthologous cyclins in their genome. In *L. major* one additional mitotic like cyclin A (CycA) is also present.[95,96] Cdk related kinase 3 (CRK3 gene) encodes cdc2 related protein kinase with activity similar to eukaryotic histone H1. The CRK3 is also active at G2/M phase of *Leishmania* cell cycle. In *Leishmania*, disruption of CRK3 leads to change in cell ploidy though ensuring that CRK3 mediated regulation of cell division is essential.[97] The chemical inhibitors of CRK3 impair the parasite viability within macrophage, thus validating CRK3 as potential drug target. The most potent inhibitor of CRK3 belongs to indirubin class, which provides pharmacophores for further drug development.[98] In *L. donovani*, it has recently observed that glycogen synthase kinase (LdGSK3) is also involved in cell cycle control and apoptosis based on indirubin test,[99] suggesting LdGSK3 as potential drug target in combination with CRK3. Likewise, other cdk may also be explored as possible targets and require serious attention.

In mammals, mitogen activated protein kinases (MAPK) act as receiver molecules that receive external stimulus and through cascades of intermediates regulate transcriptional, proliferative and differentiation status of a cell.[100] MAPK regulates all aspects of immune response from initiation of innate immunity to activation of adaptive immunity. In *L. mexicana*, 15 MAPK have been identified.[101] *Leishmania* mutant lacking MAPK gene has shown their significance in transformation and cellular growth. The MAPK gene deleted promastigote after differentiation into amastigote loses proliferative capacity.[102] MAPK are equally important for amastigotes and promastigotes.[103] However, they are least explored in *Leishmania* for probable drug targets and invite attention for the identification of *Leishmania* specific sequences of MAPK to obtain an effective compound.

6. Natural Products as Promising Antileishmanial Agents

The leishmanial research has made significant progress during the recent years however; a safe, effective, inexpensive, and true antileishmanial drug is still missing. Due to the lack of better alternate therapies, identification of novel drugs, compounds, and targets is still a serious business for medicinal chemists, biochemists, and physicians worldwide. Natural products are potential source of new and selective agents for the treatment of neglected tropical disease especially protozoans parasites. Utility of plant products in drug discovery and development is not surprising as humans are using many plant derived materials/secondary metabolites since centuries.[104] The secondary metabolites of plants are quite beneficial against diverse group of pathogens such as viruses, bacteria, and fungi. They also provide protection against herbivores like arthropod and vertebrates.[105] Various secondary metabolites of plants like quinones, alkaloids, terpenes, saponins, phenolic and their derivatives which are quite beneficial for human being due to their antiparasitic properties and highly selective mode of action. In addition to direct applicability of secondary metabolites like morphine, quinine, etc., the skeletols of many metabolites has been successfully utilized to design pharmacologically more active compounds.[106] To date many compounds from plant sources have shown potential of antileishmanial lead activity however, due to lack of serious interest (leishmaniasis is a neglected disease) none of them has undergone clinical evaluation. This article, hereafter, will present some important plant products that have shown excellent antileishmanial properties with special attention on their IC/EC values and mode of action to the world of leishmanial research.

6.1. Quinones

A bis-naphthoquinone, diospyrin (**1**), isolated from the bark of *Diospyros Montana* (Ebenaceae), exhibits significant activity against promastigotes of *L. donovani* with an MIC of 2.67 μM.[107] The metabolite **1** exerts its leishmanicidal action by binding to the parasite's topoisomerase I, thus inhibits the catalytic activity of the enzyme, or by stabilizing the topoisomerase I-DNA binary complex.[108] The hydroxylated derivative of **2** successfully eliminates 73.8% of amastigotes in infected macrophages at a concentration of 3 μM. The perturbation of the electron transport chain in the mitochondria of the parasite or generation of free radicals during the interaction between the metabolite and the respiratory chain of the parasite appears to be the probable mechanism of action for compound **2**.[109] Likewise, burmanin A, B, and C (**3–5**), isolated from *Diospyros burmanica*, exhibit significant activity against *L. major*. Among these metabolites, compound **3** shows strongest inhibitory activity with an IC_{50} of $0.053 \pm 2.7 \times 10^{-3}$ μM compared to dimeric analogues **4** ($IC_{50} = 0.18 \pm 5.4 \times 10^{-3}$ μM) and **5** ($IC_{50} = 0.15 \pm 11 \times 10^{-3}$ μM).[110]

The naphthoquinone, plumbagin (**6**), isolated from *Plumbago* species, shows activity against amastigotes of *L. donovani* and *L. amazonensis* with IC_{50} of 2.24 and 5.87 μM, respectively.[111,112] The compound **6** at 2.5 mg kg^{-1} day^{-1} concentrations exhibits in vivo activity against *L. amazonensis* while against *L. venezuelensis*

it display activity at concentrations of 5 mg kg^{-1} day^{-1}.[113] The plumbagin derivative **7**, isolated from *Diospyros burmanica*, exhibits significant antileishmanial activity against *L. major* with an IC_{50} of 3.3 ± 0.19 mM.[114] A prenyloxy-naphthoquinone, 2-methyl-5-(3′-methyl-but-2′-enyloxy)-[1,4]naphthoquinone (**8**), isolated from roots of *Plumbago zeylanica*,[115] exhibits significant activity against promastigote and amastigote forms of *L. donovani* with an EC_{50} of 1.9 ± 0.076 and 3.46 ± 0.63 μM, respectively and when compared with miltefosine as reference drug, it was found more effective.[116] The mechanism of the action of naphthoquinones **6–8**, is supposed to be due to their ability to perturb the electron transport chain (ETC) in the mitochondria of the parasite or in the generation of free radicals during the interaction between the metabolite and the respiratory chain of the parasite, something that the parasites are not able to defend.

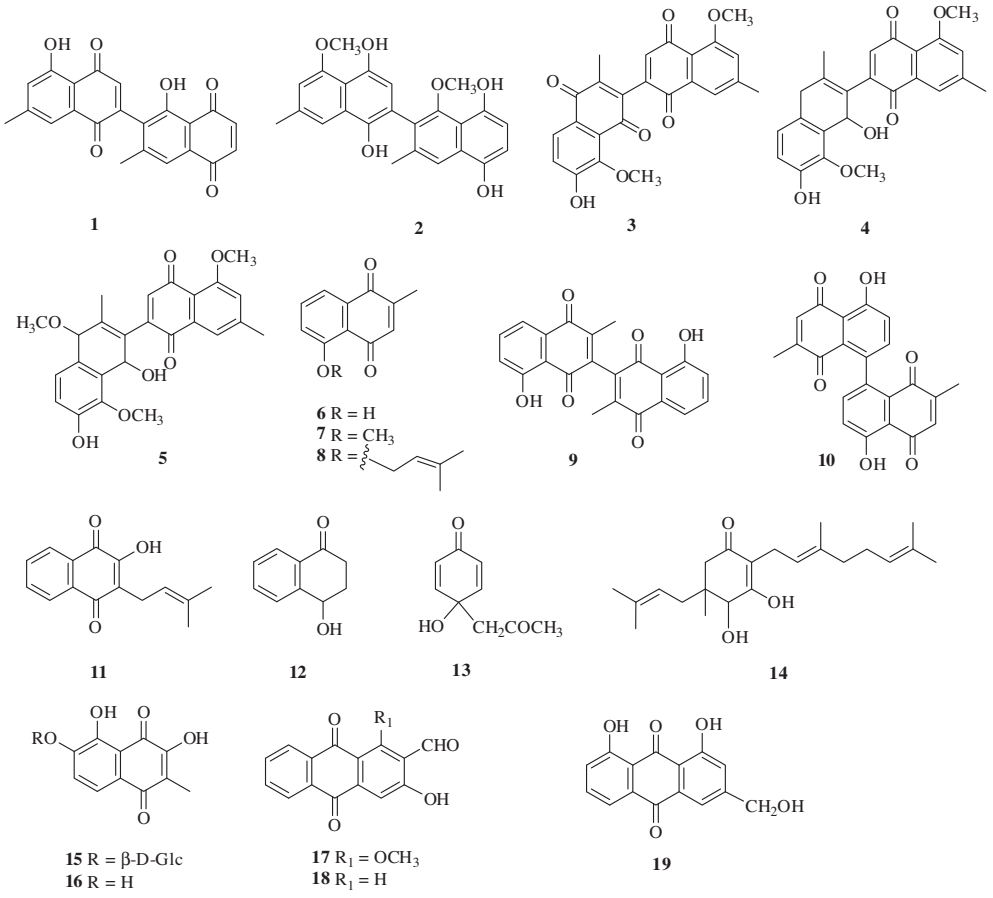
The dimeric naphthoquinones, 3,3′-biplumbagin (**9**) and 8,8′-biplumbagin (**10**), isolated from the bark of *Pera benensis* (Euphorbiaceae), show activity against promastigotes of *L. braziliensis*, *L. amazonensis*, and *L. donovani* with an IC_{90} of 133.56 and 13.35 μM.[110] A prenylated hydroxynaphthoquinone, lapachol (**11**), isolated from a species of *Tecoma* (*Bignoniaceae*), displays weak activity against amastigotes of *L. donovani* in peritoneal mice macrophages.[117] The 4-hydroxy-1-tetralone (**12**), isolated from *Ampelocera edentula* (Ulmaceae), exhibits activity against promastigotes of *L. braziliensis*, *L. amazonensis* and *L. donovani* with an IC_{90} of 10 61.65 μM. The subcutaneous treatment with compound **12** results in the in vivo activitity similar to Glucantime® (25 mg $kg-^{1}$ day^{-1} vs 56 mg$Sb^{V}kg^{-1}$ day^{-1}) in BALB/c mice infected with *L. amazonensis* or *L. venezuelensis*. However, cytotoxicity, carcinogenic and mutagenic effects associated with **12** limits its use.[118]

Jacaranone (**13**), isolated from *Jacaranda copaia* (Bignoniaceae), shows strong activity against promastigotes of *L. amazonensis* at an ED_{50} of 0.02 mM, however displays toxicity to peritoneal mice macrophages at this concentration.[119] The compound **13** shows a weak activity in vivo when administered subcutaneously to mice infected with *L. amazonensis* and a strong cutaneous toxicity when applied inside the lesion. Hydropiperone **14**, a prenylated dihydroquinone isolated from *Peperomia galioides* (Piperaceae), shows activity against promastigote forms of *L. braziliensis*, *L. donovani*, and *L. amazonensis* at 72.15 μM concentrations.[120]

Dioncoquinones A (**15**) and B (**16**) isolated from *Triphyophyllum peltatum* (Dioncophyllaceae), exhibit good—and specific—activity against *L. major*, while they remain inactive against other protozoic parasites.

The quinones **15** and **16** at 2.0 μM and 4.41 μM concentrations result in 49.6% and 79.2% growth inhibition, respectively against *L. major* compared to miltefosine as reference drug (53.0% growth inhibition at a concentration of 0.53 μM). Moreover, treatment with **15** and **16** strongly induce apoptosis in human tumor cells derived from two different B cell malignancies, B cell lymphoma, and multiple myeloma, without any significant toxicity towards normal peripheral mononuclear blood cells.[121]

The anthraquinone-2-carbaldehydes **17** and **18**, obtained from the roots of *Morinda lucida* (Rubiaceae), exhibit selective activity against promastigote forms of *L. major*. The presence of an aldehyde group at C-2 and of a phenolic hydroxy group at C-3 of both structures, suggests that these functional groups are essential for the antiprotozoal activity.[122] Similarly, aloe-emodin (**19**), an anthraquinone isolated from the aerial parts of *Stephania dinklagei* (Menispermaceae), exhibits leishmanicidal activity against promastigotes and amastigotes of *L. donovani* with IC_{50} of 185.1 and 90 μM, respectively.[123]



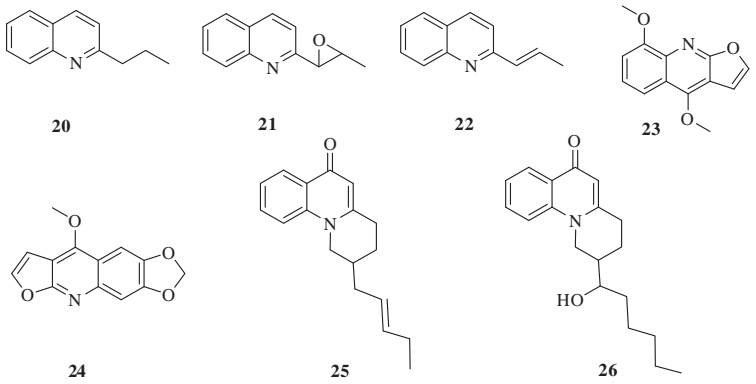
6.2. Alkaloids

6.2.1. Quinolines

Subcutaneous treatment with quinoline alkaloids has been found effective against New World cutaneous leishmaniasis (i.e. *L. amazonensis* and *L. venezuelensis*) in BALB/c mice. The 2-*n*-propylquinoline (**20**), chimanine-D (**21**) and B (**22**), isolated *Galipea longiflora* Krause (Rutaceae), display significant activity against promastigotes of *L. braziliensis* with IC_{90} of 291, 134.97 and 147.73 μM, respectively. Oral administration of compound **20** alone suppresses 99.9% of liver parasites. Subcutaneous treatment with **21** for 10 days at 0.54 mmol kg^{-1} results in 86.6% parasite suppression while oral administration of **21** for 5 days causes lower parasite suppression (72.9%). However, parenteral administration does not produce a similar effect on mice infected with *L. donovani*.[124–126]

The furoquinolines, γ-fagarine (**23**), and masculine (**24**), isolated from stem bark of *Helietta apiculata*, exhibit significant in vitro antileishmanial activity against promastigote forms of different *Leishmania* species with IC_{50} values > 74.16 μM. Among these, the oral treatement of **23** in Balb/c mice infected with *L. amazonensis* demonstrates same efficacy as amphotericin B (reference drug) by reducing 97.4% parasite loads in the lesion.[127]

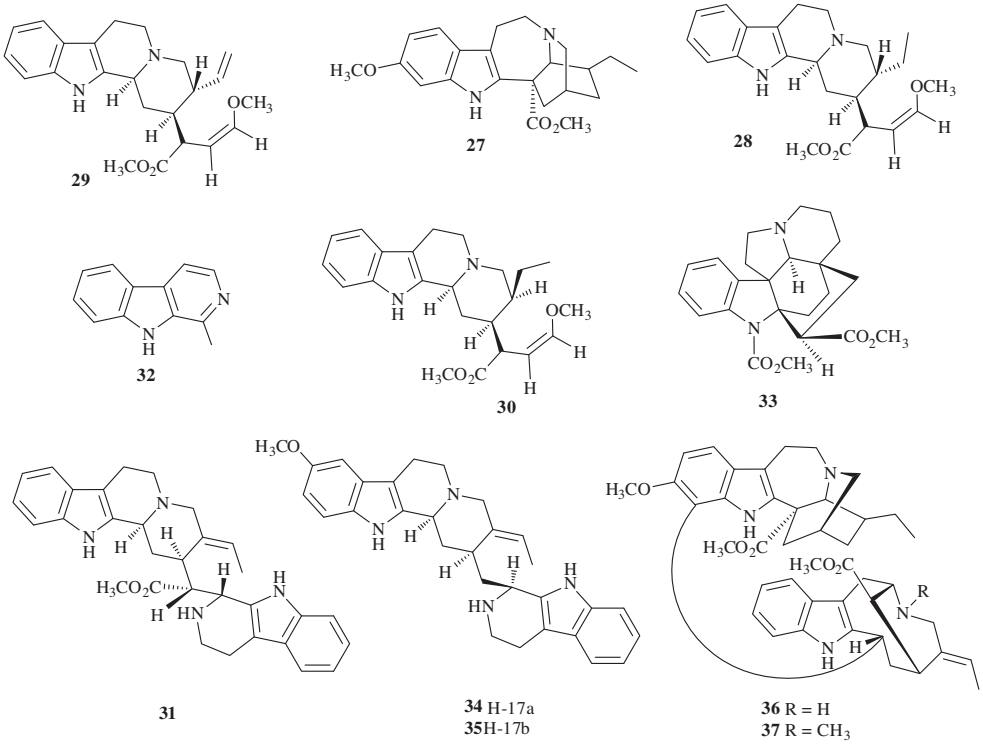
The quinoline alkaloids, dictylomide A (**25**) and B (**26**) isolated from *Dictyoloma peruviana* (Rutaceae) result in complete lyses of *L. amazonensis* promastigotes at 374.01 and 333.43 μM concentrations respectively. However, they display minor activity against promastigotes of *L. braziliensis* at same concentration.[128]



6.2.2. Indoles

Numerous indole-alkaloids have been reported to exhibit significant antileishmanial activities. Coronaridine (**27**), an iboga-type indole alkaloid isolated from *Peschirea australis* (Apocynaceae), shows activity against promastigote of the *L. amazonensis* with an IC_{50} value of 32.56 μM.[129] Similarly, dihydrocorynantheine (**28**), corynantheine (**29**) and corynantheidine (**30**) isolated from the bark of *Corynanthe pachyceras* (Rubiaceae), exhibit excellent in vitro antileishmanial activity with an IC_{50} ~3 μM against *L. major*. The mechanism of action of these metabolites is based on the inhibition of the respiratory chain of the parasite.[130]

Among alkaloids isolated from *Kopsia griffithii* (Apocynaceae), buchtienine (**31**) shows highest activity against *L. donovani* promastigotes with IC_{50} < 3.15 μM as compared to harmane **32** (IC_{50} = 34.29 μM) and pleiocarpine **33** (IC_{50} = 63.05 μM).[131] Ramiflorine A (**34**) and B (**35**), the monoterpenoid indoles isolated from *Aspidosperma ramiflorum* (Apocynaceae), exhibit activity against



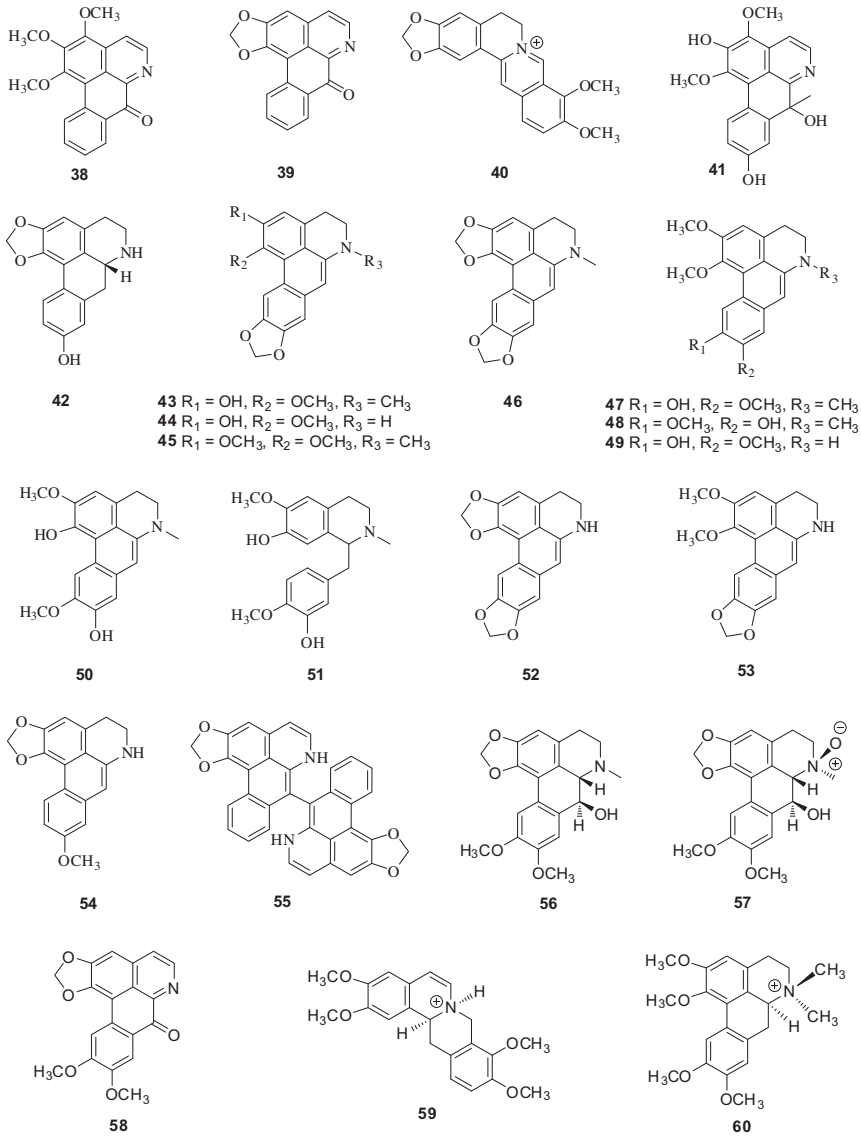
L. amazonensis promastigotes with LD_{50} of 34.93 and 10.5 μM, respectively as compared to pentamidine (LD_{50} = 16.87 μM).[132]

Gabunine (**36**), a bis-indole alkaloid isolated from *Peschiera van heurkii* (Apocynaceae), shows in vitro activity with a survival index (SI) of 3% against amastigotes of *L. amazonensis*. Similarly, conodurine (**37**) isolated from *Conopharyngia durissima* (Apocynacea), shows strong in vitro activity against promastigotes of *L. amazonensis*. However, compound **37** remains unable to display significant in vivo efficacy against *L. amazonensis* as compared to Glucantime® (IC_{50} = 40 $mgkg^{-1}$ day^{-1}, BALB/c mice).[133]

6.2.3. Isoquinolines

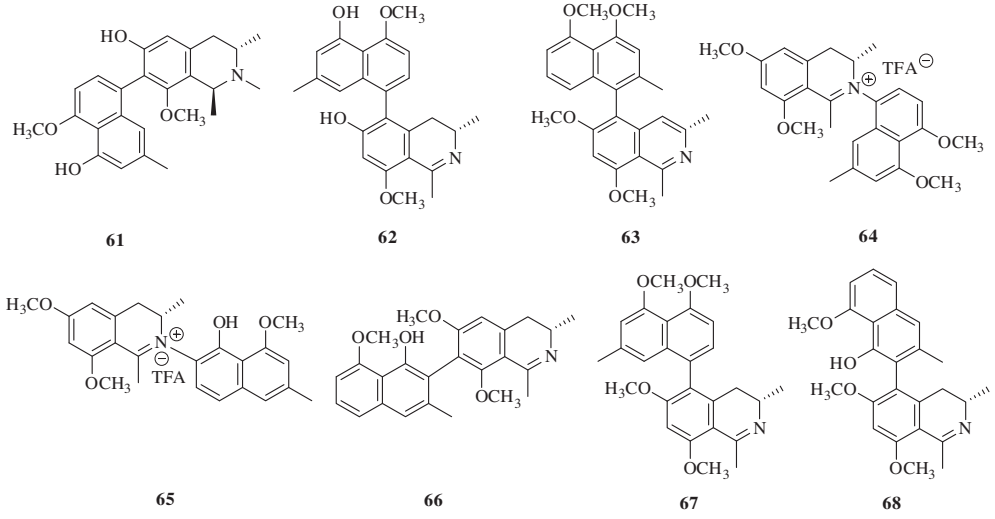
The oxoaporphine alkaloids, *O*-methylmoschatoline (**38**) and liriodenine (**39**), isolated from *Annona foetida* (Annonaceae), exhibit in vitro activity against *L. braziliensis* promastigotes with IC_{50} < 60 - μM.[134] SAR studies suggest that the metabolite **39** having methylenedioxy functionality displays eight times more activity (IC_{50} of 21.5 μM) against *L. braziliensis* and *L. guyanensis* than metabolite **38**. Besides, metabolite **39** when isolated from stem bark of *Rollinia emarginata* (Annonaceae), shows activity against promastigotes of *L. braziliensis*, *L. donovani* and *L. amazonensis* with IC_{100} of 18.16 μM.[135] Use of biphasic media or liquid media for the evaluation of leishmaniasis possibly causes variation in the biological activity of metabolite **39**.

Berberine (**40**), occurring in many plant families (Annonaceae, Menispermaceae, and Berberifaceae) eliminates *L. major* parasites in peritoneal mice macrophages at 29.73 μM concentration.[136,137] Isoguattouregidine (**41**), isolated from the bark of *Guatteria foliosa* (Annonaceae), shows activity at 294.68 μM concentration against *L. donovani and L. amazonensis.*[138] Similarly, anonaine (**42**), (+)-isodomesticine (**43**), (+)-norisodomesticine (**44**), (+)-nantenine (**45**), (+)-neolitsine (**46**), (+)-lirioferine (**47**), (+)-N-methylaurotetanine (**48**), (+)-norlirioferine (**49**), (+)-isoboldine (**50**) and (+)-reticuline (**51**), isolated from *Guatteria dumetorum* (Annonaceae), show leishmaniciadal activity against the promastigotes of *L. maxicana.*[139] SAR studies on metabolites **43–51** along with aporphine alkaloids, cryptodorine (**52**), nor-nantenine (**53**), and xylopine (**54**), isolated from same plant species, establish the methylenedioxy moiety solely responsible for high antileishmanial activity. The metabolites **46** and **52** at a concentration of 15 and 3 μM, respectivelysignificantly reduce the parasite burden as compared to amphotericin B. Further replacement of methoxy group results in significant decrease



of activity among these alkaloids (**47**:210 μM; **48**:395 μM; **49** > 916 μM and **50** > 916 μM). Also, the appearance of 1,2-metylenedioxy function (i.e. **54**:3 μM) significantly increases the activity as compared to 9,10-methylenedioxy functionality (**43**:73 μM; **44**:48 μM; **45**: 41 μM and **53**:15 μM). The presence of *N*-methyl group results in moderate decrease in activity. The alkaloids **43–54** show low toxicity (>300 μM) to murine macrophages and VERO cells while **46** shows highest selectivity index to *L. maxicana* over murine macrophages.[140]

A dimeric aporphine alkaloid, unonopsine (**55**), isolated from the *Unonopsis buchtienii* (Annonaceae), exhibits significant antileishmanial activity against the promastigotes of *L. donovani* with an IC_{100} of 48.02 μM as compared to pentamidine (IC_{100} = 8.43 - μM).[141] Among the alkaloids, duguetine (**56**), duguetine β-*N*-oxide (**57**), dicentrinone (**58**), *N*-methyltetrahydropalmatine (**59**) and *N*-methylglaucine (**60**), isolated from *Duguetia furfuracea* (Annonaceae), the compound **58** exhibits strongest antileishmanial acitivity (IC_{50} of 0.01 μM) while **57** with an IC_{50} of 0.11 μM shows promising leishmanicidal effect. The metabolites **56**, **60** and **59** show good to moderate activity with IC_{50} of 4.32, 4.88 and 17.03 μM, respectively.[142]



6.2.4. Naphthylisoquinolines

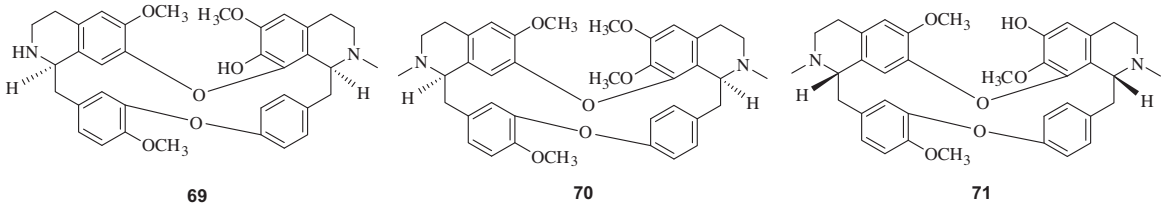
Ancistrogriffine A (**61**), isolated from *Ancistrocladus griffithii* (Ancistrocladaceae), exhibits in vitro antileishmanial activity with an IC_{50} of 7.6 μM against *L. donovani*.[143] Ancistrolikokine D (**62**), isolated from roots of *Ancistrocladus likoko* (Ancistrocladaceae), possess efficient activity with an IC_{50} of 15.07 μM against *L. donovani* promastigotes as compaired to pentamidine (IC_{50} = 9.27 - μM).[144] Ancistroealaine A (**63**) isolated from *Ancistrocladus ealaensis* (Ancistrocladaceae), displays significant activity against promastigotes of *L. donovani* with IC_{50} of 4.1 IC_{50} of 9.82 μM but remains inactive against *L. major* at the same concentration.[145] The metabolite ancistrocladinium A (**64**) and configurationally semistable B (**65**) isolated from *Ancistrocladaceae* sp., show significant activity against *L. major* promastigotes with IC_{50} of 62.06 and 3.74 μM, respectively. SAR studies suggest that the alkaloids bearing a *C*,*C*-biaryl axis connecting the naphthyl and isoquinoline moiety together exhibit weak or no leishmanicidal effect while in *N*,*C*-coupled alkaloids that is **64** and **65**, presence of a hetero biaryl axis is responsible for significantly higher leishmanicidal activities. Furthermore, compounds **64** and **65** share structural features with miltefosine, thus an apoptosis-like death pathway in the parasite is the possible mode of action. The compounds **64** and **65** also display more toxicity against J774.1 macrophage cell lines & peritoneal macrophages as compared to amphotericin B.[146]

Ancistrocladidine (**66**) isolated from *Ancistrocladus tanzaniensis* (Ancistrocladaceae) shows activity against *L. donovani*. Its activity in comparison to the highly active ancistrotanzanine B **67** (IC_{50} = 3.81 μM) is weaker only by a factor of two, and in comparison to miltefosin by a factor of ten. Ancistrotanazanine A (**68**), another alkaloid of the same group that exhibits high activity against *L. donovani*.[147]

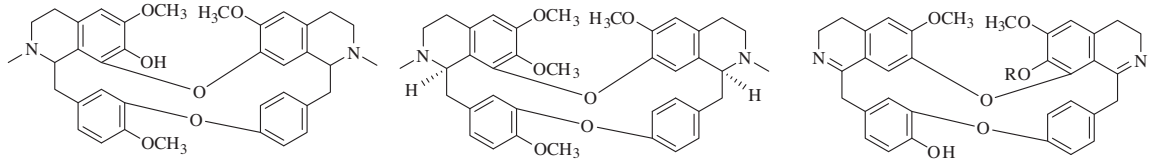
6.2.5. Benzylisoquinolines

Bisbenzylisoquinolinic alkaloid daphanandrine (**69**), isolated from *Albertisia papuana* (Menispermaceae), obaberine (**70**), obtained from *Pseudoxandra sclerocarpa* (Annonaceae), gyrocarpine (**71**) produced by *Gyrocarpus americanus* (Hernandiaceae), limacine (**72**), isolated from *Caryomene olivasans* (Menispermaceae), display anti-leishmanial activity at IC_{100} ~82.13 μM. The metabolite **71** also shows in vitro activity against the promastigote forms of *L. braziliensis*, *L. amazonensis,* and *L. donovani* at 16.42 μM concentrations. However, compound **71** displays low efficacy in vivo (100 $mgkg^{-1}$ day^{-1}) against *L. amazonensis* as compared to Glucantime® (56 $mgSbv^{-1}kg^{-1}$ day^{-1}).[148]

The bis-benzylisoquinoline alkaloid, isotetradrin (**73**), isolated from *Limaciopsis loangensis* (Menispermaceae), shows in vivo activity against *L. amazonensis* at 100 $mgkg^{-1}$ day^{-1} in the BALB/c mice.[149] Similarly, puertogaline A (**74**) and B (**75**), isolated from *Guatteria boliviana* (Annonaceae), exhibit in vitro inhibition of *L. donovani, L. amazonensis* and *L. braziliensis* promastigotes at 177.74 and 173.42 μM, respectively. SAR studies on bis-benzylisoquinoline series of alkaloids establish that the oxidation state and nature of substitution on the nitrogen atom is important for activity. The alkaloids with methylated nitrogen atoms show greater activity compared to unsubstituted or aromatic nitrogens. Quaternization of one or more nitrogen atoms results in a loss of antileishmanial activity.[150]



69 **70** **71**

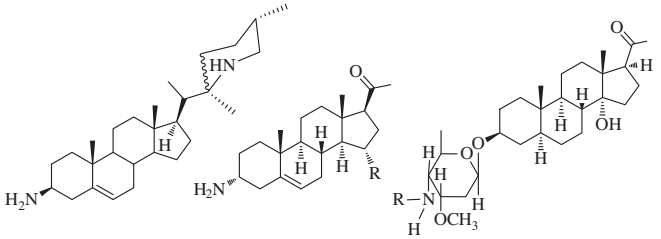


72 **73**

74 R = H
75 R = CH_3

6.2.6. Steroidal alkaloids

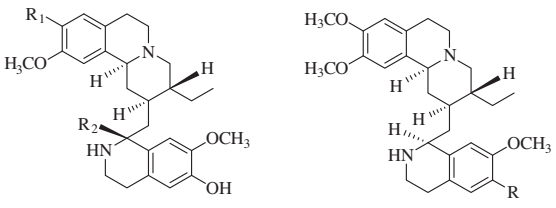
Sarachine (**76**), a steroidal alkaloid isolated from *Saracha punctata* (Solanaceae), inhibits the growth of *L. baziliensis, L. donovani and L. amazoensis* promastigotes at 24.23 μM concentrations. However, this compound also exhibits toxicity at a same concentratin against mice peritonaeal macrophages.[151] Similarly, holamine (**77**), 15-α-hydroxyholamine (**78**), holacurtine (**79**), and *N*-desmethylholacurtine (**80**), isolated from *Holarrhena curtisii* (Apocynaceae), exhibit significant activity against promastigotes of *L. donovani*. Among these metabolites, the compound **77** shows highest activity at a concentration rage of 4.94 > IC_{50} > 1.23 μM, while metabolites **78**, **79** and **80** display concentration range of 18.85 > IC_{50} > 3.17 μM.[152]



76 **77** R = H, **78** R = OH **79** R = CH_3, **80** R = H

6.2.7. Benzoquinolizidine alkaloids

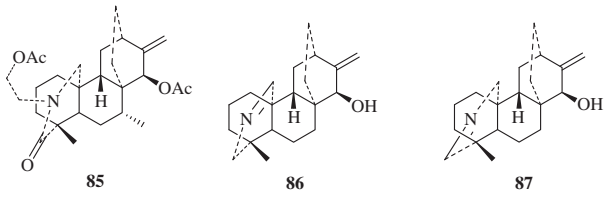
Among alkaloid klugine (**81**), cephaeline (**82**), isocephaeline (**83**) and emetine (**84**), isolated from *Psychotria klugii* (Rubiaceae), the metabolite **82** shows efficient in vitro antileishmanial activity against *L. donovani* with > 20- and > 5-fold greater potency in compared to pentamidine and amphotericin B, respectively. Similarly, benzoquinolizidine alkaloid **81** shows < 13- and < 15-fold less potent activity than **82** and **83**, respectively. The metabolite **84** also shows efficient activity but at the same time displays toxicity against VERO cells.[153,154]



81 $R_1 = R_2 = OH$
82 $R_1 = OCH_3$, $R_2 = H$
83 R = OCH_3
84 R = OH

6.2.8. Diterpene alkaloids

Among diterpene alkaloids 15,22-*O*-diacetyl-19-oxo-dihydroatisine (**85**), azitine (**86**) and isoazitine (**87**) isolated from *Aconitum*, *Delphinium* and *Consolida* species, the compound **87** exhibits highest toxicity to the extracellular *L. infantum* parasites with IC_{50} of 44.6, 32.3 and 24.6 μM at 24, 48 and 72 h of culture, respectively using pentamidine-isothionate as reference drug. The metabolites **86** and **85** exhibit activity against promastigotes of *L. infantum* with IC_{50} of 33.7 and 27.9 μM, respectively after 72 h of culture. The metabolites **87** and **86** with IC_{50} value < 1001.8 and < 667.8 μM, respectively are non-toxic to murine macrophages, while **85** with an IC_{50} of 162.3 μM shows weak toxicity.[155]

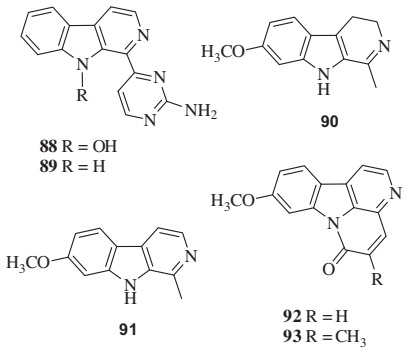


85 **86** **87**

6.2.9. Pyrimidine-β-carboline alkaloid

The *N*-hydroxyannomontine (**88**) and annomontine (**89**) isolated *Annona foetida* (Annonaceae), exhibit significant antileishmanial activity against *L. braziliensis*. In SAR study the metabolite **89** (IC_{50} = 34.8 μM) shows 6 times more activity against promastigotes of *L. braziliensis* than compound **88**. However, metabolite **88** exhibits activity against promastigote forms of *L. guyanensis* while **89** remaining inactive.[156] Harmaline (**90**), isolated from *Peganum harmala* (Nitrariaceae) exerts antiparasitic activity by DNA intercalation and displays significant leishmanicidal activity against amastigotes (IC_{50} = 1.16 μM) as compared to promastigotes (IC_{50} = 116.8 μM) of parasite. The metabolite also produces psychopathic effect by inhibition of monoamino oxidase, which limits its use as therapeutic agent.[157] The metabolite harmine (**91**) isolated from same plant species exhibits in vivo activity in mice model and effectively reduces spleen parasite load by 40%, 60%, 70%, and 80% in free, liposomal, niosomal and nanoparticular forms, respectively. The mechanism of action for **90** and **91** on the promastigote forms of the parasite involves interactions with DNA metabolism leading to an accumulation of parasites in the S-G(2)M phases of the cell-cycle.[158] Similarly, canthin-6-one (**92**) and 5-methoxycanthin-6-one (**93**) occurring in several plant species of families Rutaceae and Simaroubaceae, exhibt in vivo activity

in BALB/c mice infected with *L. amazonensis*. In a study comprising oral (14 days) or intralesional (4 days) treatment of mice at doses of 10 $mgkg^{-1}$ daily after 5 weeks post infection, the intralesional administration of **92** produces no significant reduction of the parasite burden as compared to untreated group while the reference drug *N*-methylglucamine antimonite reduces 91% parasite load.[159]



88 R = OH
89 R = H

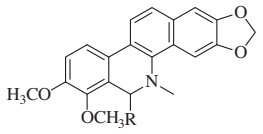
90

91

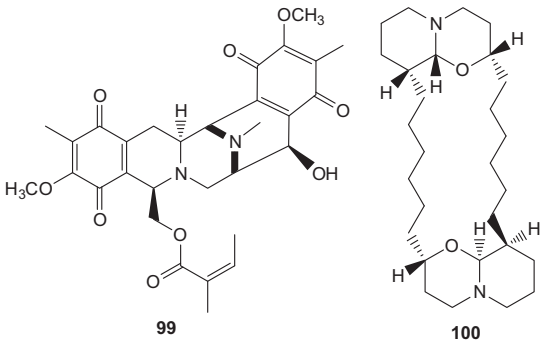
92 R = H
93 R = CH_3

6.2.10. Benzo[*c*]phenanthridine alkaloid

The benzo[*c*]phenanthridine alkaloids, dihydrochelerythrine (**94**), 6-acetonyldihydrochelerythrine (**95**), isolated from the stem bark of *Garcinia lucida* (Clusiaceae), exhibit significant activity against *L. donovani* along with little toxicity to Vero and host cells. The compound **94** with IC_{50} value of 2.0 μM shows promising antileishmanial activity, while compounds **95** with IC_{50} values of 6.6 μM exhibits comparatively low activity against *L. donovani* axenic amastigotes.[160]

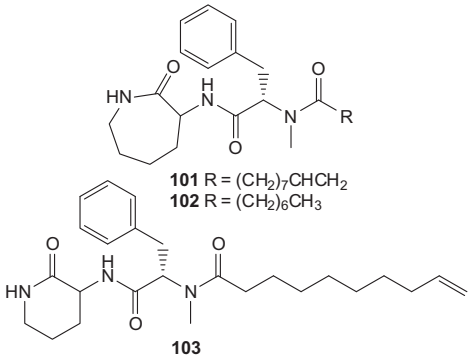


94 R = H
95 R = CH_2COCH_3



99

100

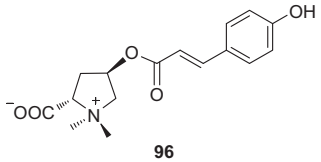


101 R = $(CH_2)_7CHCH_2$
102 R = $(CH_2)_6CH_3$

103

6.2.11. Pyrrolidinium alkaloid

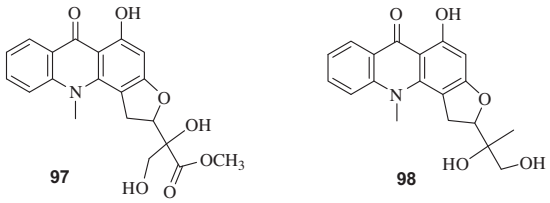
The pyrrolidinium derivative (2*S*,4*R*)-2-carboxy-4-(*E*)-*p*-coumaroyloxy-1,1-dimethylpyrrolidinium inner salt **96** obtained from *Phlomis brunneogaleata* (Lamiaceae), exhibits antileishmanial activity against *L. donovani* axenic amastigotes with an IC_{50} 29.8 μM.[161]



96

6.2.12. Acridone alkaloids

Rhodesiacridone (**97**) and gravacridonediol (**98**) isolated from *Thamnosma rhodesica* (Rutaceae), result in 69%, and 46% inhibition of promastigote forms of *L. major* at 10 μM concentrations. Interestingly, compounds **97** and **98** show grater activity on amastigote form of the parasites and cause over 90% inhibition at 10 μM and around 50% at 1 μM concentration. In addition, the metabolites do not show toxicity to murine macrophages at these concentrations.[162]



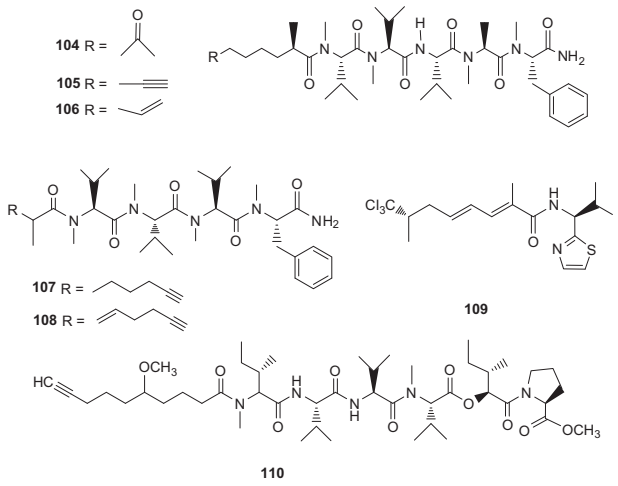
97

98

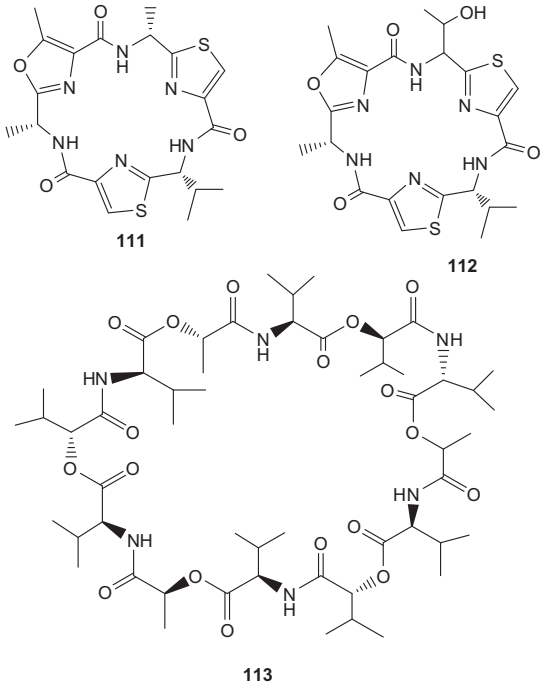
6.2.13. Alkaloids from marine sources

Renieramycin A (**99**) isolated from *Neopetrosia* species, is a La/egfp (enhanced green fluorescent protein) inhibitor that shows efficient antileishmanial activity against *L. amazonensis* with IC_{50} 0.35 μM.[163] Araguspongin C (**100**), isolated from a marine sponge *Haliclona exigua*, displays leishmanicidal activity at 214.26 μM concentrations against promastigotes as well as amastigotes.[164] Among the ciliatamides A–C (**101–103**) isolated from *Aaptos ciliate*, the compounds **101** and **102** inhibit 50% growth *L. major* promastigotes at 22.65 and 24.07 μM, respective concentrations. However, **102** also exhibit marginal cytotoxicity to HeLa cells.[165]

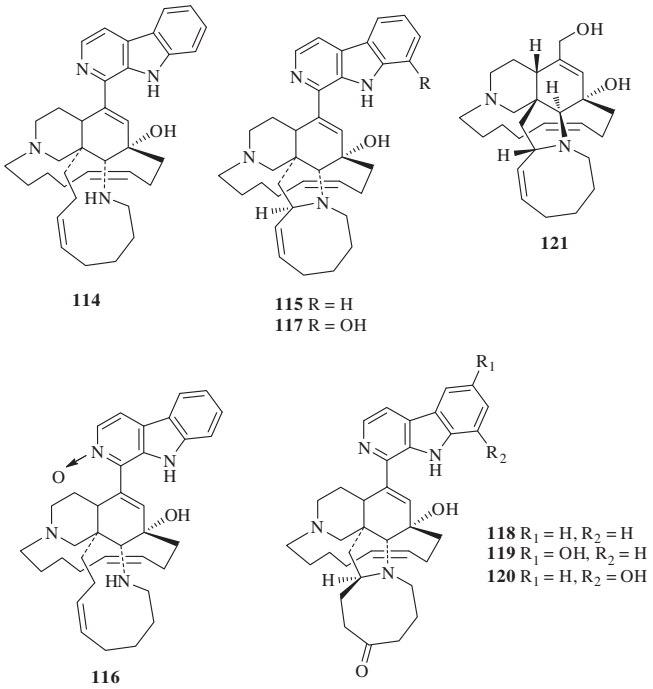
The lipopeptides, almiramides A–C (**104–106**) isolated from cyanobacterium *Lyngbya majuscule*, exhibit significant in vitro antileishmanial activity against *L. donovani*. The SAR studies among these peptides suggest that **105** and **106** exhibit strong activity against *L. donovani* with EC_{50} values of 2.4 and 1.9 μM, respectively. The metabolites **105** and **106** also display weak cytotoxicity to mammalian Vero cells at 52.3 and 33.1 μM concentrations, respectively.[166] Dragonamide A (**107**), E (**108**) and herbamide B (**109**), isolated from same cyanobacterium strain, exhibit in vitro activity against *L. donovani* with EC_{50} of 6.5, 5.1 and 5.9 μM, respectively.[167] Viridamide A (**110**) isolated from *Oscillatoria nigro-viridis*, shows activity against *L. mexicana* with EC_{50} of 1.5 μM.[168]



Venturamides A (**111**) and B (**112**) obtained from cyanobacterium *Oscillatoria* species, exhibit activity against *L. donovani* with EC_{50} > 19.0 μM.[169] Valinomycin (**113**), a dodecadepsipeptide isolated from *Streptomyces* strains, exhibits activity against promastigotes of *L. major* with EC_{50} < 0.11 μM, but at the same time shows cytotoxicity to 293T kidney epithelial cells and J774.1 macrophages.[170]



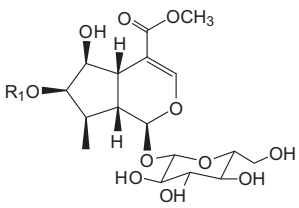
The manzamine-alkaloids, manzamine J (**114**), manzamines A (**115**), manzamine A *N*-oxide (**116**), (+)-8-hydroxymanzamine A (**117**), manzamine E (**118**), 6-hydroxymanzamine E (**119**), manzamine F (**120**), and ircinol A (**121**), isolated from sponge of genus *Acanthostrongylophora* show significant in vitro antileishmanial activity against *L. donovani*. Comparison of antileishmanial activity among **115** and **114** suggests that the bond between *N*-27 and *C*-34 appears to be crucial for the leishmanicidal effect and provides valuable insight into the structural moieties required for activity against*Leishmania* parasites. The metabolites **115** and **121** with IC_{50} values of 1.64 and 2.18 μM display excellent activity against parasites of *L. donovani* while **114** with IC_{50} value of of 45.39 μM exhibits lowest activity among all. Likewise, **116**, **119**, **118**, **120**, and **117** with IC_{50} values of 1.94, 4.30, 6.72, 7.23, 10.97 μM, respectively display good to moderate activity against *L. donovani* parasites.[171]



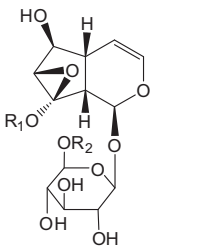
6.3. Iridoids

Iridoids, a class of monoterpenoid glycosides often serve as intermediates in the biosynthesis of indole alkaloids are well known for significant leishmanicidal activity. The arbortristosides-A (**122**), B (**123**), C (**124**) and 6-β-hydroxyloganin (**125**), isolated from *Nyctanthes arbortristis* (Oleaceae) exhibit in vitro activity against *L. donovani* amastigotes. The in vivo studies using intraperitoneal and oral treatment (10 and 100 $mgkg^{-1}$ concentrations for 5 days) of hamsters infected with *L. donovani*, the metabolite **122** displays significant leishmanicidal activities.[172] The compounds, picroside I (**126**) and kutkoside (**127**), obtained from *Picrorhiza kurroa*, exhibits a high degree of protection against the infection of promastigotes of *L. donovani* in hamsters. Picroliv, a standardized fraction of iridoid glycosides **126** and **127**, increases the nonspecific immune response and induces a high degree of protection against the infection of promastigotes of *L. donovani* in hamsters. Picroliv is an adjuvant proposed to increase the efficacy of leishmanicidal drugs and has demonstrated excellent therapeutic index in Phase I and II clinical trials.[173]

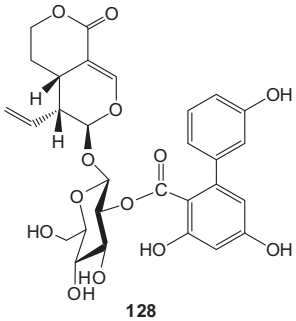
Amarogentin (**128**), a secoiridoid glycoside isolated from *Swertia chirata* (Gentiaceae), produces leishmaincidal effect at a concentration >60 μM against *L. donovani* through inhibition of catalytic activity of topoisomerase I.[174] The metabolite **128** exerts inhibitory effect with a mechanism of action similar to Pentostam® that is by binding to the enzyme and preventing the formation of a binary complex with DNA. The evaluation of **128** in the form of liposomes and niosomes shows an enhanced leishmanicidal activity (without toxic effects) than those observed for free **128** when tested in hamsters. The niosomal formulation reduces the splenic parasite load by 90% (2.5 mg kg^{-1} body weight × 6 days).[175]



122 R_1 = *p*-Methoxycinnamoyl
123 R_1= Caffeoyl
124 R_1 = Coumaroyl
125 R_1 = H



126 R_1 = Vanilloyl, R_2 = H
127 R_1 = H, R_2 = Cinnamoyl



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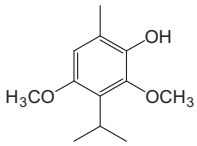
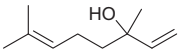
6.4. Terpenoids

6.4.1. Monoterpenes

Espintanol (**129**), isolated from the bark of *Oxandra espintana* (Annonaceae), shows antileishmanial activity against promastigotes of twelve *Leishmania* species.[176] However, the metabolite **129** exhibits only a weak activity in vivo in mice infected with *L. amazonensis*. The monoterpene, linalool (**130**) isolated from *Croton cajucara* (Euphorbiaceae), exhibits strong antileishmanial activity with IC_{50} values 28 nM and 143 nM against promastigotes and intracellular amastigotes of *L. amazonensis*, respectively. An in vitro study indicates that treatment with 15 ngmL^{-1} of **130** rich essential oil to pre-infected murine macrophages reduces interaction between macrophages and parasites by 50% along with an increased nitric oxide production.[177] In addition, further treatment with **130** for 1 h destroys 100% of promastigotes and intracellular amastigotes, while exhibits no cytotoxicity to murine macrophages. The compound **130** exerts antileishmanial activity possibly by destruction of kinetoplastid and mitochondrial swelling followed by cell lysis in parasite.[178]



129

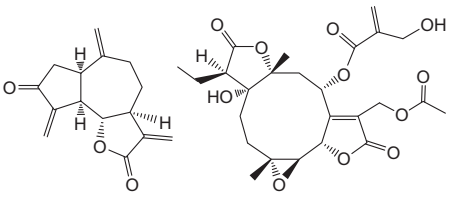


130

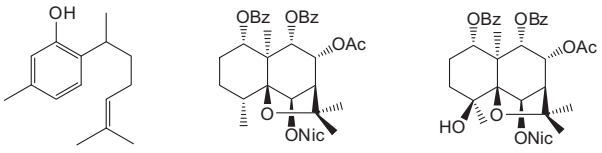
6.4.2. Sesquiterpenes

A sesquiterpene lactone, dehydrozaluzanin C (**131**), isolated from the leaves of *Munnozia maronii* (Asteraceae), shows activity at concentrations between 10.23 and 40.93 μM against promastigotes of eleven *Leishmania* species. The in vivo test using the metabolite **131** in BALB/c mice results in reduction of the lesions caused by *L. amazonensis*.[179] Sesquiterpene dilactone, 16,17-dihydrobrachycalyoxide (**132**), isolated from *Vernonia brachycalyx* (Asteraceae), exhibits activity (IC_{50} = 33.45 μM) against *L. major* promastigote but also inhibits the proliferation of human lymphocytes.[180]

Kudtriol (**133**), a sesquiterpene alcohol isolated from the aerial parts of *Jasonia glutinosa* (Asteraceae), shows toxic activity against promastigotes of *L. donovani* at 982.82 μM concentration. SAR study with metabolite **133** indicates that the presence of a *C*-5 hydroxy group in the α-orientation is essential for the expression of the leishmanicidal activity.[181] The (+)-curcuphenol (**134**), isolated from sponge *Myrmekioderma styx*, exhibits in vitro anti-leishmanial activities against *L. donovani* with an EC_{50} of 11.0 μM.[182] The natural dihydro-β-agarofuran sesquiterpenes from *Celastraceae* plants show potent and specific inhibition of Pgp. The sesquiterpenes (1*S*, 4*S*, 5*S*, 6*R*, 7*R*, 8*S*, 9*R*, 10*R*)-8-acetoxy-1,9-dibenzoyloxy-6-nicotynoyloxy-dihydro-β-agarofuran (**135**) and (1*S*,4*R*,5*R*,6*R*,7*R*,8*S*,9*R*,10*R*)-8-acetoxy-1,9-dibenzoyloxy-4-hydroxy-6-nicotynoyloxy-dihydro-β-agarofuran (**136**) isolated from root barks of *Maytenus apurimacensis*, exhibit high MDR reversing activity in the protozoan parasite *L. tropica*. The compound **136** at 7 μM concentration shows 90% growth inhibition compared to **135** that requires 1.5-fold concentration to produce similar reversal effects.[183]



131 **132** **133**



Nic = nicotynoyloxy

134 **135** **136**

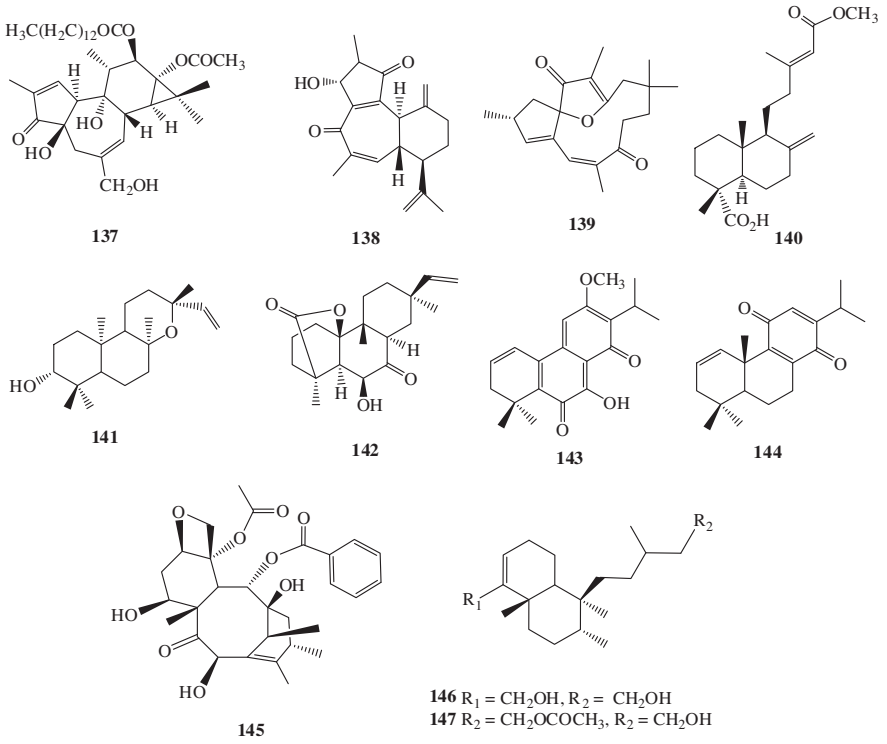
6.4.3. Diterpenes

A phorbol diester, 12-*O*-tetradecanoyl phorbol-13-acetate (**137**), also known as phorbol 12-myristate 13-acetate (PMA), was originally identified from the croton plant, which at a concentration of 32.42 nM displays ability to cause a variety of structural changes in the parasites of *L. amazonensis* by activation of protein kinase C, an important enzyme in the development of several cellular functions.[184] Among the other diterpenoids isolated from Euphorbiaceae species with leishmanicidal potentials are jatrogrossidione (**138**) and jatrophone (**139**). These metabolites possess toxic activity against the promastigote forms of *L. braziliensis*, *L. amazonensis*, and *L. chagasi*. SAR studies with these metabolites revealed that **138** with IC_{100} value of 2.4 μM displays activity higher than **139** (IC_{100} = 15.90 μM), but remains inactive in vivo.[185]

The 15-monomethyl ester of dehydropinifolic acid (**140**), obtained from the stem bark of *Polyalthia macropoda* (Annonaceae), and ribenol (**141**), an *ent*-manoyl oxide derivative isolated from *Sideritis varoi* (Lamiaceae), show in vitro activity against promastigotes of *L. donovani*.[186] In addition, the different derivatives of this metabolite, obtained through chemical or biological transformations, exhibit strong leishmanicidal activity. Additionally, 6-β-hydroxyrosenonolactone (**142**), a diterpene isolated from the bark of *Holarrhena floribunda* (Apocynaceae), has a moderate and weak activity against promastigotes and amastigotes of *L. donovani*, respectively.[187]

6.4.4. Triterpenes

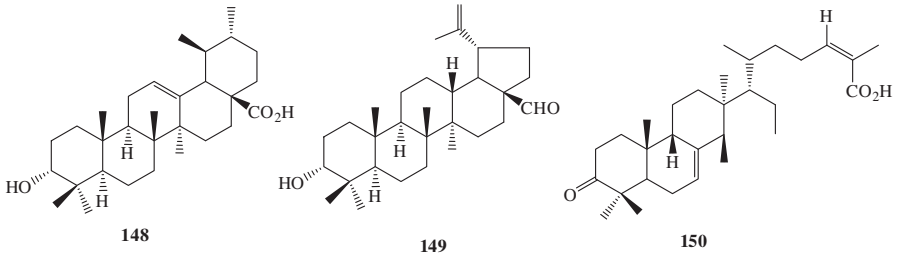
The ursolic acid (**148**) and betulinaldehyde (**149**), obtained from the bark of *Jacaranda copaia* and the stem of *Doliocarpus dentatus* (Dilleniaceae), respectively show activity against the amastigotes of *L. amazonensis*.[191,192] However, the metabolite **149** exhibits toxicity to peritoneal macrophages in mice while **148** displays limited activity in vivo. The triterpenes, (24*Z*)-3-oxotirucalla-7,24-dien-26-oic acid (**150**) and *epi*-oleanolic acid (**151**), isolated from the leaves of *Celaenododendron mexicanum* (Euphorbiaceae), display leishmanicidal activity against *L. donovani* with IC_{50} values of 13.7 and 18.8 μM, respectively.[193] The quassinoids, simalikalactone D (**152**)



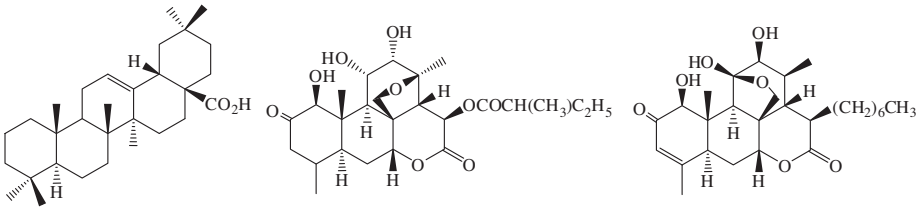
The diterpenes, 7-hydroxy-12-methoxy-20-nor-abieta-1,5(10),7,9,12-pentaen-6,14-dione (**143**) and abieta-8,12-dien-11,14-dione (**144**), isolated from roots of *Salvia cilicica* (Lamiaceae), show appreciable in vitro antileishmanial activity against intracellular amastigote forms of both *Leishmania donovani* (IC_{50} values of 170 and 120 nM, respectively) and *Leishmania major* (IC_{50} values of 290 and 180 nM, respectively).[188] The compound 10-deacetylbaccatin III (**145**), a precursor molecule of taxol, isolated from *Taxus baccata* (Taxaceae), shows strong anti-leishmanial activity selectively against *L. donovani* intracellular amastigotes with an IC_{50} of 70 nM. Additinally, the compound **145** displays no cytotoxic effects to macrophages up to a concentration of 5 μM. The activity of **145** is probably due to stimulation of nitric oxide production in macrophages.[189] The clerodane type diterpenes, 18-acetoxy-cis-clerod-3-en-15-ol (**146**), 15,18-diacetoxy-cis-clerod-3-ene (**147**), isolated from *Cistus monspeliensis,* exhibit potent and selective leishmanicidal activity against promastigotes of *L. donovani* with IC_{50} values of 10.98 and 9.70 μM, respectively.[190]

and 15-β-heptylchaparrinone (**153**), obtained from species of Simaroubaceae family show activity against promastigotes of *L. donovani* but at the same time exhibit toxicity to macrophages.[194] Triterpene glycosides obtained from marine sources for example holothurins A (**154**), isolated from the sea cucumber *Actinopyga lecanora*, causes 73.2 ± 6.8% and 65.8 ± 6% inhibition of *L. donovani* promastigotes and amastigotes, respectively at 86.08 μM concentration. The other isomer B (**155**) obtained from same source shows 82.5 ± 11.6% and 47.3 ± 6.5% inhibition against promastigotes of *L. donovani* at 113.33 and 56.66 μM concentrations, respectively.[195]

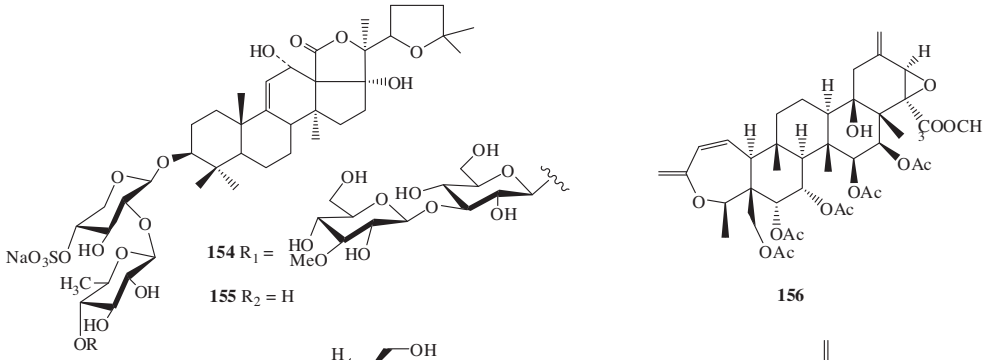
A nor-triterpene, 6α,7α,15β,16β,24-pentacetoxy-22α-carbometoxy-21β,22β-epoxy-18β-hydroxy-27,30-bisnor-3,4-secofriedela-1,20 (29)-dien-3,4 *R*-olide (**156**), isolated from *Lophanthera lactescens*, exhibits promising antileishmanial activity against amastigote forms of *L. amazonensis* with an IC_{50} of 0.50 μM. The metabolite **156** inhibits the parasite survival in a dosedependent manner with 75%, 55%, and 46% inhibition of *Leishmania* growth at 12.71, 1.27, and 0.127 μM, respectively compared to Glucantime, a reference drug.[196]



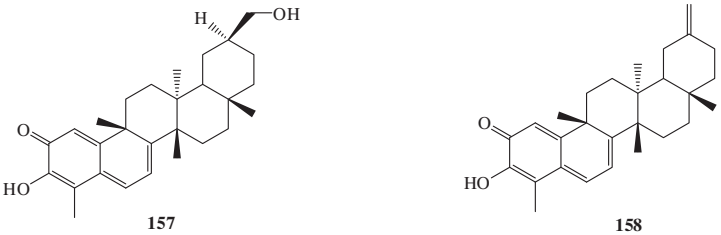
148 149 150



151 152 153



154 R_1 = 155 R_2 = H 156



157 158

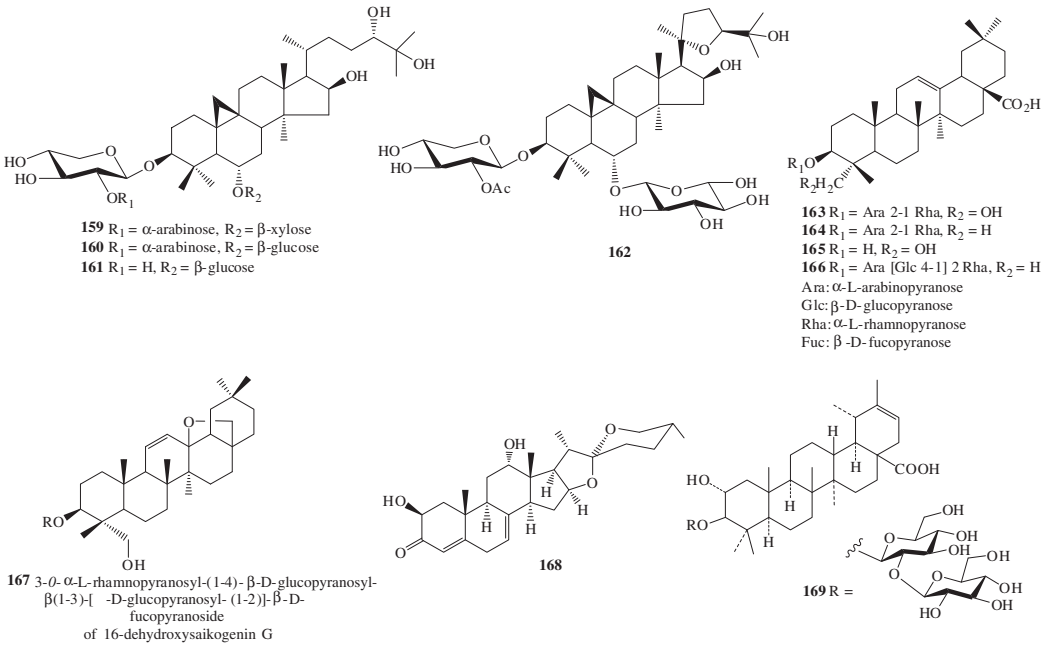
The isoiguesterin (**157**), isolated from *Salacia madagascariensis* (Celastraceae), exhibits potent leishmanicidal activity against *L. donovani* and *L. mexicana* with IC_{50} values 0.198 and 0.082 μM, respectively. Likewise, 20-epi-isoiguesterinol (**158**) isolated from same plant species shows better activity compared to isoiguesterin with an IC_{50} value of 0.079 μM against *L. donovani*.[197]

6.4.5. Saponins

The cycloartane-type glycosides oleifoliosides A (**159**), and B (**160**), cyclocanthoside E (**161**), astragaloside II (**162**), isolated from *Astragalus oleifolius*, show notable growth inhibitory activity against amastigotes of *L. donovani* with IC_{50} values ranging from 16.42 to 28.67 μM. Moreover, none of these compounds possesses cytotoxicity on mammalian L6 cells, indicating their leishmanicidal effect to be selective.[198]

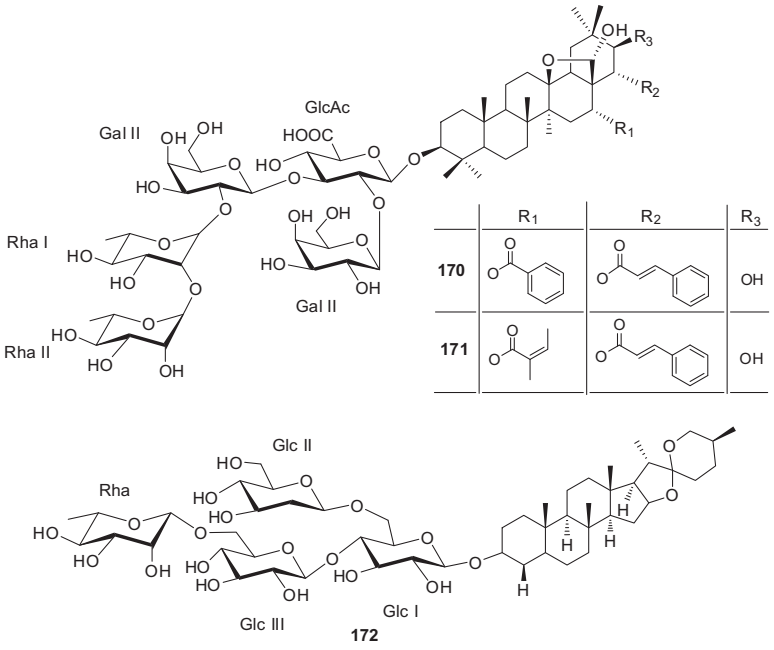
The α-hederin (**163**), β-hederin (**164**) and hederagenin (**165**), obtained from the leaves of *Hedera helix* (Araliaceae), show leishmanicidal activity against *L. infantum* and *L. tropica*. Among these, the metabolite **165** also shows significant activity against the amastigote forms while both **163** and **164** exhibit strong anti-proliferative activity on human monocytes.[199] The saponins **163–165** appear to inhibit the growth of *Leishmania* promastigotes by acting on the membrane of the parasite with induction of a drop in membrane potential.[200] The hederecolchiside-A1 (**166**), isolated from *Hedera colchica*, shows strong activity against the promastigote and amastigote forms of *L. infantum*, however it also displays a notable activity on human monocytes.

The saponin, mimengoside-A (**167**), isolated from the leaves of *Buddleja madagascariensis* (Loganiaceae),[201] exhibits activity against promastigotes of *L. infantum*. Muzanzagenin (**168**), obtained from the roots of *Asparagus africanus* (Liliaceae), displays activity with an IC_{50} value 70.04 μM against the *L. major* promastigotes. However, the metabolite **168** also inhibits the proliferation of human lymphocytes.[202] A saponin, 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-2α,3β-dihydroxy-taraxast-20-en-28-oic acid (**169**), isolated from leaves of *Careya arborea*, exhibits significant in vitro antileishmanial activity against promastigote and amastigote forms of *L. donovani* with an IC_{50} of 15.4 μM compared to AmB (IC_{50} = 0.10 μM).[203]

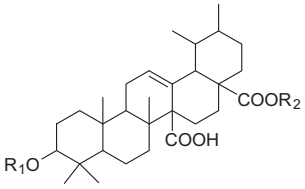


The oleane triterpenoid saponins, mesabalides III (**170**) and IV (**171**) isolated from *Maesa balansae* (Myrsinaceae), show strong anti-leishmanial activity against *L. infantum* intracellular amastigotes with IC_{50} of 5 and 9 nM, respectively.[204,205] Administration of the purified extract (PX-6518) containing these saponin maesabalides (0.4 mg kg^{-1} body weight × 1 day) reduces the parasite burden of the liver by 95% in a BALB/c mice model one day after infection (1.6 mg kg^{-1} body weight is needed after two weeks to obtain comparable results). However, at a concentration of 0.60 μM it shows cytotoxicity towards macrophage host cells and MRC-5 cells (>21.19 μM). A comparative study shows comparable efficacy of mesabalide III (0.8 mg kg^{-1} body weight × 1 day) to amphotericin B (5 mg kg^{-1} body weight × 1 day) in an *L. donovani* infected hamster model.[206,207]

A steroidal saponin, racemoside A (**172**) isolated from *Asparagus racemosus* (Liliaceae), shows potent anti-leishmanial activity against antimonial-sensitive (strain AG83) and -unresponsive (strain GE1F8R) *L. donovani* promastigotes, with IC_{50} values of 1.09 and 1.25 μM, respectively. The metabolite **172** causes morphological alterations including cell shrinkage, an aflagellated ovoid shape, and chromatin condensation. The compound **172** exerts leishmanicidal effect through the induction of programmed cell death mediated by the loss of plasma membrane integrity as detected by binding of annexin V and propidium iodide, loss of mitochondrial membrane potential culminating in cell-cycle arrest at the sub-G_0/G_1 phase, and DNA nicking shown by deoxynucleotidyltransferase-mediated dUTP end labelling (TUNEL). The metabolite **172** also shows significant activity against intracellular amastigotes of AG83 and GE1F8R at a 7 to 8-fold lower dose, with IC_{50} values of 0.16 and 0.15 μM, respectively.[208]



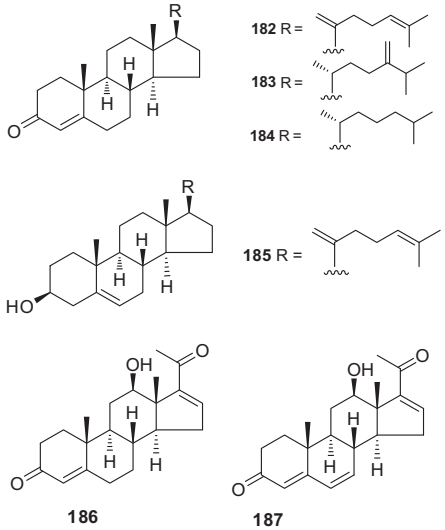
The quinovic acid glycosides (**173–181**) isolated from *Nauclea diderrichii* (Rubiaceae), exert in vitro anti-leishmanial activity against intracellular amastigotes of *L. infantum* with IC_{50} values between 1.1 and 85 μM. However, all compounds remain non-toxic to the promastigote forms of *Leishmania*. The compounds also show weak toxicity towards human macrophages at concentrations higher than 100 μM.[209]



173 R_1 = β-D-Glu(1-4)β-D-Fuc-, R_2 =β-D-Glu
174 R_1 = β-D-Glu(1-4)α-L-Rham-, R_2 =β-D-Glu
175 R_1 = β-D-Glu-, R_2 =β-D-Glu
176 R_1 = β-D-Fuc-, R_2 =β-D-Glu
177 R_1 = α-L-Rham-, R_2 =β-D-Glu
178 R_1 = β-D-Glu(1-4)α-L-Rham-, R_2 = H
179 R_1 = β-D-Glu-, R_2 = H
180 R_1 = β-D-Fuc-, R_2 = H
181 R_1 = α-L-Rham, R_2 = H

6.5. Sterols

The sterols cholest-4,20,24-trien-3-one (**182**), 24-methylcholest-4,24(28)-dien-3-one (**183**), cholest-4-en-3-one (**184**), cholest-5,20,24-trien-3b-ol (**185**), 6,7-dihydroneridienone (**186**) and neridienone (**187**), isolated from Mexican plant *Pentalinon andrieuxii*, exhibit significant in vitro antileishmanial activity against promastigote and amastigote forms of *L. Mexicana*. Among these, the compounds **182**–**185** and **187**, show strong antileishmanial activity at IC_{50} values ranging between 1.4 and 14.5 μM against amastigotes of *L. Mexicana* while **186** displays most potent activity with an IC_{50} of 0.03 μM compared to reference compound, pentostam. In cytotoxicity assay using noninfected bone marrow-derived macrophages from C57BL/6 mice, none of compounds **182**–**187** displays activity at IC_{50} > 226.48 μM.[210]

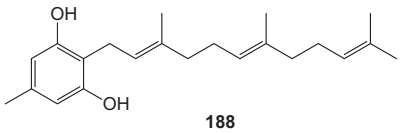


186 **187**

6.6. Phenolics

6.6.1. Simple phenols

Grifolin (**188**) and piperogalin (**189**) obtained from *Peperomia galoides*, causes total lysis of *L. braziliensis*, *L. donovani*, and *L. amazonensis* promastigotes at 304.42 μM concentrations. At 30.4 μM concentration, metabolite **189** causes more than 90% lysis of the promastigotes.[211]



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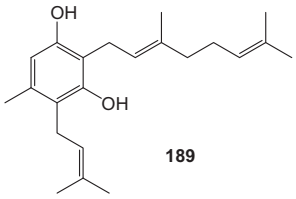

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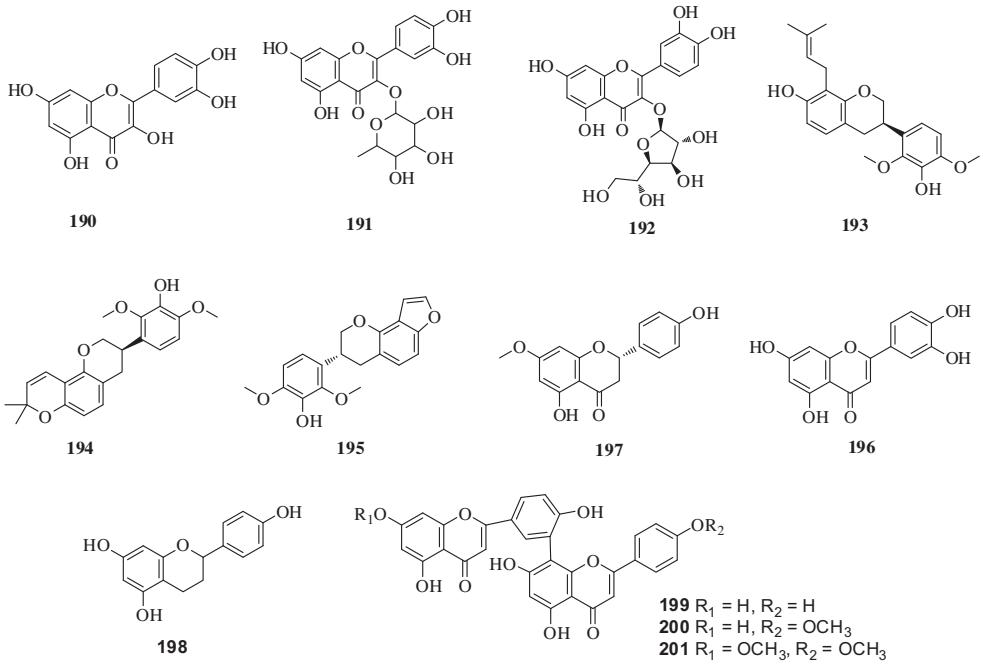
6.6.2. Flavonoides

The flavonoides can induce strong antileishmanial activity via inhibition of enzymes of polyamine biosynthesis, hence, they are promising drugs candidates for the treatment of all forms of leishmaniasis.[212] Arginase, a metallohydrolase, is the first target enzyme of polyamine pathway, is effectively targeted by quercetin (**190**), quercitrin (**191**), and isoquercitrin (**192**) that shows IC_{50} values 3.8, 10 and 4.3 μM, respectively. Among these, the **190** exerts antileishmanial activity by interaction with the substrate L-arginine and the cofactor Mn^{2+}. Docking analysis reveals a novel mechanism of action for flavonols **190–192** through cathecolic group interact with Asp129, which is involved in metal bridge formation for the cofactors Mn^{2+} in the active site of enzyme arginase.[213]

The flavanols 8-prenylmucronulatol (**193**), glyasperin H (**194**), and smiranicin (**195**) isolated from *Smirnowia iranica*, inhibit the growth of *L. donovani* promastigotes with IC_{50} values 6.9, 22.9 and 25.3 μM, respectively. The compounds **193**–**195** exert activity possibly through induction of topoisomerase II mediated kinetoplastid DNA cleavage and cell cycle arrest in the G0/G1 phase followed by apoptotic cell death in both, *L. donovani* promastigotes and intracellular amastigotes.[214] The flavanol luteolin (**196**) isolated from *Vitex negundo* (Verbenaceae) and *Fagopyrum esculentum* (Polygonaceae), inhibits the growth of *L. donovani* intracellular amastigotes with an IC_{50} value of 12.5 μM. In an in vivo study, **196** reduces splenic parasite load by 80% when administered at a dose of 3.5 $mgkg^{-1}$ body weight and **190** by 90% at a dose of 14 $mgkg^{-1}$ body weight. In addition, compound **196** is also non-toxic to human cells but **190** induce cell cycle arrest in host cell.[215,216] In addition, **196** and **190** are specific inhibitors of topoisomerase I, which is an unusual bisubunit topoisomerase in *Leishmania*.[217] Similaryl, sakuranetin (**197**) isolated from the leaves of *Baccharis retusa*, shows antileishmanial activity against various species such as *L. amazonensis*, *L. braziliensis*, *L. major*, and *L.chagasi* with their IC_{50} values 150.30–181.76 μM.[218]

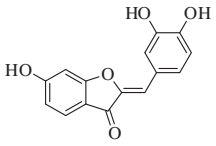
Likewise, the compound 5,7,4′-trihydroxyflavan (**198**) isolated from Amazonian shrub *Faramea guianesis*, exhibits toxic activity

on amastigotes of *L. amazonensis*[219] while the biflavonoids amentoflavone (**199**), podocarpusflavone A (**200**) and B (**201**), isolated from the leaves of *Celanodendron mexicanum*, only show a weak activity against promastigotes of *L. donovani*.[193]



6.6.3. Aurones

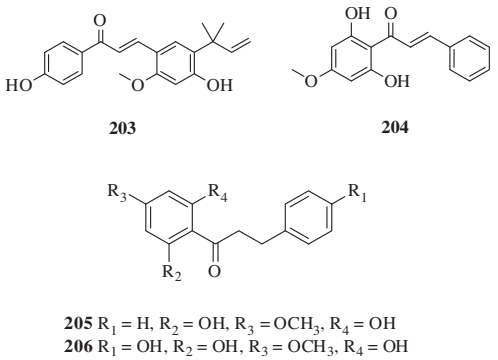
The aurones, a group of metabolites related biosynthetically to the chalcones, have demonstrated antileishmanial activity against the promastigote forms of *L. major*, *L. donovani*, *L. infantum,* and *L. enrietti*. These metabolites have also been reported to be active against the amastigote forms of *L. donovani*, but some of them are toxic to bone marrow-derived macrophages. The sulfuretin, 2-[(3,4-dihydroxyphenyl)methylene]-6-hydroxybenzofuran-3(*2H*)-one (**202**), is an aurone with activity against promastigotes of *Leishmania* spp (EC_{50} = 0.33–0.40 μM) and against amastigotes of *L. donovani* (EC_{50} = 4.58 μM), but non-toxic to bone marrow-derived macrophages.[220]



6.6.4. Chalcones

Chalcones, a diverse group of aromatic ketones, have been observed to possess potent antileishmanial activity. Licochalcone A (**203**), an oxygenated chalcone, strongly inhibits in vitro growth of *L. major* promastigotes and *L. donovani* amastigotes. The observed IC_{50} value against *L. donovani* intracellular amastigotes was 2.7 μM while 21 μM against *L. major* promastigotes.[221] In addition, it reduces parasite load up to 96% in the spleen and liver when administered at a dose of 20 mg kg^{-1} body weight for 6 days.[222] It also exhibits a remarkable capacity to eliminate amastigotes of *L. major* in human peripheral blood monocyte-derived macrophages and in U937 cells, and intraperitoneal administration prevents the development of lesions in BALB/c mice infected with *L. major* and both its intraperitoneal and oral administration reduce the parasite load in the spleen and liver of hamsters infected with *L. donovani*. The proposed mechanism of action for compound **203** involves the alteration of the ultrastructure and the function of mitochondria, thus exerting its effect on the parasite respiratory chain without damaging the organelles of macrophages or their phagocytic function. Another chalcone 2′,6′-dihydroxy-4′-methoxychalcone (**204**), obtained from *Piper aduncum*, has shown significant antileishmanial activity against both promastigote and amastigote forms of *L. amazonensis* with an IC_{50} values of 1.9 and 89 μM, respectively.[223]

The dihydrochalcones **205** and **206** isolated from *Piper elongatum,* display good to moderate in vitro antileishmanial activity against *L. braziliensis, L. tropica,* and *L. infantum* promastigotes. The compound **205** with IC_{50} of 99.23, 77.92, and 56.23 μM shows moderate in vitro activity against the promastigotes forms of *L. braziliensis, L. tropica,* and *L. infantum*, respectively. Similarly, the compound **206** with an IC_{50} of 98.58 μM shows relatively weak activity aginst *L. braziliensis*, however exhibits efficient inhibition at IC_{50} of 13.19 and 21.86 μM against *L. tropica,* and *L. infantum* promastigotes, respectively.[224]



6.6.5. Coumarins

Coumarins are an important class of polyphenolic compounds, which possess excellent antileishmanial properties. The coumarin **207** isolated from *C. brasiliense*, shows significant in vivo antileishmanial activity when administered at a dose of 18 $mgkg^{-1}$ day^{-1} intramuscularly or 0.2% topically to the *L. amazonensis* infected mice. Its efficacy was also comparable to standard drug Glucantime® when administered intramuscularly. The compound 7-geranyloxycoumarin (**208**) isolated from *Esenbeckia febrifuga* (Rutaceae), displays leishmanicidal activity with LD_{50} of 30 μM against *L. major*, a causative agent of cutaneous leishmaniasis.[225] Likewise, the isomeric coumarin epoxides, 2-epicycloisobrachycoumarinone (**209**), and cycloisobrachycoumarinone (**210**), isolated from *Vernonia brachycalyx* (Asteraceae), exhibit selective activity against promastigotes of *L. major*.[226]

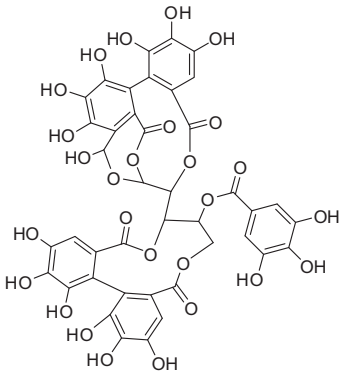
The prenylated coumarins auraptene (**211**), umbelliprenin (**212**), and galbanic acid (**213**), isolated from *Ferula szowitsiana* (Apiaceae), show in vitro antileishmanial activity against promastigotes of *L. major* compared to the negative control (DMSO). Among these, metabolites **211** and **212** inhibit the growth of promastigotes with IC_{50} of 13.3 μM and 17.1 μM after 48 h incubation, respectively. However, compound **213** exhibits a weak inhibitory effect on promastigotes (IC_{50} = 164.8 μM).[227]

The coumarin (−) mammea A/BB (**214**) isolated from *Calophyllum brasiliense* and its derivatives mammea B/BB (**215**), 5,7-dihydroxy-8-(2-methylbutanoyl)-6-(3-methylbutyl)-4-phenyl-chroman-2-one (**216**), 7-hydroxy-5-methoxy-8-(2-methylbutanoyl)-6-(3-methylbut-2-en-1-yl)-4-phenylcoumarin (**217**), 5,7-dimethoxy-8-(2-methylbutanoyl)-6-(3-methylbut-2-en-1-yl)-4-phenylcoumarin (**218**), 5,7-dimethoxy-8-(1-methoxy-2-methylbutyl)-6-(3-methylbut-2-en-1-yl)-4 phenylcoumarin (**219**), and 5,7-dihydroxy-4-phenylcoumarin (**220**), exhibit significant activity against promastigote and intracellular amastigote forms of *L. amazonensis*. Among these, compounds **214**, **216**, **217,** and **219** show activity not just against promastigote forms of *L. amazonensis*, but also against intracellular amastigote forms with IC_{50} of 14.3, 0.6, 34.0, and 22.2 μM, respectively. SAR study reveals that the compounds **216**, **217**, and **219** display higher activity than **214** (IC_{50} = 7.4 μM) with IC_{50} values of 0.9, 2.4 and 1.9 μM, respectively. The compounds **214**, **218**, and **220** show less activity compared to **215** with IC_{50} of 30.1, 15.1 and 60.2 μM respectively while **220** displays lowest antileishmanial activity among all.[228,229]

6.6.6. Tannins

Tannins, a unique group of polyphenolic secondary metabolites found exclusively in numerous woody, herbaceous higher plant species, are widely used as herbal medicine for the treatment of broad spectrum of infectious diseases. The *C*-glycosidic ellagitannins found in many plant families, including Lythraceae, Myrtaceae, Combretaceae, Melastomataceae, and Punicaceae, as well as Fagaceae, Betulaceae, Casuarinaceae, Rosaceae, Theaceae, and Elaeagnaceae, are very promising candidates for antileishmanial therapy as they have shown remarkably diverse range of biochemical and pharmacological activities.[230–232]

Kolodziej et al. evaluated 27 different tannins for in vitro antileishmanial activity against *Leishmania donovani*, wherein casuarinin (**221**) occurring in *Punica granatum*, *Casuarina,* and *Stachyurus* species demonstrate promicing antileishmanial activity with EC_{50} values of 0.52 μM. Likewise, castalagin (**222**) isolated from *n*-butanol extracts of *Anogeissus leiocarpus* and *Terminalia avicennoides* exhibits significant activity with EC_{50} values of 2.88 μM in compared to Pentosam® (EC_{50} 13.32 μM). Both of these tannins exhibit low cytotoxicity against murine host cells (EC_{50} > 26.74 μM).[233,234]



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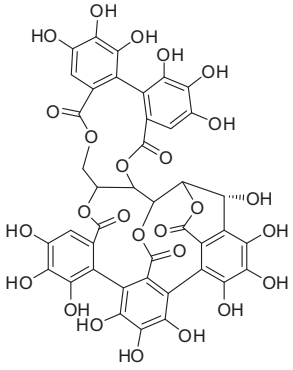
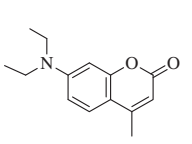


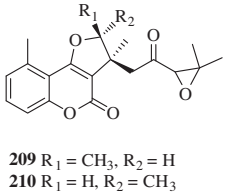
222

6.6.7. Lignans

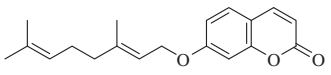
The lignans (+)-medioresinol (223), (−)-lirioresinol B (224), and (+)-nyasol (225), show activity against the amastigotes of *L. amazonensis*. Among these, the norneolignan 225 also exhibits



207



209 $R_1 = CH_3$, $R_2 = H$
210 $R_1 = H$, $R_2 = CH_3$



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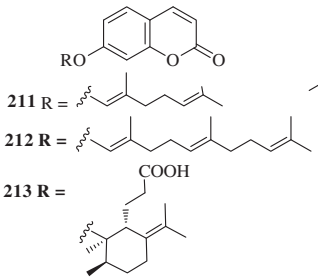
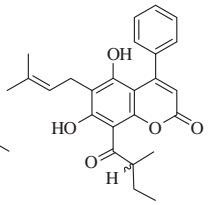
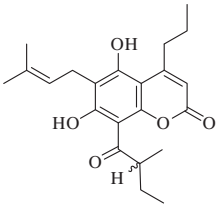


211 R =
212 R =
213 R =



214 $R_1 = R_2 = H$
217 $R_1 = CH_3$, $R_2 = H$
218 R_1, $R_2 = CH_3$



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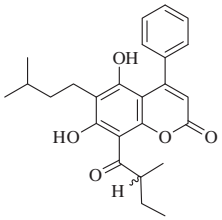

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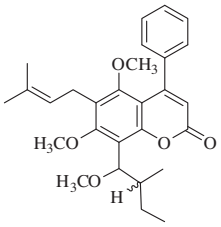

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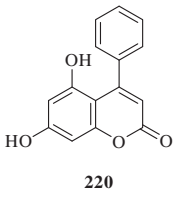
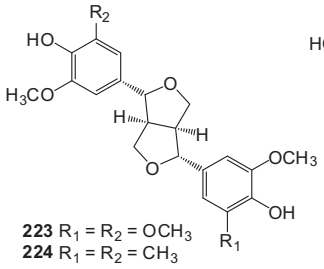
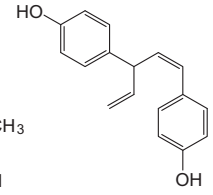


220

high selectivity in its activity against the promastigotes of *L. major*.[106]



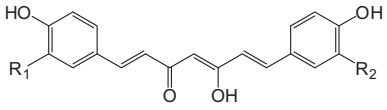
223 $R_1 = R_2 = OCH_3$
224 $R_1 = R_2 = CH_3$



225

6.6.8. Diarylheptanoid

The curcumins, curcumin (**226**), desmethoxycurcumin (**227**) and bis-desmethoxycurcumin (**228**), isolated from the rhizomes of *Curcuma longa*, show significant anti-leishmanial activity against promastigotes of *L. major*. However, these metabolites also inhibit the proliferation of human lymphocytes.[235]

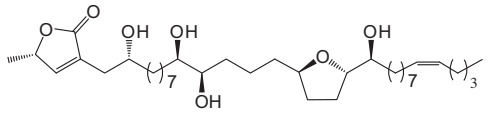


226 $R_1 = R_2 = OCH_3$
227 $R_1 = H$, $R_2 = OCH_3$
228 $R_1 = H$, $R_2 = H$

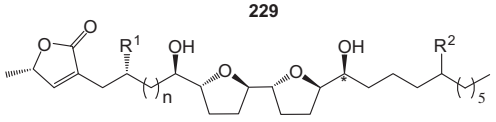
6.7. Other metabolites

Acetogenins like senegalene (**229**), squamocine (**230**), asimicine (**231**) and molvizarine (**232**), isolated from the seeds of *Annona senegalensis* (Annonaceae), show activity against promastigotes of *L. major* and *L. donovani* at concentrations that vary between 38.60 and 160.63 μM. However, these metabolites also show cytotoxicity greater than that of vinblastine against KB and VERO cell lines.[106] Other acetogenins such as rolliniastatin-1 (**233**), isolated from *Rollinia emarginata* (Annonaceae), annonacin A (**234**) and goniothalamicin (**235**), obtained from *Annona glauca* (Annonaceae), display promicing activity against the promastigote of *L. braziliensis*, *L. donovani*, *L. amazonensis*, however a clear SAR has not been established.[236]

Klaivanolide, a bisunsaturated seven-membered lactone 5-acetoxy-7-benzoyloxymethyl-7*H*-oxepin-2-one (**236**), isolated from *Uvaria klaineana* (Annonaceae), shows potent in vitro antileishmanial activity against both sensitive and amphotericin B-resistant promastigote forms of *L. donovani* with IC_{50} values of 1.75 and 3.12 μM, respectively.[237]

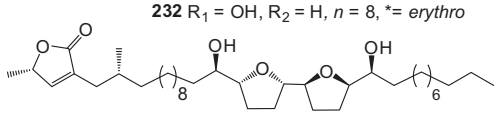


229

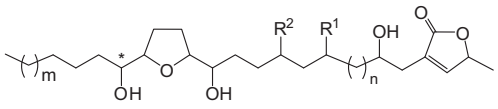


*threo-trans-threo-trans-**

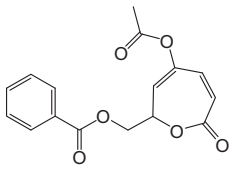
230 $R_1 = H$, $R_2 = OH$, $n = 10$, *= *erythro*
231 $R_1 = OH$, $R_2 = H$, $n = 10$, *= *threo*
232 $R_1 = OH$, $R_2 = H$, $n = 8$, *= *erythro*



233



234 $R_1 = OH$, $R_2 = H$, $n = 5$, $m = 8$, *= *erythro*
235 $R_1 = H$, $R_2 = OH$, $n = 3$, $m = 10$, *= *threo*



236

7. Combating multi drug resistance (MDR) naturally

Drug resistance is an important problem in parasitic diseases, which is further exacerbated by the limited number of drugs available especially for neglected tropical diseases. Major challenges of antileishmanial chemotherapy are increasing incidence of drug resistance particularly against pentamonials. During the recent years combination therapy approach is being applied to achieve better cure rate as well as to treat resistant cases. Although, clinical trails have showed faster cure rate but it cannot overrule the possibility of resistance development. Drug resistance mechanisms in leishmanial species are not well understood, which further complicates the issue as the knowledge of mechanisms can also suggest novel therapeutic strategies. A common mechanism of drug resistance to metal ions in *Leishmania* is active efflux of the metal ions and their reduced uptake. This involves an ABC transporter PGPA however; other metal binding thiols are also responsible.[238,239] Thus, strategies and inhibitors that modulate the activity of efflux pumps could be useful in the treatment of leishmaniasis. The in vitro studies indicate that PGPA mediated antimony resistance in *Leishmania* can be reduced by using specific thiol biosynthesis inhibitors such as buthionine sulfoximine (BSO) **237**, a specific inhibitor of γ-glutamylcysteine synthetase, an enzyme involved in glutathione and trypanothione biosynthesis, offers candidate for combination therapy.[240]

A number of studies demonstrate that natural products from plants such as flavonoids are potential drugs to overcome MDR

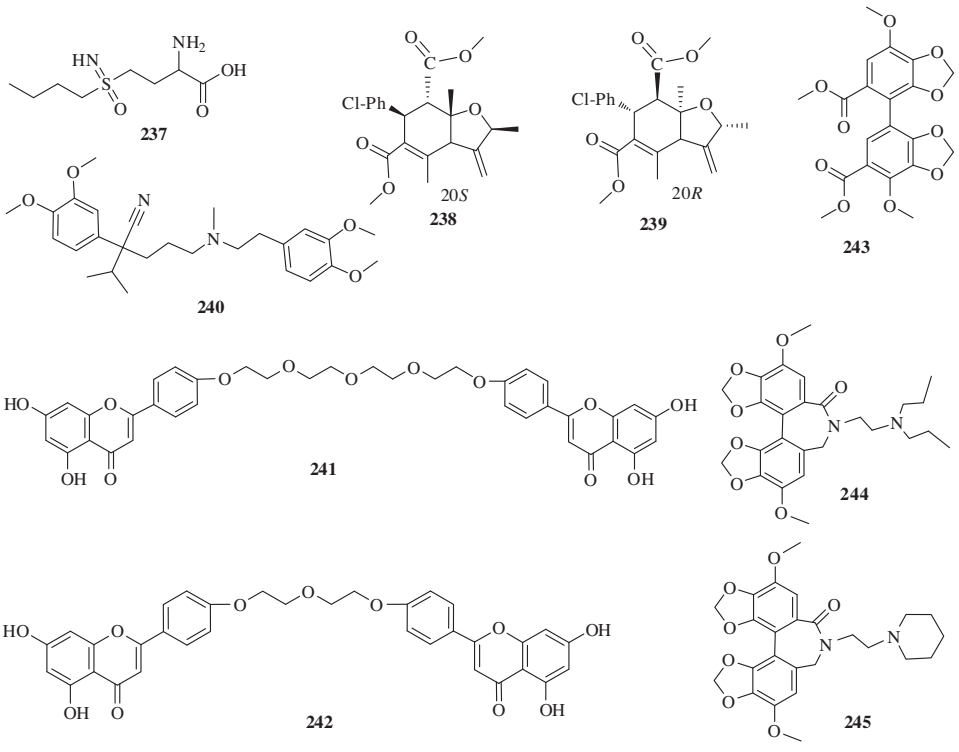
in many multi drug resistant cells and many flavonoids are active ABC transporter inhibitor.[241–244] The 1,4-dihydropyridine family of compounds exhibit ability to overcome leishmanial resistance to common drugs in MDR. The compounds containing oxazolo [3,2-*a*]pyridines unit that is enantiomers 20*S* (**238**) and 20*R* (**239**) display significant reversion of resistance to daunomycin and miltefosine in *L. tropica* strain with 6.7-fold and 8.7-fold reversion indexes respectively. Most surprisingly, the enantiopure compound **238** reverts the resistance to both drugs and fairly more significantly than **239**. Thus, these compounds can be used in combination with antilieshmanial drugs to overcome the problem of drug resistance.[245]

Verapamil (**240**), a P-glycoprotein (PGP) inhibitor, exhibits ability to reverse the resistance caused by PGP in cancer cells and hence, further studies are required for its applicability in leishmaniasis. Likewise, chalcones, the precursors of flavonoids, display similar inhibitory effect on PGP function and would be studied for reverting drug resistance.[246] The synthetic flavonoid bivalent dimers **241** and **242** containing three and four ethylene glycol unit exhibit significantly higher reversing activity than other shorter or longer ethylene glycol-ligated dimers, in pentamidine and sodium stibogluconate (SSG) resistance. The monomers do not exhibit modulatory effect, which suggest that bivalent nature of flavonoid is significant for resistance reversal. Thus, bivalency would be an useful strategy for the development of more potent ABC transporter modulators for overcoming pentamidine and SSG resistance in *Leishmania* species.[247]

8. Key issues of leishmanial research and concluding remarks

Leishmaniasis is a poorly investigated disease that mainly affects people in developing countries. Research aimed at identification and validation of drug targets is in one of the important aspect for *Leishmania* specific drug development. The cell biology of parasite differs considerably as compared to host mammalian cells and this distinctness extends to the biochemical level as well. Thus, many of the parasitic proteins and enzymes can be successfully exploited for drug targets.[250]

In the last two decades several 'interesting drug targets' have been proposed including many proteins and enzymes that differ from mammalian counterpart. Screening of natural products seems to be an attractive approach, which can result in the efficient elucidation of new lead compounds or drugs.[251,252] Although, large numbers of antileishmanial compounds have been evaluated with excellent activities in last decade however, none has ended in fruitful result. One of the major hurdles in the drug development process is the lack of knowledge about chemical and biological interaction mainly due to less interest and poor collaboration between diverse research groups. The above documented natural compounds has been mainly evaluated on inhibition of parasite growth and proliferation but unfortunately, only few studies reports the mechanisms of action. The complete sequence data of *L. major* and *L. infantum* have been elucidated thus genomic and proteomic approaches should be used to identify pathways that have already been targeted in similar organisms. In addition, approaches like structure-based drug/inhibitors designing against potential drug targets using bioinformatics tools can be utilized to develop true antileishmanial drugs. More chemical syntheses are required to obtain analogues of lead natural compounds or other enzyme inhibitors so that lead compounds can be progressed into drug development. The following key issues may be considered for a goal-oriented research on drug development against leishmaniasis.



Bifendate (**243**) and its derivatives **244** & **245** are potential inhibitor of PGP-mediated multidrug resistance. The in vitro low intrinsic cytotoxicity of **245** bearing 6,7-dihydro-dibenzo[*c*,*e*]azepine unit represents it as a promising lead for developing therapeutics targeting PGP mediated MDR in combinational chemotherapy.[248,249] These evidences suggest that combination therapy drugs along with inhibitors of MDR will be extremely useful for antileishmanial chemotherapy in drug resistant endemic areas.

- Limited drug options and possibility of resistance development is major and serious hurdle in the elimination of leishmaniasis in disease endemic countries.
- Significant research progress that has made during the last decades but exact mechanism of resistance against pentamonials is not yet elucidated. Hence, extensive studies are required to resistance mechanisms as well as to increase the efficacy of pentamonials.
- Notwithstanding combination therapy approach is providing initial success; however, due to lack of information about the precise mode of action of drugs, combinatorial approach is restricted and associated with possible emergence of multidrug resistance *Leishmania* strains.
- In absence of synthetic antileishmanial chemistry, screening and identification of natural products as novel drug agents is urgently required. Most of the studies conducted so far are mainly preliminary in vitro studies. Hence, more in vivo studies are required in order to obtain an effective and true antileishmanial compound.
- Further, serious cellular toxicity validation studies of possible antileishmanial compounds are required for their safety to meet human compliance.
- Since all biochemical, immunological and molecular mechanisms of parasites are known; attempts should be made to understand the mechanisms of action of antileishmanial compounds.
- Many of the newly identified compounds posses immunemodulatory nature along with antileishmanial activity, which is a very important benefit. Because leishmaniasis is characterized by compromised immune response possible effort should be made to identify these compounds to develop an effective drug candidate.
- Multidrug resistance (MDR) against first line antileishmanial drugs can be solved by using MDR inhibitory compounds, however effect of these drugs on immunemodulation of host is needed to be explored further.

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