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Rational Approaches for Drug Designing Against Leishmaniasis

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Abstract Leishmaniasis has been ignored for many years mainly because it plagues remote and poor areas. However, recently, it has drawn attention of several investigators, and active research is going on for antileishmanial drug discovery. The current available drugs have high failure rates and significant side effects. Recently, liposomal preparations of amphotericin B are available and have proved to be a better drug, but they are very expensive. Miltefosine is one of the few orally administered drugs that are effective against *Leishmania*. However, it has exhibited teratogenicity, hence, should not be administered to pregnant women. Thus, the search for novel and improved antileishmanial drugs continue. A rational approach to design and develop new antileishmanials can be to identify several metabolic and biochemical differences between host and parasite that can be exploited as drug target. Moreover, many natural products also have significant antileishmanial activity and are yet to be exploited. In the current review, we aim to bring together various drug targets of *Leishmania*, recent development in the field, future prospects, and hope in the area.

Keywords Leishmaniasis · Drug development · Drug targets · Metabolic pathways · Proteins

Introduction

Leishmaniasis is mainly a poverty-related disease caused by 20 species of genus *Leishmania*, a protozoa transmitted by the bite of a 2- to 3-mm long insect vector female sandfly. Two genera of sandfly transmit *Leishmania* to humans: *Lutzomyia* in the New

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World and *Phlebotomous* in the Old World. This disease is formerly known as Orient Boil, Baghdad Boil, black fever, sand fly disease, Dum-Dum fever, or espundia [1]. As per World Health Organization statistics, leishmaniasis currently threatens 350 million men, women, and children in 88 countries around the world. The vector condition of the disease is most favorable in some parts of Third World countries due to poor living conditions and sanitation [2]. Over 90% of the cases of leishmaniasis occur in different parts of India, Bangladesh, Nepal, Sudan, and Brazil [3]. In India, the state of Bihar accounts for more than 90% of the total reported cases [4]. However, human migration and climate changes has broadened the ecologic niche of *Leishmania's* vector and, currently, human infections are found in 16 countries in Europe, including France, Italy, Greece, Malta, Spain, and Portugal [5]. Recently, India has launched a program to eliminate visceral leishmaniasis (VL) from the country by 2010.

In many of the endemic areas, dogs are considered as the major reservoir for human disease, while in other regions, people are the principal reservoir for further human infections [6]. Dogs appear naturally resistant to this parasite and may remain asymptomatic despite infection [7].

There are three forms of leishmaniasis depending on the infecting species, which results in wide range of clinical symptoms. These forms are cutaneous (CL), mucocutaneous (MCL), and VL. The most of the VL, which is also known as kala-azar, is caused by *Leishmania donovani* and is fatal if untreated. In case of VL, the parasites reside in the liver, spleen, and bone marrow causing a severe systemic disease. Other two forms are less serious, CL and MCL, and causes ulcers on the face, arms, and legs. They heal slowly but leave scars or cause disfiguring. The causative *Leishmania* species of CL are *Leishmania major*, *Leishmania tropica*, *Leishmania ethiopica*, *Leishmania braziliensis*, *Leishmania panamensis*, *Leishmania amazonensis*, and *Leishmania mexicana* [8].

The life cycle of *Leishmania* parasite involves two forms, promastigote and amastigote. Promastigote stage of parasite is injected into the human host by infected sandfly. The promastigotes are first phagocytosed by macrophages where they are transformed into amastigote, multiplied, and released to systemic circulation. Non-motile amastigotes subsequently infect different tissues, depending on the *Leishmania* species, which causes the corresponding clinical manifestation of the disease. When sandflies bite an infected host, there is intake of amastigotes. In the vector fly's midgut, these parasites differentiate into the so-called promastigote form, which multiplies and finally migrates to the fly's proboscis [9].

The treatment of leishmaniasis strongly relies mainly upon pentavalent antimonials, sodium stibogluconate (Pentostam), and meglumine antimoniate (Glucantime) introduced in the first half of the century. The mechanism of action of these drugs is not clearly understood, but they are reported to have significant side effects [10]. Moreover, failure rates of these drugs are approximately in 10–25% of cases, except in state of Bihar (India), where the failure rate is >60%. The new and first ever oral drugs Miltefosine, a phosphocholine analog originally developed as antimalignant drug, has been found to be highly active against *Leishmania* in vitro as well as in animal model. Miltefosine and Amphotericin B appear to be better treatment options, but problem of resistance and side effect remains uncracked [11]. High cost precludes the use of Miltefosine and the latter, Amphotericin B, is teratogenic, thus not recommended for pregnant women [12]. Another drug, paromomycin sulfate (Humatin), approved for use recently, achieved cure rates similar to those with amphotericin B in patients with VL with similar side effects [13].The above discussion is indicative of the urgent need to identify new antileishmanial compounds.

Antileishmanial Drug Discovery by Targeting Various Metabolic Pathways

Trypanothione Metabolism

A rational approach to design and develop new antileishmanials has identified several metabolic and biochemical differences between host and parasite that can be exploited as drug target. One such validated drug target is trypanothione reductase [14]. In mammals, glutathione (γ-glutamyl-cysteinyl-glycine; GSH) metabolism is the principle route of removal of reactive oxygen intermediates, such as the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), and the hydroxyl radical (HO^-) produced by cellular respiratory process or by external agents like host immune system. Glutathione synthetase is one of the important enzyme involved in synthesis of glutathione. The enzyme that converts it from its oxidized form, glutathione reductase, (nicotinamide adenine dinucleotide phosphate (NADPH)-dependent), is constitutively active and inducible upon oxidative stress. Oxidized glutathione is reduced immediately and is found mainly in its reduced form. Selenium-dependent glutathione peroxidase reduces reactive oxygen intermediates and, in the process, converts GSH to GSSH [15]. However, *Leishmania* parasite lacks this antioxidant defense mechanism and relies on cascade of three enzymes: trypanothione synthetase trypanothione-recycling flavoprotein trypanothione reductase (TR) and tryparedoxin-recycling enzyme tryparedoxin peroxidase (TP) working in concert with trypanothione (N^1, N^8-bis (glutathionyl)-spermidine), tryparedoxin (TryX) and NADPH. Trypanothione synthetase catalyzes trypanothione synthesis from glutathione and spermidine. The synthesized trypanothione is maintained in reduced form by trypanothione reductase in the presence of NADPH, which in turn reduces tryparedoxin followed by reduction of 2 Cyc-peroxiredoxin tryparedoxin peroxidase. As shown in Fig. 1, The active tryparedoxin peroxidase is then used to catalyze the reduction of hydrogen peroxide and organic hydro-peroxides to water and alcohol [16]. Thus, trypanothione reductase and tryparedoxin peroxidase, which are analogous to mammalian glutathione reductase and glutathione peroxidase, respectively, and trypanothione synthetase, which is analogous to glutathione synthetase, are the novel targets for development of new drug by rational inhibitor design. Dumas et al. [14] have investigated further the physiological role of TR in *Leishmania* and tried to create TR-knockout mutants by gene disruption in *L. donovani* and *L. major* strains using the selectable markers neomycin and hygromycin phosphotransferases. The experiment has established that the enzyme is essential for survival of the parasite [14]. Lots of research is focused on identification of specific inhibitors of TR. However, most of research is focused on other trypanosomal species and may not be inhibitory against TR from *Leishmania* species due to minute difference in structure. Bonse et al. [17] have evaluated series of 9-amino and 9-thioacridines as inhibitors of trypanothione reductase (TR) from *Trypanosoma cruzi.* The compounds are structural analogs of the acridine drug mepacrine (quinacrine), which is a competitive inhibitor of the parasite enzyme, but not of human glutathione reductase, the closest related host enzyme. 9-Aminoacridines are competitive inhibitors of TR with more than one binding site, while 9-thioacridines inhibit TR with mixed-type kinetics [17]. Recently, quaternized analogs of the 2-chlorophenyl phenyl sulfides are also reported to be antileishmanial and suggested to be the inhibitor of TR [18]. The bisbenzylisoquinoline alkaloids were also shown to be potent inhibitors of TR and trypanocidal agents [19]. Antimicrobial chlorhexidine {1, 1′-hexamethylenebis [5-(4-chlorophenyl) biguanide]} is also shown to be an inhibitor of TR [20]. Only limited animal studies have been reported on these compounds. Only the phenothiazines and related tricyclic antidepressants were shown

to reduce parasite burden in infected mice. Most of the other compounds are not effective, most likely because they get metabolized in the body. Thus, search for new class of TR inhibitor is still on. Moreover, inhibitory effect of these compounds against *Leishmania* TR needs to be evaluated.

Methylglyoxal Metabolism

Methylglyoxal, a highly active dicarbonyl glycolytic byproduct, causes mutagenesis and cytotoxic effects by reacting with nucleophilic centers of RNA, DNA, and proteins. In all mammalian cells, the detoxification of methylglyoxal solely depends on a universal glyoxalase system which involves a sequential action of glyoxalase I (Glx1; EC 4.4.1.5) and glyoxalase II (Glx2; EC 3.1.2.6) in concert with a tripeptide glutathione as cofactor [21]. However, for a similar purpose, the trypanosomatid relies on an analogous trypanothione-dependent glyoxalase system in which Glx1 catalyzes formation of S-D-lactoyltrypanothione from the hemithioacetal formed non-enzymatically from methylglyoxal and reduced trypanothione. In the next step, Glx2 catalyzes an irreversible hydrolysis of S-D-lactoyltrypanothione to D-lactate and regenerates trypanothione [22].

Although the trypanosomatids and their mammalian host both have similar glyoxalase system, their difference in substrate specificity indicates that Glx1 or Glx2 could be a potential target for antitrypanosomatid chemotherapy. Moreover, the glyoxalase system has

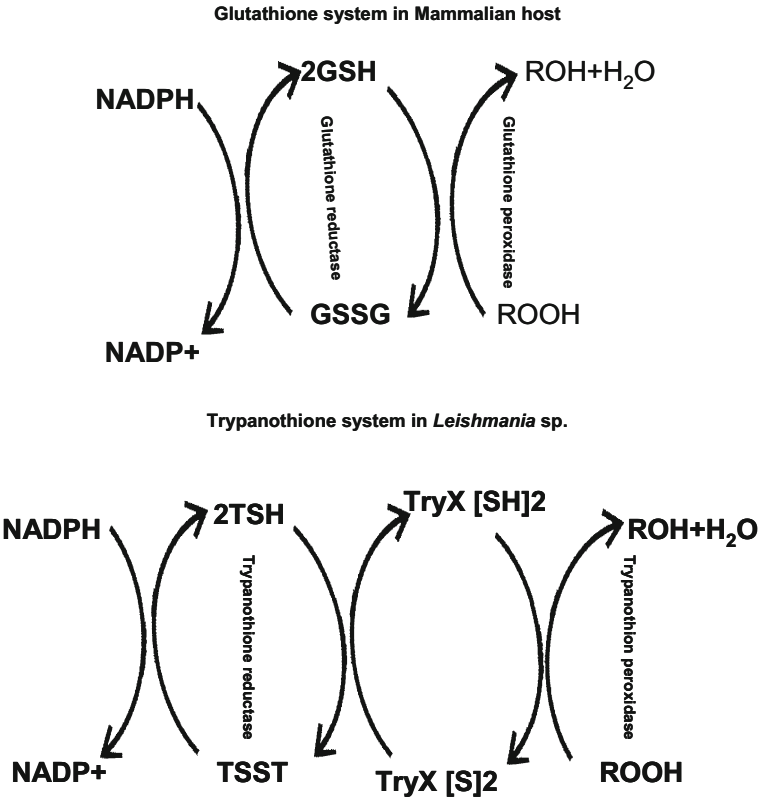

Fig. 1 Difference is oxidative stress removal metabolism between mammalian host and *Leishmania* parasite

already been proposed as an interactive target for anticancer [21] and antimalarial [22] chemotherapy, thus the inhibition of the trypanothione-dependent glyoxalase pathway of *Leishmania* sp. may be a possible solution of leishmaniasis.

Parasite Glycolysis

The energy metabolism of trypanosomatids solely depends on the available carbon sources in their hosts. The amastigote forms of *Leishmania* species use glucose of mammalian bloodstream and fatty acids of lysosomal compartment of macrophages as energy source. Whereas, the insect stage of *Leishmania* species (promastigote) encodes an amylase to obtain energy from amino acids catabolism and a sucrase-like protein for digestion of disaccharides taken in by the sand flies feeding on nectar or honeydew. The metabolic adaptation of the rapid environmental change, caused by parasite transmission between vertebrates host to insect, is regulated by peroxisomes-like organelles called glycosomes [23]. The net ATP production in glycolysis pathway from glucose to pyruvate results in autocatalysis of hexokinase (HXK) or phosphofructokinase (PFK) that in-turn causes the accumulation of hexose phosphate intermediates to lethal level. In trypanosomatids, the unique compartmentalization of glycolysis within glycosomes regulates HXK and PFK catalysis, in order to avoid the negative side effect of autocatalytic design of glycolysis, whereas in other organisms, this is performed by a feedback inhibition of HXK and PFK [24].

Glycosomes contain the seven glycolytic enzymes catalyzing glucose to 3-phosphoglycerate conversion, where the consumption and production of ATP is balanced. The net ATP synthesized in cytosolic portion of remaining glycolysis pathway is inaccessible through glycosomal membrane for HXK or PFK. Hence, the compartmentalization of glycolysis provides an alternative mechanism to prevent the accumulation of toxic level of glycolysis intermediates, glucose 6-phosphate, fructose 6-phosphate, and fructose 1,6-bisphosphate [25]. This unique organization of glycolytic pathway in *Leishmania* sp. and its large evolutionary distance to mammalian host indicates the occurrence of unique structural and functional features of intermediate enzymes in parasite. Thus, these enzymes could be potential targets for antileishmanial drug.

Purine Salvage Pathway

All parasitic protozoa, including *Leishmania* sp. lack the ability to synthesize purine nucleotides de novo and absolutely rely on a unique salvage enzyme system to obtain purine bases from their mammalian hosts. In *Leishmania* sp., at the first step of this process, purine bases are transported across parasite membrane by two kind of nucleoside transporters, LdNT1 and LdNT2, present on cell surface [26]. LdNT1 is present in both promastigote and amastigote stages of the parasite life cycle and transports adenosine/pyrimidine nucleoside, while LdNT2, present at the amastigote stage, specifically transports only purine nucleosides [27]. Since these purine transporters have an important role in nucleoside transportation within cells, they could be a potential route for delivery of analogous cytotoxic drug delivery in *Leishmania* species.

Within parasite, the enzymes adenine deaminase and guanine deaminase catalyze the conversion of adenine and guanine to hypoxanthine and xanthine, respectively, which in turn are converted to inosine monophosphate (IMP) and xanthine monophosphate (XMP), respectively, by phosphoribosyl transferase (PRTase) activity of adenine phosphoribosyl transferase (APRTase), hypoxanthine-guanine phosphoribosyl transferase (HGPRTase), and xanthine phosphoribosyl transferase (XPRTase). Adenylosuccinate synthetase and adeny-

losuccinate lyase convert IMP to AMP, whereas XMP is converted to GMP by GMP synthetase [28].

Moreover, it has also been reported that *Leishmania* sp. contains trace amount of nucleoside kinase, which can directly convert nucleosides to mononucleotides [29]. Thus, various alternative purine salvage pathways exist in the parasite, so inhibition of a single enzyme would not be lethal for them. Therefore, a chemotherapeutic agent that can inhibit more than one enzyme of purine salvage pathway could be an alternative solution of antileishmaniasis drug.

Natural Products as Antileishmanial Compound

Natural products have played an important role in the drug discovery process. Several medicinal plants have been traditionally used for treatment of leishmaniasis. Many compounds were isolated from plant source and found to have antileishmanial activity, although the exact mechanism of action of these plants extract against *Leishmania* parasite remains unclear. It is likely that these natural products may have inhibitory effects against one or more parasite-specific enzyme or parasitic enzyme whose mammalian counterpart is significantly different.

A variety of aromatic ketones that forms the central core for many biological compounds, known collectively as chalcones, is shown to have antileishmanial activity. Licochalcone A is one of such compounds, isolated from the roots of licorice, *Glycyrrhiza inflate.* The compound strongly inhibited amastigotes state of *L. major.* It has been demonstrated that licochalcone A alters the ultrastructure and function of the mitochondria of *Leishmania* parasites. Licochalcone A is reported to inhibit parasite-specific fumarate reductase enzyme [30]. Another chalcone, 2′,6′-Dihydroxy-4′-methoxychalcone was purified from the dichloromethane extract of *Piper aduncum* and showed significant activity against promastigotes and intracellular amastigotes of *L. amazonensis* [31]. The antileishmanial activity of 2′,6′-dihydroxy-4′-methoxychalcone was further enhanced after encapsulation in polymeric nanoparticles. Recently, Torres-Santos et al. [31] have shown that the compound alters the sterol composition of *L. amazonensis* and suggests that the parasite target is different from other known sterol inhibitors.

Two chalcones, 2,2′,4′-trihydroxy-6′-methoxy-3′,5′-dimethylchalcone and 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone, from methanolic extract of *Psorothamnus polydenius* have also shown to have antileishmanial activity [32].Three isoflavans, isolated from the Iranian plant *Smirnowia iranica* (Fabaceae), 8-prenylmucronulatol, lyasperin H, and smiranicin, inhibited the growth of *L. donovani* promastigotes. *S. iranica* appears to have a rich source of antileishmania isoflavans. However, these compounds isolated from *S. iranica* show little host cell toxicity, which limits their pharmaceutical value.

The antileishmanial activity of these flavonoids was correlated to minicircle linearization in parasites and inhibition of leishmanial topoisomerase II catalytic activity [32]. Similarly, several other plant secondary metabolites are shown to have antileishmanial activity and listed in Table 1. Many plant extracts are shown to have antileishmanial activity, but the chemical nature of the compound is not defined in such cases. The chloroform fraction extract of *Peschiera australis* was shown to have antileishmanial activity [33]. Aqueous onion extracts shows antileishmanial effect against many *Leishmania* species [34]. Hexane, dichloromethane, ethyl acetate, and methanol extracts of *Warburgia ugandensis* (Canellaceae), a Kenyan medicinal plant, shows strong antileishmanial activity [35]. Crude ethanolic extract of Indian medicinal plant, *Desmodium gangeticum*, also shows significant activity against *Leishmania* [36].

Table 1 Various natural products shown to have antileishmanial activity.

S No	Compound	Comments	Plant	Reference
1	Argentilactone	Lactones	*Annona haematantha* (Annonaceae)	[43]
2	Klaivanolide	Lactones	*Uvaria klaineana* (Annonaceae)	[43]
3	Rolliniastatin-1	Acetogenins	*Rollinia emarginata* (Annonaceae)	[44]
5	Sylvaticin	Acetogenins	*Rollinia emarginata* (Annonaceae)	[44]
6	Quamocin	Acetogenins	*Rollinia emarginata* (Annonaceae)	[44]
7	Minquartynoic acid	Acetylenes	*Minquartia guianensis*	[45]
8	Actinodaphnine[a]	Alkaloid	*Cassytha filiformis* (Lauraceae)	[46]
9	Sarachine	Aminosteroid	*Saracha punctata* (Solanaceae)	[47]
10	2-n-Propylquinoline	Quinoline alkaloids	*Golipea longiflora* (Rutaceae)	[48]
11	Chimanine B	Quinoline alkaloids	*Golipea longiflora* (Rutaceae)	[48]
12	Chimanine D	Quinoline alkaloids	*Golipea longiflora* (Rutaceae)	[48]
13	Canthin-6-one-alkaloids	Alkaloids	*Ailanthus excelsa*	[49]
14	Amarogentin[b]	Secoiridoid glycoside	*Picrorhiza kurrooa*	[50]
15	Liriodenine	Alkaloid	*Stephania dinklagei* (Menispermaceae)	[51]
16	N-methylliriodendronine	Alkaloid	*Stephania dinklagei* (Menispermaceae)	[51]
17	Xylopine	Alkaloid	*Guatteria amplifolia* (Annonaceae)	[52]
18	Berberine	Alkaloid	*Hydrastis canadensis*	[53]
19	Benzoquinolizidine cephaeline	Alkaloid	*Psychotria klugii* (Rubiaceae)	[54]
20	Pendulone	Quinones	*Millettia pendula*	[55]
21	1-Hydroxybenzoisochromanquinone	Quinones	*Psychotria* (*Cephaelis*) *camponutans*	[56]
22	Benzo[g]isoquinoline-5,10-dione	Anthranoid	*Vismia orientalis* (Clusiaceae)	[56]
23	Emodin	Anthranoid	*Vismia orientalis* (Clusiaceae)	[57]
24	Vismione D	Anthranoid	*Vismia orientalis* (Clusiaceae)	[57]
25	3-Geranyloxy-6-methyl-1, 8-dihydroxyanthraquinone	Anthraquinones	*Vismia orientalis* (Clusiaceae)	[57]
26	Diospyrin[c]	Naphthoquinones	*Diospyros montana*	[50]
27	Yuccasaponin MC3	Saponin	*Yucca filamentosa* (agavaceae)	[58]
28	3-Oxotirucalla-7, 24Z-dien-26-oic acid	Triterpene carboxylic acid	*Celaenodendron mexicanum*	[59]
29	Isoiguesterin	Triterpenes	*Salacia madagascariensis* (Celasteraceae)	[60]
30	Simalikalactone D	Triterpenes	*Simaba orinocensis* (Simaroubaceae)	[61]

[a] The alkaloid was shown to interfere with the catalytic activity of DNA topoisomerases.

[b] Inhibits the activity of DNA topoisomerase I.

[c] Selective inhibition of topoisomerase I (does not inhibit topoisomerase II).

Table 2 Various pathways/proteins targeted by different inhibitors and may be potential drug targets.

Pathway	Proteins targeted	Inhibitors/compounds	Mode of Inhibition	Reference
Trypanothione metabolism	Trypanothione reductase	Pentavalent antimonial compounds, e.g., sodium stibogluconate and meglumine antimoniate	It boosts IFN-gamma and T-cell production, inducing a strong Th1 response	[62]
		Melarsoprol	Melarsoprol forms covalent complex with trypanothione reductase thus inactivating it and exposing parasite to oxidative stress	[8]
		Kukoamine A (a natural hypertensive agent), isolated from *Lycium chinense*	Mixed-type inhibition of trypanothion reductase (TR)	[63]
		Acridine drug mepacrine (quinacrine)	Competitive inhibitor of TR	[17]
		9-Aminoacridines and 9-thioacridines	9-Aminoacridines are competitive inhibitors of TR with more than one binding site while 9-thioacridines inhibit TR with mixed-type kinetics	[19]
		Clomipramine	Competitive inhibitor of TR	[64]
		Altenusin (a biphenyl from *Alternaria* sp.)	Inhibitor of TR	[65]
Cell signal transduction	Phospholipids and sterols	Miltefosine	Inhibition of phospholipid and sterol biosynthesis	[8]
Polyamine biosynthesis	Ornithine decarboxylase	Pentamidine (Pentam-300)	Iinteracts with trypanosomal kinetoplast DNA	[66]
Cell growth and proliferation	Protein and phospholipids	Nebulizer	Nebulizer inhibits incorporation of nucleic acids into RNA and DNA, causing inhibition of protein and phospholipid synthesis	[8]
Microbial respiration	Fumarate reductase	Licochalcone A	Inhibits the conversion of fumarate to succinate and disrupts ultrastructure of mitochondria of amastigote stage of *Leishmania* lifecycle	[30]
Ergosterol biosyntesis	Sterols	2′,6′-dihydroxy-4′-methoxychalcone	Alters the sterol composition	[31]
DNA Replication	DNA topoisomerase II	Flavonoids (e.g., 8-prenylmucronulatol, lyasperin H, and smiranicin)	Inhibits DNA topoisomerase II catalytic activity	[32]
Immune system	–	KMP-11 DNA vaccine	Elevates levels of IFN-gamma and thus the immune response	[67]
Oxidative pathway	Amastigotes	Redox active dinitrodiphenylthioethers	Generation of reactive oxygen species in microphages containing amastigotes	[68]

Conclusion

As discussed earlier in the review, several metabolic pathways like trypanothione metabolism, methylglyoxal metabolism, parasite glycolysis, purine salvage pathway, and target enzymes/molecules of the pathways have been identified for antileishmanial drug discovery. Extensive research is being carried out to identify compounds which can interfere with parasite-specific pathway and can be further developed in the drug against leishmaniasis. We have summarized such studies in Table 2. Moreover, several plant contain antileishmanial compound. An extensive list is given in Table 1. However, in most of the cases, exact mode of action of the plant product is not known. Few of the compounds from the plant origin has been studied in more detail, and mode of action/or target molecule is known. As mentioned in Table 1, diospyrin, a naphthoquinones from *Diospyros montana*, and amarogentin, a secoiridoid glycoside from *Picrorhiza kurrooa*, are selective inhibitor of topoisomerase I. Moreover, the compound vasicine or peganine, found in the plant *Peganum harmala*, has been tested in vitro against the promastigote stage of *L. donovani.* It was shown that this compound induces apoptosis in *Leishmania* promastigotes. Peganine hydrochloride dehydrate is more safe and induces apoptosis in all stages of life cycle of *L. donovani* by eliminating transmembrane potential of mitochondria [37]. Moreover, betulinic acid, a naturally occurring pentacyclic triterpenoid, and Luteolin, a flavonoid isolated from *Salvia tomentosa*, are found to inhibit topoisomerase of *Leishmania* [38, 39]. An anticarcinogen, 3,3′-diindolylmethane which is derived from indole-3-carbinol found in *Brassica* vegetables is found to have antileishmaniasis activity by inhibition of F_0F_1-ATP synthase [40]. Recently, our group has focused the attention on development of novel therapeutics against leishmaniasis by targeting parasite-specific trypanothione metabolic pathway [41, 42].

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