Fighting Against Leishmaniasis: Search of Alkaloids as Future True Potential Anti-Leishmanial Agents

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Abstract: Leishmaniasis, a group of tropical diseases caused by protozoan parasites of genus *Leishmania*, is a major health problem worldwide that affects millions of people especially in the developing nations. Generic pentavalent antimonials have been the mainstay for therapy in the endemic regions due to efficacy and cost effectiveness, but the growing incidence of their resistance has seriously hampered their use. In many cases the drugs employed for the treatment are toxic, marginally effective, given by injection and, compromised by the development of resistance. Therefore, the development of new mechanism based safe, effective and affordable chemotherapeutic agents to fight leishmaniasis would be an urgent priority research. The recent researches focused on natural products have shown a wise way to get a true and potentially rich source of drug candidates against leishmaniasis, where alkaloids have been found more effective. The present review briefly illustrates an account on current status of leishmaniasis, life cycle of parasites and biology, synergy of the disease with HIV, therapeutic options available to cure this disease and, highlights why natural products especially alkaloids as folk medicines are so important? Additionally, the outlines for the leishmanicidal activities of various alkaloids including indole, quinoline, isoquinoline, pyrimidine-β-carboline, steroidal and diterpene alkaloids from various plants as well as alkaloids from marine sources have been provided with their mechanistic studies.

Key Words: Leishmaniasis, treatment, natural products and alkaloids.

[#] Dedicated to Late Professor Arya K Mukherjee, Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi-221005.

1. INTRODUCTION

The leishmaniasis has been considered a tropical affliction that together constitutes one of the six entities on the World Health Organization tropical disease research list of the most important diseases [1]. It occurs in 88 countries of tropical and temperate regions, while 72 of them are developing or least developed. An estimated 350 million population is at risk and 10 million peoples are affected from this disease worldwide [2]. Two million cases occur annually however, there is a gross under reporting of the cases from endemic regions. There has been a progressive increase in the cases of leishmaniasis, and it is being reported from the newer areas as well [3,4].

Leishmaniasis is caused by 20 species of *Leishmania* and transmitted by 30 species of sand fly [5]. *Leishmania* species are divided into Old World and New World species by geographic location of endemic species. Most of *Leishmania* infections are zoonotic, rodents and canids are reservoir host. The parasite is carried by the female phlebotamine sandfly of the genus *Phlebotamus* in the Old World or *Lutzomyia* in the New World [6]. Only two *Leishmania* species can maintain anthroponotic human-human cycle. They are *L. donovani*, responsible for visceral leishmaniasis (VL) in Indian subcontinent & East Africa, and *L. tropica*, responsible for cutaneous leishmaniasis (CL) in the Old World [7].

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The annual global burden of VL (also known as *kala-azar*) is about 500,000. Out of these, about 90% cases occur in India, Nepal, Bangladesh and Brazil. In these countries VL is endemic and epidemics are quiet frequent, which leads to considerable mortality. Population migration leads to epidemic of disease in non-endemic areas. The epidemic of VL in Western Upper Nile and Southern Sudan that led to about 100,000 deaths among 280,000 populations in this area is an example of the devastating nature of the disease [8]. In India, about 100,000 cases of VL are estimated to occur annually, and epidemiology of the disease is changing due to widespread migration of population and emerging HIV/VL co-infection. The risk of VL among AIDS patients increases by 100-1000 times in endemic areas while VL accelerates the onset of AIDS in HIV infected people [6].

VL comprises broad range manifestations of infection that remains asymptomatic or sub-clinical in many cases or can follow acute or chronic courses. The clinical symptoms are characterized by prolonged and irregular fever often associated with rigor and chills, splenomegaly, lymphadenopathy, hepatomegaly, pancytopenia, progressive anemia, weight loss and hypergammaglobulinemia (mainly IgG from polyclonal B cell activation) with hypoalbunemial. It is always fatal if left untreated. After recovery, some patients (50% in Sudan and 1-3% in India) develop post kala-azar dermal leishmaniasis (PKDL) [9].

2. TAXONOMY OF LEISHMANIA SPP.

Among the different genera of *Trypanosomatidae* family, the taxonomic classification [3] of genera *Leishmania* may be summarized form Fig. (**1**).

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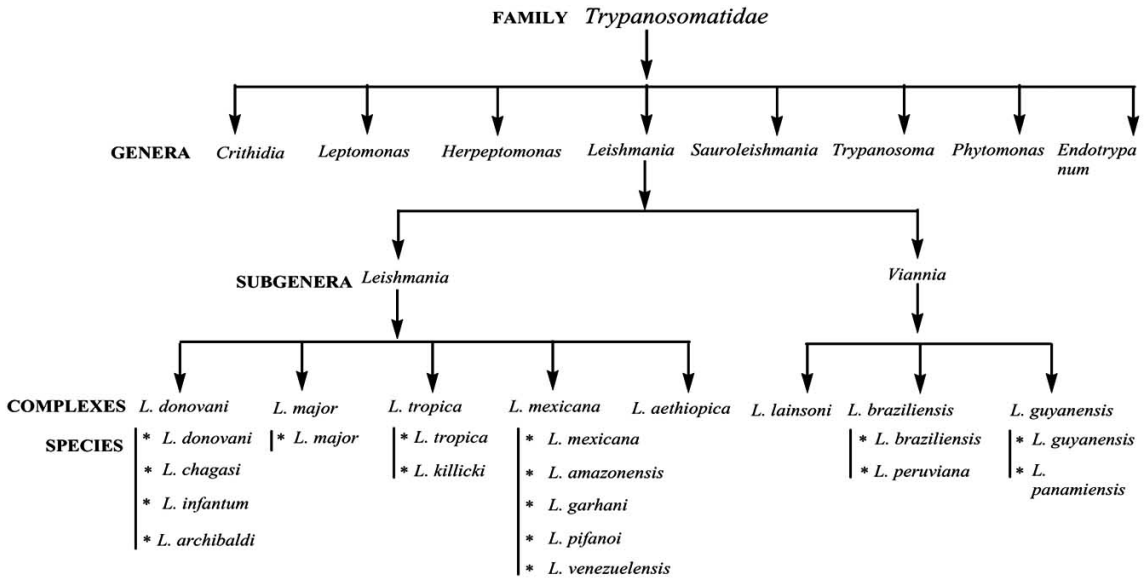


Fig. (1). Taxonomic classification of *Leishmania* spp.

3. MORPHOLOGY

The *Leishmania* parasites are unicellular protozoa that exist in two distinct morphologic forms. In the alimentary tract of insect vectors, the parasite exists extracellularly as the flagellated motile promastigote. In the phagolysosomal system of the vertebrate host mononuclear phagocytes, the parasite occurs intracellularly as oval shaped non-flagellated amastigotes [10]. The environment of the phagosome becomes acidified after parasites or bacteria are ingested by polymorphonuclear leukocytes. In contrast, promastigotes perform their metabolic processes maximally at 7.0 pH. Since these parasites belong to the order Kinetoplastidae, they are characterized by the presence of a cytoplasmic organelle called, kinetoplast. It is a giant mitochondrion located adjacent to the basal body of the flagellum, containing intertwined circular DNA molecules, called maxicircles and minicircles, which make up 5-10% of total DNA and, are termed as kinetoplast DNA (kDNA). The function of maxicircles is to encode mitochondrial proteins. Minicircles are present in larger numbers and are highly heterogeneous in most of the species. They encode the guide RNAs that participate in the process of RNA editing of maxicircle transcripts [11].

All species of *Leishmania* parasite are essentially indistinguishable morphologically, except the *L. enriettii*, which is not a human pathogen and, infects guinea pigs. Promastigotes have spindle-shaped body with a single anterior flagellum and, are 10 to 15 pm in length measuring 1.5 to 3.5 μM at their widest part. The monoflagellated amastigotes have spherical shape with a diameter of approximately 2 to 3

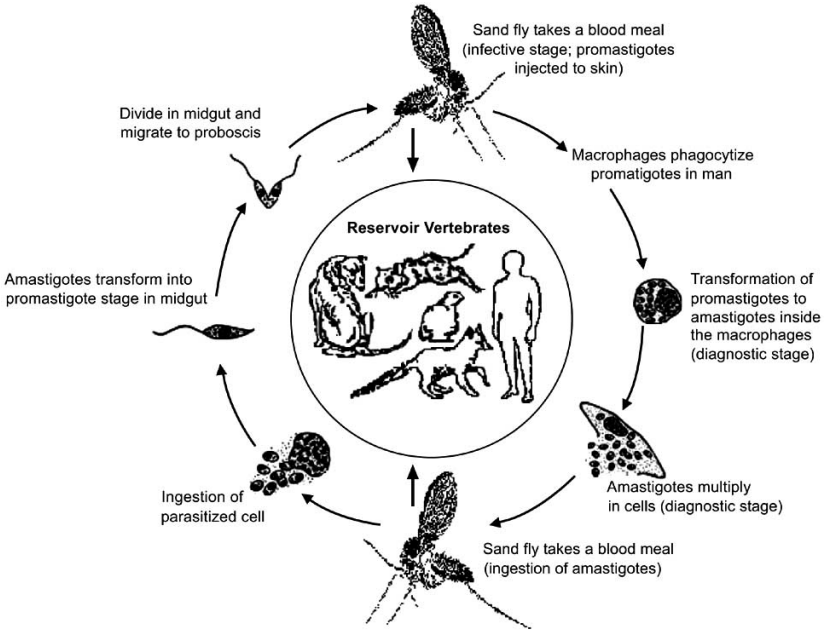


Fig. (2). Life cycle of *Leishmania* parasite.

pm. The average amount of DNA per cell is 91 x 106 and 242 x 106 base pairs for the promastigotes of *L. donovani* and *L. braziliensis*, respectively. For *L. mexicana*, the quantity of protein per cell is 5.3 pg for promastigote and 1.3 pg for amastigote [12].

4. LIFECYCLE OF LEISHMANIA PARASITE

Sand flies of the genera *Phlebotamus* in the Old World or *Lutzomyia* in the New World are the proven vectors for the leishmaniasis. The amastigotes, after ingestion by the sand fly from infected vertebrate host, migrate to the midgut of sand fly and transform into the promastigote. After a period of days to weeks, as a result of replication by means of binary fission and subsequent migration to foregut of the insect, promastigotes partially obstruct the digestive tract of the insect. Infected sand fly during second blood meal regurgitates the infectious promastigotes from its pharynx into the bloodstream of host vertebrate (e.g., canines, marsupials, edentates, and rodents). Once inside the bloodstream of reservoirs for disease, promastigotes are phagocytosed by mononuclear phagocytic cells of the host. They transform into the amastigotes and, begin to replicate within the modified phagolysosomes (designated as arasitopherous vacuoles), eventually, the host cells lyse, releasing the free parasites that spread to new cells and tissues, causing lesions and tissue destruction. Amastigotes present in the blood and tissues of an infected person are ingested by a biting sand fly and the life cycle repeats itself [12].

5. TREATMENT

In the beginning of the 20th century, generic pentavalent antimonial compounds (Sb^v) have been the main drug to treat the infected persons in India and other part of world. But the resistance of *Leishmania* parasites against pentavalent antimonials has made the current situation terrific, as there is no true anti-leishmanial drug. Moreover, all of the drugs available needs to be given parentally instead of recently developed miltefosine, and are potentially toxic.

The organic pentavalent antimonials were first used in 1912, soon after the recognition of fact (in 1904) that *Leishmania* species were the cause of leishmaniasis. Pentostam"®" (sodium stibogluconate) and Glucantime"®" (meglumine antimoniate) has been the mainstay therapy for VL. However, due to high cost (approx 200USD per patients) of branded sodium stibogluconate, a generic sodium antimony gluconate (SAG, Albert David Ltd, India, 13USD per patients) is presently being used to treat patients satisfactorily without any significant difference in final cure [13,14].

In India, SAG has been used successfully for several years to treat patients suffering from VL, but during the last few years parasite has become resistant for Sb^v. Till late 1970, a small daily dose (10 mg per kg, 600 mg maximum) for short duration (6-10 days) was considered adequate for the treatment of disease. However, unconfirmed reports have suggested a treatment failure of 30% with this regimen [15]. Based on an expert committee revised recommendation to use Sb^v in two 10 days (20 mg/kg, 600 mg total) courses with interval of 10 days [16], an improvement in cure rates (99%) were noted [17]. Few years later, Thakur *et al.* (1984) randomized patients to receive Sb^v 20 mg/kg (maximum 600 mg) either for 20 days or longer, and found only 86% cure rate [18]. In the same year WHO expert committee recommended that Sb^v be used in doses of 20 mg/kg per day up to maximum of 850 mg for 20 days, and a reported similar regimen for 20 days in cases of treatment failure [19]. However, following the above recommendation, a significant decline in cure rate from 60-70% has been reported from hyper endemic regions of India [20,21].

The reason for the emergence of resistance is widespread misuse of the drug, as SAG is freely available in India and is easily accessible over the counter. In the endemic regions, VL affected patients (73%) first consult unqualified quacks that might not use the drug appropriately [22]. It has been observed that only a minority (26%) were treated according to prescribed guidelines. Irregular use and incomplete treatments were common occurrence [23,24]. Further, it's a common practice to start with small dose, and gradually building up the dose over a week. Drug free intervals are given with the belief that it will prevent renal toxicity. Many times the daily dose of drug is split into two injections, to be given twice daily. These practices presumably expose the parasites to drug pressure, leading to progressive tolerance of parasite to SAG [25].

Unfortunately, little is known about the mechanism of underlying drug resistance as seen in human visceral leishmaniasis. After administration, pentavalent antimonials are converted into trivalent compounds for the antileishmanial effects. The reduction of pentavalent to trivalent compound takes place either in macrophages or in the parasite. In the later case, loss of reductase activity of parasite may lead to resistance. It is supported by the observation that Sb^v resistant *L. donovani* amastigotes loose their reductase activity. Molecular studies have identified an ATP binding cassette (ABC) transporter system, P-glycoprotein A (PGPA) involved in the metal resistance [25,26]. PGPA is a member of multidrug resistance protein family, whose substrate includes organic anions and drugs conjugated to glutathione, glucoroate or sulphate. The recently identified gene in some of our antimony unresponsive isolates, clearly suggests for being involved in conferring resistance to the parasites. However, the exact mechanism by which it produces resistance is yet to be elucidated [27]. Since the amplified sequence in clinical isolates did not hybridize with PGPA gene or multidrug resistance gene (MDR), suggesting involvement of some other mechanism in drug resistance in these strains.

6. THERAPEUTIC OPTIONS

SAG is no longer be used in VL hyper endemic regions of India because of its declining efficacy. During the past few years various other drugs have been tried with varying efficacy however, these drugs are not true anti-leishmanials.

Pentamidine was the first drug to be used for patient refractory to Sb^v [28]. The pentamidine regimen consisted of 4 mg/kg, given three times per week until initial parasitological cure was achieved. Initially high cure rate was reported, but its efficacy has gradually declined over years and, now it cures only 70% of patients [29,30]. Moreover, this drug is associated with serious adverse effects like shock, hypoglycemia and occasional death in significant proportion of patients.

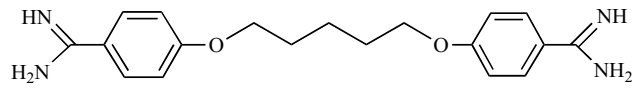


Fig. (3). Structure of pentamidine.

Amphotericin B, a pollen antibiotic, is now being more widely used for VL and, has established a major advance in antileishmanial chemotherapy during the last 20 years. The target of amphotericin B is ergosterol like sterols, which are the major membrane sterols of *Leishmania* species. Due to high affinity of amphotericin B for sterols, aqueous pores are formed in the membrane leading to increased membrane permeability and, killing of *Leishmania* parasite. At a dose of 0.75-1 mg/kg for 15 infusions on alternate days, it cures more than 97% of patients [31,32]. Occasional relapse (1%) may occur with amphotericin B that would be treated successfully with the same drug. It has been recommended as the first line drug in India by National Expert Committee for Sb^v refractory regions of VL for the last seven years [33].

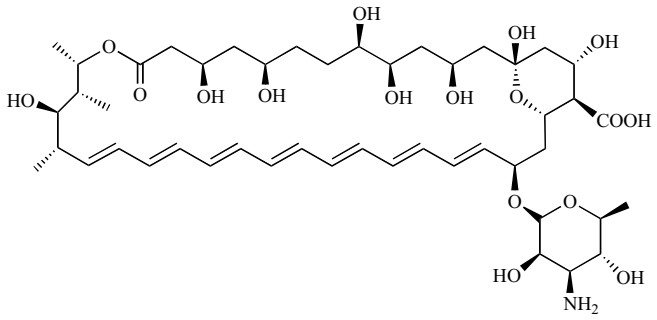


Fig. (4). Structure of amphotericin B.

The need to develop less toxic, more effective formulation of amphotericin B has led to the development of three new clinical formulations of amphotericin B in which deoxycholate has been replaced by other lipids. These formulations are liposomal ampho B (L-AmB: Ambiosome®), amphotericin B colloidal dispersion (ABCD: Amphocil®) and amphotericin B lipid complex (ABL: Abelcit®). These substitutes are well taken up by reticuloendothelial system, and poorly taken up by kidney, the major target of organ toxicity. Safety of liposomal amphotericin B permits administration of total dose requirement in a single infusion [34,35]. However, prohibitively high cost makes these compounds unaffordable in VL endemic countries like India.

Alkyl phospholipids, miltefosine, originally developed as an anti tumour agent has excellent potency against leishmaniasis too. In all clinical studies, a cure rate $\geq$ 94% has been found consistently with this drug. Unfortunately, the drug has mild gastrointestinal adverse events like vomiting and diarrhea in 40% and 20% patients, respectively. Miltefosine has been approved in India for treatment of VL at a dose of 50-100 mg (~2.5 mg/kg) for four weeks [36,37]. It has also been found safe and effective in VL affected children [38,39]. However, there are certain limitations of this drug. Miltefosine has a median long terminal half-life of 154 hours that would encourage the development of clinical resistance as well as teratogenic and abortafacient effects. In spite of all hype and trials, the drug is not in use by medical advisors either at district level or at periphery in Indian sub continent probably due to high cost and serious side effects.

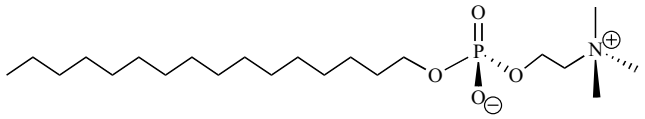


Fig. (5). Structure of miltefosine (hexadecylphosphocholine).

Paromomycin is an aminoglycoside antibiotic with unique antileishmanial activity. It acts synergistically with antimonials *in vitro*, and the combination has been used effectively in India. The drug is effective, well tolerated and as cheap as conventional amphotericin B. Its efficacy has been demonstrated in India and a dose of 16 mg/kg intramuscularly for 21 days has cured 93% of patients [40,41]. Recently, this drug has been shown to be noninferior to amphotericin B for the treatment of visceral leishmaniasis in India in phase III open-label studies [42].

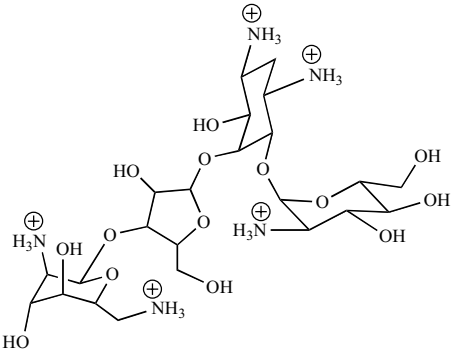


Fig. (6). Structure of paromomycin.

Sitamaquine, a primaquine analogue (8-aminoquinolene), is another orally administrable compound. However, its development has been very slow and, little is known about the efficiency and toxicity. It has been under development for over 8 years by SmithKline Becham (now Glaxo SmithKline) and Walter Reed Army Institute of Research, USA [43]. In a randomized, open label and multicenter phase II trial in India and Kenya, the drug has been found efficacious and well tolerated at various dose levels [44,45].

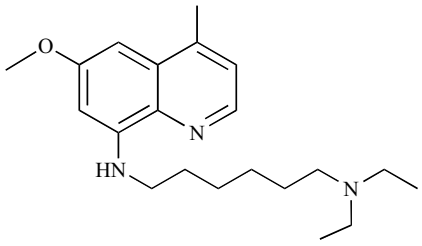


Fig. (7). Structure of sitamaquine.

7. USE OF NATURAL PRODUCTS: ALKALOIDS AS FOLK MEDICINES

As we have discussed that all the available drugs excluding SAG, are not the true anti-leishmanials to cure leishmaniasis. Moreover, they are not only expensive but also have

several side effects. Hence, search of a true antileishmanial drug is still a paramount interest for medicinal chemist.

Natural products are potential sources of new and selective agents for the treatment of important tropical diseases caused by protozoans and other parasites [46]. Utility of natural products in drug discovery and development is not surprising, as many of plant derived alkaloids have shown antileishmanial activity. Alkaloids, the so-called secondary metabolites, a characteristic feature of plants, are especially important since they protect plants against a wide variety of microorganisms (viruses, bacteria, fungi) and herbivores (arthropods, vertebrates). These are equally important to man kinds, due to their diverse range of effects on parasite biology. In addition to naturally occurring alkaloids like morphine, vinblastine, vincristine and quinine, many alkaloids have provided the skeletal basis for designing of more pharmacologically useful compounds *e.g.* pethidine, buprenorphine, verapamil, mefloquine etc.

Natural product literature provides a growing research on plant derived antileishmanial alkaloids. Many of alkaloids are reported to have excellent antileishmanial activity. However, none of them have been evaluated in clinical studies or projected to reach the clinical applications in near future. We feel useful to consider the importance of plants derived alkaloids in view of their antileishmanial potentials. This review is focused to cover the entire formal and constant research on leishmanicidal alkaloids from the mid-1980 to late 2007 with special attention on structure-activity relationship (SAR) based activity and mechanism of action for most of the alkaloids, in addition to a number of bioassay procedures reported in literature. Hence, in the coming sections we will discuss different alkaloids, which have been shown to be effective against leishmaniasis (Tables **1** & **2**).

7.1. Indole Alkaloids

Among some of the indole alkaloids, harmaline (**1**) is reported to possess significant antileishmanial activity [49]. The metabolite **1**, exhibits weak activity against promastigotes with an IC_{50} of 116.8 μM, but shows strong amastigote-specific activity with an IC_{50} of 1.16 μM [50]. The mechanism of action for compound **1** is based on its ability to intercalate DNA or interfere with the metabolism of amino acids in the parasite. However, due to monoamino oxidase inhibitory action, compound **1** produces psychopathic effect that prevents its use as therapeutic agent [51].

An iboga-type indole alkaloid, coronaridine (**2**), isolated from *Peschirea australis* (Apocynaceae) shows activity against promastigote and amastigote forms of the *L. amazonensis* parasite. The metabolite **2** also occurs in other species of the *Peschirea* genus, i.e. *P. laeta*, *P. van heurkii*, *E. coronaria* and also has been synthesized. The metabolite **2** displays antipromastigote activity (97% killing) with an IC_{50} of 12 μg/mL. The metabolite possibly effects on energy metabolism of the parasite, but the exact mechanism responsible for parasite killing has not been specified [52].

Among the indole alkaloids isolated from the bark of *Corynanthe pachyceras* (Rubiaceae), the dihydrocorynantheine (**3**), corynantheine (**4**) and corynantheidine (**5**) exhibit excellent *in vitro* antileishmanial activity against *L. major* with IC_{50} ~3 μM [49]. The mechanism of action of these metabolites is based on the inhibition of the respiratory chain of the parasite [53].

The indole analogues harmane (**6**), pleiocarpine (**7**) and buchtienine (**8**), obtained from stem bark and leaf of *Kopsia griffithii* (Apocynaceae), display significant *in vitro* an-

Table 1. Summary of Medicinal Plants with Leishmanicidal Alkaloid Fractions

Plant Family	Botanical Name of Plant	Plant Parts	Organism Tested
Annonaceae [48]	*Cardiopetalum calophyllum* Schldl.	LF + SM	*L. amazonensis* *L. braziliensis* *L. donovani*
	Duguetia spixiana Mart.	SB	*L. amazonensis* *L. braziliensis* *L. donovani*
	Guatteria foliosa Benth	SB	*L. amazonensis* *L. braziliensis* *L. donovani*
	Guatteria schoburgkiana Mart.	RB	*L. amazonensis* *L. braziliensis* *L. donovani*
	Oxandra espintana (Spruce)	SB	*L. amazonensis* *L. braziliensis* *L. donovani*
	Xylopia aromatica (Lam.)	LF + SB	*L. amazonensis* *L. braziliensis* *L. donovani*

(Table 1. Contd….)

Plant Family	Botanical Name of Plant	Plant Parts	Organism Tested
Berberidaceae [48]	*Berberis boliviana* Lechl.	BK + SM	*L. amazonensis* *L. braziliensis* *L. donovani*
	Berberis bumeliaefolia Schum	BK	*L. amazonensis* *L. braziliensis* *L. donovani*
	Berberis cf. laurina Epl.	SM	*L. amazonensis* *L. braziliensis* *L. donovani*
	Berberis aff. paucidentata	SB	*L. amazonensis* *L. braziliensis*
Menispermaceae [48]	*Abuta pahni* Mart.	SM	*L. amazonensis* *L. braziliensis*
	Abuta rufescens Aublet	BK	*L. amazonensis*
	Anomospermum bolivianum Kruk. & Mold	BK	*L. amazonensis* *L. braziliensis*
Papaveraceae [48]	*Bocconia integrifolia* H and B	LF + SB	*L. amazonensis* *L. braziliensis* *L. donovani*
	Bocconia pearcei Hutch.	LF	*L. amazonensis* *L. braziliensis* *L. donovani*
Rutaceae [48]	*Dictyoloma peruvianum* Planch.	SB	*L. amazonensis* *L. braziliensis*
	Galipea longiflora Kr	LF	*L. amazonensis* *L. braziliensis* *L. donovani*
		LF + RB	*L. amazonensis* *L. braziliensis* *L. donovani*
		RB	*L. amazonensis* *L. braziliensis* *L. donovani*
Apocynaceae [97]	*Aspidosperma ramiflorum* Muell. Arg.	SB	*L. amazonensis* *L. braziliensis*
Nitrariaceae[98]	*Peganum harmala*	AP	*L. major*

LF, leaves; SB, stembark; SM, stem; AP, aerial part; BK, bark; RB, rootbark.

tileishmanial activity against promastigotes of *L. donovani*. Among these, metabolite **8** shows highest antileishmanial activity against promastigotes of *L. donovani* with IC_{50} less than 1.56 μg/mL in compared to **6** (6.25 μg/mL) and **7** (25 μg/mL) [54].

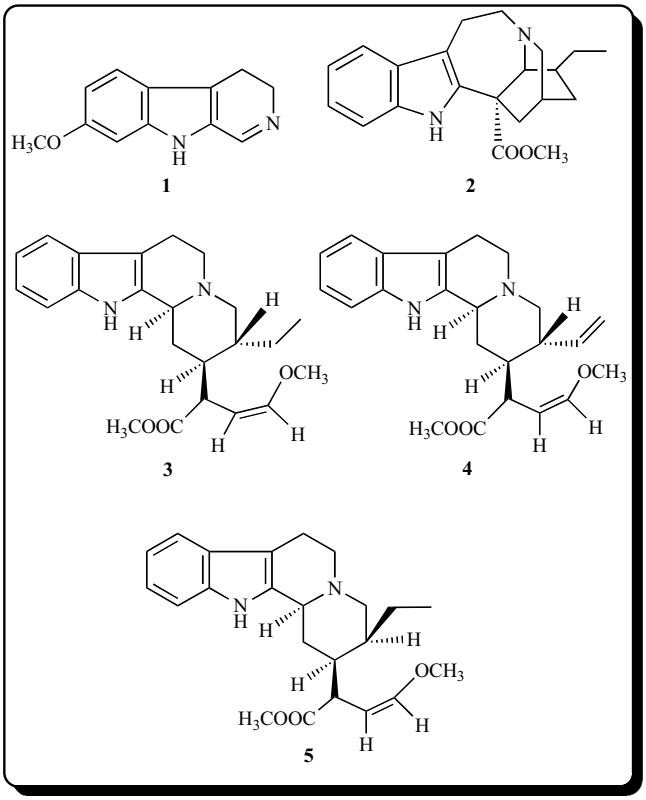
Monoterpenoid indole alkaloids ramiflorine A (**9**) and B (**10**), isolated from stem bark of *Aspidosperma ramiflorum* (Apocynaceae), show significant antileishmanial activity against promastigotes of *L. amazonensis* with LD_{50} of 16.3± 1.6 μg/mL and 4.9 μg/mL, respectively. These metabolites

display high activity in compared to pentamidine, a drug used in clinical practices (LD_{50} = 10.0 μg/mL) [55].

Table 2. Alkaloid and Their Synthetic Analogues with Leishmanicidal Activity

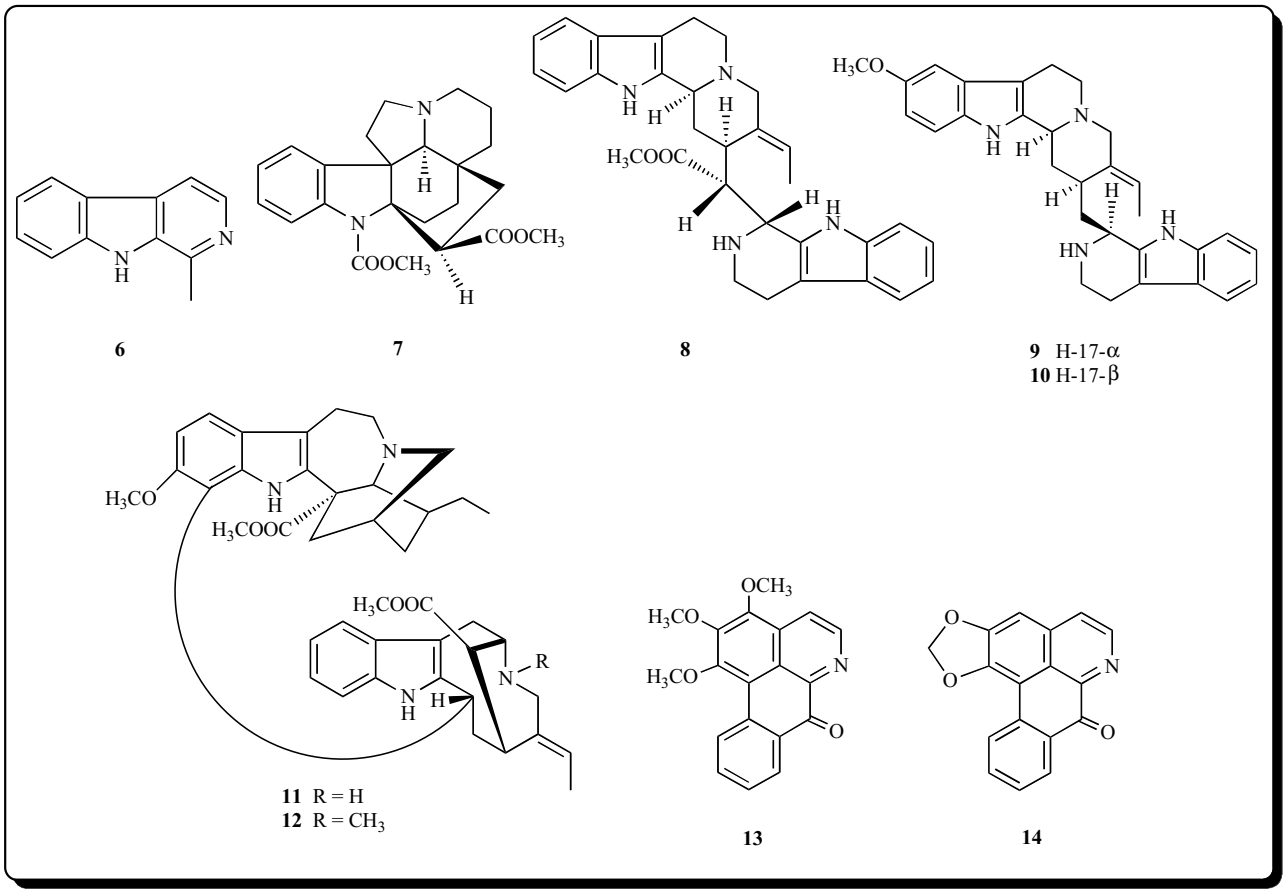
Indole Alkaloids	Limacine (42)[a]
Harmaline (1) [a, b]	Isotetradrin (43) [c]
Coronaridine (2) [a, b]	Puertogaline A (44) [a]
Dihydrocorynantheine (3) [a, b]	Puertogaline A (45) [a]
Corynantheidine (5) [a]	**Quinoline alkaloids**
Corynantheine (4) [a]	2-n-propylquinoline (46) [a, c]
Harmane (6) [a]	Chimanine D (47)[a, c]
Pleiocarpine (7) [a]	Chimanine B (48)[a]
Buchtienine (8) [a]	2-Styrylquinoline (49) [c]
Ramiflorine-A (9) [a]	(2-(2'-Hydroxypropyl) quinoline (50)[c]
Ramiflorine-B (10) [a]	Dictylomide A (51) [a]
Gabunine (11) [a, b, c]	Dictylomide B (52) [a]
Conodurine (12) [a, c]	Quninoline (53) [c]
Isoquinoline alkaloids	Quninoline (54) [c]
O-methylmoschatoline (13) [a]	Quninoline (55) [c]
Liriodenine (14) [a, b]	Quninoline (56) [c]
Berberine (15) [a, c]	Quninoline (57) [c]
Isoguattouregidine (16) [a]	Quninoline (58) [c]
Anonaine (17) [a]	**Steroidal alkaloids**
(+)-Isodomesticine (18) [a]	Sarachine (59) [a]
(+)-Norisodomesticine (19) [a]	Holamine (60) [a]
(+)-Nantenine (20) [a]	15-α-hydroxyholamine (61) [a]
(+)-Neolitsine (21) [a]	Holacurtine (62) [a]
(+)-Lirioferine (22) [a]	N-desmethylholacurtine (63) [a]
(+)-N- methylaurotetanine (23)[a]	**Benzoquinolizidine alkaloids**
(+)-Norlirioferine (24) [a]	Klugine (64) [a]
(+)-Isoboldine (25) [a]	Cephaeline (65) [a]
(+)-Reticuline (26) [a]	Isocephaeline (66) [a]
Cryptodorine (27) [a]	Emetine (67) [a]
Nor-nantenine (28) [a]	**Diterpene alkaloids**
Xylopine (29) [a]	15,22-O-Diacetyl-19-oxo-dihydroatisine (68) [a]
Unonopsine (30) [a]	Azitine (69) [a]
Naphthylisoquinoline alkaloids	Isoazitine (70) [a]
Ancistrogriffine A (31) [a]	**Pyrimidine-*β*-carboline alkaloids**
Ancistrolikokine D (32) [a]	*N*-hydroxyannomontine (71) [a]
Ancistroealaine A (33) [a]	Annomontine (72) [a]
Ancistrocladinium A (34) [a]	**From marine invertebrates**
Ancistrocladinium B (35) [a]	Renieramycin A (73) [a]
Ancistrocladidine (36) [a]	Araguspongin C (74) [a, b, c]
Ancistrotanzanine B (37) [a]	**Other alkaloids**
Ancistrotnazanine A (38) [a]	Piperine (75) [a]
Bisbenzylisoquinoline alkaloids	Benzoxazol-2(3H)-one (76) [a]
Daphanandrine (39) [a]	Canthin-6-one (77) [c]
Obaberine (40) [a, c]	5-Methoxycanthin-6-one (78) [c]
Gyrocarpine (41) [a, c]	

In vitro activity against promastigotes, [a] *In vitro* activity against amastigotes, [b] *In vivo* activity[c].



The bis-indole alkaloid gabunine (**11**), obtained from stem bark of *Peschiera van heurkii* (Apocynaceae), shows strong *in vitro* activity against amastigotes of *L. amazonensis* with a survival index (SI) of 3% at 25 μg/mL concentration. Unfortunately, in the *in vivo* assay the metabolite **11** displays no activity possibly due to inactivation in the host [56].

Similarly, the metabolite conodurine (**12**) obtained from same plant sp. shows strong *in vitro* activity against promastigotes of *L. amazonensis* with IC_{50} of 50 μg/mL. However, *in vivo* activity of compound **12** against *L. amazonensis* is



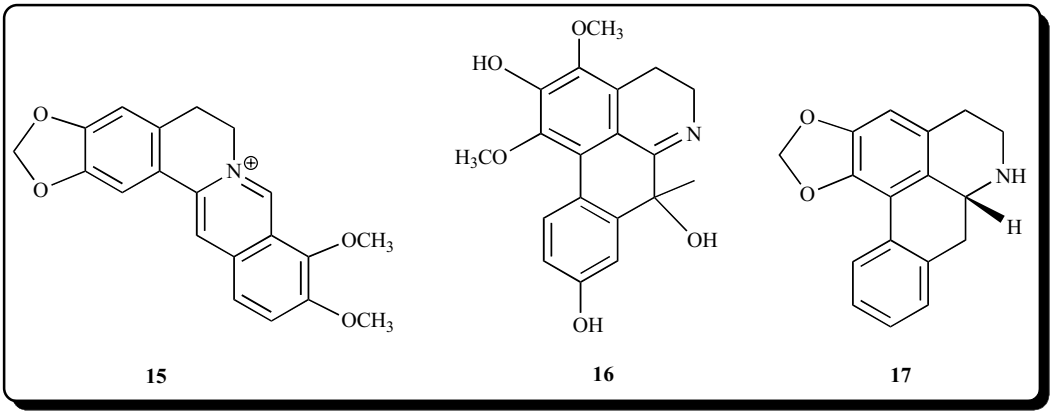
found less than Glucantime"®" (IC_{50} of 40 mg/kg/day, BALB/c mice) and doses of **12** at 200 mg/kg are toxic [57].

7.2. Isoquinoline Alkaloids

The two oxoaporphine alkaloids namely *O*-methylmoschatoline (**13**) and liriodenine (**14**), isolated from *Annona foetida* (Annonaceae) exhibit *in vitro* antileishmanial activity against promastigote forms of *L. braziliensis* with an IC_{50} less than 60 μM. The metabolite **14** also shows efficient activity against promastigotes of *L. guyanensis* with an IC_{50} of 21.5 μM [58]. SAR based study has shown that the alkaloid **14**, possessing a methylenedioxy moiety displays eight times more activity against *L. braziliensis* and *L. guyanensis* than the metabolite **13**.

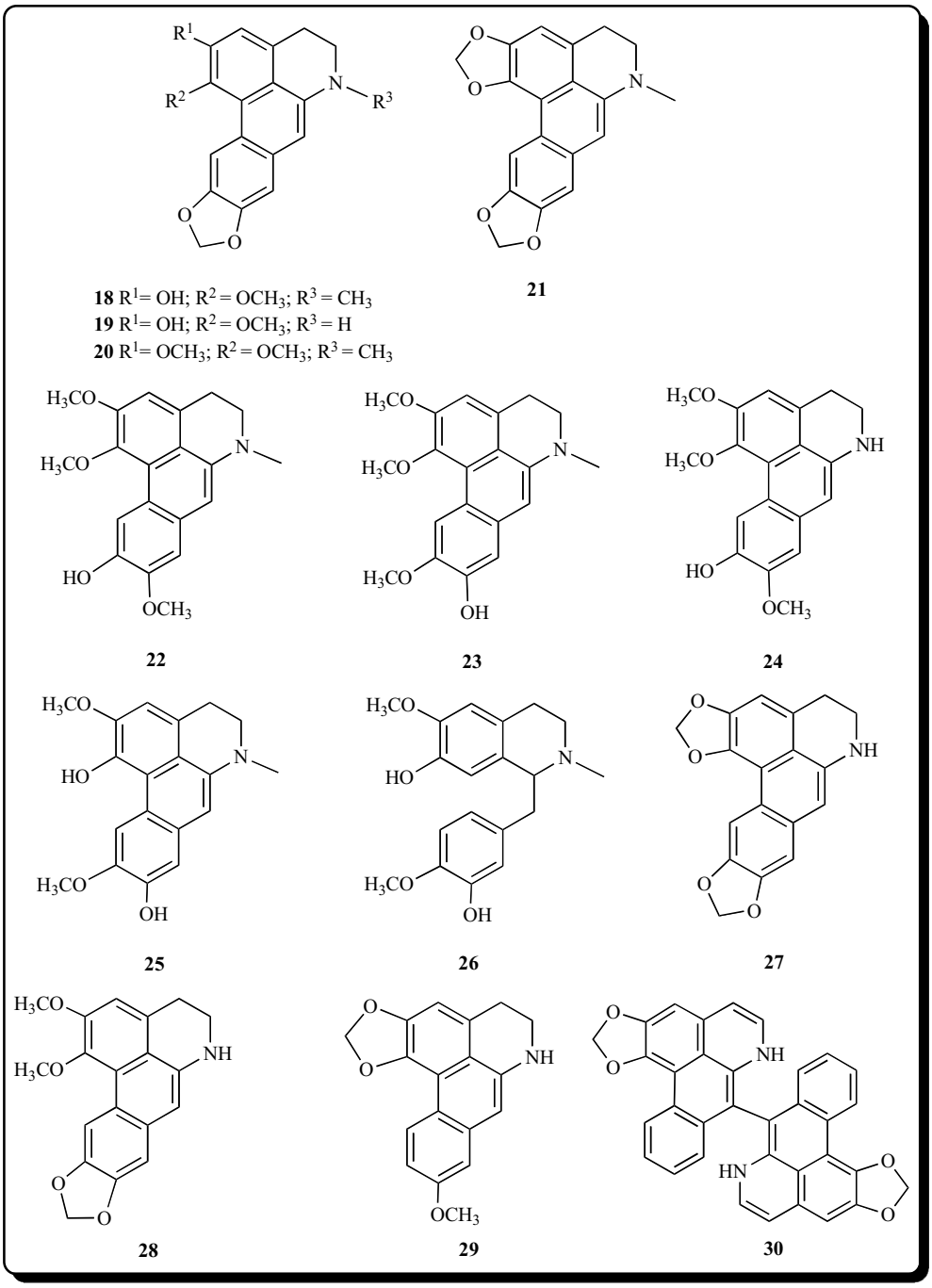
Among the other isoquinoline analogues, the berberine (**15**), an active leishmanisidal constituent of many plant families (*e. g.* Annonaceae, Menispermaceae, Berberifaceae) exhibits highest leishmanisidal activity [59,60]. At a concentration 10 μg/mL, the metabolite **15** effectively eliminates *L. major* parasites in peritoneal mice macrophages. The metabolite isoguattouregidine (**16**), isolated from the bark of *Guatteria foliosa* (Annonaceae), shows antileishmanial activity at a concentration 100 μg/mL against parasites of *L. donovani and L. amazonensis* [49].

Anonaine (**17**) and metabolite **14** isolated from the trunk bark and root of *Annona spinescens* (Annonaceae) exhibit activity against promastigotes of *L. braziliensis, L. donovani*



and L. amazonensis [61]. However, the metabolite **14** when isolated from stem bark of *Rollinia emarginata* (Annonaceae), shows activity against promastigotes of same parasites with an IC_{100} of 5 μg/mL [62]. Use of biphasic media or liquid media for the evaluation of leishmaniasis might be responsible for variation in the biological activity of metabolite **14** against the same species of *Leishmania*.

Several alkaloids containing isoquinoline skeleton have been isolated from the young leaves of plant *Guatteria dumetorum* (Annonaceae) including (+)-isodomesticine (**18**), (+)-norisodomesticine (**19**), (+)-nantenine (**20**), (+)-neolitsine (**21**), (+)-lirioferine (**22**), (+)-N- methylaurotetanine (**23**), (+)-norlirioferine (**24**), (+)-isoboldine (**25**) and (+)-reticuline (**26**) show leishmaniciadal activity against the promastigotes of *L. maxicana* [63]. *In vitro* studies of metabolites **18-26** along with aporphine alkaloids namely, cryptodorine (**27**), nor-nantenine (**28**) and xylopine (**29**) obtained from *Guatteria sp.* [64], have shown for the highest antileishmanial activity associated with the methylenedioxy functionalities. The metabolites **21** and **27** effectively reduce the parasite burden at a concentration 15 μM and 3 μM, respectively, in compared to amphotericin B used for positive control. Studies on SAR have revealed that the replacement of methoxy group leads to a significant decrease in activity among these alkaloids (**22**:210 μM; **23**:395 μM; **24** > 916 μM and **25** > 916 μM). Additionally, a 1,2-metylenedioxy function (i.e. in **29**:3 μM) increases the activity more than a

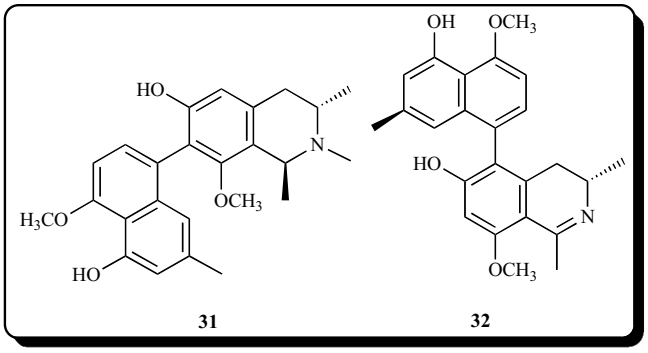


9, 10-methylenedioxy functionality (**18**:73 μM; **19**:48 μM; **20**: 41 μM and **28**:15 μM). Moreover, *N*-methyl group moderately decreases the activity. Alkaloids **18-29** on evaluation for toxicity to murine macrophages, the normal host cell type for the leishmania parasites and to VERO cells (a cell line derived from African green monkey kidney) have shown low toxicity (> 300 μM) in both cell types. Among these the most potent antileishmanial compound **21**, shows high selectivity index to *L. maxicana* over murine macrophages (25 fold).

A dimeric aporphine alkaloid, unonopsine (**30**), isolated from the *Unonopsis buchtienii* R. E. Fries (Annonaceae), displays excellent antileishmanial activity against the promastigotes of *L. donovani* with an IC_{100} of 25 μg/mL in comparison to pentamidine (IC_{100} of 5 μg/mL) used for positive control [65].

7.3. Naphthylisoquinoline Alkaloids

The naphthylisoquinoline alkaloid ancistrogriffine A (**31**), isolated from leaves and twigs of *Ancistrocladus griffithii* (Ancistrocladaceae) displays significant *in vitro* activity against *L. donovani* with an IC_{50} of 3.1 μg/mL [66]. The metabolite ancistrolikokine D (**32**), isolated from roots of *Ancistrocladus likoko* J. Leonard (Ancistrocladaceae) shows efficient antileishmanial activity against the promastigote forms of *L. donovani* with an IC_{50} of 5.9 μg/mL, in compaired to pentamidine (IC_{50} of 5.5 μg/mL) used for positive control [67].

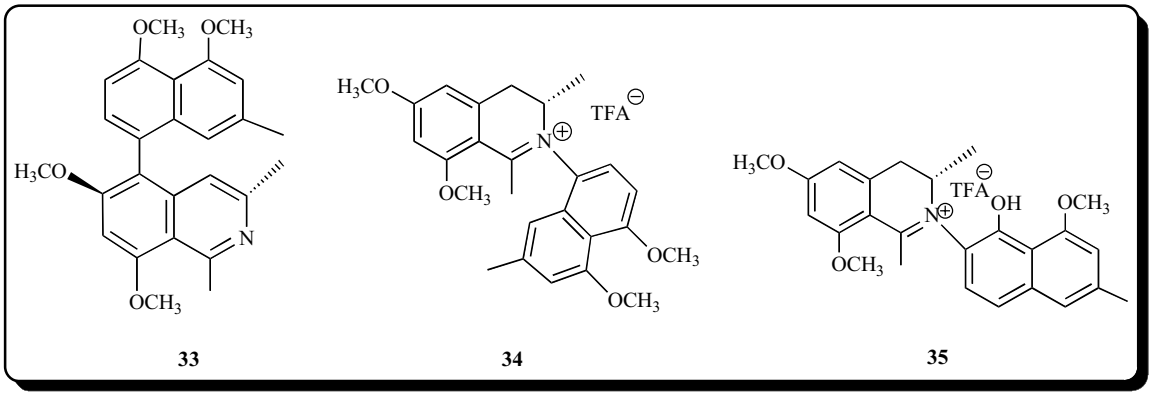


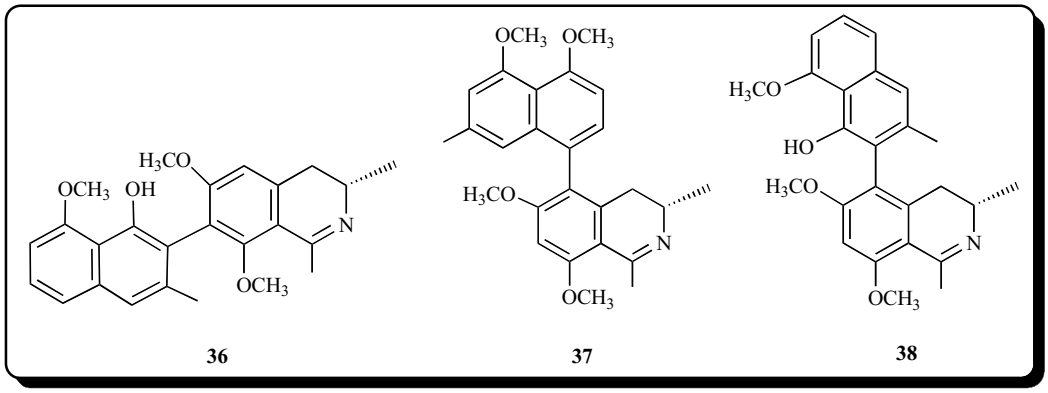
Among the other naphthylisoquinoline alkaloids, ancistroealaine A (**33**) isolated from *Ancistrocladus ealaensis* (Ancistrocladaceae) shows antileishmanial activity against promastigotes of *L. donovani* with IC_{50} of 4.1 μg/mL [68]. The metabolite remains inactive against *L. major* at the same concentration. On making comparison of compound **33** with the other naphthylisoquinoline alkaloids ancistrocladinium A (**34**) and configurationally semistable B (**35**) isolated from yet undescribed Congolese *Ancistrocladaceae sp.*, a same range of concentrations is reportedly needed for compounds i. e. **34** (2.61 μg/mL), **35** (1.52 μg/mL) to reach the IC_{50} towards *L. major* promastigotes [69]. In general, SAR based studies have revealed that the naphthylisoquinoline alkaloids, having a *C*,*C*-biaryl axis connecting the naphthyl and isoquinoline moiety exhibit weak or no leishmanicidal activity. Since the alkaloids **34** and **35** are *N*,*C*-coupled and thus, are equipped with a hetero biaryl axis and display significantly higher leishmanicidal activities. As compounds **34** and **35** share structural features with miltefosine, which has been proposed to induce an apoptosis-like death pathway in the parasite, the same is also reported to be the possible mode of action for these alkaloids. However, compounds **34** and **35** display more toxicity against *J774.1* macrophage cell lines & peritoneal macrophages than amphotericin B used for positive control and, are also moderately toxic against the BMDC. Finally, structural modifications of the lead compounds **34** and **35** should be performed in order to decrease their cytotoxicity assisted by toxicity-guided quantitative structure-activity relationship (QSAR) investigations [69].

Among other naphthylisoquinoline alkaloids obtained from *Ancistrocladus tanzaniensis* (Ancistrocladaceae), the 7, 3'-coupled naphthylisoquinoline alkaloid ancistrocladidine (**36**) shows activity against *L. donovani* [70]. Its activity in comparison to the highly active ancistrotanzanine B (**37**) (IC_{50} of 1.6 μg/mL) is reportedly weaker only by a factor of 2 and, in comparison to miltefosin (used for positive control) by a factor of 10. Ancistrotanazanine A (**38**), another alkaloid of the same group exhibits a similar high activity against *L. donovani* [70,71].

7.4. Bisbenzylisoquinoline Alkaloids

Few bisbenzylisoquinolinic alkaloids from different plant species are known for their strong activity against *L. donovani, L. braziliensis* and *L. amazonensis*. The metabolite daphanandrine (**39**), isolated from *Albertisia papuana* (Menispermaceae), obaberine (**40**), obtained from *Pseudoxandra sclerocarpa* (Annonaceae), gyrocarpine (**41**) produced by *Gyrocarpus americanus* (Hernandiaceae), limacine (**42**), isolated from *Caryomene olivasans* (Menispermaceae), display anti-leishmanial activity at IC_{100} ~50 μg/mL. At 10 μg/mL concentration, the metabolite **41** also shows *in vitro*

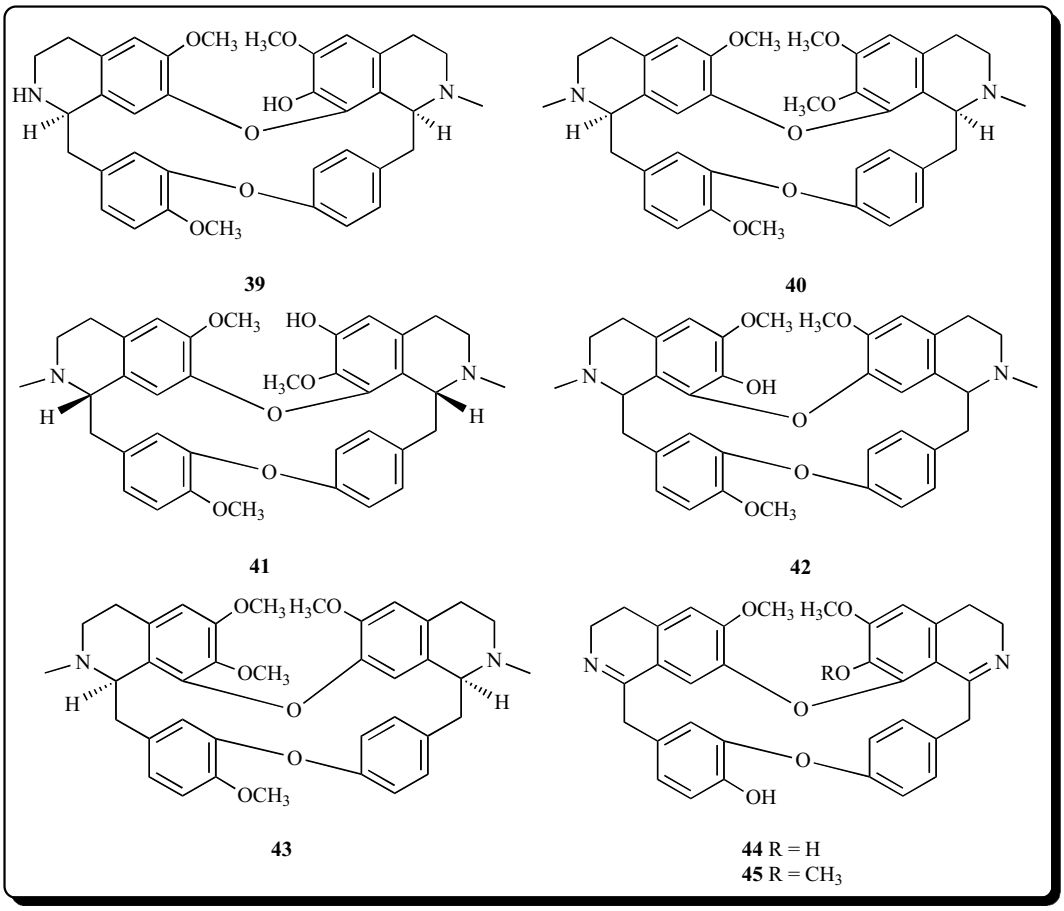




activity against the promastigote forms of *L. braziliensis*, *L. amazonensis* and *L. donovani* [49]. However, in comparison to Glucantime"®" (56 mg/Sb^v/kg/day), the *in vivo* test for compound **41** (100 mg/kg/day) against *L. amazonensis*, shows less effectiveness [72].

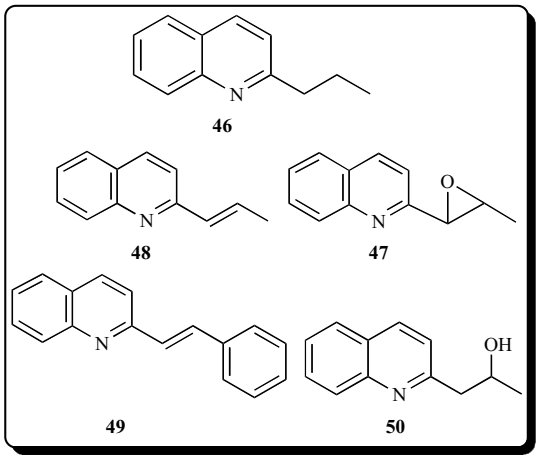
Isotetradrin (**43**), a *bis*-benzylisoquinoline alkaloid, isolated from *Limaciopsis loangensis* (Menispermaceae), shows antileishmanial *in vivo* activity at 100 mg/kg/day in the BALB/c mice. The metabolite is comparable to that of Glucantime"®" (56 mg Sb^v/kg) when tested against *L. amazonensis*, and is slightly less effective against *L. venezuelensis* [73].

Similarly, puertogaline A (**44**) and B (**45**), isolated from stem bark of *Guatteria boliviana* (Annonaceae) exhibit moderate *in vitro* inhibition of promastigotes at 100 µg/mL concentration on *Leishmania sp.* (*L. donovani, L. amazonensis* and *L. braziliensis*) [74]. SAR studies on *bis*-benzylisoquinoline series of alkaloids have shown that the oxidation state and nature of substitution on the nitrogen atoms are important for activity. Alkaloids with methylated nitrogen atoms are found more active than those with non-substituted or aromatic nitrogens. Quaternization of one or more nitrogen atoms results in a loss of antileishmanial activity [47].

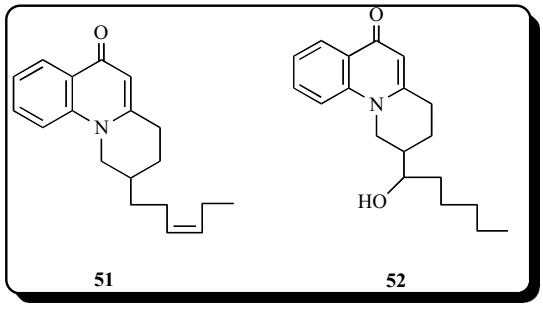


7.5. Quinoline Alkaloids

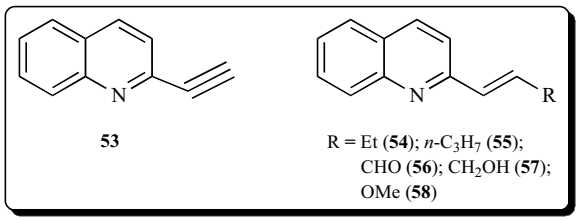
The quinoline alkaloids, 2-n-propylquinoline (**46**), 2-(1', 2'-trans-epoxypropyl) quinoline or chimanine-D (**47**) and chimanine-B (**48**), isolated from the Bolivian plant, *Galipea longiflora* Krause (Rutaceae), exhibit activity against promastigotes of *L. braziliensis* with IC_{90} of 50, 25 and 25 μg/mL, respectively. Some synthetic analogues 2-styrylquinoline (**49**) and (2-(2'-hydroxypropyl) quinoline (**50**) are also found to be active. Subcutaneous treatment with quinoline alkaloids is found effective against New World cutaneous leishmaniasis (i.e. *L. amazonensis* and *L. venezuelensis*) in BALB/c mice. Oral administration of compound **46** alone suppresses 99.9% of liver parasites. Compound **47** results in 86.6% parasite suppression, when it is given for 10 days at 0.54 mmol/kg by the subcutaneous route [49,75]. In contrast, suppression of *L. donovani* by 97.4% in the liver has been achieved on treatment with meglumine antimonate through subcutaneous route for 10 days at 56 mg/Sb^v/kg/day. Oral administration of **47** for *5* days results in lower parasite suppression (72.9%). However, parenteral administration does not produce a similar effect on mice infected with *L. donovani*. The quinoline **49** alone suppresses 79.6% parasites in the liver [76-78].



The two alkaloids namely dictylomide A (**51**) and dictylomide B (**52**) isolated from the bark of *Dictyoloma peruviana* (Rutaceae) exhibit leishmanicidal activity against promastigotes of *L. amazonensis*. At 100 μg/mL concentration, the metabolites result in total lyses of *L. amazonensis* promastigotes. Minor activity is observed for promastigotes of *L. braziliensis* at same concentration [79].

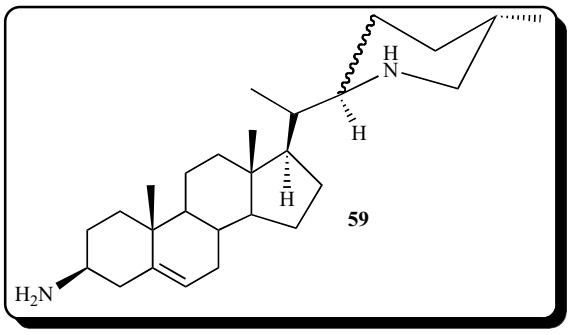


Some synthetic 2-substituted quinolines reportedly exhibit leishmanicidal activities. In the *in vivo* model of *L. amazonensis*, the quinolines **53**, **54**, **55** and **59** on oral administration at 25 mg/kg twice daily for 15 days, reduce 80 to 90% parasite burdens in the lesion, whereas *N*-methylglucamine antimoniate (Glucantime®), on administration by subcutaneous injections at 100 mg [28 mg Sb^v] per kg of body weight daily, reduce the parasite burdens by 98%. In visceral leishmaniasis caused by *L. infantum*, an oral treatment of mice at 25 mg/kg daily for 10 days with quinolines **53**, **56**, **57** and **58** display a significant reduction of parasite burdens in the liver and spleen. An study comprising the evaluation of oral *in vivo* activities of three quinolines (compounds **56**, **57** and **46**) against *L. donovani* (LV 9) were determined at 12.5 and 25 mg/kg for 10 days and compared with that of miltefosine at 7.5 mg/kg. Miltefosine, compound **46** and quinoline **56** at 12.5 mg/kg significantly reduce the parasite burdens in the liver by 72, 66 and 61%, respectively. Among these quinoline, **56** is the most promising compound against both cutaneous and visceral leishmaniasis [80].



7.6. Steroidal Alkaloids

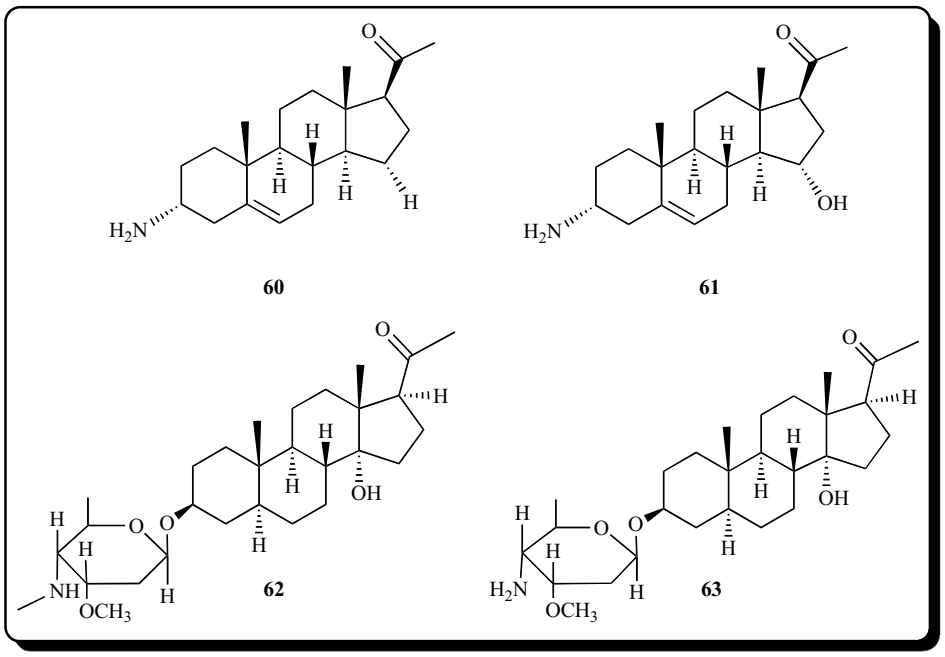
A steroidal alkaloid sarachine (**59**), obtained from the leaves of the Bolivian plant *Saracha punctata* (Solanaceae), at a concentration of 10 μg/mL, effectively inhibits the growth of the promastigotes of *L. baziliensis, L.donovani and L. amazoensis*. But at the same concentration it presents a strong toxic activity against mice peritonaeal macrophages [81].



Four steroidal alkaloids namely, holamine (**60**), 15-α-hydroxyholamine (**61**), holacurtine (**62**) and *N*-desmethylholacurtine (**63**) obtained from the leaves of *Holarrhena curtisii* (Apocynaceae) exhibit cytotoxic activity against the HL-60 cell line and promastigotes of *L. donovani.* Among these metabolites, the compound **60** shows highest activity at a concentration rage of $1.56 > IC_{50} > 0.39$ μg/mL, while metabolites **62**, **61** and **63** display same concentration range of $6.25 > IC_{50} > 1.56$ μg/mL [82].

7.7. Benzoquinolizidine Alkaloids

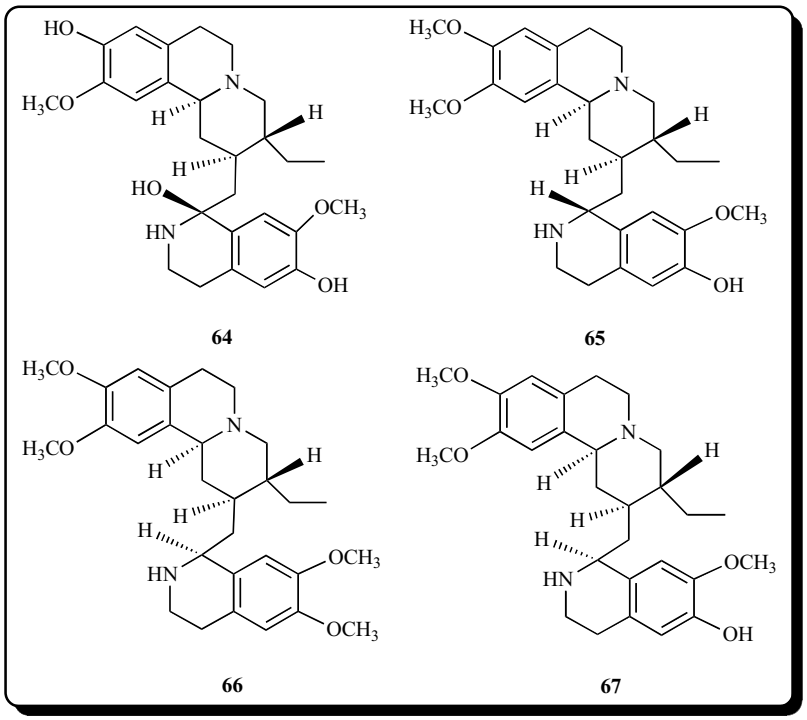
Among the benzoquinolizidine alkaloids obtained from *Psychotria klugii* (Rubiaceae), klugine (**64**), cephaeline (**65**), isocephaeline (**66**) and emetine (**67**), exhibit the potentials

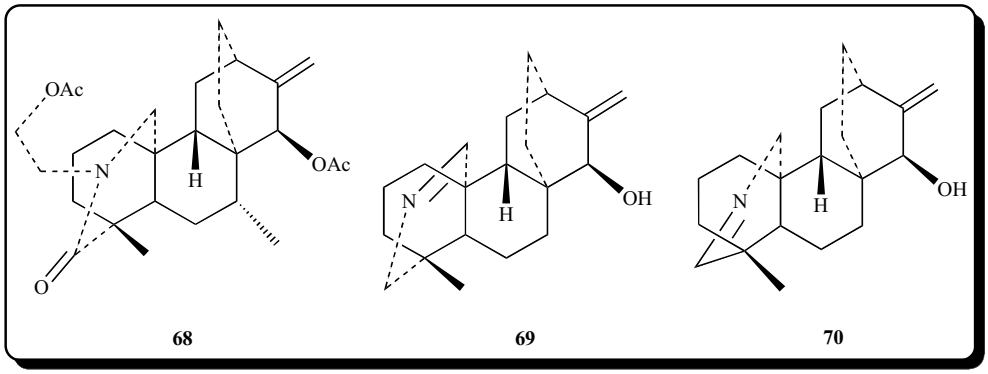


for leishmanicidal activity against *L. donovani.* The metabolite **65** shows *in vitro* antileishmanial activity against *L. donovani* with an IC_{50} of 0.03 μg/mL. It shows >20- and >5-fold more potency than pentamidine and amphotericin B, respectively. Similarly, metabolite **64** with an IC_{50} of 0.40 μg/mL and **66** with an IC_{50} 0.45 μg/mL shows <13- and <15-fold less potent activity than **65**. The alkaloid **67**, with an IC_{50} of 0.03 μg/mL shows leishmanicidal activity, but also display >12- fold more toxic activity against VERO cells with IC_{50} of 0.42 *vs* 5.3 μg/mL than cephaeline. Moreover, the metabolite **67** shows high activity, but toxic for the treatment of cutaneous leishmaniasis caused by *L. major* [82-84].

7.8. Diterpene Alkaloids

The plant species of genera *Aconitum*, *Delphinium* and *Consolida* (Ranunculaceae) are the known sources of C_{19}-norditerpene and C_{20}-diterpene alkaloids (NDAs and DAs, respectively) [85-88]. The three diterpene alkaloids namely, 15,22-*O*-diacetyl-19-oxo-dihydroatisine (**68**), azitine (**69**) and isoazitine (**70**) obtained from *Aconitum*, *Delphinium* and *Consolida* sp., exhibit leishmanicidal activities against promastigotes of *L. infantum*. Among these metabolites, compound **70** exhibits highest toxicity to the extracellular *L. infantum* parasites (IC_{50} 44.6, 32.3 and 24.6 μM at 24, 48 and

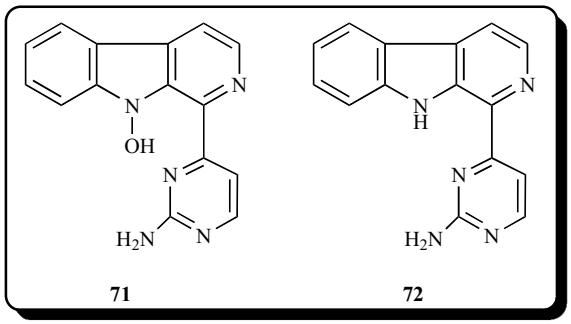




72 h of culture, respectively) with IC_{50} values lower than those obtained for the reference drug pentamidine-isothionate. The metabolites **69** and **68** also show activity against promastigotes of *L. infantum* (IC_{50} 33.7 and 27.9 μM, respectively, at 72 h of culture). The **70** with an IC_{50} < 1001.8 μM and **69** with an IC_{50} < 667.8 μM are reportedly devoid of toxicity to murine macrophages, while **68** with an IC_{50} of 162.3 μM shows weak toxicity [89].

7.9. Pyrimidine-*β*-Carboline Alkaloid

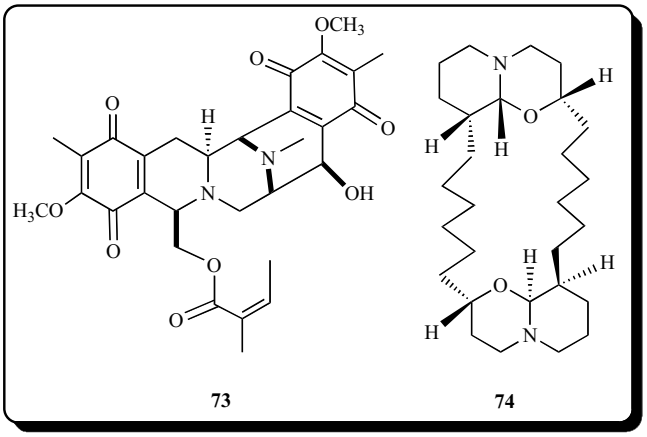
The bark extract of *Annona foetida* (Annonaceae) yields antileishmanial pyrimidine-*β*-carboline alkaloid *N*-hydroxyannomontine (**71**), together with the annomontine (**72**). Based on SAR, the metabolite **72** with an IC_{50} of 34.8 μM, shows 6 times more activity against promastigotes of *L. braziliensis* than the metabolite **71**. However, compound **71** shows activity against promastigotes of *L. guyanensis* too, while **72** remaining inactive [58].



7.10. From Marine Invertebrates

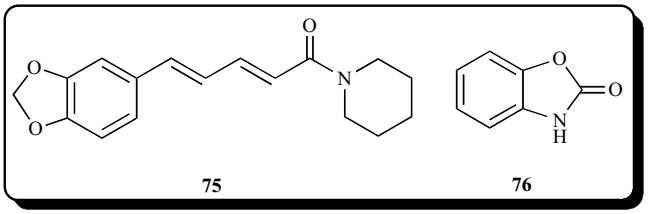
Some marine sponges are reported to possess significant antileishmanial activity e.g. *Amphimedon viridis*, *Acanthostrongylophora sp.*, *Neopetrosia sp.*, *Plakortis angulospiculatus* and *Pachymatisma johnstonii*. However, the numbers of antileishmanial compounds isolated from marine resources are still limited [90]. The newly developed assay system using recombinant *Leishmania amazonensis* expressing enhanced green fluorescent protein (La/*egfp*) has been applied to the screening of Japanese marine sponges for antileishmanial activity. Bioassay-guided fractionation of an active sponge *Neopetrosia* sp. afforded an active compound renieramycin A (**73**) that inhibits La/*egfp* with IC_{50} of 0.2 μg/mL [91].

The metabolite araguspongin C (**74**), an alkaloid isolated from *n*-butanes fraction of a marine sponge *Haliclona exigua*, shows moderate inhibition of promastigotes and intracellular amastigotes at 100 μg/mL concentration, but shows weak antileishmanial action *in vivo* [92].

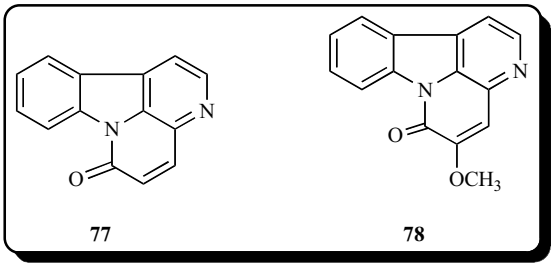


7.11. Other Alkaloids

Alkaloids piperine (**75**) and benzoxazol-2(3H)-one (**76**), isolated from leaves of *Acanthus illicifolius* (Acanthaceae), present activity against promastigotes of *L. donovani* [93, 94].



Two metabolites, canthin-6-one (**77**) and 5-methoxycanthin-6-one (**78**), isolated from stem bark of *Zanthoxylum chiloperone var. angustifolium* (Rutaceae) possess leishmanicidal activity in assay *in vivo* on BALB/c mice infected with *L. amazonensis*. The sub-cutaneous treatment with antimonial drug at 28 mg/kg of Sb for 10 days reduce the lesion weight by 28.5% and parasite burden in lesion by 90.9% ($P<0.05$) *vs* the untreated mice. The intralesional treatment with compound **77** results in decrease of lesion weight by 15.0% and the parasite load by 77.6% in compared with the group of untreated mice. Through the intraperitoneal route, metabolite **77** shows effectiveness and low toxicity, its lethal dose kills 50% of mice (LD_{50}) is found to be upward of 400 mg/kg [95,96].



77 **78**

8. CONCLUDING REMARKS

Advances in the parasitological and biochemical research on various species of *Leishmania* and host-parasite interactions, the available treatment options are far from satisfactory. To eliminate this problem from every corner of world, a safe, non-toxic and cost-effective drug is urgently required. Truly speaking, a little attention has been paid by scientific community all around the globe for this neglected disease as evident by current situation. Moreover, more attention has been paid on biochemical and immunological researches and only few laboratories are involved in drug evaluation and development against this devastating disease. The potentials of plant products as a source of anti-leishmanial drugs are needed to be discussed with respect to biochemical differences between protozoa and hosts. Intensive research works are needed to have a better understanding of the leishmanial targets for plants products especially alkaloids.

A new drug, which would allow a safer, shorter and cheaper treatment and would be easier to administrate orally, a non invasive alternative to the invasive method of parasitological diagnosis, identification of the most cost effective surveillance system and control strategies to reduce the mortality rate, a most suitable vector control approach, identification and quantification of risk factors to better focus the control activities and prevent epidemics, are the most important aspect for the control and complete eradication of the disease from India as well as from other developing and developed countries.

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REFERENCES

[1] Berman, J.D. Human leishmaniasis: clinical, diagnostic and chemotherapeutic developments in the last 10 years. *Clin. Infect. Dis*., **1997**, *24*, 684-703.

[2] Killick-Kendrick, R. Phlebotomine vectors of the leishmaniases: a review. *Med. Vet. Entomol*., **1990**, *4*, 1-24.

[3] Chance, M.L.; Peters, W. Shchory, L. Biochemical taxonomy of leishmania. I. Observations on DNA. *Ann. Trop. Med. Parasitol*., **1974**, *68*, 307-16.

[4] Desjeux, P. The increase in risk factors for leishmaniasis worldwide. *Trans. R. Soc. Trop. Med. Hyg.*, **2001**, *95*, 239-43.

[5] Desjeux, P. Human leishmaniases: epidemiology and public health aspects. *World Health Stat. Q.*, **1992**, *45*, 267-75.

[6] Bora, D. Epidemiology of visceral leishmaniasis in India. *Natl. Med. J. India*, **1999**, *12*, 62-8.

[7] Magill, A.J. Epidemiology of leishmaniases. *Dermatol. Clin*., **1995**, *13*, 505-23.

[8] Ashford, R.W. The leishmaniases as model zoonoses. *Ann. Trop. Med. Parasitol*., **1997**, *91*, 693-701.

[9] Zijlstra, E.E.; el-Hassan, A.M.; Ismael, A. Endemic kala-azar in eastern Sudan: post-kala-azar dermal leishmaniasis. *Am. J. Trop. Med. Hyg.*, **1995**, *52*, 299-305.

[10] Saraiva, E.M.; Pinto-Da-Silva, L.H.; Wanderley, J.L.M.; Bonomo, A.C.; Barcinski, M.A.; Moreira, M.E.C. Flow cytometric assessment of *Leishmania* spp metacyclic differentiation: validation by morphological features and specific markers. *Exp. Parasitol.*, **2005**, *110*, 39-47.

[11] McConville, M.J.; Souza, D.; Saunders, E.; Likic, V.A.; Naderer, Thomas. Living in a phagolysosome; metabolism of leishmania amastigotes. *Trends Parasitol.*, **2007**, *23*, 368-75.

[12] Glew R.H.; Saha, A.K.; Das, S.; Remaley, A.T. Biochemistry of the Leishmania species. *Microbiol. Rev*., **1988**, *52*, 412-32.

[13] Veeken, H.; Ritmeijer, K.; Seaman, J.; Davidson, R. A randomized comparison of branded sodium stibogluconate and generic sodium stibogluconate for the treatment of visceral leishmaniasis under field conditions in Sudan. *Trop. Med. Int. Health*, **2000**, *5*, 312-17.

[14] Moore, E.; O'Flaherty, D.; Heuvelmans, H.; Seaman, J.; Veeken, H.; de-Wit, S.; Davidson, R.N. Comparison of generic and proprietary sodium stibogluconate for the treatment of visceral leishmaniasis in Kenya. *Bull. W. H. O.*, **2001**, *79*, 388-93.

[15] Peters, W. The treatment of kala-azar--new approaches to an old problem. *Indian J. Med. Res.*, **1981**, *73*, Suppl. 1-18.

[16] Indian Council of Medical Research. Anonymous: Proceeding of the meeting of an Expert Group on Kala-azar, Indian Council of Medical Research Headquarters, New Delhi, **1977**.

[17] Aikat, B.K.; Sahaya, S.; Pathania, A.G.; Bhattacharya, P.K.; Desai, N.; Prasad, L.S.; Mishra, S.; Jain, S. Clinical profile of cases of kala-azar in Bihar. *Indian J. Med. Res.*, **1979**, *70*, 563-70.

[18] Thakur, C.P.; Kumar, M.; Singh, S.K.; Sharma, D.; Prasad, U.S.; Singh, R.S.; Dhawan, P.S.; Achari, V. Comparison of regimens of treatment with sodium stibogluconate in kala-azar. *Br. Med. J. (Clin. Res. Ed.)*, **1984**, *288*, 895-97.

[19] World Health Organisation, Report of a WHO expert committee. Report: the leishmaniasis, *W. H. O. Tech. Rep. Ser.*, **1984**, *701*, 1-140.

[20] Thakur, C.P.; Kumar, M.; Pandey, A.K. Evaluation of efficacy of longer durations of therapy of fresh cases of kala-azar with sodium stibogluconate. *Indian J. Med. Res*., **1991**, *93*, 103-10.

[21] Sundar, S.; Rosenkaimer, F.; Lesser, M.L.; Murray, H.W. Immunochemotherapy for a systemic intracellular infection: accelerated response using interferon-gamma in visceral leishmaniasis. *J. Infect. Dis*., **1995**, *171*, 992-96.

[22] Sundar, S.; More, D.K.; Singh, M.K.; Singh, V.P.; Sharma, S.; Makharia, A.; Kumar, P.C.; Murray, H.W. Failure of pentavalent antimony in visceral leishmaniasis in India: report from the center of the Indian epidemic. *Clin. Infect. Dis.*, **2000**, *31*, 1104-7.

[23] Sundar, S.; Thakur, B.B.; Tandon, A.K.; Agrawal, N.R.; Mishra, C.P.; Mahapatra, T.M.; Singh, V.P. Clinicoepidemiological study of drug resistance in Indian kala-azar. *B. M. J*., **1994**, *308*, 307.

[24] Sundar, S. Drug resistance in Indian visceral leishmaniasis. *Trop. Med. Int. Health*, **2001**, *6*, 849-54.

[25] Perez-Victoria, J.M.; Di-Pietro, A; Barron, D.; Ravelo, A.G.; Castanys, S.; Gamarro, F. Multidrug resistance phenotype mediated by the P-glycoprotein-like transporter in leishmania: a search for reversal agents. *Curr. Drug. Targets*, **2002**, *3*, 311-33.

[26] Legare, D.; Richard, D.; Mukhopadhyay, R.; Stierhof, Y.D.; Rosen, B.P.; Haimeur, A.; Papadopoulou, B.; Ouellette, M. The leishmania ATP-binding cassette protein PGPA is an intracellular metal-thiol transporter ATPase. *J. Biol. Chem.*, **2001**, *276*, 26301-7.

[27] Singh, N.; Singh, R.T.; Sundar, S. Novel mechanism of drug resistance in kala azar field isolates. *J. Infect. Dis*., **2003**, *188*, 600-7.

[28] Jha, T.K. Evaluation of diamidine compound (pentamidine isothionate) in the treatment resistant cases of kala-azar occurring in North Bihar, India. *Trans. R. Soc. Trop. Med. Hyg.*, **1983**, *77*, 167-70.

[29] Thakur, C.P.; Kumar, M.; Pandey, A.K. Comparison of regimes of treatment of antimony-resistant kala-azar patients: a randomized study. *Am. J. Trop. Med. Hyg.*, **1991**, *45*, 435-41.

[30] Jha, S.N.; Singh, N.K.; Jha, T.K. Changing response to diamidine compounds in cases of kala-azar unresponsive to antimonial. *J. Assoc. Physicians India*, **1991**, *39*, 314-16.

[31] Mishra, M.; Biswas, U.K.; Jha, D.N.; Khan, A.B. Amphotericin versus pentamidine in antimony-unresponsive kala-azar. *Lancet*, **1992**, *340*, 1256-57.

[32] Thakur, C.P.; Singh, R.K.; Hassan, S.M.; Kumar, R.; Narain, S.; Kumar, A. Amphotericin B deoxycholate treatment of visceral leishmaniasis with newer modes of administration and precautions: a study of 938 cases. *Trans. R. Soc. Trop. Med. Hyg*., **1999**, *93*, 319-23.

[33] Indian Council of Medical Research. Anonymous: minutes of the expert group meeting on drug therapy in lesihmaniasis, *ICMR*, New Delhi, **2000**.

[34] Thakur, C.P. A single high dose treatment of kala-azar with ambisome (amphotericin B lipid complex): a pilot study. *Int. J. Antimicrob. Agents*, **2001**, *17*, 67-70.

[35] Sundar, S.; Jha, T.K.; Thakur, C.P.; Mishra, M.; Singh, V.P.; Buffels, R. Single-dose liposomal amphotericin B in the treatment of visceral leishmaniasis in India: a multicenter study. *Clin. Infect. Dis*., **2003**, *37*, 800-4.

[36] Jha, T.K.; Sundar, S.; Thakur, C.P.; Bachmann, P.; Karbwang, J.; Fischer, C.; Voss, A.; Berman, J. Miltefosine, an oral agent for the treatment of Indian visceral leishmaniasis. *N. Engl. J. Med.*, **1999**, *341*, 1795-800.

[37] Sundar, S.; Jha, T.K.; Thakur, C.P.; Engel, J.; Sindermann, H.; Fischer, C.; Junge, K.; Bryceson, A.; Berman, J. Oral miltefosine for Indian visceral leishmaniasis. *N. Engl. J. Med.*, **2002**, *347*, 1739-46.

[38] Bhattacharya, S.K.; Jha, T.K.; Sundar, S.; Thakur, C.P.; Engel, J.; Sindermann, H.; Junge, K.; Karbwang, J.; Bryceson, A.D.; Berman, J. Efficacy and tolerability of miltefosine for childhood visceral leishmaniasis in India. *Clin. Infect. Dis*., **2004**, *38*, 217-21.

[39] Sundar, S.; Jha, T.K.; Sindermann, H.; Junge, K.; Bachmann, P.; Berman, J. Oral miltefosine treatment in children with mild to moderate Indian visceral leishmaniasis. *Pediatr. Infect. Dis. J*., **2003**, *22*, 434-38.

[40] Jha, T.K.; Olliaro, P.; Thakur, C.P.; Kanyok, T.P.; Singhania, B.L.; Singh, I.J.; Singh, N.K.; Akhoury, S.; Jha, S. Randomised controlled trial of aminosidine (paromomycin) v sodium stibogluconate for treating visceral leishmaniasis in North Bihar, India. *Biol. Med. J*., **1998**, *316*, 1200-5.

[41] Thakur, C.P.; Kanyok, T.P.; Pandey, A.K.; Sinha, G.P.; Zaniewski, A.E.; Houlihan, H.H.; Olliaro, P. A prospective randomized, comparative, open-label trial of the safety and efficacy of paromomycin (aminosidine) plus sodium stibogluconate versus sodium stibogluconate alone for the treatment of visceral leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.*, **2000**, *94*, 429-31.

[42] Sundar, S.; Jha, T.K.; Thakur, C.P.; Sinha, P.K.; Bhattacharya, S.K. Injectable paromomycin for visceral leishmaniasis in India. *N. Engl. J. Med.*, **2007**, *356*, 2571-81.

[43] Sherwood, J.A.; Gachihi, G.S.; Muigai, R.K.; Skillman, D.R.; Mugo, M.; Rashid, J.R.; Wasunna, K.M.; Were, J.B.; Kasili, S.K.; Mbugua, J.M. Phase 2 efficacy trial of an oral 8-aminoquinoline (WR6026) for treatment of visceral leishmaniasis. *Clin. Infect. Dis*., **1994**, *19*, 1034-39.

[44] Jha, T.K.; Sunder, S.; Thakur, C.P.; Felton, J.M.; Sabin; A.J.; Horton, J. A phase II dose-ranging study of sitamaquine for the treatment of visceral leishmaniasis in India. *Am. J. Trop. Med. Hyg*., **2005**, *73*, 1005-1011.

[45] Wasunna, M.K.; Rashid, J.R.; Mbui, J.; Kirigi, G.; Kinoti, D.; Lodenyo, H.; Felton, J.M.; Sabin, A.J.; Horton, J. A phase II dose-increasing study of sitamaquine for the treatment of visceral leishmaniasis in Kenya. *Am. J. Trop. Med. Hyg.*, **2005**, *73*, 871-876.

[46] Wright, C.W.; Phillipson, J.D. Natural products and the development of selective antiprotozoal drugs. *Phytother. Res.*, **2006**, *4*, 127-39.

[47] Manar, M.S.; Karl, A.W. Natural Products from plants as drug candidates and lead compounds against leishmaniasis and trypanosomiasis. *Curr. Med. Chem.*, **2006**, *13*, 2571-98.

[48] Rocha, L.G.; Almeida, J.R.G.S.; Macedo, R.O.; Barbosa-Filho, J.M. A review of natural products with antileishmanial activity. *Phytomedicine*, **2005**, *12*, 514-35.

[49] Chan-Bacab, M.J.; Pena-Rodriguez, L.M. Plant natural products with leishmanicidal activity. *Nat. Prod. Rep*., **2001**, *18*, 674-88.

[50] Giorgio, C.D.; Delmas, F.; Ollivier, E.; Elias, R.; Balansard, G.; Timon-David, P. *In vitro* activity of the *β*-carboline alkaloids harmane, harmine and harmaline toward parasites of the species *Leishmania infantum*. *Exp. Parasitol.*, **2004**, *106*, 67-74.

[51] Mahiou, V.; Roblot, F.; Hocquemiller, R.; Cave, A.; Arias, de A.R.; Yaluff, G.; Fournet, A.; Angelo, A. New aporphine alkaloids from *Guatteria Foliosa*. *J. Nat. Prod.*, **1994**, *57*, 890-95.

[52] Delorenzi, J.C.; Attias, M.; Gattass C.R.; Andrade M.; Rezende, C.; Pinto, A.C.; Henriques, A.T.; Bou-Habib, D.C.; Saraiva, E.M.B. Antileishmanial activity of an indole alkaloid from *Peschiera australis. Antimicrob. Agents Chemother*., **2001**, *45*, 1349-54.

[53] Staerk, D.; Lemmich, E.; Christensen, J.; Kharazmi, A.; Olsen, C.E.; Jaroszewski, J.W. Leishmaniacidal, antiplasmodial and cytotoxic activity of indole alkaloids from *Corynanthe pachyceras*. *Planta Med.*, **2000**, *66*, 531-36.

[54] Kam, T.; Sim, K.; Koyano, T.; Komiyama, K. Leishmanicidal alkaloids from *Kopsia griffithii*. *Phytochem*., **1999**, *50*, 75-79.

[55] Tanaka, J.C.A.; da Silva, C.C.; Ferreira, I.C.P.; Machado, G.M.C.; Leon, L.L.; de Oliveira, A.J.B. Antileishmanial activity of indole alkaloids from *Aspidosperma ramiflorum*. *Phytomedicine*, **2007**, *14*, 377-80.

[56] Munoz, V.; Morretti, C.; Sauvain, M.; Caron, C.; Porzel, A.; Massiot, G.; Richard, B.; Men-Oliver, L.L. Isolation of bis-indole alkaloids with antileishmanial and antibacterial activities from *Peschiera van heurkii* (syn. *Tabernaemontana van heurkii*). *Planta Med*., **1994**, *60*, 455-59.

[57] Carvalho, P.B.; Ferreira, E.I. Leishmaniasis phytotherapy: nature leadership against an ancient disease. *Fitoterapia*, **2001**, *72*, 599-618.

[58] Costa, E.V.; Pinheiro, M.L.B.; Xavier, C.M.; Silva, J.R.A.; Amaral, A.C.F.; Souza, A.D.L.; Barison, A.; Campos, F.R.; Ferreira, A.G.; Machado, G.M.C.; Leon, L.L.P. A pyrimidine-*β*-carboline and other alkaloids from *Annona foetida* with antileishmanial activity. *J. Nat. Prod*., **2006**, *69*, 292-94.

[59] Phillipson, J.D.; Wright, C.W. Medicinal plants in tropical medicine. 1. Medicinal plants against protozoal diseases. *Trans. R. Soc. Trop. Med. Hyg.*, **1991**, *85*, 18-21.

[60] Birdsall, T.C.; Kelly, G.S. Berberine: therapeutic potential of an alkaloid found in several medicinal plants. *Altern. Med. Rev.*, **1997**, *2*, 94-103.

[61] Queiroz, E.F.; Roblot, F.; Cave, A.; Paulo, M.Q.; Fournet, A. Pessoine and spinosine: two catecholic berbines from *Annona spinescens*. *J. Nat. Prod*., **1996**, *59*, 438-40.

[62] Fevrier, A.; Ferreira, M.E.; Fournet, A.; Yaluff, G.; Inchausti, A.; Rojas de Arias, A.; Hocquemiller, R.; Waecher, A.I. Acetogenins and other compounds from *Rollinia emarginata* and their antiprotozoal activities. *Planta Med*., **1999**, *65*, 47-49.

[63] Correa, J.E.; Rios, C.H.; Castillo, A.R.; Romero, L.I.; Ortega-Barria, E.; Coley, P.D.; Kursar, T.A.; Heller, M.V.; Gerwick, W.H.; Rios, L.C. Minor alkaloids from *Guatteria dumetorum* with antileishmanial activity. *Planta Med.*, **2006**, *72*, 270-72.

[64] Montenegro, H.; Gutierrez, M.; Romero, L.I.; Ortega-Barria, E.; Capson, T.L.; Cubilla, L. Aporphine alkaloids from *Guatteria* spp. with leishmanicidal activity. *Planta Med*., **2003**, *69*, 677-79.

[65] Waechter, A.I.; Cave A.; Hocquemiller, R.; Bories, C.; Munoz, V.; Fournet, A. Antiprotozoal activity of aporphine alkaloids isolated from *Unonopsis buchtienii* (Annonaceae). *Phytother. Res*., **1999**, *13*, 175-77.

[66] Bringmann, G.; Wohlfarth, M.; Rischer, H.; Schlauer, J.; Brun, R. Extract screening by HPLC coupled to MS–MS, NMR and CD: a dimeric and three monomeric naphthylisoquinoline alkaloids from *Ancistrocladus griffithii*. *Phytochemistry*, **2002**, *61*, 195-204.

[67] Bringmann, G.; Saeb, W.; Ruckert, M.; Mies, J.; Michel, M.; Mudogo, V.; Brun, R. Ancistrolikokine D, a 5,8'-coupled naphthylisoquinoline alkaloid and related natural products from *Ancistrocladus likoko*. *Phytochemistry*, **2003**, *62*, 631-36.

[68] Bringmann, G.; Hamm, A.; Gunther, C.; Michel, M.; Brun, R.; Mudogo, V. Ancistroealaines A and B: two new bioactive naphthylisoquinolines and related naphthoic acids from *Ancistrocladus ealaensis*. *J. Nat. Prod*., **2000**, *63*, 1465-70.

[69] Ponte-Sucre, A.; Faber, J.H.; Gulder, T.; Kajahn, I.; Pedersen, S.E.H.; Schultheis, M.; Bringmann, G.; Moll, H. Activities of naphthylisoquinoline alkaloids and synthetic analogs against *Leishmania major. Antimicrob. Agents Chemother*., **2007**, *51*, 188-94.

[70] Bringmann, G.; Dreyer, M.; Faber, J.H.; Dalsgaard, P.W.; Staerk, D.; Jaroszewski, J.W.; Ndangalasi, H.; Mbago, F.; Brun, R.; Chris-

tensen, S.B. Ancistrotanzanine C and related 5,1' and 7,3'–coupled naphthylisoquinoline alkaloids from *Ancistrocladus tanzaniensis*. *J. Nat. Prod.*, **2004**, *67*, 743-48.

[71] Bringmann, G.; Dreyer, M.; Faber, J.H.; Dalsgaard, P.W.; Staerk, D.; Jaroszewski, J.W.; Ndangalasi, H.; Mbago, F.; Brun, P.; Reichert, M.; Maksimenka, K.; Christensen, S.B. Ancistrotanzanine A, the first 5,3'-coupled naphthylisoquinoline alkaloid and two further 5,8'-linked related compounds from the newly described species *Ancistrocladus tanzaniensis*. *J. Nat. Prod.,* **2003**, *66*, 1159-65.

[72] Fournet, A.; Munoz, V.; Manjon, A.M.; Angelo, A.; Hocquemiller, R.; Cortes, D.; Cave, A.; Bruneton, J. Activite antiparasitaire d'alcaloides bisbenzylisoquinoleiques. I: activite *in vitro* sur des promastigotes de trois souches de *Leishmania. J. Ethnopharmacol.*, **1988**, *24*, 327-35.

[73] Fournet, A.; Angelo, A.; Munoz, V.; Hocquemiller, R.; Cave, A. Effect of some bisbenzylisoquinoline alkaloid on American *leishmania* sp. in BALB/c mice. *Phytother. Res*., **1993**, *7*, 281-84.

[74] Mahiou, V.; Roblot, F.; Fournet, A.; Hocquemiller, R. Bisbenzylisoquinoline alkaloids from *Guatteria boliviana* (Annonaceae). *Phytochemsitry*, **2000**, *54*, 709-16.

[75] Fournet, J.C.; Gantier, A.; Gautheret, L.; Leysalles, M.H.; Munos, J.; Mayrargue, H.; Moskowitz, A.C.; Hocquemiller, R. The activity of 2-substituted quinoline alkaloids in BALB/c mice infected with *Leishmania donovani*. *J. Antimicrob. Chemother.*, **1994**, *33*, 537-44.

[76] Munos, M.H.; Mayrargue, J.; Fournet, A.; Gantier, J.C.; Hocquemiller, R.; Moskowitz, H. Synthesis of an antileishmanial alkaloid isolated from *Galipea longiflora* and of related compounds. *Chem. Pharm. Bull*., **1994**, *42*, 1914-16.

[77] Fournet, A.; Hocquemiller, R.; Roblot, F.; Cave, A.; Richomme, P.; Bruneton, J. Les chimanines, nouvelles quinolines substituees en 2, isolees d'une plante bolivienne antiparasitaire: *Galipea longiflora*. *J. Nat. Prod.*, **1993**, *56*, 1547-52.

[78] Fournet, A.; Angelo, A.; Munoz, V.; Hocquemiller, R.; Cave, A.; Bruneton, J. 2-substituted quinoline alkaloids as potential antileishmanial drugs. *Antimicrob. Agents Chemother.*, **1993**, *37*, 859-63.

[79] Lavaud, C.; Massiot, G.; Vasquez, C.; Moretti, C.; Sauvain, M.; Balderrama, L. 4-quinolinone alkaloids from *Dictyoloma peruviana*. *Phytochemistry*, **1995**, *40*, 317-320.

[80] Nakayama, H.; Loiseau, P.M.; Bories, C.; Torres de Ortiz, S.; Schinini, A.; Serna, E.; Rojas de Arias, A.; Fakhfakh, M.A.; Frank, X.; Figadere, B.; Hocquemiller, R.; Fournet, A. Efficacy of orally administered 2-substituted quinolines in experimental murine cutaneous and visceral leishmaniases. *Antimicrob. Agents Chemother*., **2005**, *49*, 4950-56.

[81] Moretti, C.; Sauvain, M.; Lavaud, C.; Massiot, G.; Bravo, J.A.; Munoz, V. A novel antiprotozoal aminosteroid from *Saracha punctata*. *J. Nat. Prod*., **1998**, *61*, 1390-93.

[82] Kam, T.; Sim, K.; Koyano, T.; Toyoshima, M.; Hayashi M.; Komiyama, K. Cytotoxic and leishmanicidal aminoglycosteroids and aminosteroids from *Holarrhena curtisii*. *J. Nat. Prod.*, **1998**, *61*, 1332-36.

[83] Cazorla, D.; Yepez, J.; Anez, N.; Sanchez de Mirt, A. Antileishmania effect of intralesional procaine and dibucaine in hamsters. *Invest. Clin*., **2001**, *42*, 5-21.

[84] Muhammad, I.; Dunbar, D.C.; Khan, S.I.; Tekwani, B.L.; Bedir, E.; Takamatsu, S.; Ferreira, D.; Walker, L.A. Antiparasitic alkaloids from *Psychotria klugii*. *J. Nat. Prod.*, **2003**, *66*, 962-67.

[85] Gonzalez-Coloma, A.; Reina, M.; Guadano, A. Structural diversity and defensive properties of norditerpenoid alkaloids. *J. Chem. Ecol*., **2004**, *30*, 1393-408.

[86] Bessonova, I.A.; Shaidkhodzaeva, S.A. Hetisane-type diterpene alkaloids. *Chem. Nat. Comp.*, **2000**, *36*, 419-77.

[87] Gonzalez-Coloma, A.; Reina, M.; Guadano, A. Antifeedant C_{20} diterpene alkaloids. *Chem. Biodivers*., **2004**, *1*, 1327-35.

[88] Luque, F.; Fernandez-Ramos, C.; Entrala, E. *In vitro* evaluation of newly synthesised [1, 2, 4] triazolo [1,5α] pyrimidine derivatives against *Trypanosoma cruzi*, *Leishmania donovani* and *Phytomonas staheli*. *Comp*. *Biochem. Physiol.*, **2000**, *126*, 39-44.

[89] Gonzalez, P.; Marin, C.; Rodriguez-Gonzalez, I.; Hitos, A.B.; Rosales, M.J.; Reina, M.; Diaz, J.G.; Gonzalez-Coloma, A.; Sanchez-Moreno, M. *In vitro* activity of C_{20}-diterpenoid alkaloid derivatives in promastigotes and intracellular amastigotes of *Leishmania infantum. Int. J. Antimicrob. Agents*, **2005**, *25*, 136-41.

[90] Kerr, R.G.; Kerr, S.S. Marine natural products as therapeutic agents. *Exp. Opin. Ther. Pat*., **1999**, *9*, 1207-22.

[91] Nakao, Y.; Shiroiwa, T.; Murayama, S.; Matsunaga, S.; Goto, Y.; Matsumoto, Y.; Fusetani, N. Identification of Renieramycin A as an antileishmanial substance in a marine sponge *Neopetrosia* sp. *Mar. Drugs*, **2004**, *2*, 55-62.

[92] Dube, A.; Singh, N.; Saxena, A.; Lakshmi, V. Antileishmanial potential of a marine sponge *Haliclona exigua* (Kirkpatrick) against experimental visceral leishmaniasis. *Parasitol. Res*., **2007**, *101*, 317–24.

[93] Kapil, A. Piperine: a potent inhibitor of *Leishmania donovani* promastigotes *in vitro*. *Planta Med.*, **1993**, *59*, 474.

[94] Kapil, A.; Sharma, S.; Wahidulla, S. Leishmanicidal activity of 2-benzoxazolinone from *Acanthus illicifolius in vitro. Planta Med.*, **1994**, *60*, 187-88.

[95] Ferreira, M.E.; Rojas de Arias, A.; Torres de Ortiz, S.; Inchausti, A.; Nakayama, H.; Thouvenel, C.; Hocquemiller, R.; Fournet, A. Leishmanicidal activity of two canthin-6-one alkaloids, two major constituents of *Zanthoxylum chiloperone* var. *angustifolium. J. Ethnopharmacol*, **2002**, *80*, 199-202.

[96] Kanchanapoom, T.; Kasai, R.; Chumsri, P.; Hiraga, Y.; Yamasaki, K. Canthin-6-one and *β*-carboline alkaloids from *Eurycoma harmandiana. Phytochemistry*, **2001**, *56*, 383-86.

[97] Ferreira, I.C.P.; Lonardoni, M.V.C.; Machado, G.M.C.; Leon, LL.; Filho, L.G.; Pinto, L.H.B.; Oliveira, A.J.B. Anti-leishmanial activity of alkaloidal extract from *Aspidosperma ramiflorum. Mem. Inst. Oswa. Cruz.*, **2004**, *99*, 325-27.

[98] Mirzaie, M.; Nosratabadi, S.J.; Derakhshanfar, A.; Sharifi, I. Antileishmanial activity of *Peganum harmala* extract on the *in vitro* growth of *Leishmania major* promastigotes in comparison to a trivalent antimony drug. *Vet. Arh*., **2007**, *77*, 365-75.

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