Chapter- 4

Chemotherapy Studies

4. CHEMOTHERAPY STUDIES

4.1. Studies of various agents

4.1.1. Antibacterial compounds

A large number of antibacterial compounds have been tested against leishmaniasis in both in vivo and in vitro at high concentrations. But not even a simple compound showed good antileishmanial of activity. Even the combination of trimethoprim and sulphadiazine did not show activity in mice infected with *L. major* [259]. The rifampicin, isoniazid potentiation was observed against mice infected with *L. mexicana amazonensis,* but high dose levels were required [260]. Russian Physician has used Monomycin against zoonotic cutaneous leishmaniasis caused by *L. major* [261].

4.1.2. Antimalarial agents

A large number of antimalarial compounds have been tested for antileishmanial activities both in vivo and in vitro. But not even a single compound showed prominent antileishmanial effects. Quinine showed moderate activity against *L. mexicana, L. major* and *L. donovani* amastigote cell line in vitro [262]. Chloroquine is reported to have low activity against *L. donovani* promastigotes in vitro [263]. Hanson et al., have reported moderate activity of two experimental antimalarials, a phosphonium (WR 179, 422) and quinazoline (WR 152, 412) derivative against hamsters infected with *L. donovani* [264]. The effect of 2–styrylquinazolines was also observed only at maximum tolerated doses against leishmaniasis [265]. 4-Nitroquinoline-1-oxide showed high activity against *L. donovani* promastigotes in vitro [266]. Cycloguanil showed less activity in vivo and in vitro against *L. mexicana mexicana, L. braziliensis paramenesis, L. donovani* [259, 267]. Pyrimethamine showed high activity in vitro against *L. mexicana* [262]. Mepacrine showed consistent but moderate activity in vivo and in vitro. It appeared to be inactive when given by local injection into cutaneous lesions in mice due to *L. major* [262, 264].

4.1.3. Antifungals and Anthelmentics

A large number of antifungal agents were tested against leishmaniasis. The highest activity was reported with ketocanazole against cutaneous leishmaniasis [268, 269]. Its use to treat patients with CL was suggested by its activity against the fungal cytochrome P450. It blocks demethylation of the enzyme substrate at C-14, an essential step in

formation of ergosterol [270]. Terbinafine is an allylamine broad-spectrum antifungal compound active against many fungal pathogens particularly dermatophytes. It is capable of inhibiting squalene-epoxidase [271], a key enzyme in the biosynthesis of ergosterol, an essential component of cell membrane.

In a murine model of *Leishmania infantum* visceral leishmaniasis, terbinafine was less effective than antimonial agents in reducing hepatic parasite load [272]. Khalil et al., observed the failure of a combination of two antifungal drugs, terbinafine and itracanazole in Sudanese PKDL [273]. The antiproliferative effects and ultra structural alterations induced in vitro by terbinafine and ketocanazole on *Leishmania amazonensis* are reported. Combination of terbinafine and ketocanazole produced additive effects on promastigote axenic growth and synergistic effects on intracellular amastigote proliferation [274].

Simoesmattos et al., studied the effect of terbinafine treatment in hamsters infected with *Leishmaina chagasi*. Terbinafine alone at the dose of 10 mg/kg had no effect on spleen parasite burden or relative spleen weight of *L. chagai* –hamsters. Glucantime alone at the dose of 80 mg/kg and combination of glucantime (80 mg/kg) plus terbinafine (100 mg/kg) significantly reduced the weight of the spleen in comparison with the infected untreated groups [275]. No high activity was found with anthelmentics such as TAC Pamoate, Trichlorphan, Niridazole, Oxamniquine etc.

4.1.4 Allopurinol and its derivatives

Allopurinol was shown to be active against promastigotes of *L. donovani* and *L. mexicana* [276]. Allopurinol ribonucleoside, metabolite of allopurinol, was found to be more effective against promastigotes and intracellular amastigotes than allopurinol [277, 278]. Thiopurinol and thiopurinol ribonucleoside were found to have similar activity to that of allopurinol against promastigotes and amastigotes [279].

4.1.5. Aurones

Aurones are the secondary metabolite natural compounds belong to the flavanoids family (Rubiaceae; Cyperaceae), and structurally are the isomers of flavones, widely distributed in flowers and fruits. They play a significant role in the pigmentation of the parts of the plant in which they occur. Aurones are plant flavanoids that provide yellow colour to the

flowers of some popular ornamental plants, such as Snapdragon (Antirrhinum majus) and cosmos. The yellow coloration is mainly provided by the 6–glucosides of aureusidin and bracteatin. Literature survey indicates that flavones, chalcones and isoflavones have been studied largely for their therapeutic potential. However, aurones still are less studied and it is only recently that these compounds have been to be investigated. Three unusual, highly oxygenated novel phenylpropanoids and two novel isoflavones, 8-prenylmucronulatol and smiranicin, were isolated from *Smirnowia iranica* together with isoflavon, glyasperin H [280].

The isoflavones significantly inhibited the growth of extracellular stages of *leishmania* in vitro their activity against the intracellular stages being considerably lower [280]. A series of aurones with drug-potential for *Leishmania* infections was identified in vitro using both a direct cytotoxicity test against extracellular promastigotes of *L. donovani*, *L. infantum*, *L. enriettii*, and *L. major*, and a test against intracellular amastigote *L. donovani* residing within murine macrophages. The compounds proved to be active at concentrations in a microgram range between 0.4 and 5.0 μg/ml. When tested against murine bone marrow–derived macrophages as a mammalian host cell control, all compounds showed only moderate cytotoxicity EC 50 (2.32-25.0 μg/ml) [281]. 6-hydroxy-2-[phenylmethylene]-3(2H)-benzofuranone had an EC_{50} of 0.45 μg/ml in the extra-, and an EC_{50} of 1.4 μg/ml in the intracellular assay against extracellular promastigotes of *L. donovani L. infantum, L. enriettii* and *L. major*, and intracellular amastigotes of *L. donovani* residing within murine macrophages [282].

A series of aurones were analyzed for the ability to inhibit respiratory functions of mitochondria of *Leishmania* parasites. In a cell-free assay mitochondrial fumarate reductase from *L. donovani* was inhibited in a concentration-dependent manner. The most active compounds were 4^{l}, 6-dihydroxyaurone and 6-methoxyaurone, which inhibited parasite enzyme activity at 25 nM by over 90% [283].

4.1.6. 8–Aminoquinolines

Studies of 6-methoxy-8-alkylpiperazinoalkyl- aminoquinolines showed good antileishmanial activity [284]. Among the 8-aminoquinolines, primaquine finally emerged as the compound of choice. From a study of the activity of over 260 compounds against hamsters experimentally infected with *L. donovani*, two compounds, viz..

Wellcome 125C and Wellcome 171C were selected for clinical trails against kala-azar in Kenya. But it was observed that the therapeutic activity of these two compounds was less than that of sodium stibogluconate [284]. The Walter Reed Army Institute of Research Group has reinvestigated the structure-activity relationship of 8-aminoquinolines. The outcome of their studies has revealed a new series of agents with activities upto several hundred times greater than pentavalent antimonial and meglumine antimoniate [285, 286]. The antileishmanial activity was also observed with 6–aminoquinolines and 7–aminoquinolines; but the activity was lower than 8–aminoquinolines [264].

4.1.7. Sitamaquine

The oral drug that might have an impact on VL is the 8-aminoquinoline derivative sitamaquine, currently in development with Glaxo SmithKline [287]. The antileishmanial activity of this compound was first identified in the 1970s at the Walter Reed Army Institute of Research. Limited Phase I/II clinical trials have been completed with varying levels of success, for instance, 67 per cent of patients were cured of *L. chagasi* in Brazil when treated with 2 mg/kg daily for 28 days [288], and 92 per cent were cured of VL when treated with 1.7 mg/kg daily for 28 days in Kenya [289] and a 89 per cent cure rate with 1.75 mg/kg daily for 28 days in India [290]. Sitamaquine is rapidly metabolized, forming desethyl and 4-CH_2OH derivatives, which might be responsible for its activity. Toxicity appears to be relatively mild, it causes mild methemoglobinaemia, and further studies are underway on this drug.

Moxipraquine showed antileishmanial activity against cutaneous leishmaniasis. The observation of foetal toxicity precluded further development [291]. Among that new series, the compound WR6026 was shown to be 474 times as active as meglumine antimoniate when given by the standard intra–muscular route. Compound WR 6026 was more active when given orally, being reported to be 708 times as active as meglumine antimoniate [285].

4.1.8. α–DFMO

Several new approaches to the chemotherapy of leishmaniasis are being explored. Kaur et al., [292] have shown that DFMO inhibits the growth of *L. donovani* in culture. Bacchi [293, 294] has reviewed the role of polyamines in trypanosomatids, including several species of *Leishmania*. He studied the effect of α-difluromethylornithine (DFMO) on

infections of *Trypanosoma brucei* in mice. This compound inhibits ornithine decarboxylase, blocking putrescine and spermidine synthesis. These compounds work in cell growth as co–factors and as membrane stabilizers. Another novel approach is based on the studies of Fairlamb and his co-workers [295-297]. They isolated a unique molecule, trypanothione, which in part controls redox potentials in trypanosomatids and protect them against oxidant stress. The two key enzymes that work together with this molecule are peroxidase and a reductase.

4.1.9. Chalcones

Chalcones exhibited potent antileishmanial and antitrypanosomial activity in vitro and in vivo [298]. Licochalcone A inhibited the activity of fumarate reductase (FRD) in the permeabilized *Leishmania major* promastigote and in the parasite mitochondria, and it also inhibited solubilized FRD and a purified FRD from *L. donovani* [299]. Two other chalcones, 2, 4-dimethoxy-4′-allyloxychalcone (24m 4ac) and 2, 4-dimethoxy-4′-butoxy chalcone (24 mbc) also exhibited inhibitory activity on the solubilized FRD in *L. major* promastigotes [299].

4.1.10. Diamidine compounds

A large number of diamidines and related cyclic compounds were tested for antileishmanial effects against *L. donovani* in vivo. Diminazene aceturate (berenil), which was developed for treating bovine trypanosomiasis, was found to have antileishmanial activity [300].

4.1.11. Emetine and its related compounds

Emetine and its related compounds showed good activity, but were highly toxic in vivo and to the cell lines in vitro Berberine, a plant alkaloid, was found to have moderate effect against promastigotes of *L. donovani* in vitro [263]. Al-Khateeb and Molan (1981) concluded that dehydroemetine showed anti-*L.donovani* properties by weight reduction of infected transfer liver and spleen [301].

4.1.12. Imiquimod

Imiquimod (Aldara, 3M Pharmaceuticals) is an antiviral compound [1-(2-methylropyl)-1H- imidazo (4,5-c) quinolin-4-amine] used extensively for the topical treatment of genital warts caused by the human papillomavirus. It is an immunomodulator, stimulating

a local immune response at the site of application, which in turn resolves the infection. Imiquimod induces the production of cytokines and nitric oxide in macrophages and has been shown to have an effect in experimental infections of cutaneous leishmaniasis [302]. It is suggested that the topical treatment activates localized macrophages to kill the parasite, while the antimonial eliminates systemic amastigotes, which are responsible for persistence of infection [303, 304].

4.1.13. Tranquillizers [Antipsychotics]

The phenothiazine tranquillizers and tricyclic antidepressants are toxic to *Leishmania* [305, 306]. Compounds of both groups kill *L. donovani* and *L. major* amastigotes within macrophages as well as extracellular promastigotes in vitro [307]. Neal and Allen [308] have shown that amitriptylin, an analog of imapramine, and chlorprothixene, a derivative of promazine, are high toxic to *L. donovani.* Previously it was suggested that antidepressants are toxic because they inhibit membrane functions, which are essential for the survival of *Leishmania* within its hosts. Evidence for this hypothesis arose from experiments, which demonstrated that imapramine and clomipramine inhibit transport of L–proline in promastigotes of *L. donovani* [305]. Proline actively accumulated in *L. donovani* promastigotes and the transport is driven by the proton electrochemical gradient across the plasma membrane [309]. Tricyclic drugs reduce proton motive force in *L. donovani* promastigotes [310].

4.1.14. Trifluralin

Antileishmanial activity studied by Rabinovitch (1989) reported that addition of L–amino acid esters to the culture media of macrophages infected with amastigotes killed the parasites [311]. Man–Ying Chan and Fong (1990) observed that leishmanial growth is inhibited by trifluralin, a dinitro aniline herbicide. At a concentration of five parts per million, it inhibited the growth of amastigotes in cultured macrophages by 50%. At lower concentrations, it prevented amastigote–promastigote transformation [312].

4.1.15. Trypanocides

Nifutrimox, benznidazole, ethidium, suramins and melarsaprol were tested for antileishmanial activity. Nifutrimox has been reported to have effective against human

cutaneous leishmaniasis in Brazil, but further development is precluded because of toxic side effects [313].

4.1.16. Plant glycoproteins

The ribosome–inactivating proteins (RIPs), such as plant glycoproteins cleave the glycosidic bond of adenine in 28SrRNA. They have been shown to inhibit the ribosomal function of *L. d. infantum* [314].

4.1.17. Sinefungin

Sinefungin [5–deoxy–5'–(1,4–diamino–4–carboxy butyl) adenosine] is a naturally occurring antifungal antibiotic nucleoside in which an ornithine residue is linked to the 5' end of adenosine by a carbon–carbon bond. Sinefungin was shown to be significantly suppressive against *L. donovani* and *L. braziliensis paramensis* infections in hamsters when compared with meglumine antimoniate [Glucantime] [315]. It inhibits the incorporation of thymidine into DNA [316]. Sinefungin was also shown to be effective in the treatment of American leishmaniasis [317].

4.1.18. Azoles and other steroid biosynthesis inhibitors

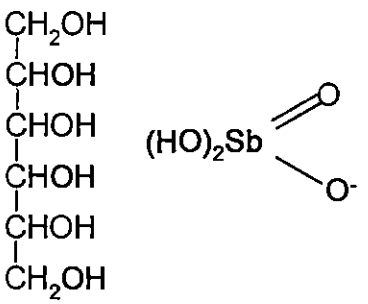
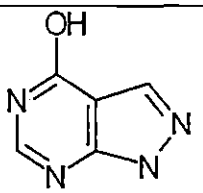
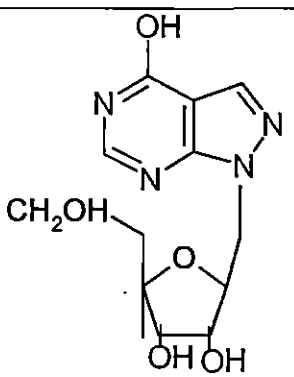
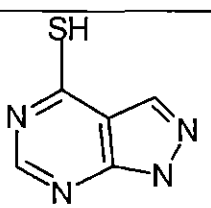
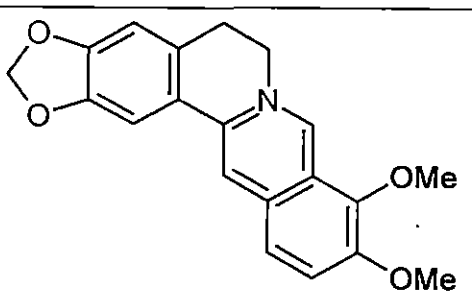
The azoles, like ketoconazole and triazoles, itraconazole and fluconazole produce an anti-leishmanial effect by blocking ergosterol synthesis [318]. Varying results have been reported from small-uncontrolled poorly designed clinical trials in both VL and cutaneous leishmaniasis (CL). In a study in Saudi Arabia, fluconazole showed a cure rate of 79 % in patients of CL caused by *L. major* [319]. Till date this drug has not been tried in India.

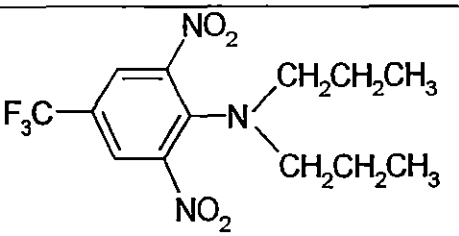
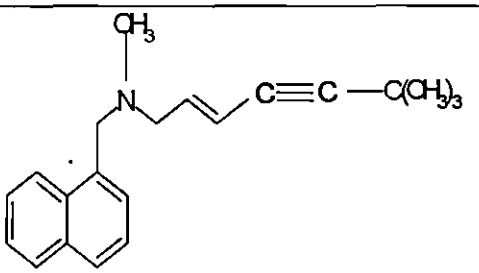
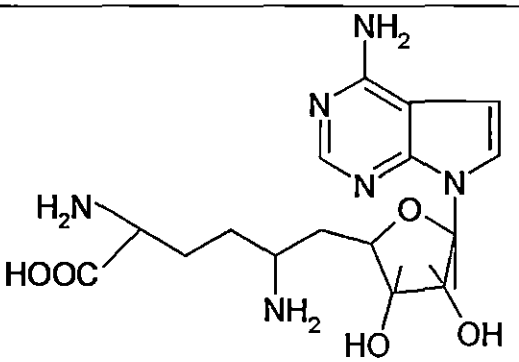
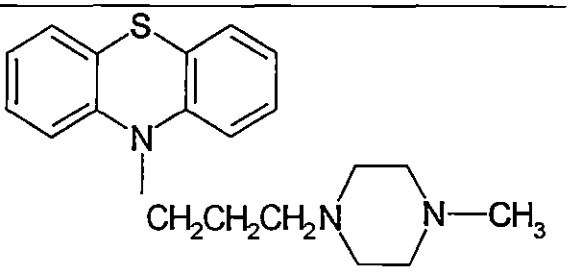
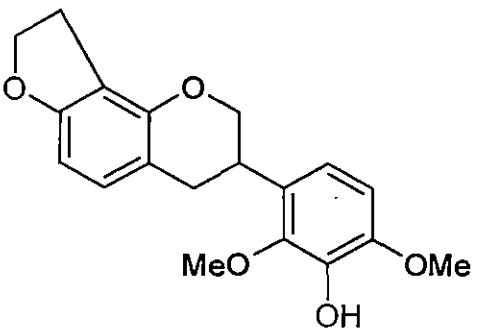
4.1.19. Cytokines

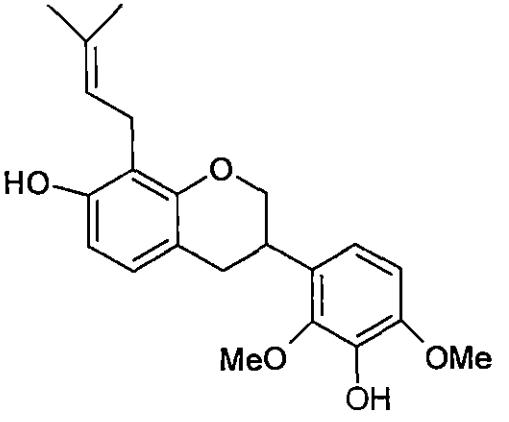
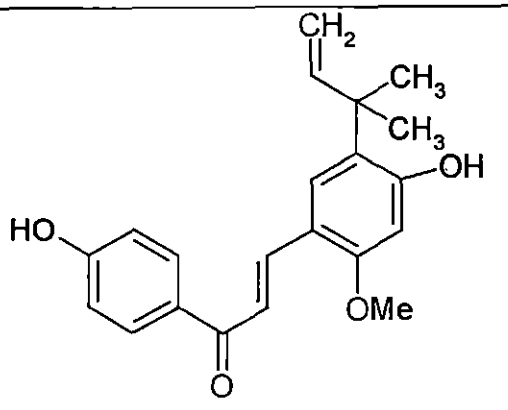
Leishmania infection progresses to kala-azar in individuals who fail to initiate Th1 response, which is mediated by IL-2 and IFN-r [320, 321]. Interferon-r is one of the principal activators of macrophages. Interferon-r as adjutants to Sbv has been used successfully in VL with high cure rate in comparison to Sbv alone [322]. Later, it was observed that interferon-r (daily dose 100 μg/m2) though improved the response rate to antimony, but overall cure rate was less than 50 per cent [323]. However, steep decline in the response rate to antimony rendered the addition of IFN-r ineffective.

Structure of some drugs studied for antileishmanial activity shown in (Table 3.1.)

Table 4.1. Name and structure of antileishmanial drugs

Name(s)	Chemical type	Structure(s)
Meglumine antimoniate (Glucantime)	Pentavalent antimonal	
Allopurinol	Xanthine oxidase inhibitor	
Allopurinol ribonucleoside	Purine analog	
Thiopurinol	Purine analog	
Berberine	Plant alkaloid	

Trifluraline	Herbicide	
Terbinafine	Antifungal	
Sinefungin	Antifungal nucleoside antibiotic	
Trifluoperazine	Phenothizine	
Smiranicin	Isoflavans	

8 - Prenylmucronulatol	Isoflavans	
Licochalcone A	Chalcones	

4.2. Drugs used in the treatment of leishmaniasis

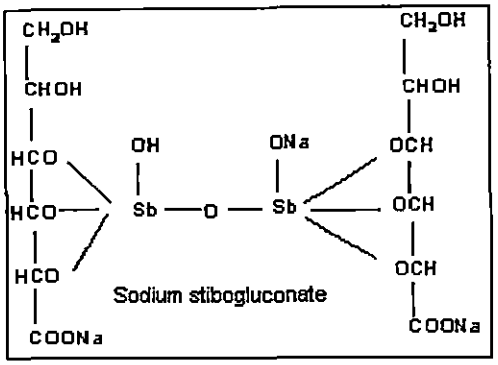
Leishmaniasis is a significant cause of morbidity and mortality in several countries. The basic treatment consists in the administration of sodium stibogluconate (Pentostam) and meglumine (Glucantime). Treatment failure, especially in kala-azar, mucosal leishmaniasis and diffuse cutaneous leishmaniasis, is becoming a common problem in many areas where diseases are endemic. There is now strong indication that treatment failure may be partly due to the drug resistance of the parasite [324-327]. In cases of treatment failure, second line agents such as pentamidine and amphotericin B are used [328, 329]. In some cases, even this agent failed to eradicate the parasite, [330-336]. In addition, the low efficacy of pentavalent antimony in the treatment of patient's co–infected with AIDS and *Leishmania* spp. is often noticed.

Table 4.2. Represents Current drugs used for the treatment of leishmaniasis [337]

	Visceral Leishmaniasis
First line drugs	Sodium stibogluconate (Pentostam, SSG)
	Meglumine antimoniate (Glucantime)
	Amphotericin B (Fungizone)
	Liposomal amphotericin B (AmBisome)
	Pentamidine
Clinical trials	Miltefosine (oral, Phase IV; registered in India)
	Paromomycin (Phase III)
	Sitamaquine (oral, Phase II)
	Other amphotericin B formulations

	Cutaneous Leishmaniasis
First line drugs	Sodium stibogluconate (Pentostam)
	Meglumine antimoniate (Glucantime)
	Amphotericin B (Fungizone)
	Pentamidine
	Paromomycin (topical formulations with methylbenzethonium chloride or urea)
Clinical trials	Miltefosine (oral, Phase III, registered in Colombia)
	Paromomycin (topical formulation with gentamicin and surfactants, Phase II)
	Imiquimod (topical immunomodulator, Phase II)
	Also anti-fungal azoles – ketoconazole, fluconazole, itraconazole

4.2.1. Antimony compounds

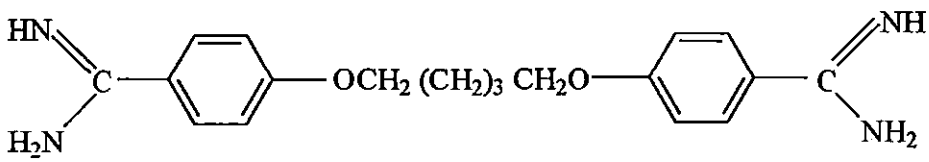


In 1937, Kikuth and Schmidt [338] have reported the antileishmanial activity of solustibosan (Sodium Stibogluconate), pentavalent antimony compound. In 1946, Durand et al [339] were the first to list pentavalent antimony, N–methylgucamine antimoniate (glucantime) in man against leishmaniasis. Today these two pentavalent antimonides (PVAs) are the most widely used leishmanicides. Although it is usually reported that both have similar efficacy and toxicity in relation to their pentavalent antimony content, sodium stibogluconate (SSG) contains 10% antimony (100 mg sb/ml, whereas meglutamine antimoniate (MA) contains about 8.5% antimony (85 mg sb/ml) [340]. The dose of pentavalents recommended by WHO is 20 mg Sb/kg body weight per day to a maximum of 600mg daily for 10–14 days. The course can be repeated in resistant cases after a resting period of 14 days [341]. Pentavalent antimonials appear to have number of modes of action against leishmaniasis. Berman et al., [342] were able to show that sodium stibogluconate inhibits the purine nucleotide triphosphate and macromolecular synthesis. Sodium stibogluconate can inhibit glucose uptake by promastigotes of *L. tropica* [343]. In addition, both aerobic and anaerobic glucose oxidation are inhibited, resulting in ATP and GTP reduction in the amastigotes exposed to sodium stibogluconate [344]. Now it is clear that the pentavalent antimonial compounds have to be converted into trivalent antimony to show their antileishmanial activity [337].

Even though pentavalents are considered to be initial treatment of choice, still their use is controversial. Treatment failures have been frequently observed [345]. Recently, strains of *Leishmania* resistant of pentavalent antimonials have been emerged, and this has reached 'alarming' proportions in some countries [346, 347]. Antimonials are

contraindicated in pregnancy and in patients with significant renal, hepatic or cardiac diseases. The first signs to toxicity are myalgia, joint stiffness, malaise, anorexia and bradycardia with ECG changes of prolongation of the QT interval and T–wave inversion. Hepatotoxicity, haemolytic anaemia, nephrotoxicity, pancreatitis and anaphylaxis are rare occurrences [348].

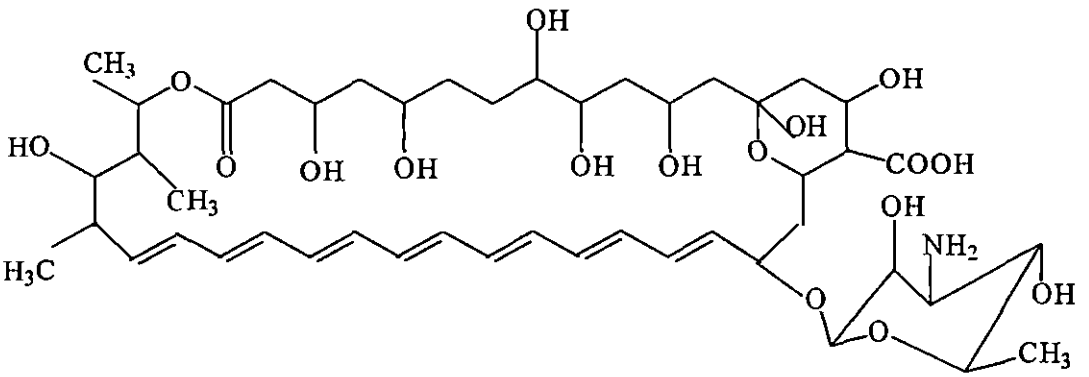
4.2.2. Pentamidine



Pentamidine is used in the treatment of African trypanosomiasis, babesiosis, leishmaniasis and pneumocystosis. In the early 1940s pentamidine emerged as the safest of the diamidines [349]. It is effective against all forms of leishmaniasis. Pentamidine become the backbone of anti kala-azar therapy. It has been used extensively for many years in the treatment of visceral leishmaniasis in India and is still being used [350]. Pentamidine was the first registered drug to be used in patient's refractory to Sbv, and high cure rates were reported [351]. Thus it is apparent that Sbv continues to be effective in the state of UP, but in North Bihar, where most (approximately 90%) of the disease occurs, it is ineffective in most patients. The magnitude of resistance varies in different areas of Bihar, peaking in the 11 districts most patients from southern districts of Bihar respond well to Sbv therapy, as do patients from West Bengal or UP. But its efficacy has declined over the years, and now it cures only approximately 70% of patients [352]. It acts against Leishmania spp. by damaging the kinetoplast DNA – mitochondria complex [353]. It is effective against all forms of leishmaniasis. Pentamidine in a dose of 3 – 4 mg/kg once or twice weekly until resolution occurs is recommended in resistant cases of leishmaniasis [341]. Although 50% of injected dose of pentamidine is excreted mainly in the urine in five days, traces can be detected in the urine up to 217 days, and in the kidney up to 240 days after a single injection. Cumulative effects, which often limit dose or frequency of administration, include weakness, nausea, vomiting and abdominal

pain, which may indicate pancreatic damage [354]. The unusually high rate of hyperglycemia (50%) associated with its use has been attributed to be high rate of pancreatic flybosis [355]. Others have also attributed the observed hypertension, tachycardia and electrocardiographic changes in T waves to its cardiotoxicity [356].

4.2.3. Amphotericin B



It is a polyene microlide antibiotic, act on sterols and phospholipids in cell membranes of *Leishmania* and fungi [357]. It has shown itself to be an effective antileishmanial drug. Amphotericin B (Amp B) amphotericin B deoxycholate (Fungizone) is the drug of choice for second-line of treatment for visceral leishmaniasis, if the patient either fails to respond to treatment with antimonial drugs or relapses thereafter [329]. Amphotericin B is the most effective antileishmanial drug; it is originally developed as an antifungal agent. The activity of the drug induces highcure rates. Use of formulation of amphotericin B, a pollen antibiotic, for treatment of leishmaniasis is biochemically rational because the target of amphotericin B is ergosterol like sterols, which are the major membrane sterols of *Leishmania* species [357]. 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* [358]. Amphotericin B is now being more widely used for VL and constitutes the major advance in antileishmanial chemotherapy during the last 10 yr. At dose of 0.75-1.0 mg per kg for 15 infusions on alternate days, it cures more than 97 per cent of patients [359, 360]. Occasional relapse (1%) might occur with amphotericin B, which can be treated successfully with the same drug. It has been recommended as first line drug in India by the National Expert Committee for Sbv in refractory regions of VL. Primary resistance to this drug is

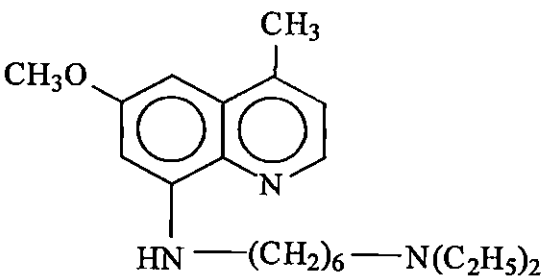
unknown. It is administered over a period 4-6 hours by slow intravenous infusion in 500 ml glucose 5%; starting at 0.1mg/kg doses and gradually increasing to 1mg/kg. It is only stable at neutral pH and is unstable when exposed to light and air.

The use of amphotericin B for leishmaniasis in human has been limited. In addition to nephrotoxicity other side effects include anaemia, thrombophlebitis and hypokalemia.

4.2.4. Lipid formulation of Amphotericin B

The need to develop less toxic, more effective formulation of amphotericin B has led to three new clinical formulation 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). Novel amphotericin B formulations have been used successfully to treat canine visceral leishmaniasis [361] and cutaneous leishmaniasis in immunocompromised patients [362] and paediatric CL [363]. These substitutes are well taken by reticuloendothelial system and poorly taken by kidney, the major target of organ toxicity [364]. Adverse effects of the conventional amphotericin B can be circumvented without compromising with the efficacy of the drug. It is possible to deliver high doses of drugs over short periods. The dose requirement varies from region to region. In Indian subcontinent a small dose (3.75 mg/kg) of ambiosome for five consecutive days induces high cure rates [365].

4.2.5. 6–Methoxy–8–(Diethylaminohexylamino) lepidine

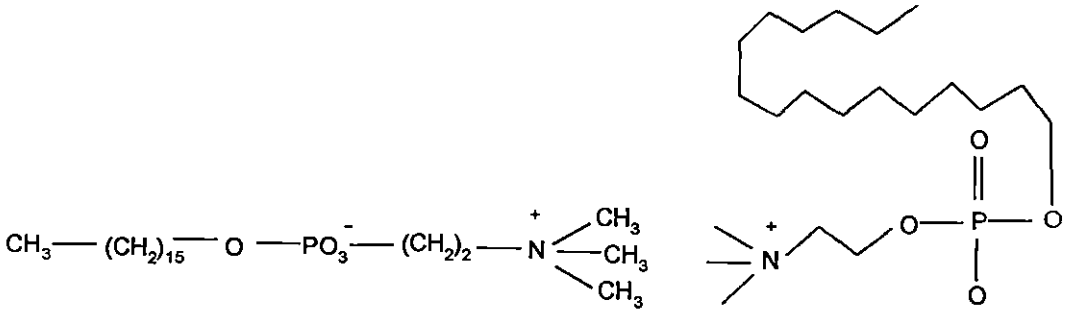


WR6026 was first synthesized and studied by scientists from the Water Reed Army Institute of Research under the code name WR6026 [366, 367]. The compound is in the public domain.

The bioavailabilities and pharmacokinetics of intravenous and oral dose of this compound were studied in beagle dogs [368]. In addition, tests conducted by Water Reed Army Institute of Research with healthy male subjects with oral doses upto 60mg revealed no significant drug related symptoms and no physical or laboratory abnormalities [369]. WR 6026 was effective for the treatment of visceral leishmaniasis in 16 patients at a dose of 0.75 to 1.00 mg/kg/day. The therapy was associated with minimal toxicity; adverse effects included gastrointestinal distress, headache and methemoglobinaemia.

4.2.6. Miltefosine

There are few new antileishmanial drugs in the pipeline. Recently a progress has been achieved in the treatment of leishmaniasis by introducing a drug called miltefosine.



Miltefosine

(N–hexadecylphosphoryl choline)

Miltefosine, an alkylphospholipid was originally developed as an oral antineoplastic agent, is the most advanced drug in the treatment of leishmaniasis. It is the first effective oral drug for the treatment of visceral Leishmaniasis and received the market registration in India in March 2002.

Miltefosine was originally developed as an anticancer agent first by ASTA Medica and since 2001 in collaboration with the Max Planck Institute in Gottingen (Prof. H. J. Eibl) and the Universitatsklinik in Gottingen (Prof. G. Nagel, Prof. C. Unger). Its antileishmanial activity was initially discovered in the mid-1980s. In 1988, Simon Croft [370] and his group reported anti–leishmanial activity of miltefosine and related compounds after parenteral use in mice. Considering the good oral bioavailability of

miltefosine, as evident from the studies in tumour patients, Kulencord, Unger and others demonstrated for the first time an excellent oral activity in their leishmaniasis models [371]. In 1995, ASTA Medica/Zentaris signed an agreement with WHO for the clinical development of miltefosine for visceral leishmaniasis. The Task Force for this development was introduced by TDR, with Prof. Anthony Bryceson as chairman, Dr. Jonathan Berman as co chair, and clinical investigators from India [372, 373].

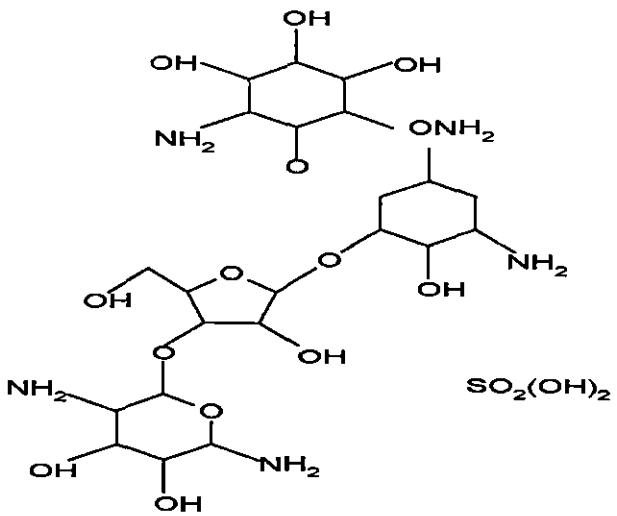
On the basis of a small scale proof of concept study in India, which was organized by ASTA Medica/Zentaris, Prof. Shyam Sundar and Prof. Henry Murray of Cornell University, a clinical development programme was initiated under the supervision of the TDR Task Force. A dose ranging and pharmacokinetic phase I/II study and a large phase III trial comparing miltefosine with amphotericin B were organised, initiated and completed in adult patients. In addition, dose ranging and pharmacokinetic study, and a confirmatory phase III study, were conducted in children. Recently, the Indian Council of Medical Research (ICMR) has involved in developing the drug and a phase IV study, to be conducted under the auspices of ICMR, has been organised jointly by ICMR, WHO, Zentaris and German Remedies Ltd. In all clinical studies, a cure rate >94 per cent has been found consistently with this drug. This drug has mild gastrointestinal adverse events like vomiting and diarrhoea in 40 and 20 per cent patients, respectively. Miltefosine has been used in India for treatment of VL at a dose of 50-100 mg (~2.5 mg/kg) for four weeks [374, 375]. It has also been found safe and effective in paediatric patients [376, 377]. This drug with mild side effects can become an important tool in containing the epidemics of VL. However, there are certain major limitations. Miltefosine has a median long terminal half-life of 154 h, which could encourage development of clinical resistance, and the best way to use this drug would be to use as a combination multi drug therapy. It is teratogenic and abortifacient, which means the drug, cannot be used in pregnancy, and females with child bearing potential must observe contraception for the duration of treatment and an additional two months. Further, rapid therapeutic response coupled with unsupervised treatment can severely affect compliance, and bring a premature end to this very important arsenal against *Leishmania*.

The Indian partner company of Zentaris received the approval for miltefosine for the treatment of visceral leishmaniasis in India. Very encouraging therapeutic responses have been seen even in patients with multiple pretreatment. In addition, a placebo

controlled phase III study in cutaneous leishmaniasis is currently ongoing in South America, with the aim to confirm the efficacy seen in an earlier dose finding study. Finally, a multinational programme is now being sponsored by the European Community to study the mechanism of action of miltefosine in leishmaniasis.

The mechanism of action of miltefosine is not well established, but it probably interacts with the cell membrane of *Leishmania* [378]. The antiproliferative effect may be mediated by an increase in cellular ceramide, which results in apoptosis. Treatment of cells with 25 μM miltefosine results in a 53% increase in ceramide concentration relative to control. The drug acts as a membrane signaling pathway inhibitor. Miltefosine inhibits CTP: phospho cholineytidyl transferase. It also possesses antimetastatic properties [379].

4.2.7. Paromomycin sulfate



Paromomycin (PM), an aminoglycoside antibiotic, was originally identified as an antileishmanial in the 1960s and has been used in clinical trials for both VL and CL. As with miltefosine, resistance to paromomycin could be induced in *L.donovani* promastigotes experimentally in vitro. The resistance was specific to PM and stable and its mechanism seems to be due to decreased drug uptake [380]. Ribosomes have been implicated as target [381] and inhibition of RNA synthesis followed by protein synthesis shown, along with induction of respiratory dysfunction [382]. Its efficacy has been demonstrated in India and a dose of 16 mg per kg intramuscularly for 21 days has cured 93 per cent of patients [383, 384].

In India under the aegis of the Institute for One World Health Founder and CEO Victoria G. Hale, and Gland Pharma Limited has registered the Paromomycin, Intramuscular (IM) Injection for the treatment of Visceral Leishmaniasis (VL), in 2006.

A combination of PM and sodium stibogluconate has been the subject of various clinical trials in Sudan and India [385] but further studies to optimize the combination and define drug-drug interactions are required. PM might also be a drug suitable for the topical treatment of CL. The report by El-On and colleagues in 1984 [386] that a topical formulation containing 15 per cent PM and 12 per cent methyl benzethonium chloride (a skin-penetrating agent) was effective against experimental CL led to the clinical trials. One such trial demonstrated that 77 per cent were cured after 20 days treatment compared with 27 per cent cured in the placebo group [387]. Other topical formulations with lower skin irritancy have recently been on clinical trial, including one containing 15 per cent PM with 10 per cent urea and another containing 15 per cent PM with 0.5 per cent gentamicin in a 10 surfactant vehicle (WR279 396) that cured 64 per cent of CL patients after 20 days treatment in Colombia [388]. In an endemic area of Iran the 15 per cent PM/10 percent urea showed no efficacy on cutaneous leishmaniasis and it was argued that the response to PM varied with the species and the type of lesion being treated [389]. These studies have also highlighted the need for a rational pharmaceutical design of formulations optimal for cutaneous leishmaniasis and the need for species-specific diagnosis [390].

4.3. Clinical manifestations

Currently, the most used methods for diagnosis of visceral leishmaniasis are direct agglutination test (DAT) and enzyme linked immunosorbent assay (ELISA). DAT was introduced about two decades ago rapidly followed by its improved version for field use [391, 392].

ELISA is now being used as potential serodiagnostic tool for visceral leishmaniasis. Though this technique is highly sensitive, its specificity depends upon the antigen used.

4.4. Drug resistance in human leishmaniasis

Treatment failures have long been observed in the chemotherapy of leishmaniasis [267, 348]. Reasons postulated for failure include derangements in the typical host-parasite

interaction, poor host immune response, improper dosing and lack of understanding of pharmacokinetics, poor drug penetration into sites of infection, insensitivity of promastigotes, species and strain differences in drug sensitivity [259]. Unfortunately, little is known about the mechanism of underlying drug resistance as seen inhuman visceral leishmaniasis. After administration, pentavalent antimonials are converted into trivalent compounds for its antileishmanial effect. The reduction of pentavalent to trivalent compound takes place either in macrophages [393] or in the parasite. In the later case, loss of reductase activity of parasite may lead to resistance. This is supported by the observation that Sbv resistant *L. donovani* amastigotes loose their reductase activity [344]. Molecular studies have identified an ATP binding cassette (ABC) transporter system, p-glycoprotein A (PGPA) involved in the metal resistance [394, 395]. PGPA is a member of multidrug resistance protein family, whose substrate includes organic anions and drugs conjugated to glutathione, glucoroate or sulphate. *Leishmania* contains glutathione as well as trypanothione (TSH) formed by conjugation of glutathione with spermidine. Transport experiments using radioactive conjugates clearly showed that PGPA recognized and actively transported the metal conjugates [394]. Thus, PGPA might be conferring resistance either through efflux from *Leishmania* [344] or by sequestering metal thiol conjugates into a vacuole [396, 397]. In a laboratory generated multidrug resistant (MDR) *L. tropica* line overexpressing a P-glycoprotein-like transporter displayed significant cross-resistance to miltefosine [398]. Defective uptake of miltefosine by resistant *L. donovani* [399] lines appeared to be through point mutations on a plasma membrane aminophospholipid translocase [400].