CHEMICAL REVIEWS

Review

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Recent Developments in Drug Discovery for Leishmaniasis and Human African Trypanosomiasis

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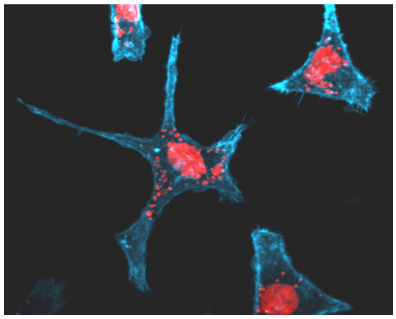
Infection of macrophages with *Leishmania donovani*



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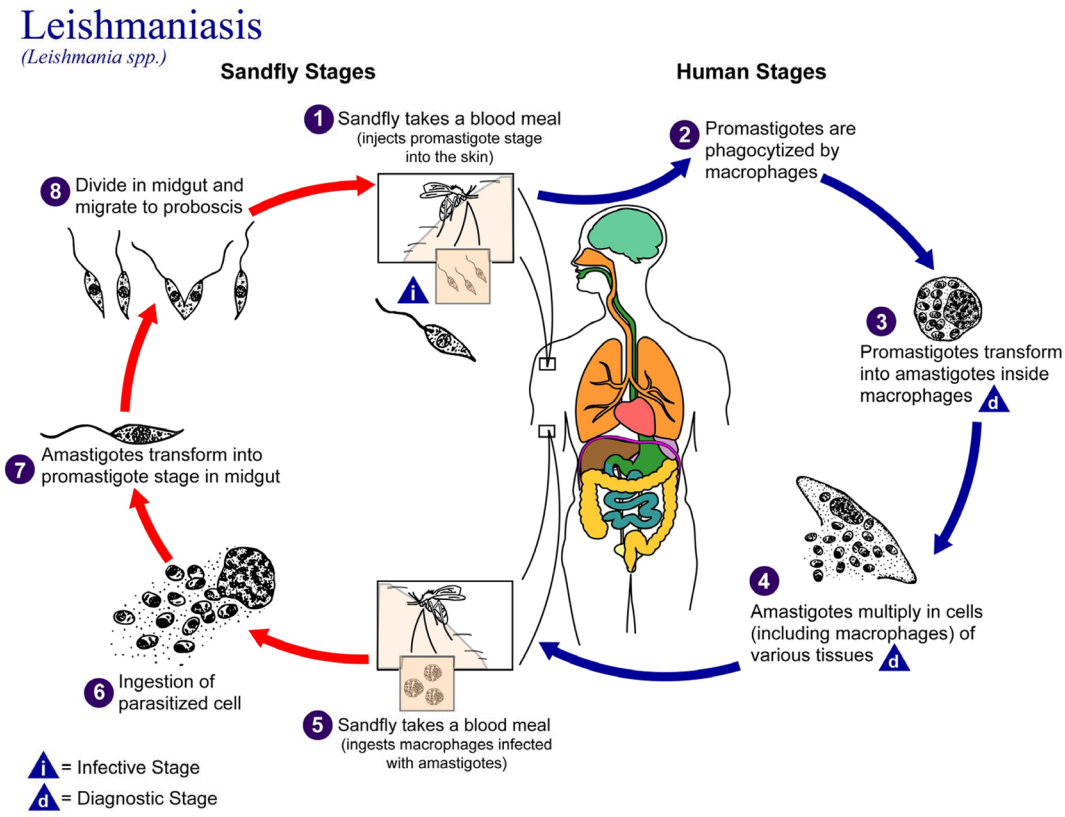
Scheme 1. Life Cycle of *Leishmania* Parasites (source: Public Health Image Library, provided by CDC- DPDx/Alexander J. da Silva, Ph.D., Blaine Mathison)



1. INTRODUCTION TO LEISHMANIASIS

Leishmaniasis is a parasitic disease that presents four main clinical syndromes: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), visceral leishmaniasis/kala azar (VL), and post kala azar dermal leishmaniasis (PKDL). Causative *Leishmania* are protozoan parasites that are transmitted among mammalian hosts by phlebotomine sandflies. In mammalian hosts, parasite cells proliferate inside the host phagocytic cells as round amastigotes. Infection of sandflies with *Leishmania* occurs during insect feeding on infected mammalian hosts. After introduction into the insect gut together with the blood meal, *Leishmania* amastigotes transform into elongated flagellated promastigotes that propagate in the insect gut. A new round of infection is initiated after the infected sandfly takes a blood meal from a naïve mammalian host and introduces *Leishmania* parasites into the bite wound in the host dermis (Scheme 1). More than 20 different *Leishmania* species have been found to cause human leishmaniasis (Table 1).

Leishmaniasis is endemic in 98 countries and is closely associated with poverty. More than a million new cases are reported per year and 350 million people are at risk of contracting the infection. For the most severe form of leishmaniasis, VL, ~300 000 new cases are estimated to occur annually resulting in ~40 000 deaths. Approximately 90% of all VL cases occur in 3 endemic foci: 1. India, Bangladesh, and Nepal; 2. East Africa; and 3. Brazil. In spite of the high prevalence, currently available treatments for leishmaniasis are inadequate. Pentavalent antimonials, the standard treatment for leishmaniasis for many decades, are not efficacious in Bihar (~60% of VL cases worldwide) any longer due to widespread resistance to the drug in this region. Several new VL treatments have emerged during the past 10−15 years, but each has serious shortcomings (summarized in Table 2). These include paromomycin (injectable, long treatment, region-dependent efficacy), miltefosine (cost, teratogenicity, long treatment), and liposomal amphotericin B (cost, hospitalization, region-dependent efficacy). An additional challenge is represented by patients with HIV/VL coinfections who are more difficult to cure (lower initial and final cure rates), have greater susceptibility to drug toxicity, and have higher rates of death and relapse.

Due to the limitations of the existing treatments, better drugs are urgently needed. Ideally, new VL drugs would be efficacious across all endemic regions, would affect cure in ≤10 days, and would cost <$10 per course (for a complete target product profile for new VL drugs, which was formulated by DNDi, see Table 4).[1] Here we describe the disease history and parasite biology followed by a summary of the currently available treatments and, finally, review reports of novel small molecules with antileishmanial activity.

2. BACKGROUND OF LEISHMANIASIS

2.1. History and Biology of Leishmaniasis

Depending on the disease symptoms, leishmaniasis diagnosis typically falls into one of four major categories: visceral (VL), mucocutaneous (MC), post kala azar dermal (PKDL), or cutaneous leishmaniasis (CL). The earliest Old World records describing lesions with CL character go back to the seventh century BCE.[2] Detailed reports from Arab physicians in the 10th century describe CL in various regions of what is today called the Middle East.[2] Old World VL, or kala azar, characterized by an enlarged spleen, was first recognized in

Table 1. *Leishmania* Species Reported to Cause Human Infections and Associated Leishmaniasis Syndromes

Species	Clinical Presentation	Epidemiology	Symptoms
L. donovani (anthroponotic)	Visceral leishmaniasis/ kala azar (VL)	• India, Bangladesh, Nepal, East Africa • 300,000 new cases per year, with India having the highest incidence • 40,000 deaths annually	• Prolonged fever, splenomegaly, hepatomegaly, pancytopenia, progressive anemia and weight loss • Darkening of the skin
L. infantum (zoonotic)	Visceral leishmaniasis	• Mediterranean region, Central and South America • Primary animal reservoir: dog	• Same as above
L. siamensis (zoonotic)	Visceral leishmaniasis	• Thailand	• Same as above
L. donovani (anthroponotic)	Post-kala azar dermal leishmaniasis (PKDL)	• East Africa (Sudan), India, Bangladesh, and Nepal • Occurs in 50-60% of Sudanese and 10-20% of Indian VL patients with 0.5 – 7 years of infection	• Severe dermatitis entailing parasite-containing facial skin lesions and plaques on body • Potential to lead to nerve damage or blindness
L. aethiopica *L. killicki* *L. major* *L. tropica* *L. turanica*	Cutaneous leishmaniasis (CL, Old World)	• Southern Europe, Middle East, and Southwest Asia and Africa • 0.7 to 1.2 million cases per year, with greatest number of infected individuals residing in Afghanistan, Algeria, Iran, Syria, and Ethiopia	• Erythematous papulae at the site of the sand fly bite, with eventual scarring • Potential for becoming more severe and diffuse
L. amazonensis *L. aristedesi* *L. braziliensis* *L. colombiensis* *L. garnhami* *L. guyanensis* *L. lainsoni* *L. mexicana* *L. naiffi* *L. panamensis* *L. peruviana* *L. pifanoi* *L. shawi* *L. venezuelensis*	Cutaneous leishmaniasis (CL, New World)	• Central and South America • Greatest number of infected individuals residing in Brazil, Colombia, Costa Rica, and Peru • Infected patients include military workers, international travelers, and endemic area migrants	• Erythematous papulae at the site of the sand fly bite (for *L. mexicana*) • Metastasizing lesions that can lead to MCL (for *L. braziliensis)* and diffuse cutaneous leishmaniasis (DCL for *L. amazonensis*) • DCL or MCL are complications that occur (90% of cases in Brazil, Bolivia, and Peru)
L. aethiopica *L. braziliensis* *L. guyanensis* *L. panamensis*	Mucocutaneous leishmaniasis (MCL)	• Ethiopia • Central and South America	• Disfiguring and destructive lesions of the mucosal membrane occurring in 1-10% of CL patients • Destruction of the oronasopharyngeal mucosa

Table 2. Overview of Existing VL Drugs

Drug	Efficacy	Advantages	Limitations	Cost
Amphotericin B (Fungizone)	>95%	• Effective against Sb^{V+} resistance	• Deoxycholate form requires hospitalization and can cause myocarditis, hypokalemia, renal toxicity and reactions at the infusion site	~$100
Liposomal amphotericin B (AmBisome)	~100%	• No documented cases of drug resistance • Effective with low toxicity profile	• High cost • Fever and rigor during infusion • Renal toxicity	$280
Miltefosine	94-97%	• Highly potent; first effective oral treatment for VL and CL	• Highly toxic (liver and kidneys) • Gastrointestinal complications • Not safe for pregnant patients (teratogenic)	~$70
Paromomycin sulfate	95% (India); 46-85% (Africa)	• Low cost	• Reversible ototoxicity (2%) • Pain at injection site (55%) • Highly hepatotoxic (6%)	$10
Pentamidine	70-80%	• Potential use in combination therapy at low dosage	• Renal toxicity • Myocarditis • Insulin-dependent diabetes mellitus as irreversible side effect (4-12% patients) • Hypoglycemia and hypotension • Fever	~$100
Pentavalent antimonials: Sodium stibogluconate Meglumine antimoniate	35-95%	• Low cost • Can be used in combination with amphotericin B in pregnant or elderly patients	• Drug Resistance in Bihar, India (>60%) • Heart ventricle complications (prolonged QTc interval, premature beats, tachycardia, fibrillation, and torsades de pointes) and fatal cardiac arrhythmias • Arthralgia, myalgia, pancreatitis, elevated hepatic enzymes • Highest toxicity in HIV patients	$50-70

India in 1824. However, the symptoms were confused with those of malaria, and attempts were then made to treat the patients with quinine.[3] Clear recognition of VL as a distinct disease was achieved in 1900 after William Leishman and Charles Donovan independently identified *Leishmania donovani* parasites in the spleens of kala azar patients.[4] At about the same time *Leishmania* parasites were also observed in samples obtained from CL lesions. In 1908, Nicolle isolated the parasite from a cutaneous lesion and established the similarity between cutaneous and visceral forms of the disease with regard to the causative agent.[5] The majority of CL cases in the Old World are caused by two *Leishmania* species: *L. major* and *L. tropica*.

In the New World, CL and MCL cause disfiguring conditions and these have been depicted on sculptures dating back to the

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fifth century. References to leishmaniasis are also found in the writings of Spanish missionaries from the 16th century.[6] In 1911, Gaspar Vianna discovered that leishmaniasis in South America was caused by a different *Leishmania* species from that in the Old World, and coined a new name, *L. brazilienses*, for this species.[7] The species name was later corrected to *L. braziliensis*.[8] In the 1960s, additional Leishmania species causing CL in Latin America, were recognized such as *L. mexicana*.[9] In 1937, the causative agent of VL in the New World was designated as a distinct species, named *L. chagasi*.[10] However, this species is indistinguishable from *L. infantum*, the species that causes VL in southern Europe.[11]

Leishmania parasites are protozoa belonging to the Kinetoplastida order and Trypanosomatidae family. Over 20 species have been shown to be pathogenic in mammals, with affected hosts including domesticated and sylvatic animals. The parasites are transmitted indirectly between hosts by two different genera of hematophagous sand flies: *Phlebotomus* and *Lutzomyia* in the Old and New Worlds, respectively.

The life cycle of the *Leishmania* parasite is characterized by two distinct morphologies (Scheme 1): the elongated and flagellated promastigote, found in the alimentary tract of the female sand fly vector, and the round nonmotile amastigote, present in the bloodstream and tissues of the mammalian host. As an infected sand fly takes a blood meal from a naïve host, it regurgitates infective promastigotes at the bite site. The parasites are subsequently taken up by host dendritic cells and macrophages in the dermal layer of the skin. Here, they differentiate into amastigotes and multiply within phagolysosomes (via binary fission) while resisting degradation by lysosomal enzymes. Upon lysis of infected macrophage and dendritic cells, the parasites disseminate via the lymph and circulatory system and go on to infect other macrophages of the reticulo-endothelial system. The parasites persist in macrophages present in the spleen, bone marrow, liver, and lymph nodes and induce extensive inflammation and increased hematopoiesis.[12]

Infected patients serve as parasite reservoirs and can infect naïve sandflies when infected macrophages are ingested as part of the sandfly blood meal. After the parasite-infected macrophage is ingested by the sandfly, the amastigotes transform into promastigotes in the insect midgut, multiply, and migrate to the proximal end of the gut, where they remain until the next cycle of vector−host infection and transmission.[12c,d,13]

2.2. Clinical Description and Diagnosis of Leishmaniasis

2.2.1. Visceral Leishmaniasis and Post Kala Azar Dermal Leishmaniasis. VL is the most severe form of the disease and typically results in death if left untreated.[14] The clinical features generally manifest 2−6 months after infection, and these include prolonged fever, splenomegaly, hepatomegaly, pancytopenia, progressive anemia, and weight loss.[12c,d,15] Latent cases may remain undiagnosed until the patient becomes immunocompromised, with symptoms then appearing only several years after infection.[12c,d,14,15b] Darkening of the skin occurs in patients (particularly in South Asia) and defines the origin of the disease synonym kala azar (black fever in the Hindi language).[12d,16]

VL patients are at high risk for bacterial coinfections, including pneumonia, tuberculosis, and gastrointestinal (GI) infection.[12c] Both *Leishmania* and HIV target the immune system, and coinfections are found in overlapping HIV/VL-endemic areas, specifically Ethiopia, Brazil, and India.[17] Furthermore, the risk of developing VL is approximately 100- to 2000-times greater in patients infected with HIV compared to non-HIV individuals.[18] HIV/VL coinfected patients have a reduced $CD4^+$ T-cell count (below 200 cells/μL) and generally present symptoms similar to those observed in HIV-negative patients, including fever, splenomegaly, pancytopenia, lymphadenopathy, lethargy, and gastrointestinal issues. Co-infections are also more refractory to treatment and often require VL rescue therapy with an alternative drug.[19]

Post kala azar dermal leishmaniasis, or PKDL, is a form of dermal leishmaniasis that may appear months to years after effective treatment of VL and exhibits distinct features based on geography (Indian and Sudanese PKDL).[12d,15b,20] The clinical symptoms include papule skin lesions on the face, which gradually increase in size to form nodules all over the body and which can further transform into large plaques (Indian) or ulcers (Sudanese). These nodules have been shown to contain *Leishmania* parasites, so that PKDL patients become a reservoir of parasites for future transmission.[12d,20,21] While most cases of PKDL present as severe dermatitis, the spread of infection can lead to blindness (via the mucosal membranes) and to nerve damage (primarily in Indian PKDL).[22]

Early detection and treatment are crucial determinants of the prognosis for infected patients, and for prevention of transmission. Diagnostic tests include direct parasite detection (by microscopic visualization), use of PCR for quantification and determination of the infecting species determination by PCR, serological tests, and antigen-detection tests.[12c,23] The presence of amastigotes can be microscopically observed in patient lymph nodes, bone marrow, or splenic aspirates, and has been used for both diagnosis and evaluation of successful therapy. Quantitative assessment of parasite burden has been improved with use of PCR to amplify *Leishmania* gene targets such as 18S rRNA, the kinetoplast (mitochondrial) DNA, ß-tubulin, and cytochrome *b*.[23a,24] While direct parasite detection is the most dependable method for disease confirmation, complications from hemorrhage during splenic aspiration (0.1% of individuals) do arise, and examination requires high fidelity, skilled expertise, and established laboratories for sample collection and evaluation.[12c,23a] Serological tests monitor specific antileishmanial antibodies and include the direct agglutination test (DAT) or fast agglutination screening test (FAST), indirect immunofluorescence assay test (IFAT), and the rK39-based immunochromatographic test (ICT).[12c,23a] The antigen-detection tests represent an alternative to antibody detection. KAtex, a latex agglutination test which detects a low molecular weight glycoconjugate antigen in the urine of patients, shows high selectivity for parasite, but has low sensitivity.[12c,23a,25] For HIV/VL coinfected patients, diagnosis by direct visualization and quantification are highly reliable and sensitive, as the parasite burden has been shown to be more than 10-fold higher in HIV-positive (versus HIV-negative) patients.[17,26]

Diagnosis of PKDL is based on previous history of VL and results from the various clinical and serological tests. As sample collection (via tissue biopsy) is quite invasive and parasite loads tend to be low in papulae, detection of infection is not always straightforward and misdiagnosis of leprosy is common.[27] The splenic aspirate collection method is less invasive and is currently shows the greatest promise for diagnosis of PKDL in a reliable and noninvasive manner.[27]

Overall, the diagnostic tests need to be improved for greater sensitivity and specificity, low cost and convenience, greater

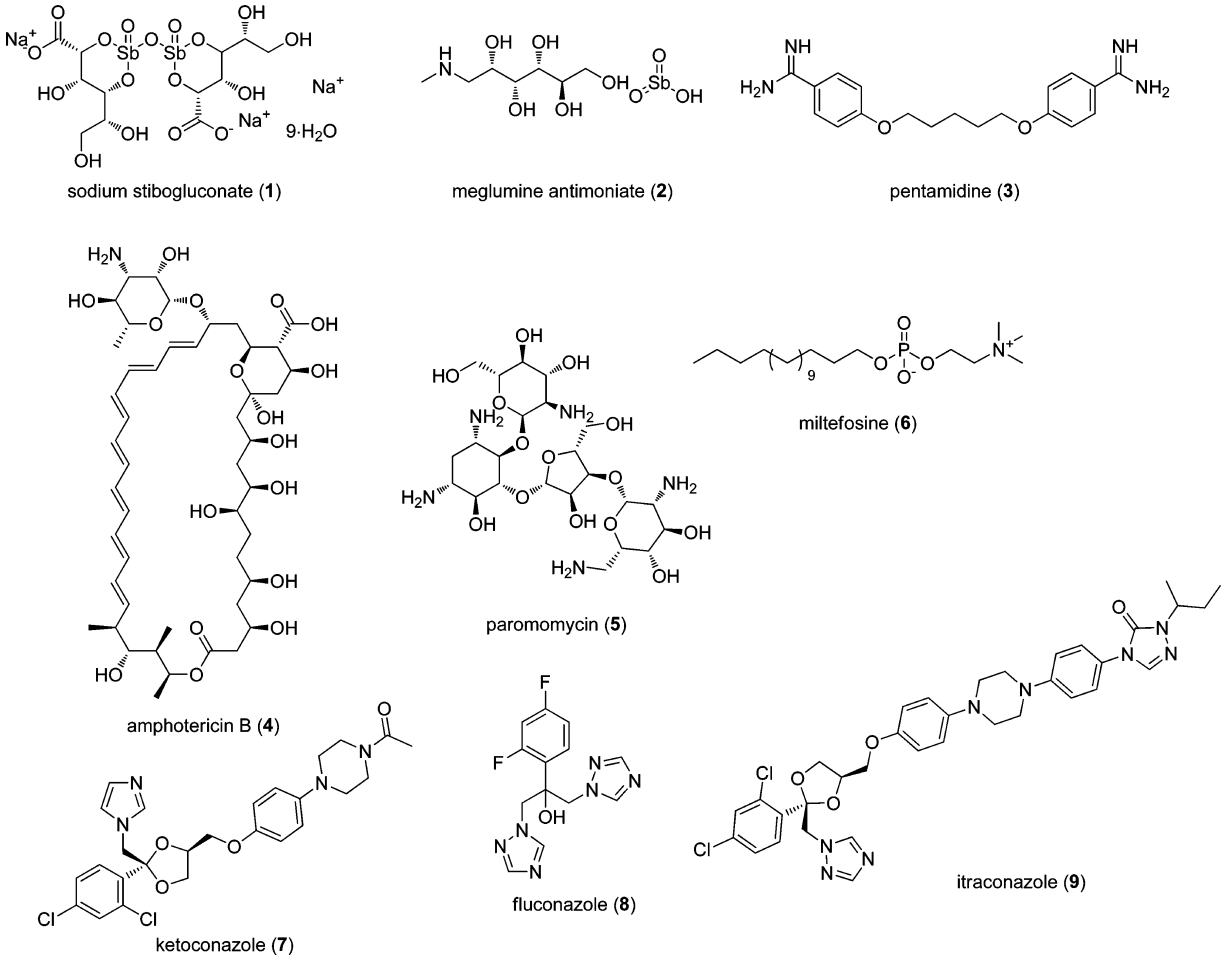

Figure 1. Current drugs used for treatment of leishmaniasis.

throughput, and ease of sample collection and test administration.[12c,14,23a]

2.2.2. Cutaneous and Mucocutaneous Leishmaniasis. The most common form of the disease, cutaneous leishmaniasis (CL) exhibits various clinical presentations dependent on the *Leishmania* species (Table 1) and the mode of transmission. CL starts with an erythematous preulcer papule at the site of the sand fly bite. This may self-cure within months or undergo slow-healing with severe scarring.[28] Rarer manifestations of CL include diffuse cutaneous leishmaniasis (DCL) and MCL, a life-threatening condition.[28a,29]

MCL is characterized by disfiguring and destructive lesions of the mucosal membranes and is usually observed months or even years after the CL lesions, in approximately 1−10% of CL patients.[13,28a,30] In addition to ulcerative lesions and erythema around the nose and lips, MCL patients initially present with nasal congestion and nasal septal granulomas (both anterior and posterior), lymphadenopathy, fever, hepatomegaly, and scars from previous CL incidence. Later stage MCL patients may exhibit additional complications within the nasal cavity (edema, septum perforation) and periodontitis, with eventual destruction of oronasopharyngeal mucosa and airway obstruction.[31]

Diagnostic tests for the various forms of CL are similar to those used to identify VL and include parasite collection (cutaneous skin scraping of center/margin of ulcer) and subsequent microscopic visualization via Giemsa staining, punch biopsy, needle aspirate and parasite culturing, serological antibody detection, and PCR quantification.[28a,29b]

2.3. Epidemiology of Leishmaniasis

2.3.1. Visceral Leishmaniasis and Post Kala Azar Leishmaniasis. There are two types of VL that are defined by the causative *Leishmania* species and the parasite reservoir. The zoonotic form, caused by *L. infantum,* occurs in the Mediterranean basin and Central and South America with dogs being the main parasite reservoir.[12d,15b,32] The more common anthroponotic form is caused by *L. donovani* and is predominant in India, Bangladesh, Nepal, and East Africa.[12c,d] VL is endemic to rural areas of developing countries and has been reported in approximately 98 countries in the world; 90% of all cases occur in six countries in tropical/subtropical regions: India, Bangladesh, Sudan, South Sudan, Brazil, and Ethiopia.[12c,d] Approximately 300 000 new cases of VL occur each year leading to an estimated 40 00 deaths. India has the highest incidence of the disease with approximately 60% of all new cases occurring in Bihar state.[12c,d,14,33] Outbreaks are common during migration or entry of naïve hosts into endemic areas and an increase in the immunosuppressed patient population (such as with HIV) has contributed to the escalation in VL incidence in East Africa.[15b] Additionally, an absence of implementation of cost-effective control strategies makes VL a major public health concern.[12c]

PKDL is prevalent in areas where *L. donovani* is endemic (India and East Africa) and occurs in 50−60% of Sudanese and

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Table 3. WHO Recommended Regimens for Treatment of VL and PKDL in Different Endemic Regions[105]

Drug	Efficacy	Advantages	Limitations	Cost
Amphotericin B (Fungizone)	>95%	• Effective against Sb^{V+} resistance	• Deoxycholate form requires hospitalization and can cause myocarditis, hypokalemia, renal toxicity and reactions at the infusion site	~$100
Liposomal amphotericin B (AmBisome)	~100%	• No documented cases of drug resistance • Effective with low toxicity profile	• High cost • Fever and rigor during infusion • Renal toxicity	$280
Miltefosine	94-97%	• Highly potent; first effective oral treatment for VL and CL	• Highly toxic (liver and kidneys) • Gastrointestinal complications • Not safe for pregnant patients (teratogenic)	~$70
Paromomycin sulfate	95% (India); 46-85% (Africa)	• Low cost	• Reversible ototoxicity (2%) • Pain at injection site (55%) • Highly hepatotoxic (6%)	$10
Pentamidine	70-80%	• Potential use in combination therapy at low dosage	• Renal toxicity • Myocarditis • Insulin-dependent diabetes mellitus as irreversible side effect (4-12% patients) • Hypoglycemia and hypotension • Fever	~$100
Pentavalent antimonials: Sodium stibogluconate Meglumine antimoniate	35-95%	• Low cost • Can be used in combination with amphotericin B in pregnant or elderly patients	• Drug Resistance in Bihar, India (>60%) • Heart ventricle complications (prolonged QTc interval, premature beats, tachycardia, fibrillation, and torsades de pointes) and fatal cardiac arrhythmias • Arthralgia, myalgia, pancreatitis, elevated hepatic enzymes • Highest toxicity in HIV patients	$50-70

Table 4. Target Product Profile for VL (Adapted from DNDi)

Specification	Optimal target profile	Minimal target profile
Leishmania species	All species	*L. donovani*
Distribution	All areas	Either India or Africa
Target population	Immunocompetent and Immunosuppressed	Immunocompetent
Clinical efficacy	> 95%	> 90%
Resistance	Active against resistant strains	Active against resistant strains
Safety and tolerability	No adverse events requiring monitoring	1 monitoring visit in mid/end - point
Contraindications	None	Pregnancy/lactation
Interactions	None – compatible for combination therapy	None for malaria, TB, and HIV therapies
Drug formulation	Oral or intramuscular depot	Oral or intramuscular depot
Drug stability	3 years in hot and humid countries (zone 4)	Stable under conditions that can be reasonably achieved in the target region (> 2 yr.)
Treatment regimen	Q.d. for 10 days p.o. or 3 shots given over 10 days	B.i.d. for < 10 days p.o. or < 3 shots over 10 days
Cost	< $10 per drug course	< $80 per drug course

10−20% of Indian VL patients within 6 months to 2−7 years after initial infection.[20,21,22a] Of these cases, approximately 15−20% (India) and 8% (Sudan) of patients do not have a history of VL, indicating the existence of an asymptomatic infection.[22a,34] Few cases of PKDL caused by *L. infantum* or *L. tropica* have been reported.[35] It has been previously shown that the presence of a small population of infected individuals (0.5%) may lead to a widespread epidemic of VL infection in India and other regions of Asia; therefore, PDKL patients play a major role in the spread of the disease, and parasite eradication should be a high priority.[36,13]

2.3.2. Cutaneous Leishmaniasis. Approximately 0.7 to 1.2 million cases of CL occur each year in the Americas, Mediterranean Basin, the Middle East, and Central Asia. A large fraction (75%) of CL patients reside in the following ten countries: Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica, and Peru.[33] The disease is caused by *L. tropica*, *L. major*, and *L. aethiopica* in the Old World (Southern Europe, Middle East, Southwest Asia, and Africa) or by *L. mexicana, L. braziliensis and additional Leishmania* species in the New World (Central and South America, Table 1).[15a,32] CL cases caused by *L. major* and *L. tropica* (anthroponotic) and by *L. mexicana* are characterized by papulae that typically heal within a few months without medical intervention, whereas CL caused by *L. braziliensis* is distinguished by lesions that frequently metastasize to mucosal tissues (MCL) and are treated with antileishmanial therapeutics.[15b,28a,32,37] DCL (*L. amazonensis*) and MCL are complications of CL that occur primarily in the New World (90% of cases found in Brazil, Bolivia, and Peru), respectively.[28a]

An increasing number of CL cases have been reported in individuals that have served in the military, international travelers, and endemic area migrants.[37,38] Travels to Central and South America account for approximately 40% of CL cases in tourists and workers in the USA.[39] While some cases of leishmaniasis introduced into industrialized nations involve VL, greater than 80% of these are caused by CL. In fact, CL is one of the most frequent skin disorders in the New World, and accounts for around 60% of all cases in nonendemic areas.[40] With increasing travel, immigration, and military work in endemic areas of this disease, the risk levels and incidence are predicted to increase hence making implementation of precautionary measures crucial in this selected group.

2.4. Current Treatments

The focus of this section is to discuss the drugs already in use for the treatment of VL. These include pentavalent antimonials,

pentamidine, various formulations of amphotericin B, paromomycin, and miltefosine (Table 2 and Figure 1). As some of the same drugs are used for treatment of CL and MCL, the corresponding regimens for these syndromes (including PKDL) are also briefly described when applicable. Treatment of VL varies from one endemic region to another; the WHO recommended regimens for major VL endemic foci are summarized in Table 3. In general, as discussed earlier, summarized in Table 2, and described in more detail in the next sections the current treatment options are inadequate and new chemical entities are urgently needed (target product profile in Table 4).

2.4.1. Pentavalent Antimonials. Antimony has been used as a therapeutic for several centuries. The first use of antimony in the modern era dates to 1905, when trivalent sodium antimonial tartrate was used to treat trypanosomiasis.[41] Use of the trivalent antimonials for the treatment of CL was first reported by Vianna, and for VL by Di Cristina and Caronia in Sicily, and Rogers in India in 1915.[42−44] Later this drug was found to be highly toxic and exhibited side effects such as cough, chest pain, and depression. The key breakthrough in the use of antimony for the treatment of leishmaniasis was achieved in 1925 by Brahmachari, who synthesized the pentavalent antimony compound urea stibamine and discovered it was an effective chemotherapeutic agent against VL.[45] This discovery saved millions of lives in India, especially in Assam state, where many villages were depopulated by VL epidemics. Further progress in antimony therapy of VL was achieved through synthesis of antimony gluconate (Solustibosan) in 1937 and sodium stibogluconate (Pentostam) in 1945.[46,47]

Currently, there are two formulations of pentavalent antimonials in use: sodium stibogluconate (**1**) (100 mg antimony(Sb^{V+})/100 mL) and meglumine antimoniate (**2**) (85 mg antimony/100 mL). Both formulations have poor oral absorption and are given via intramuscular injections or intravenous infusions.[48] Common side effects of pentavalent antimonials include prolonged QTc interval, ventricular premature beats, ventricular tachycardia, ventricular fibrillation, and torsades de pointes.[38b,49] Prolongation of QTc interval (>0.5 s) is often associated with serious or even fatal cardiac arrhythmias.[50] Arthralgia and myalgia, elevated hepatic enzymes and pancreatitis are other common adverse events.[51] Antimonial use causes more toxicity and mortality in HIV-positive patients, compared to HIV-positive patients treated with miltefosine or AmBisome, or HIV-negative patients treated with antimonials.[52]

In India, sodium stibogluconate was initially administered at low doses of 10 mg/kg/day for 6−10 days.[53] These regimens were successful in curing most of the patients until the late seventies, when several unconfirmed reports of unresponsiveness appeared. In the eighties, clinical studies were done to determine the most effective regimen and these concluded in the recommendation in 1992 to treat VL in India with 20 mg Sb^{V+}/kg for 28−30 days.[39,54] During the 1990s and 2000s, the clinical efficacy of antimonials in Bihar state (where ∼90% of VL cases in India occur) gradually declined, and more than 60% of VL cases in this state are now refractory to this treatment although the drug continues to be effective in surrounding areas (e.g., Uttar Pradesh state).[55] It is not established with certainty what factors drove the emergence of antimony-resistant *L. donovani* in Bihar. According to one hypothesis, the resistance to antimonials emerged as the result of large scale misuse of the drug in Bihar, where in one survey only 26% of patients were treated according to the WHO guidelines.[56] The alternative hypothesis is based on the observation that exposure of *L. donovani* to low concentration of arsenic leads to emergence of parasite resistance to pentavalent antimonials. Starting in the 1970s, there was a large scale tapping of aquifers in Bihar to provide clean drinking water. The Bihari population was at risk from arsenic exposure due to contamination from naturally occurring trivalent arsenic in the groundwater. Thus, chronic exposure of the Bihar population to arsenic in drinking water could have driven emergence of antimony-resistant *L. donovani* strains.[57] Even though pentavalent antimonials continue to be efficacious in other parts of Southeast Asia, the WHO currently recommends alternative drugs (AmBisome infusion) as the first line therapy options in this region.[32]

As in India, VL in Africa is caused by *L. donovani* with major disease foci in Sudan, South Sudan, and Ethiopia, and a lower number of cases found in Kenya and Uganda. Recommended treatment consists of 20 mg/kg of sodium stibogluconate for 30 days.[58] This regimen typically yields >90% cure rates in HIV-negative patients across the East Africa region.[59] However, monotherapy with pentavalent antimony is not considered the first line treatment in East Africa according to the WHO, which recommends combination treatment with pentavalent antimony and paromomycin.[32]

Unlike in India and Africa, VL in South America is caused by *L. infantum* (formerly referred to as *L. chagasi*). There is no evidence of significant resistance to pentavalent antimonials in Brazil and meglumine antimoniate is the first choice for the treatment of mild and moderate cases of VL.[60] For severe cases (age less than six months or over 65 years with signs of malnourishment, renal or hepatic insufficiency) and pregnant women, the Brazilian Health Ministry recommends treatment with liposomal amphotericin B (AmBisome).[61] A recent retrospective study focusing on a cohort of children treated with 20 mg/kg per day meglumine antimoniate for 20−40 days reported efficacy of 96.9% in mild-to-moderate cases, and over 60% in severe cases.[60]

VL in the Mediterranean countries is caused by *L. infantum* as well. During the 1990s, antimonials were the first-line of treatment in most countries of this region (France, Greece, Italy, Malta, Spain, Portugal, Albania, Israel, Turkey, Morocco, Algeria, and Tunisia) with cure rates >95% in immunocompetent patients using regimens of 20 mg Sb^{V+}/kg for 20−30 days.[62] More recently, pentavalent antimonials have been replaced by AmBisome as the first line of treatment in European countries.[63]

Most countries endemic for VL also have HIV-infected populations with the highest coinfection rates found in East Africa (up to 25−40% in parts of Ethiopia) followed by Brazil (∼5%) and India (2−5%).[64] Use of pentavalent antimonials in HIV-infected patients is no longer recommended by most experts in the field due to their unacceptable toxicity in this patient group and high rates of treatment failure.[52a,65] However, because of their low cost, antimonials at a dose of 20 mg/kg for 28−30 days are still used when alternative treatments are prohibitively expensive. HIV infection has consistently been a predictor of poor outcome of VL treatment (e.g., only 44% cure rate in HIV-positive versus 92% in HIV-negative patients in one trial in Ethiopia) and associated with high rates of relapse (15−57%).[65]

Antimonials have also been used extensively as the primary treatment option for CL and ML, particularly in the New World where there is a greater risk of mucosal involvement.[66]

Administration is either by intralesion injections (limited to Old World CL infections - up to 5 individual doses separated by 3−7 days) or systemically (20 mg/kg for 20 days for CL and 28−30 days for MCL). Several studies of this drug therapy indicate differences in effectiveness, with 85−90% cure rates in Old World CL and 26%−100% in South America, depending on country and parasite species.[67]

2.4.2. Pentamidine. Pentamidine (**3**) has been in use since the 1940s for treatment of sleeping sickness.[68] The first use for VL treatment was reported in India in 1949 and in Spain in 1950.[69,70] Most regimens are based on intramuscular injection or intravenous infusion of 4 mg/kg of pentamidine (isethionate or methanosulfonate) per day for a variable number (up to 30) of days. Safety is a major concern with insulin-dependent diabetes mellitus being the most feared and irreversible adverse event.[71] This complication, while not uniformly reported, occurs in 4−12% of cases. Additional side effects include hypoglycemia, hypotension, fever, myocarditis and renal toxicity.[72]

Pentamidine was used as the second line therapy for treatment of antimony-refractory cases of VL in India. However, due to its toxicity and rapidly emerging resistance (frequently to both pentamidine and antimonials), pentamidine use in India was abandoned in the 1990s and replaced with amphotericin B deoxycholate as the recommended treatment.[73] During the early years of increased pentamidine use in India (1978), 10 injections were sufficient to effect cure in all treated patients. By the early 1990s, 15 or more injections were required to produce cure in only 67−77% patients.[74] More recently, pentamidine was successfully used in several cases of HIV-positive patients to prevent VL relapse following the initial treatment with an alternative drug.[75]

Pentamidine is the first option for treatment of CL caused by *L. guyanensis* and is recommended as the first-line treatment in French Guiana, and in Suriname, where it is the only available antileishmanial. The typical treatment consists of a single intramuscular injection of 7 mg/kg of pentamidine isethionate and can be repeated 48 h later in complicated cases. In one study these regimens yielded 78.8 and 83.6% cure rates, respectively.[76]

2.4.3. Amphotericin B. Amphotericin B (**4**) is a polyene antibiotic isolated from *Streptomyces nodosus* in 1955, which was identified because of its antifungal activity.[77] In vitro activity of amphotericin B on *Leishmania* was for the first time reported in 1960 and the first successful treatment of patients with VL was reported in 1963 in Brazil.[78,79] The drug increases membrane permeability by binding to ergosterol present in the *Leishmania* plasma membrane.[80] Amphotericin B is used in complex with deoxycholate or various lipids and all formulations are administered by intravenous infusion. The deoxycholate form of the drug has many adverse effects including infusion reactions, nephrotoxicity, hypokalemia, and myocarditis, and needs close monitoring and hospitalization for 4−5 weeks. Lipid formulations of amphotericin B are efficacious at lower doses and have reduced toxicity, but the high cost complicates treatment of patients in low income settings.[81]

In India, amphotericin B was traditionally a second line treatment for VL, but decreased efficacy of antimonials and pentamidine led to recommendation for use as a first-line treatment starting in 1990s in Bihar. Amphotericin B deoxycholate has been used with different dosing regimens, with a total dose ranging from 7 to 20 mg/kg, and treatment administered on alternate days or daily for up to 43 days at either constant or incremental dosing. Amphotericin B regimens typically produce high cure rates (close to 100%) for both antimony-sensitive and refractory infections.[82] Several lipid formulations of amphotericin B (liposomal-AmBisome, lipid complex-Abelcet, colloidal dispersion-Amphocil, lipid emulsion - Amphomul) have also been tested; all enabling regimens with ∼100% cure rates.[83] Lipid formulations lead to the rapid concentration of the drug in organs such as liver and spleen.[84] This greatly reduces adverse effects including nephrotoxicity and allows delivery of large doses of the drug over short periods of time. In an open label study in Bihar in 2010, a single dose of 10 mg/kg of AmBisome produced a 96.3% cure rate.[85] The outcome prompted the WHO to recommend this regimen as the first line treatment for VL in South Asia.[32]

Efficacy of amphotericin B deoxycholate in East Africa (Uganda) was extensively evaluated in 2003−2004 during an interruption in supply of antimonial drugs. The regimen consisted of slow infusion of 1 mg/kg of amphotericin B on alternate days for 30 days (total dose 15 mg/kg) and produced a 92.4% cure rate.[86] Experience with AmBisome treatment in East Africa suggests that higher total doses than in India are required to achieve >90% cure rates. Treatment with 30 mg/kg AmBisome in 6 doses on alternate days in Sudan produced a 92.6% initial cure rate in HIV-negative patients but only 59.5% in HIV-positive group. AmBisome was even less effective in HIV-positive VL relapses (38.0% initial cure, 55.7% parasitological failure). Of additional interest, a study to determine the optimal single dose of AmBisome (tested doses include 7.5, 10, 12.5, and 15 mg/kg) in HIV-negative patients in East Africa was concluded and the results are expected to be published soon.[87]

In Latin America, there is much less data on AmBisome's efficacy. In Brazil, a total dose of 20 mg/kg has been proven to be efficacious.[88] The Pan American Health Organization guidelines for treatment of leishmaniasis in the Americas have established liposomal amphotericin B (3−5 mg/kg per day IV for 3−6 days, with a total dose of 20 mg/kg) as one of the first-line therapeutic options.

In Southern Europe, doses of 3−5 mg/kg per day, up to a total of 20 mg/kg in different regimens, have been demonstrated to be effective in up to 99−100% of patients. Total doses of 15, 18, and 24 mg/kg were tested in Italy, with response rates of 91, 98 and 100%, respectively. In Greece, one study administered a total dose of 20 mg/kg in a short regimen of 2 days, with a cure rate of 98%, versus 90%, when it was administered over 5 days. Because of the large number of published case series, there is an important accumulation of evidence regarding the use of liposomal amphotericin B in pediatric populations in Europe, with high response rates (97% with total doses of 18−24 mg/kg in different regimens).[89] It has been shown that liposomal amphotericin B reduces the average duration of hospitalization when compared with antimonials and that it was effective in cases that did not respond to treatment with antimonials.[90] For all of these reasons and despite the absence of randomized clinical trials, liposomal amphotericin B is considered a reference treatment for VL in the Mediterranean countries in both adults and children.

Amphotericin B deoxycholate (0.7 mg/kg per day, by infusion, for 25−30 doses) and AmBisome (2−3 mg/kg per day, by infusion, up to 20−40 mg/kg total dose) are also used for treatment of CL and MCL infections caused by *L.*

braziliensis and other species, including *L. guyanensis*, *L. infantum*, and *L. aethiopica*.[91] In a study completed by Solomon and colleagues, a dosage of 18 mg/kg total given to patients afflicted with *L. braziliensis* CL resulted in an approximately 85% complete cure in patients within two months.[92]

2.4.4. Paromomycin. Paromomycin (**5**) is an aminoglycoside broad-spectrum antibiotic, first isolated in the 1950s from *Streptomyces krestomuceticus*. Paromomycin inhibits proteosynthesis by binding to 16S rRNA.[93] It was shown to be efficacious for the treatment of CL in 1966 and for VL in 1990 in Kenya.[94] The most common adverse event with paromomycin is injection site pain (55%); however, this typically does not lead to the discontinuation of therapy. A small fraction of patients experience reversible ototoxicity (2%) and a rise in hepatic transaminases (6%).[95]

In a phase III study in Bihar in 2003−2004, a paromomycin regimen of 11 mg/kg (15 mg/kg as the sulfate) i.m. for 21 days was shown to be noninferior to amphotericin B (1 mg/kg i.v. alternate day for 30 days) with final cure rates of 94.6 versus 98.8%, respectively.[95] The cure rate among those previously treated with Sb^{V+} or miltefosine was 98%. The cure rate in pediatric patients was 96% and in females 95%. The main advantage of paromomycin is its affordability: the cost of the treatment is only ∼$10 per patient.

A study conducted in 5 centers in Sudan, Kenya, and Ethiopia compared the efficacy of paromomycin as monotherapy at a dose of 15 mg/kg per day for 21 days, antimonials (20 mg/kg per day) as monotherapy for 30 days or the combination of both drugs for 17 days. At 6 months after the end of treatment, paromomycin monotherapy provided only a 63.8% average cure rate with very low cure rates observed in 2 Sudan centers (14.3% and 46.7%).[96] A follow-up study in East Africa evaluated paromomycin regimens of 15 mg/kg per day for 28 days, and 20 mg/kg per day for 21 days with final cure rates of 81% and 80%; however, both regimens were still inferior to the standard treatment (20 mg/kg of sodium stibogluconate for 30 days yielded a 94.1% cure rate).[97] There are no reports on paromomycin use in VL treatment in Latin America and Mediterranean countries.

Paromomycin in a form of ointment (15% paromomycin/12% methylbenzethonium chloride) is also used for the local treatment of noncomplicated Old World CL by application to the lesion twice daily for 20 days. Experience with paromomycin ointment for the treatment of New World CL is limited. In one trial, 20 day treatment twice daily produced 70−90% cure rates for CL caused by *L. mexicana*, *L. panamensis*, and *L. braziliensis* in Ecuador and Guatemala.[98] More recently, a novel paromomycin ointment was described (15% paromomycin, 0.5% gentamycin) and found efficacious for CL treatment caused by *L. major*.[99]

2.4.5. Miltefosine. Miltefosine (**6**; hexadecylphosphocholine) was originally developed as an anticancer drug. In the 1990s, several laboratories discovered that miltefosine has antileishmanial activity,[100] and in 2002, it was approved in India as the first oral treatment of VL. The most common adverse events include gastrointestinal side effects and occasional hepato- and nephrotoxicity. Another miltefosine limitation is teratogenicity, and women of child-bearing age have to take contraceptives for the duration of treatment and for an additional 3 months afterward due to the long half-life of miltefosine (∼1 week).[101]

In 2002 a phase III trial in India with a regimen of 50−100 mg/day for 28 days resulted in a 94% cure rate, and miltefosine was selected for the VL elimination program in India, Nepal, and Bangladesh.[102] However, a recent study suggests that miltefosine efficacy is starting to decline and a study in 2012 yielded a reduced cure rate of 90.3%.[101] Miltefosine is also efficacious for treatment of PKDL cases and the recommended regimen includes treatment with 50−100 mg/day for 12 weeks.[103]

The efficacy of miltefosine in East Africa was determined during a trial in Ethiopia in 2006. A regimen of 100 mg/kg per day of miltefosine for 28 days was found to be equivalent to sodium stibogluconate treatment (20 mg/kg per day for 40−60 days) in HIV-negative patients (final cure rate of ∼94% for patients who could be traced during follow up).[52a] A phase II trial to evaluate the efficacy of miltefosine in Sudan and Kenya is ongoing.

Miltefosine is considered to be the first effective oral treatment regimen for CL, with greater accessibility and lower toxicity compared to antimonials.[104] Miltefosine at a dose of 2 mg/kg per day for 28 days is effective against CL in Colombia caused by *L. panamensis* (70−90% cure rate), but has only limited effect against the disease caused by *L. braziliensis* and *L. mexicana* (<60% cure rate). Treatment extension to six months for CL in Brazil originating from *L. braziliensis* infection resulted in a 75% cure rate compared to the 53% cure rate following treatment with antimony, with efficacy shown to be greater in adults compared to children.[104]

In Table 3 the WHO regimens for the treatment of VL and PKDL in various endemic regions are described.

2.4.6. Ketoconazole. Azoles are oral antifungal drugs that inhibit fungal ergosterol biosynthesis at the lanosterol demethylase step resulting in the accumulation of 14 α-methyl sterols. As *Leishmania* parasites rely on ergosterol for their sterol needs and share this biosynthetic pathway with fungi, azoles have been explored for their therapeutic potential against *Leishmania* infections. For CL, the efficacy of compounds varies depending on species.[106] Ketoconazole (**7**) was tested for a month in both adults and children on CL caused by *L. braziliensis* (either 600 mg or 100 mg daily, respectively, for 28 days) and resulted in a 76% cure with mild side effects.[107]

Similar testing in patients afflicted with CL caused by *L. mexicana* resulted in 89% cure in another study completed by Navin and colleagues.[108] Another ergosterol biosynthesis inhibitor, fluconazole (**8**) (200 mg daily for 6 weeks), was also previously tested in patients with CL originating from *L. major* and resulted in 59% cure and shorter healing time for patients residing in Saudi Arabia.[109] In the case of itraconazole (**9**), minimal response rates were observed in cases of CL resulting from *L. major* and in MCL originating from *L. braziliensis*.[110] Among the several azole drugs tested (fluconazole, itraconazole, ketoconazole), only ketoconazole was found to be consistently efficacious and is now used for treatment of CL infections caused by *L. mexicana* (600 mg per day for 28 days).

2.4.7. Treatments with Drug Combinations. There are only a limited number of new chemical entities in the drug development pipeline to address the limitations of the current VL treatments. Instead, treatments with combinations of existing drugs have become the main short to medium term strategy to combat emerging drug resistance, reduce adverse events, and shorten therapy duration. The earliest attempts to explore this approach occurred in the early 1990s, with a combination of sodium stibogluconate and paromomycin tested in Kenya, Bihar state, and Sudan. A study in Bihar

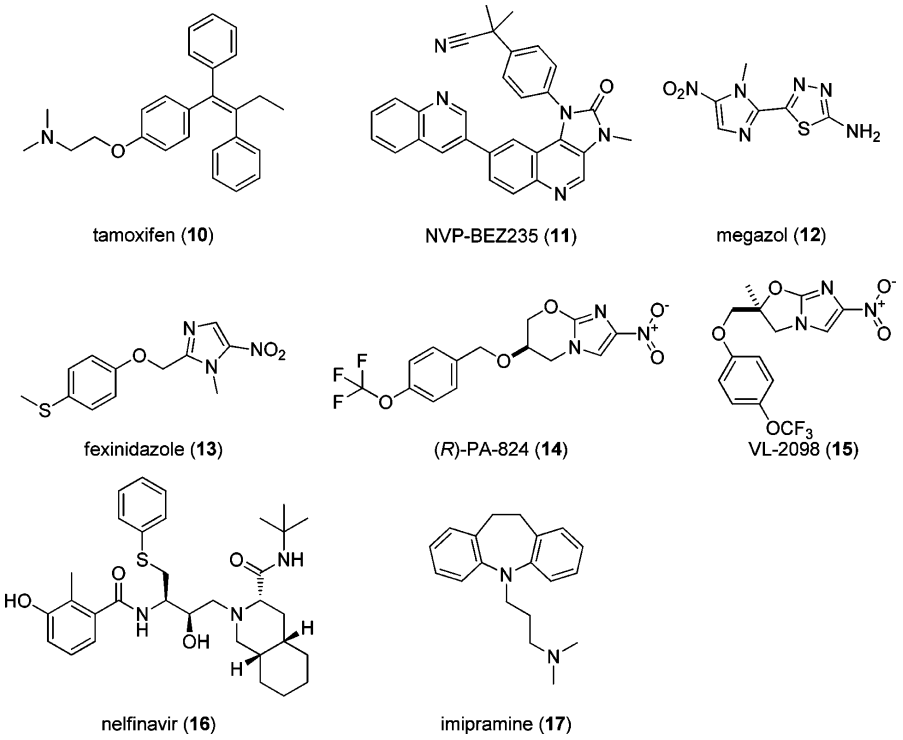

Figure 2. Drugs that have been repurposed for the treatment of leishmaniasis.

evaluating combinations of various paromomycin and sodium stibogluconate doses found that a combination of 12 mg/kg of paromomycin and 20 mg/kg of sodium stibogluconate (both administered daily) for 20 days yielded an 88% cure rate.[111]

Seventeen day treatment with the combination of sodium stibogluconate (20 mg/kg) and paromomycin (15 mg/kg) in Sudan affected a 97% initial cure rate and was found to be superior to sodium stibogluconate alone (20 mg/kg for 30 days).[112] Similar results were also observed in a subsequent East Africa multicenter trial, and this combination regimen is now the preferred treatment in this region.[32,59]

Another approach to combination treatment relies on sequential use of 2 different drugs. During recent trials in India it was established that a single infusion of 5 mg/kg of AmBisome followed by either 7 days of 50 mg/kg per day of miltefosine or 10 days of 11 mg/kg per day of paromomycin both yielded 97.5% cure rates 6 months after the end of treatment. As a part of this trial, a treatment arm with daily coadministration of miltefosine and paromomycin (50 mg/kg and 11 mg/kg per day, respectively) for 10 days was also evaluated and yielded a 98.7% final cure rate.[101] In summary, combination therapies have been established as safe and effective treatment options and their implementation into primary treatment centers in India and East Africa is ongoing.

Combination therapy with antimonials has been used to enhance efficacy for CL.[66] Allopurinol supplementation led to a 2-fold reduction in the required antimony dosage and resulted in a cure rate of 75−80% in Iranian patients infected with *L. major* and improved treatment outcomes for patients treated with a single agent while infected with *L. tropica*.[113] To treat *L. braziliensis*, pentavalent antimony (15−20 mg/kg daily) has been used in conjunction with pentoxifylline (400 mg, three times a day) for a month to cure 90% of patients with MCL and lesions resistant to single agent therapy.[114]

3. DRUG DISCOVERY FOR LEISHMANIASIS

In spite of a large patient population, leishmaniasis drugs have led to poor economic returns as endemic areas are typically impoverished. As a consequence there have been limited funds available to support the research and development of new antileishmaniasis treatments to address the liabilities of the current standard of care according to the target profile shown in Table 4.

In order to bolster the pipeline a significant effort has been applied in repurposing drugs from different indications. The repurposing of drugs offers a short and fast path to reach patients and the cost of development is greatly reduced. The drug repurposing strategy has been summarized in the literature in several reviews and has been shown to be very successful. Indeed several current treatments such as miltefosine, amphotericin B, and pentamidine were previously approved or primarily designed for other indications.[115,116] In section 3.1, we summarize the main drugs and compound classes that have been recently considered for repurposing in leishmaniasis.

As in other areas of infectious diseases most of the novel chemical entities are coming from phenotypic drug discovery campaigns rather than target based efforts. Until recently, the screening of large libraries using phenotypic readouts was nonexistent in the antileishmanial field because of the complexity of biology as well as lack of resources. New technological advancements have allowed the screening of large libraries using phenotypic readouts and it is anticipated that these screening efforts will yield new structurally diverse antileishmanial compounds and will help identify new critical targets. Section 3.2 will describe the recent advancements from phenotypic efforts including compounds identified from screening of synthetic compound libraries as well as natural product extracts followed by isolation and chemistry modification. Efforts related to the modification of existing anti-infective scaffolds are also described. Finally, tremendous

efforts have been put in the understanding of the *Leishmania* biology leading to identification of numerous putative targets. Section 3.3 discusses the proposed essential targets and the compounds used as tools to validate them.

3.1. Repurposing Efforts for Leishmaniasis

3.1.1. Tamoxifen. Tamoxifen (**10**) (Figure 2) is an estrogen receptor antagonist which has been in clinical use for the treatment of breast cancer. Tamoxifen has in vitro activity against *L. braziliensis* and *L. infantum* intracellular amastigotes with an EC_{50} of 1.9 ± 0.2 and 2.4 ± 0.3 μM, respectively.

Treatment of *L. braziliensis*-infected mice with tamoxifen at a dose of 20 mg/kg led to significant reductions in lesion size and a 99% decrease in parasite burden when compared with vehicle controls.

Treatment of *L. infantum*-infected hamsters with tamoxifen led to significant reductions in liver parasite load and a 95% to 98% reduction in spleen parasite burden. Furthermore, there was a 100% survival rate for all animals treated with tamoxifen. In contrast, all the vehicle-treated animals perished by 11 weeks.[117] In a similar experiment carried out for cutaneous leishmaniasis, the infected mice were treated with tamoxifen (**10**), orally, at a dose of 20 mg/kg/day for 15 days. Results indicated that untreated infected mice suffered from autoamputation of the inoculated foot pad. In comparison, the treated mice exhibited marked improvement of the cutaneous lesions and reduction of overall parasite load. However, the treated male mice showed scrotal swelling with evident histopathological changes in the testes that could seriously compromise fertility of the male mice.

In conclusion, while tamoxifen (**10**) is able to cure leishmaniasis infection in laboratory animals, it also causes significant side effects to the male reproductive system in the mouse model.[118]

3.1.2. PI3 Kinase Inhibitors. A series of human phosphoinositide-3-kinase (PI3K) and mammalian target of rapamycin (mTOR) inhibitors were investigated for activity against the kinetoplastid parasites (*Trypanosoma brucei*, *T. cruzi*, and *Leishmania* sp). The rationale behind this study was based on the premise that both parasites and humans express similar kinase enzymes. Thus, one could exploit the extensive research on the human targets to repurpose compounds to kinetoplastid infections. Among the inhibitors examined, NVP-BEZ235 (**11**), was found to have potent antileishmanial activity in parasite cultures in submicromolar concentration. However, despite its activity against *L. donovani* axenic amastigotes, no efficacy was observed in in vivo mouse models at tolerated doses.[119]

3.1.3. Nitroimidazoles. Nitroimidazoles are a well-known class of pharmacologically active compounds, most notably in the field of anaerobic bacterial and parasitic infections.[120]

The most profiled antitrypanosomal drug candidate in this class was megazol (**12**) (Figure 2), though development was stopped due to mutagenicity issues.[121] Continuing exploration of this class of compounds led to the identification of fexinidazole (**13**) as an effective antitrypanosomal agent. Fexinidazole is currently in clinical trials for stage 2 HAT (see section 6.2.). Fexinidazole is rapidly oxidized in vivo in mice, dogs, and humans to the sulfoxide and sulfone metabolite. While the parent compound is devoid of activity, both metabolites of fexinidazole are active against intracellular *L. donovani* amastigotes. A q.d. regimen for 5 days at 200 mg/kg dose led to a 98.4% suppression of parasites in a mouse model of visceral leishmaniasis which is equivalent efficacy to that seen with miltefosine. Overexpression of the leishmanial nitroreductase homologue in *L. donovani* led to an increase in sensitivity to fexinidazole by 19-fold, indicating that reductive activation, via an NADH dependent bacterial-like nitroreductase, is responsible for the activity.[122] Based on the impressive efficacy, fexindazole is currently in phase II clinical trials for visceral leishmaniasis.

Bicyclic nitroimidazole derivative (R)-PA-824 (**14**) shows potent cidal activity against *L. donovani* with an EC_{50} of 160 nM and 930 nM against promastigotes and intracellular amastigotes, respectively. In a murine model, (R)-PA-824 exhibits >99% suppression of parasite burden at a dose of 100 mg/kg b.i.d when administered orally for 5 days. In contrast to fexinidazole, transgenic parasites overexpressing the leishmania nitroreductase are not oversensitive to (R)-PA-824 (**14**) indicating that this enzyme is not involved in the mechanism of action of this compound and some other unknown nitroreductase specific to leishmania species might be involved. Thus, (R)-PA-824 offers the promise of being a potential candidate for late lead optimization for VL.[123] Indeed, similar compound VL-2098 (**15**) is already in preclinical development for the treatment of visceral leishmaniasis and has the potential to further bolster the pipeline.

3.1.4. Nelfinavir. Reports of visceral leishmaniasis co-occurring in individuals infected with human immunodeficiency virus type 1 (HIV-1) are well documented.[124] A series of protease inhibitors (nelfinavir, ritonavir, and saquinavir) were examined for their activity against various *Leishmania* species. While it was observed that these protease inhibitors do not inhibit the growth of *Leishmania infantum* promastigotes alone in culture, they were found to significantly inhibit the intracellular survival of parasites in phorbol myristate acetate-differentiated THP-1 macrophages and human primary monocyte-derived macrophages (MDMs) (65−79% inhibition). Furthermore, these compounds were found to be equally active against a field isolate of *Leishmania donovani* resistant to sodium stibogluconate (SbV), suggesting that resistance to SbV does not result in cross-resistance to protease inhibitors. Additionally, the ability of nelfinavir (**16**) (Figure 2) to reduce the intracellular growth of *Leishmania* parasites is also observed in MDMs coinfected with HIV-1.[125] Further work into the mechanism of action suggests that nelfinavir (**16**) induces oxidative stress in *Leishmania* amastigotes, leading to caspase-independent apoptosis, in which DNA is degraded by endonuclease G. These studies provide a rationale to test nelfinavir (**16**) as a potential antileishmanial agent as well as for possible future use in *Leishmania*/HIV-1 coinfections.[126]

3.1.5. Imipramine. Imipramine (**17**) is a cationic amphiphilic drug commonly used for the treatment of depression in humans. Previous studies have shown that this compound was able to decrease the mitochondrial transmembrane potential of *L. donovani* promastigotes and purified amastigotes as opposed to miltefosine where only a marginal change in potential was observed.[127] Moreover it was found to inhibit trypanothione reductase, an enzyme which is upregulated in antimony resistant strains.[128] In addition, as an effective immunomodulator, it was known to upregulate TNF-α, which plays an important role in cytokine defense.[129] Different groups of hamsters infected with antimony sensitive and resistant isolates were treated with imipramine at doses of 0.05, 0.5, and 5 mg/kg/day respectively for 4 weeks and while there was no clearance of splenic and hepatic parasite load at 0.05 mg/kg,

50% clearance was observed at 0.5 mg/kg and there were no detectable parasites in animals dosed at 5 mg/kg. More importantly, organ parasite clearance was similar for all isolates irrespective of their sensitivity toward antimonials. No further development work has been reported on this compound.

3.2. Antileishmanials from Phenotypic Efforts

Phenotypic drug discovery has proven to be a successful approach for identifying new chemotypes and starting points for medicinal chemistry optimization.[130] Moreover, the poor understanding of relevant targets in the parasite field has led to poor success rates when using a target based drug discovery approach, making a phenotypic strategy particularly attractive in this context.[131] This broad approach offers the potential to identify agents acting on a previously undescribed target or by acting on multiple targets in tandem. The prospect of establishing new mechanisms of action for antileishmanial activity is of growing importance as drug treatment pressure has resulted in emerging parasite resistance.[132]

Advances in chemical proteomics have made subsequent target elucidation and the evaluation of therapeutic intervention via this approach a viable alternative to target-based approaches. However, it is important to discern between inhibitory activity of interest and general cytotoxicity. Complementary assays allow for the selection of candidate compounds with an adequate selectivity index (SI), where the in vitro cytotoxicity in mammalian cells is significantly less than the antiparasitic activity. However, while general cytotoxicity can be easily established via such assays, a particular drawback of phenotypic approaches is the uncertainty related to mechanism-based toxicity.

In general, the utilization of phenotypic assays and screens for the identification of novel lead structures can be of greatest use when using the appropriate parasite form under the relevant physiological conditions, allowing for a reasonable probability that efficacious compounds can be obtained.

3.2.1. Lead Structures Resulting from Phenotypic Screens. In the context of *Leishmania* drug discovery efforts, compound screens using promastigotes, axenic amastigotes, or intramacrophage amastigotes have been explored. Each of these assays offers a unique set of advantages and drawbacks. Promastigotes have been used extensively for the purpose of screening compounds; still the relevance of compounds active against them is in question as they are only prevalent in the insect life-stage. Alternatively, screening against axenic amastigotes offers the advantage of being more similar to the disease-relevant parasite stage; however, there are several cited examples of differences between axenic amastigotes and intracellular amastigotes as well in terms of protein expression and in terms of drug susceptibility. There are multiple reports of high false positive rates from axenic amastigote screens where the activity of the hits was not confirmed by using an intramacrophage assay.[133] Ideally, one should employ an intracellular assay which is the most physiological relevant assay to find high quality hits. In one such effort, Mckerrow and co-workers carried out a comparative screen on a 909-member library of bioactive compounds against *Leishmania donovani*. The results revealed 59 hits in the promastigote primary screen and 27 in the intracellular amastigote assay, with 26 compound hits shared by both screens. This result clearly indicated the promiscuity of promastigote stage screens as well as the failure to identify all active compounds. Interestingly compound **18** (Figure 3) inhibits intracellular but not axenic parasites, suggesting a host cell-dependent mechanism of action and would have been missed in a screening using axenic amastigotes.[134]

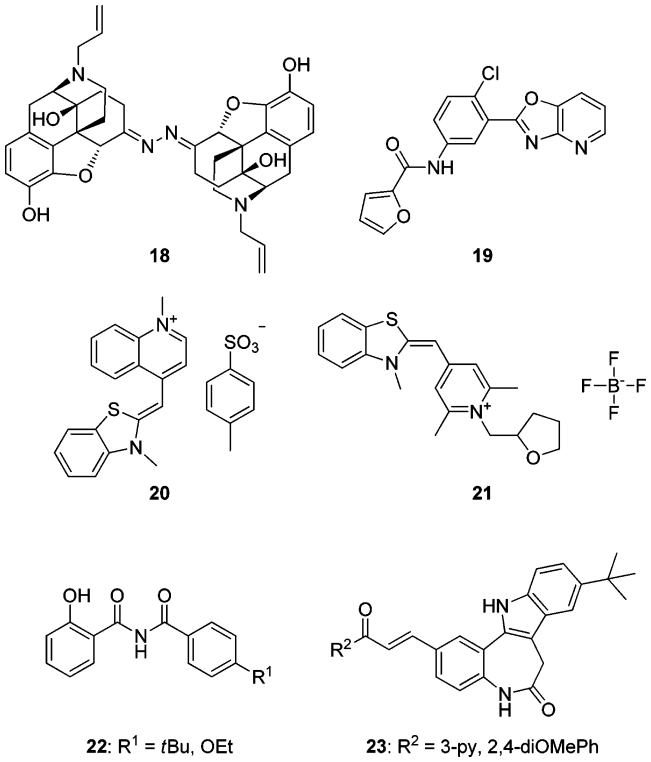

Figure 3. Novel scaffolds resulting from phenotypic screens.

Cognizant of the technological difficulties associated with the screening of a large number of compounds with an intracellular macrophage assay, GNF adopted the strategy of running an axenic amastigote screen on 2.2 million compounds using a 1536 well format. This was followed-up with a physiologically relevant intracellular assay to confirm the activity of the hits. As expected, a low hit confirmation was obtained with intracellular amastigotes, validating the observations about the poor translatability of the axenic amastigote assay. Despite the limitations of the assay, we are satisfied with the screen which resulted, after reconfirmation, in a significant number of novel hits. One such antileishmanial hit (**19**) has been pursued by several groups for human African trypanosomiasis.[135]

Despite many reports describing phenotypic assays against various leishmanial forms, there are few published reports of follow up to these initial efforts.[133,134,136] In one such effort, a *L. major* promastigote screen was carried out by Sharlow et al. and 31 compounds were picked up for further characterization and were evaluated for in vitro activity against intracellular *L. donovani* and *L. amazonensis* parasites. Compounds **20** and **21** exhibited exceptional activity against intracellular *L. donovani* in vitro with EC_{50} values of 21 and 260 nM, respectively. Moreover, the benzothiazole derivative **20** demonstrated low cytotoxicity against Vero cells indicating that the compound does not affect the mammalian cells at submicromolar concentration. Administration of **20** and **21** at a dose of 1 mg/kg intraperitoneally (i.p.) as a single dose for 5 days resulted in 44% and 42% reduction of liver parasitemia in *L. donovani*-infected BALB/c mice. In this study, the control groups also showed a 27−30% reduction in parasites, indicating

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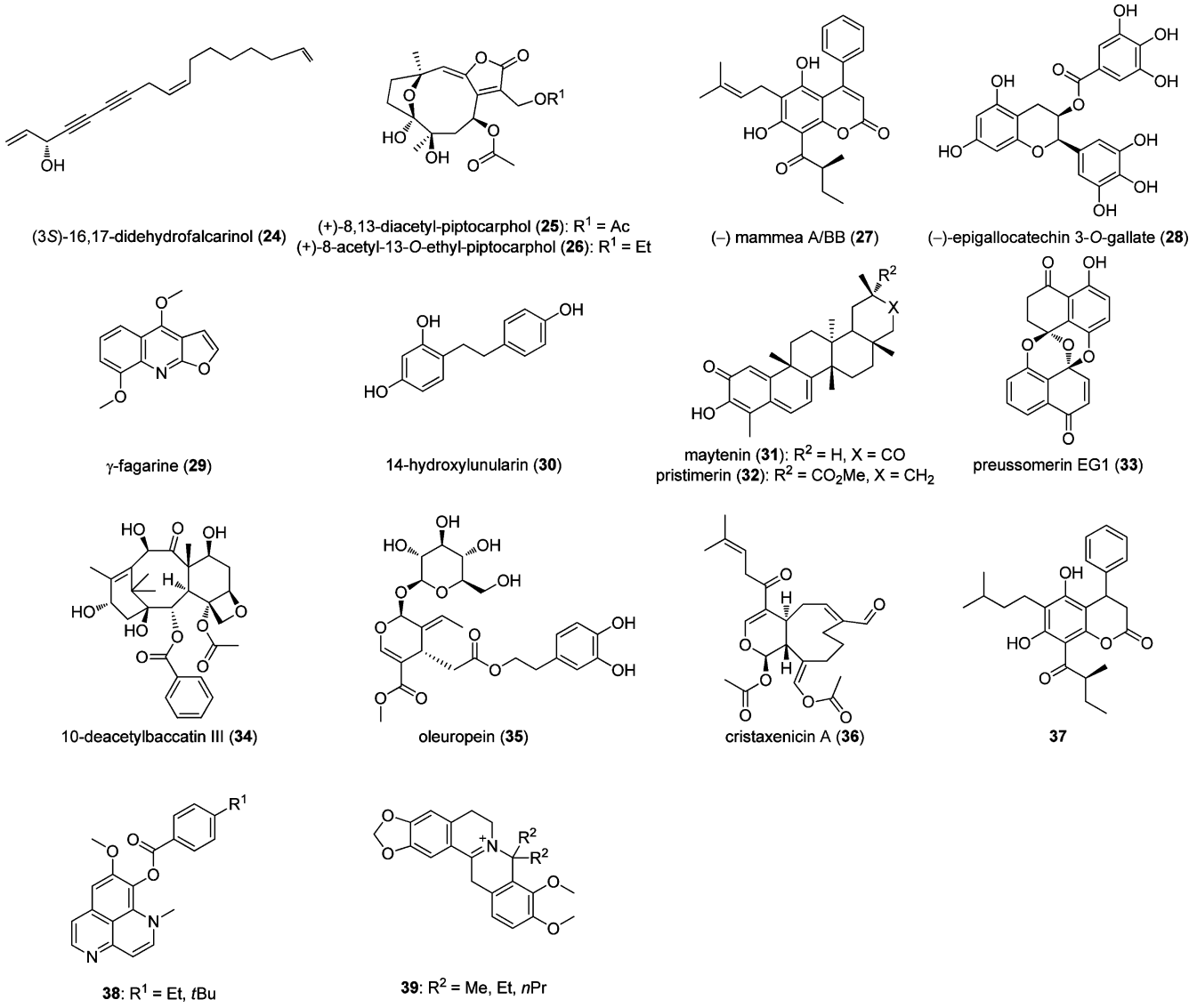

Figure 4. Antileishmanial natural products and derivatives.

that the vehicle (2-hydroxypropyl)-β-cyclodextrin solution (HPβCD) had antileishmanial activity on its own.[136a,137] In conclusion, benzothiazole-containing cyanine dyes do have some activity; however, their potential as drugs remains in question given the report of their interaction with DNA.[138]

In another effort, the repurposing of a narrow range of *N*-benzoyl-2-hydroxybenzamides from a *Toxoplasma gondii* tachyzoite screen led to two salicyclamide examples (**22**) (Figure 3) with reasonable activity (<0.5 μg/mL) against *L. donovani* axenic amastigotes.[139]

A small compound library that was screened at a single concentration using *L. donovani* axenic amastigotes, revealed a paullone chemotype. The initial paullone hits from this phenotypic screen, however, were found to be inactive in an *L. donovani* intracellular assay. Cursory structural optimization of the parent scaffold resulted in two 9-*tert*-butyl-paullone chalcones (**23**) with demonstrated growth inhibition of *L. donovani* axenic and also intracellular amastigotes, however, through the integration of a well-established antileishmanial chalcone moiety (vide infra), these specific compounds became less structurally novel.[140]

Based on the scarcity of literature reports so far, one could infer that to date very little progress has been made in identifying optimization-ready leads from phenotypic screens. The lack of new leads could be attributed to technological hurdles in running relevant biological screens or due to the lack of resources with which to carry out the screening of larger libraries and the subsequent synthetic follow-up necessary to achieve a development candidate. However, there are indications of active medicinal chemistry programs being pursued by various organizations including us at GNF, the University of Dundee, and DNDi. This brings hope that more starting points would be delivered from phenotypic and other approaches in the near future. Based on the public profile, DNDi is following up on numerous chemotypes in addition to backup nitroimidazoles.[141]

3.2.2. Natural Products. Natural products derived from plants and animals have been of great interest in the search for novel antileishmanial compounds. This interest can be attributed to the potential identification of unique chemical architectures and pharmacophores, and the often inherent "drug-like" properties of isolates. Over the last three decades, 69% of all new small molecule drugs for the treatment of

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infectious diseases have been derived from, or inspired by, natural products.[142] While, natural products represent interesting starting points for further follow up, the complexity of the molecules often prevents broad optimization efforts to further improve their properties. The greatest potential of natural product hits lie in the identification of novel targets which in turn can spur targeted drug discovery efforts. The extensive antileishmanial potential of plant and marine based natural products has been previously reviewed in the literature.[143−145]

3.2.2.1. Plant- and Fungal-Derived Natural Products. Natural products from plants and fungi have proven to be a valuable source of chemical matter for anti-infective programs. Typically, the natural product of interest is isolated using activity based fractionation. With large numbers of samples produced from typical plant extract fractionations, it can be of great benefit to proceed with the use of axenic parasites, where assays often have quick turnaround times.

In efforts searching for novel treatments for cutaneous leishmaniasis, 3(*S*)-16,17-didehydrofalcarinol (**24**) (Figure 4) was identified by the axenic bioassay-guided fractionation of the plant *Sarcococca hookeriana* and *Tridax procumbens*.[146] Similarly, axenic *L. amazonensis* parasite-based assays demonstrated the antileishmanial potential of the natural product parthenolide,[147] in addition to the two potent sesquiterpene lactones (+)-8,13-diacetyl-piptocarphol (**25**) and (+)-8-acetyl-13-*O*-ethyl-piptocarphol (**26**) (Figure 4), isolated from the extract of the traditional medicine *Pseudelephantopus spicatus*.[148] As a result of described leishmanicidal potential of *Calophyllum brasiliense* crude extracts,[149] and further investigations thereof, the coumarin natural product (−)-mammea A/BB (**27**) was determined to be a potent active component of *C. brasiliense*, with an EC_{50} of 0.88 μg/mL against *L. amazonensis* axenic amastigotes.[150] Dosing of (−)-mammea A/BB (**27**) for 30 days intramuscularly led to significant reduction of lesion size compared to vehicle with no observed side effects.[151] Efficacy in *L. amazonensis*-based in vivo models has also been demonstrated with (−)-epigallocatechin 3-*O*-gallate (**28**), the most abundant flavanol constituent of green tea, where dosing in mice (30 mg/kg/d, 5 d/wk. over 52 days, p.o.) resulted in substantial lesion size reduction.[152] Similarly, oral dosing of γ-fagarine (**29**) (10 mg/kg, 14 d), from *Helietta apiculata*, led to a 97% reduction in parasite burden of *L. amazonensis*-infected mice, and treatment of *L. amazonensis*-infected mice with the bryophyte constituent 14-hydroxyunularin (**30**) (Figure 4), resulted in a 93% reduction in lesion parasite load (10 mg/kg, 15 days, s.c.).[153]

Numerous natural products have also been reported to successfully affect in vitro those *Leishmania* parasites that are the causative agents of visceral leishmaniasis. Examples include the protoberberine natural product palmatine, active against *L. infantum*,[154] and the quinonemethide natural products maytenin (**31**) and pristimerin (**32**) (Figure 4), with demonstrated activity against *L. chagasi*.[155] Screens employing *L. donovani* promastigotes are also prevalent and have identified a wide range of promising antileishmanial natural products (EC_{50} < 1 μg/mL) from the plants *Plumbago zeylanica*,[156] *Septoria pistaciarum*,[157] *Abrus schimperi*,[158] *Prosopis glandulosa* var. *glandulosa*,[159] *Clerodendrum eriophyllum*,[160] and *Uvaria grandiflora*.[161] Reports utilizing axenic amastigotes validated the potent natural product preussomerin EG1 (**33**).[162] A *L. donovani* intracellular amastigote assay revealed the potential of the taxoid 10-deacetylbaccatin III (**34**) (Figure 4), isolated from *Taxus baccata*, with demonstrated potent in vitro activity (EC_{50} value of 0.07 μM) and an SI value of >10, contrary to taxol, which is cytotoxic at nanomolar concentrations.[163]

3.2.2.2. Animal-Derived Natural Products. Among the many animal-derived isolates with observed antileishmanial activity, most are derived from marine invertebrates or associated bacteria. As a result of screening a range of marine organism extracts against *L. amazonensis* promastigotes, the promising natural product cristaxenicin A (**36**) (Figure 4) was isolated (EC_{50} = 0.09 μM).[164] Analogous investigation of the bioactive crude extract of the sponge *Plakortis angulospiculatus* afforded small amount of the natural product plakortide P, with good activity against *L. chagasi* intracellular amastigotes and respectable cytotoxic selectivity.[165] There are also reports of antileishmanial activity from the venom of the scorpion *Tityus discrepans* against *L. mexicana* promastigotes,[166] and crude venom from the snake *Bungarus caeruleus*.[167] However, no active components were isolated.

3.2.3. Natural Product Derived Compounds. Systematic exploration of the structure−activity relationship of antileishmanial natural products has led to a variety of semisynthetic efforts, where defined natural pharmacophores effectively provided a pedestal for synthetic manipulation and were leveraged for potential compound improvement. A comprehensive approach toward obtaining appropriate clinical candidates via this method is often hindered, however, by the structural complexity of isolated natural products and the relatively small amount that can be isolated in certain cases (vide supra).

Hydrogenation of the antileishmanial coumarin natural product (−)-mammea A/BB (**27**) (EC_{50} = 3.0 μg/mL), obtained from the extract of *C. brasiliense*, provided the more potent synthetic derivative **37** (Figure 4), with an EC_{50} of 0.37 μg/mL against *L. amazonensis* promastigotes.[168] Similarly, synthetic esterification of the phenolic marine natural product isoaaptamine (EC_{50} = 0.7 μg/mL), available from the sponge *Aaptos* sp. in gram quantities, resulted in two derivatives (**38**) with improved EC_{50} values (0.4 μg/mL and 0.1 μg/mL) against *L. donovani*.[169] Due to potent activity against intracellular *L. donovani* amastigotes, 8,8-dialkyldihydroberberine derivatives (**39**) (Figure 4) were further explored in vivo. Unfortunately subpar efficacy was observed upon i.p. dosing in a murine model for 5 days likely due to poor pharmacokinetic properties.[170] In many such cases further optimization is impaired by the resources required for structural modification of such complex molecular architectures.

3.2.4. Derivatives of Anti-Infective Scaffolds. Due to the limited understanding of leishmanial biology, it has been typical to proceed in the rational design of leishmanicidal agents through the inspiration and modification of structural classes already known to possess anti-infective activity.

3.2.4.1. Benzoxazoles. The report of potent antibacterial activity demonstrated by the natural product A-33853 (**40**) (Figure 5) prompted the hypothesis that similar compounds could be evaluated as novel anti-infective agents. Synthetic analogs of A-33853 (**40**) were subsequently found to be remarkably potent against *L. donovani* axenic amastigotes with compound **41** demonstrating an EC_{50} value of 0.31 μM and SI value of 99.[171]

3.2.4.2. Imidazoles. Imidazole-containing compounds have received considerable attention in the search for leishmaniasis chemotherapy due to the success of agents such as ketoconazole, miconazole, econazole, and clotrimazole in treating fungal infections, thus lending credence to the possible

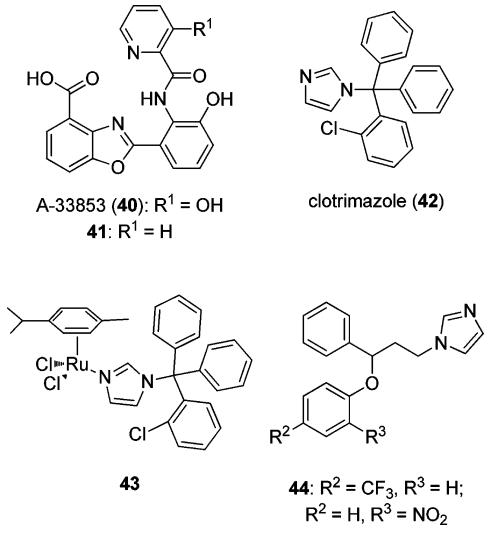

Figure 5. Antileishmanial benzoxazole and imidazole derivatives.

utility of this broad class of compounds in other types of infection. Analogs of the imidazole antifungal agent clotrimazole (**42**) have demonstrated effective *Leishmania* inhibition when combined with metals. Clotrimazole (**42**) was incorporated into a pseudo-octahedral ruthenium-clotrimazole complex, [$Ru^{II}(\eta^6$-p-cymene)Cl_2(clotrimazole)] (**43**), (Figure 5) that was found to exhibit very good in vitro activity against *L. major* promastigotes (EC_{50} = 0.015 μM) and intracellular amastigotes (EC_{70} = 0.029 μM) with an SI value of >500.[172]

Analogs of the antifungal agents miconazole and econazole have also been explored. A report that examined the anti-infective nature of analogs of miconazole and econazole found that a range of synthesized imidazoles tested against *L. donovani* intracellular amastigotes provided good antileishmanial activity, with two examples (**44**) exhibiting EC_{50} values of <0.5 μg/mL. Efficacy of both of these compounds in vivo demonstrated moderate reduction of parasitemia (52% and 60%) in a hamster model (50 mg/kg, 10 d, i.p.), suggesting some potential for this compound class.[173]

3.2.4.3. Chalcones. Chalcones demonstrate a wide range of pharmacological anti-infective activity, making this substructure an attractive pharmacophore from which to explore anti-protozoal SAR. A small set of chloro-substituted 1-(6-methoxy-2*H*-chromen-3-yl)-3-phenylpropen-1-ones were found to have significant antileishmanial activity. Three such compounds (**45**) (Figure 6) demonstrated EC_{50} values of <1 μM against the promastigote form of *L. major*.[174] A synthetic polysubstituted chalcone **46** demonstrated good activity against *L. amazonensis* promastigotes (EC_{50} = 1.1 μM) and intracellular amastigotes (EC_{50} = 0.9 μM). The compound was also found to be active against *L. braziliensis* (EC_{50} = 1.4 μM) and *L. peruviana* (EC_{50} = 4.0 μM). However, upon dosage in vivo, **46** led to only a 25% reduction in parasite burden in *L. amazonensis*-infected mice when treated for 6 weeks intralesionally (5 mg/kg). Compound **47** was much less active against intracellular *L. amazonensis* amastigotes (EC_{50} = 24.0 μM) but resulted in a 92% reduction in parasite burden in vivo upon intralesional dosing for 4 weeks (5 mg/kg). Despite the in vivo reductions in parasite burden for this group of synthetic chalcones, no significant differences in lesion diameter were observed relative to untreated controls.[175]

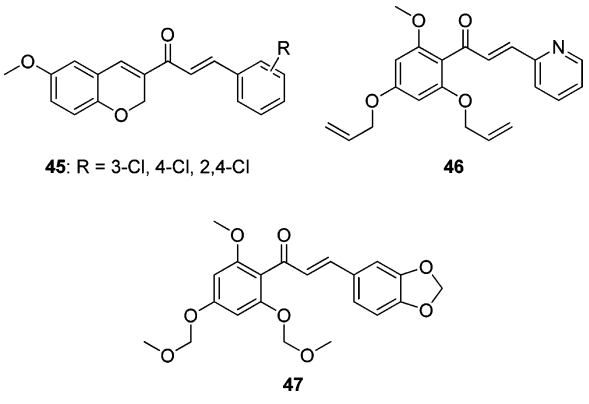

Figure 6. Antileishmanial chalcone derivatives.

3.2.4.4. Diamidines. The effective treatment of leishmaniasis with the diamidine drug pentamidine has led to the investigation of a wide range of diamidines for their anti-infective potential. The diamidine pharmacophore has been researched extensively and its role in medicinal chemistry has been previously reviewed.[176]

The synthesis of pentamidine analogs, where the amidine moiety is cyclized into a benzimidazole substructure akin to the known anthelminthic agents mebendazole and albendazole, yielded hybrid structures with antiparasitic activity (**48**) (Figure 7).[177] In a reported antiprotozoal SAR study where the diamidine moiety was incorporated into an imidazoline substructure and a cadaverine linker was utilized, the resulting derivative **49** displayed broad antiparasitic activity.[178]

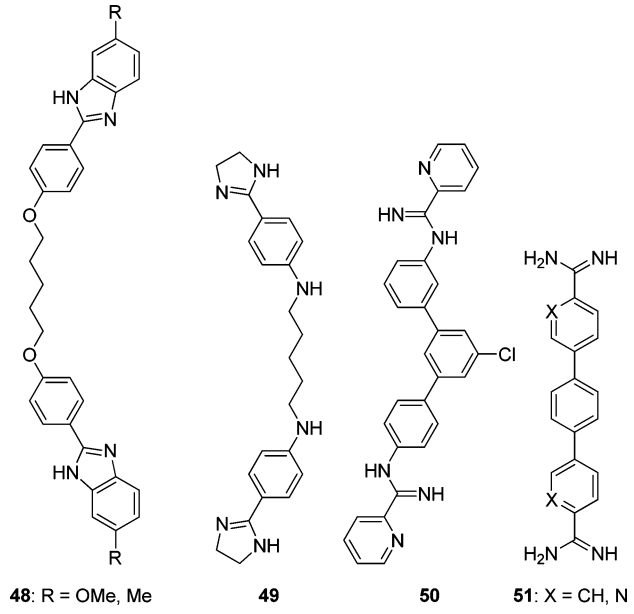

Figure 7. Diamidine-containing leishmanicidal derivatives.

Conformationally restricted diamidine derivatives have also shown to inhibit *Leishmania* growth. Synthesized diamidine compounds with an *m*-terphenyl core displayed encouraging activity in vitro; however, when these promising compounds were tested in vivo in *L. donovani*-infected mice, two led to adverse effects in uninfected animals, and dosing with a third compound (**50**) resulted in only a 23% inhibition of liver parasitemia (30 mg/kg, 5 d, i.p.). Also disappointingly, two promising compounds (**51**) that demonstrated good activity

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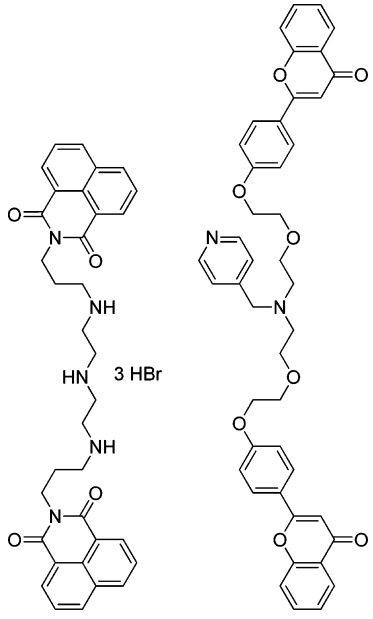
against *L. donovani* axenic amastigotes, showed no activity in *L. amazonensis*-infected macrophages.[179]

3.2.4.5. Amino and Aminoalcohol Linkers. Napthalimide and its derivatives are generally known to have anticancer activity in a variety of human and murine cell lines.[180] In an effort to discover antiparasitic compounds with adequate aqueous solubility, nitrogen- and oxygen-containing linkers have been utilized to enhance conformationally flat and otherwise insoluble chromophores. When this strategy was employed to join naphthalimide groups via nitrogen-containing linkers of various lengths, one compound (**52**) (Figure 8) was



52 **53**

Figure 8. Dimers containing amino and aminoalcohol linkers.

found to demonstrate selective activity against *L. infantum* promastigotes.[181] Similarly, flavones are known to have a wide ranging activity and known to perturb a variety of enzymes.[182] The synthesis of dimers containing flavonoid chromophores joined by PEG- and amino PEG-linkers resulted in the identification of a highly active lead compound (**53**) with 0.13−0.21 μM activity (EC_{50}) in wild-type, sodium stibogluconate-resistant, and pentamidine-resistant *L. donovani* promastigote strains, making it an attractive foundation for the development of a visceral leishmaniasis treatment, irrespective of parasite drug sensitivity.[183]

3.2.4.6. Nitroheterocycles. The synthesis of structural hybrids, utilizing the nitro-furan moiety as found in the antitrypanosomal agent nifurtimox and the antileishmanial benzamidine pharmacophore, yielded two highly potent derivatives (**54**) (Figure 9) with activity against *L. major* promastigotes and intracellular amastigotes.[184] Additionally, the synthesis of hybrids of the nitro-containing antiprotozoal agent megazol and the antileishmanial combretastatin-type pharmacophore, yielded compound **55** that demonstrated potent activity against *L. donovani* axenic amastigotes with an EC_{50} value of 0.08 μg/mL and an SI value of 240.[185]

3.2.4.7. Phospholipids. The teratogenic nature of miltefosine and the potential for resistance as a result of its long half-life (extended presence of subtherapeutic concentrations) have prompted the desire to find more efficacious and/or less toxic

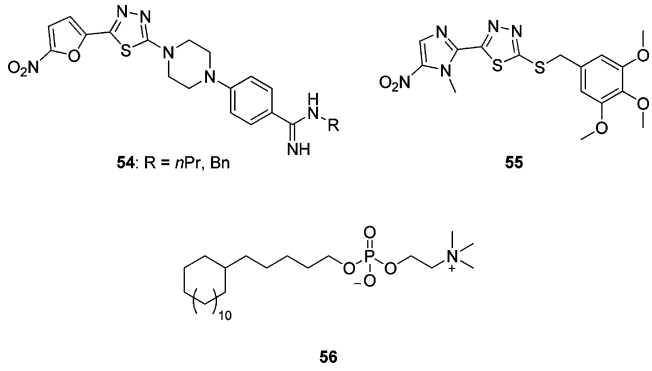


Figure 9. Nitroheterocycles and organophosphate antileishmanial agents.

congeners. As miltefosine was originally developed as an antitumor agent, there is only limited knowledge of miltefosine's SAR with respect to antiparasitic activity. One of the more promising analogs is compound **56** (Figure 9), which demonstrated a >9 fold improvement in potency relative to miltefosine in the promastigote assay, as well as lower cytotoxicity and hemolysis improvements when compared to the parent drug.[186] However, while the gains in potency of newer analogs might seem impressive, very little is known about these compounds with respect to their teratogenicity or their activity on isolates which are resistant to miltefosine.

3.2.4.8. Triphenylmethanes. The known anthelmintic and antifungal compound gentian violet (**57**) (Figure 10) has been previously demonstrated to exhibit antileishmanial properties.[187] Synthetic efforts incorporating the triphenylmethane pharmacophore, have yielded a range of compounds with EC_{50} values of <1 μM. When examined in vivo, compound **58** also demonstrated a 1000-fold in vivo reduction in parasite burden in a murine cutaneous leishmaniasis (*L. amazonensis*) model, and analogous application of gentian violet (**57**) led to a complete elimination of parasites (1% gel, b.i.d., 20 days, topical), highlighting the therapeutic potential of triphenylmethanes and structurally similar electron carriers.[188]

3.2.4.9. Rhodacyanines. The antileishmanial potential of rhodacyanines has been previously described,[189] prompting the further investigation of the SAR of this class of delocalized lipophilic cation compounds. In a recent report, synthesized rhodacyanine compounds **59** and **60** demonstrated highly potent activity in vitro against *L. donovani* intracellular amastigotes with EC_{50} values of 0.35 and 0.08 μM, respectively. Despite being less potent, the efficacy of compound **59** was found to be superior to **60** in vivo when the compounds were dosed intraperitoneally at 50 mg/kg in *L. donovani*-infected mice (31% versus 18%). The efficacy of compound **59** could also be enhanced with intravenous administration, leading to a 97% (4.1 mg/kg, i.v.) reduction in liver parasitemia after dosing for 5 days.[190]

3.2.4.10. β-Carbolines. The natural product canthin-6-one has been demonstrated as an active leishmanicidal agent both in vitro and in vivo,[191] rendering the β-carboline pharmacophore attractive for the investigation of further antiparasitic SAR. Examination of a range of synthesized canthin-6-ones and 1-phenyl-β-carbolines revealed that, in general, leishmanicidal activity was more pronounced for the latter class of compounds. In particular, compound **61** (Figure 11) displayed significant antileishmanial activity, with an EC_{50} value of 0.25 μM against *L. amazonensis* promastigotes.[192]

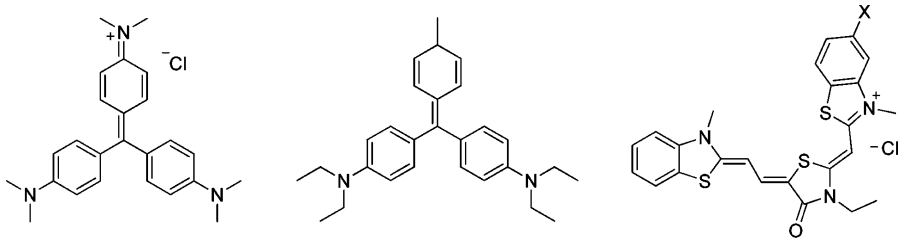


Figure 10. Antiparasitic triphenylmethane and rhodacyanine compounds.

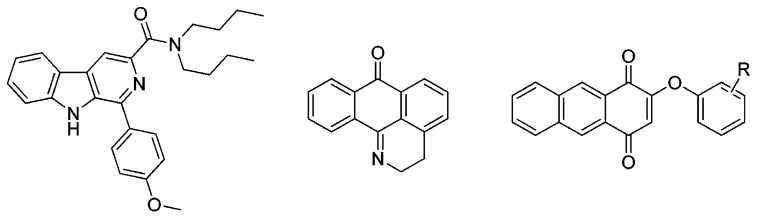


Figure 11. Antileishmanial β-carboline and quinone derivatives.

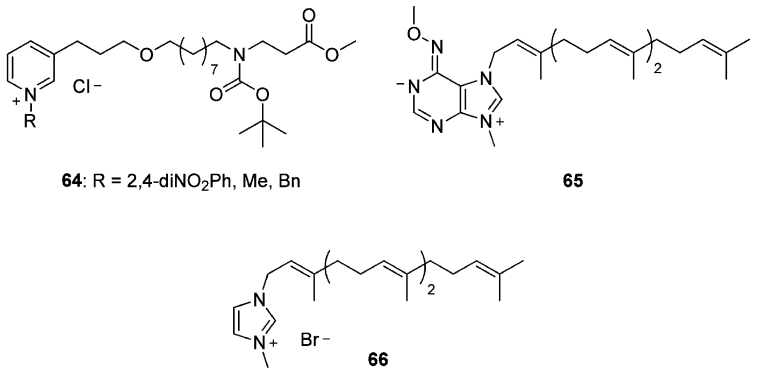


Figure 12. Heterocyclic salts active against *Leishmania*.

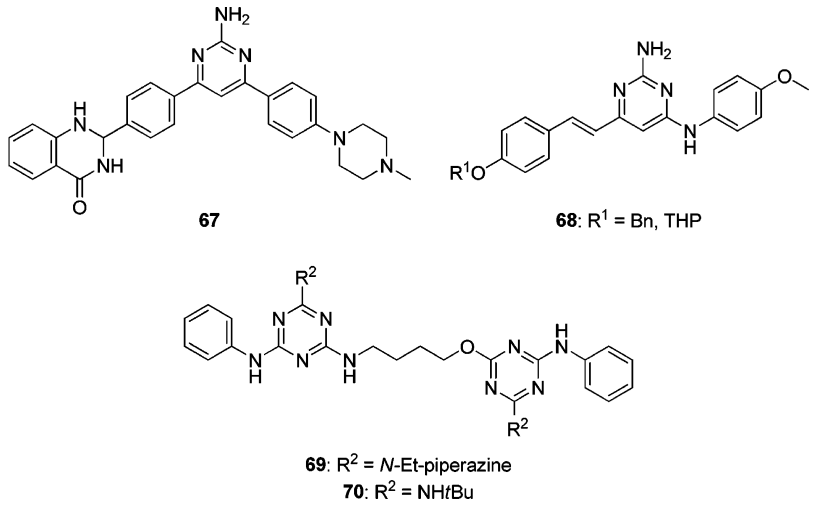


Figure 13. Compounds containing pyrimidine/triazine pharmacophore.

3.2.4.11. Quinones and Iminoquinones. The quinone moiety and analogous derivatives are pharmacophores that have been previously shown to exhibit significant activity against *Leishmania*. The antileishmanial naphthoquinone compounds diospyrin, plumbagin, lapachol, and buparvaquone exemplify the potential of this structural class[193] and have made the derivatization of quinone-type architectures alluring. In a recent report, synthesized iminoquinone compound **62** (Figure 11) resulted in 99% and 78% reductions in the murine parasite (*L. infantum*) burden in the liver and spleen, respectively (10

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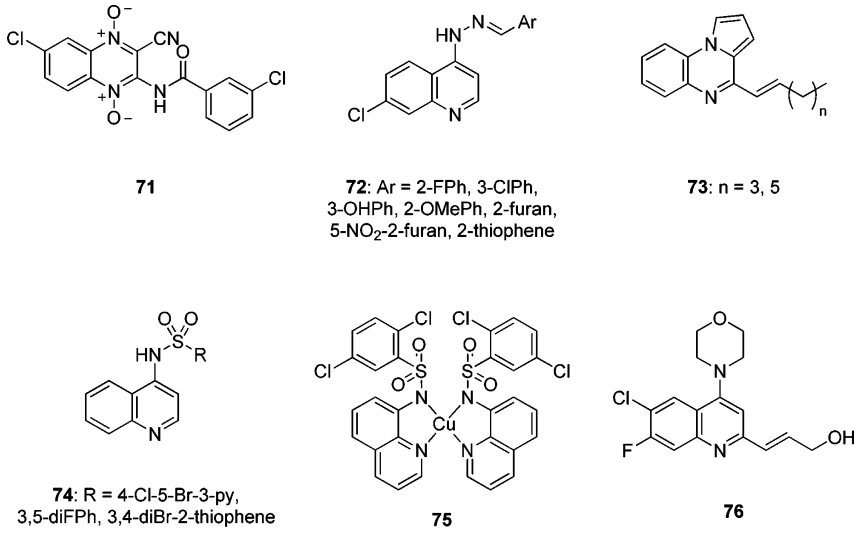

Figure 14. Antiparasitic quinoline compounds.

mg/kg, i.p.), and was found to be relatively nontoxic. Additional iminoquinone derivatives, however, were found to be ineffective in reducing parasitemia.[194] In another report, a small library of 2-phenoxy-1,4-anthraquinones (**63**) was synthesized via a parallel approach, with the intent of combining the naphthoquinone pharmacophore with the substituted phenolic moiety present in the structure of the known antibacterial and antifungal agent triclosan. These hybrids (**63**) were observed to have reasonable activity against *L. donovani* axenic amastigotes; however, the SI values for this group of compounds were generally poor (<15).[195]

3.2.4.12. Heterocyclic Salts. A small set of synthesized pyridinium salts (**64**) (Figure 12), similar in structure to the bioactive marine natural products viscosaline and theonelladin C, were found to be only moderately active against *L. amazonensis* and *L. braziliensis* promastigotes; however, they were remarkably specific against the intracellular form of the parasite.[196] Similarly, an antileishmanial screen of synthetic derivatives of the bioactive natural product agelasine D led to the discovery of two potent imidazolium compounds **65** and **66** with EC_{50} values of 0.09 and <0.11 μg/mL, respectively, against *L. infantum* intracellular amastigotes.[197]

3.2.4.13. Pyrimidines and Triazines. Pyrimidine- and triazine-type scaffolds have been of great interest in the search for novel antiprotozoal agents due to the successes of representative anti-infective compounds like pyrimethamine, cycloguanil, and trimethoprim. Inspiration from these architectures, and subsequent hybrids thereof, has yielded a series of promising synthetic antileishmanial derivatives. The synthesis of quinazolinone-pyrimidine derivatives led to the discovery of the reasonably potent compound **67** (Figure 13) with an EC_{50} value of 0.65 μM against intracellular *L. donovani* amastigotes.[198] Additional synthetic pyrimidines (**68**) have been demonstrated to be efficacious in vivo, with each compound leading to 78% parasite inhibition when dosed in *L. donovani*-infected hamsters for 5 days (50 mg/kg, i.p.).[199] Efforts to hybridize the triazine pharmacophore with that of the ether linkage of pentamidine led to the synthesis of two compounds (**69** and **70**) with good activity on intracellular *L. donovani* amastigotes (EC_{50} values of <1 μM). Efficacy of compound **69** in vivo, however, was only found to be moderate, with a 63% reduction in hamster splenic parasite (*L. donovani*) burden when animals were dosed for 5 days (50 mg/kg, i.p.).[200]

3.2.4.14. Quinolines. The clinically relevant antileishmanial therapeutics sitamaquine, primaquine, and imiquimod highlight the significant potential of quinoline substructures. The role of quinolines as leishmanicidal chemotherapeutic agents has been specifically reviewed.[201] Taking advantage of the privileged quinoline-type core, a substituted quinoxaline 1,4-di-*N*-oxide (**71**) (Figure 14) was found to effectively inhibit the growth of *L. amazonensis* axenic amastigotes with an EC_{50} of 0.7 μM.[202] Similarly, a class of 7-chloro-4-quinolinyl hydrazones was found to be broadly active against a range of *Leishmania* promastigotes with seven compounds (**72**) demonstrating EC_{50} values of <0.5 μg/mL.[203] Additionally, the synthesis of 4-substituted pyrrolo[1,2-*a*]quinoxalines resulted in two compounds (**73**), with EC_{50} activity against promastigotes of 0.5 (*L. amazonensis*) and 0.6 μM (*L. infantum*).[204]

Synthesis and evaluation of *N*-quinolin-8-yl-arylsulfonamides, structurally similar to sitamaquine, yielded three compounds (**74**) with good activity against *L. amazonensis* (EC_{50} = 2−3 μM) and *L. chagasi* (EC_{50} = 0.4−0.6 μM) promastigotes. Moreover when a similar compound, 2,5-dichloro-*N*-(quinolin-5-yl)benzenesulfonamide, was employed as a ligand in the formation of a copper complex, the resulting organometallic species **75** was found to be highly active with an EC_{50} value of 0.35 μM on *L. braziliensis* intracellular amastigotes, and an SI value of >100.[205] Also, the synthesis of a series of 2-substituted quinoline derivatives revealed the promising compound **76**, which demonstrated an IC_{50} of 0.22 μM against *L. donovani* intracellular amastigotes and was also found to inhibit parasitemia in hamsters by 84% when dosed orally (50 mg/kg, b.i.d.) over 5 days, despite exhibiting very low bioavailability in mice.

3.3. Targeted Approaches toward Novel Leishmaniasis Therapies

Species within the genus *Leishmania* have been the focus of target based drug discovery by numerous groups. A large number of targets have been proposed; however, there have been relatively few medicinal chemistry campaigns. This subsection attempts to capture relevant efforts that have been reported in the literature over the last five years. Recent reviews

describing *Leishmania* targets have also been published (Table 5).[143a,156,206]

Table 5. List of Targets Identified (Not Described in Review) for *Leishmania* Species

pathway/target	*Leishmania* species	refs
DNA binders	*L. amazonensis, L. mexicana*	207
protein synthesis	*L. donovani, L. major*	208
sterol 24-methyltransferase	*L. amazonensis, L. donovani*	209
CYP P450 enzyme 14-α-demethylase	*L. tropica, L. amazonensis, L. braziliensis*	116
farnesyl pyrophosphate	*L. major*	210
glyoxalase pathway	*L. donovani*	211
Glycosylphosphatidylinositol (GPI) pathway	*L. mexicana*	212
Leishmania β-1,2-mannosyltransferase	*L. mexicana*	213
oligopeptidase-B	*L. donovani*	214
pyruvate kinase	*L. mexicana*	215
Leishmania MAP kinase homologue (LMPK)	*L. mexicana*	216
N-myristoyl transferase	*L. donovani*	217
nitroreductase	*L. donovani*	218
nucleoside hydrolase	*L. donovani*	219
adenosine kinase	*L. donovani*	220
nucleoside diphosphate kinase b	*L. major*	221
protein disulfide isomerase	*L. major*	222
S-adenosylhomocysteine hydrolase	*L. donovani*	223
methionyl-tRNA synthetase	*L. major*	224
tyrosyl-tRNA synthetase	*L. major*	224a
uridine-5′-monophosphate synthase	*L. donovani*	225
deoxyuridine triphosphate nucleotidohydrolase	*L. major*	226
dihydroorotate dehydrogenase	*L. major*	227
aldolase	*L. mexicana*	228
glucose-6-phosphate isomerase	*L. mexicana*	229
glycerol-3-phosphate dehydrogenase	*L. mexicana*	230
phosphomannomutase	*L. mexicana*	231
nicotinamidase	*L. infantum*	232
triosephosphate isomerase	*L. donovani*	233
thiol-dependent reductase	*L. major*	234
cysteine synthase	*L. major*	234
deoxyhypusine synthase	*L. donovani*	206b
sphingolipid biosynthetic pathway	*L. amazonensis*	235
metacaspase	*L. donovani*	236
cytochrome-*c*-oxidase	*L. donovani*	237

3.3.1. Kinases. The relevance of kinases in drug discovery is well documented, particularly in the field of oncology. Aberrant activation of kinases has been linked to proliferation of certain cancer cells. For example, activation of Abelson tyrosine kinase (Abl) has been linked to chronic myeloid leukemia. Small molecules such as imatinib (**77**) (Figure 15) are known to inhibit Abl thus leading to cancer cell death by apoptosis. Inspired by the successes in oncology, a number of efforts have been carried out in the field of parasitic diseases targeting kinases.[238] Based on homology studies, the kinome of *L. major* contains 179 genes encoding putative homologues of eukaryotic protein kinases (ePKs) and 17 encoding atypical protein kinases.[239] While druggability of the kinase target is not in question, questions persist regarding achieving a selective kinase inhibitor which targets only the leishmania species and not the host. This is not an impossible task as there are recent examples from the antimalarial field where selective compounds have been achieved.[240]

Berberine chloride (**78**), a quaternary isoquinoline alkaloid, is known to have antileishmanial activity both in vitro as well as in vivo in hamster models.[241] Recently, it was unraveled by Western blot phosphorylation studies that berberine chloride (**78**) was responsible for time dependent activation of p38 MAPK along with deactivation of ERK1/2.[242] While berberine chloride has proven to be a valuable tool compound in understanding the mechanism of action its development as a drug is hampered by its poor physicochemical properties. However, validation of the MAPK pathway opens up the possibility of a target based approach in the future.

Cyclin dependent kinases or CDKs represent another interesting subclass of kinases as potential drug targets because of their ability to affect the cell cycle. Analysis of the genome from *L. major* has revealed the existence of 11 CDKs. Moreover, 11 putative cyclins (CYC2-11 and CYCA) have also been identified. Interestingly, among the kinetoplastids, only *Leishmania* possesses cyclin CYCA, a cdc-2 related serine/threonine protein kinase, which is essential for transition through the G2-M phase of the *Leishmania* cell cycle. A CRK3:CYC6 protein kinase assay was developed and two groups followed up on this target leading to the identification of potent enzymatically active compounds **79** and **80** (Table 6). However, there was a poor correlation between the observed enzymatic activity and cellular potency.[238c,243]

3.3.2. Folate Biosynthesis. The folate biosynthesis pathway has been a successful target for cancer and malarial chemotherapy. Folates are essential cofactors in a variety of metabolic pathways such as DNA and RNA synthesis and amino acid metabolism. Two enzymes which are of particular interest in this pathway are thymidylate synthetase (TS) and dihydrofolate reductase (DHFR). In trypanosomatids these enzymes exist as single polypeptides (DHFR-TS), with the DHFR domain on the amino terminus and the TS domain on the carboxy terminus. It was discovered that most of the known DHFR inhibitors are inactive against *Leishmania*. This can be explained by the amplification of the PTR1 gene in some mutants. PTR1 can reduce both pterins and folates and is much less susceptible to inhibition by antifolates.[244] In order to

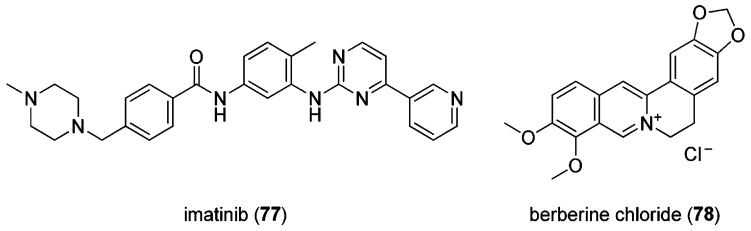

Figure 15. Structures of imatinib (77) and berberine chloride (78).

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Table 6. Kinase Inhibition and Antileishmanial Activity of 79 and 80

Assay (μM)	79	80
CRK3:CYC6	5.3	0.06
CDK4:CYCD1	9.7	
L. major promastigote	8.6	
L. major amastigote	>50	

overcome this bypass mechanism, it was envisaged to design compounds which inhibit both DHFR-TS and PTR1 enzymes. Hardy and co-workers were able to identify compounds which were effectively able to inhibit both enzymes. However, there was little correlation between potencies on PTR1 or DHFR-TS and activity in the whole cell assay.[245] Compounds **82** and **83** are more potent in the promastigote assay, while exhibiting mediocre potency on the targets of interest (Table 7). This suggests that there may be other targets for this class of compounds. In contrast, compound **81** exhibits whole cell potency which is in agreement with the enzymatic assays. Recently, Gilbert and co-workers were able to design and optimize 2,4-diaminoquinazolines, such as **84**, as inhibitors of dihydrofolate reductase. While the synthesized compounds exhibited potent activity against *L. major* DHFR, there was only relatively weak inhibition of *L. donovani* axenic amastigotes despite activity on *T. cruzi* and *T. brucei*. Lack of cellular activity on *L. donovani* can possibly be explained by the low pH of the medium which prevented diffusion of basic compounds into the parasites.[246] The activity of **84** on the PTR1 enzyme was not examined in the study (Table 7).

In order to overcome PTR1 resistance, a series of inhibitors of PTR1 (quinazolines **85** and **86**) were synthesized and tested in combination with pyrimethamine (a known DHFR inhibitor) (Table 8). Both **85** and **86** were only weakly active on *L. mexicana* as well as other *L. major* strains when tested alone but showed a profound parasite reduction when tested in combination with pyrimethamine.[247]

Leishmania protozoans are autotrophic for folates and unconjugated pteridines and rely on their host and insect vectors to provide them. Unlike other organisms there are no choke point enzymes and multiple bypass mechanisms exist. A

Table 8. Inhibition Constants on *Leishmania pteridine* Reductase Inhibitors

Assay (μM)	85: X = CH	86: X = N
K_i LmPTR1	0.10	0.21
K_i hDHFR	10	>30
K_i hTS	>190	>50

suitable molecule has to target DHFR-TS and PTR1 enzymes simultaneously while maintaining selectivity against mammalian targets. Despite numerous efforts, such an inhibitor with good efficacy in vivo remains elusive.

3.3.3. Trypanothione Pathway. The trypanothione pathway is downstream to the polyamine pathway which synthesizes spermidine, a key molecule for the synthesis of trypanothione. Trypanothione (bis(glutathionyl) spermidine) is an essential molecule for modulating oxidative stress in parasites. Trypanothione synthesis is catalyzed by two key enzymes, namely trypanothione synthetase (TS) and trypanothione reductase (TR). TS is responsible for the synthesis of trypanothione from spermidine and two molecules of glutathione.[248] Trypanothione is then maintained in its reduced state by the enzyme trypanothione reductase using NADPH as the cofactor. Trypanothione in reduced form then reduces tryparedoxin (TX) which is then followed by reduction of tryparedoxin recycling enzyme tryparedoxin peroxidase (TP). It has been shown that TR, TS, and TP are essential targets for the survival as well as infectivity of parasites.[249] However, trypanothione reductase has structural similarity with its human homologue glutathione reductase, which could make it difficult to design selective analogues against this enzyme.[206b,250]

The efforts on trypanothione pathway enzymes have been the focus of several past reviews.[250,251] Recently pyrrole compound **87** (Figure 16) was identified to be a competitive inhibitor of trypanothione reductase with a K_i of 4.6 μM. The compound also showed activity on *L. donovani* intracellular amastigotes with an EC_{50} of 13 μM. However, the compound was equally cytotoxic on KB cells. The X-ray structure of the compound with the trypanothione complex shows that compound **87** binds to the trypanothione binding site, thereby impeding substrate entry which explains the competitive nature of its inhibition.[251a]

In a separate effort, a combinatorial library of quinone–polyamine conjugates was designed based on phenotypic *T. brucei* hits and conjugated with polyamine derivatives to optimize their antitrypanosomatid profile. The best compound from this series (compound **88**) was found to have

Table 7. Activity of 2,4-Di-aminoquinazolines on Folate Pathway Enzymes

Assay (μM)	81	82	83	84
IC_{50} LmPTR1	0.4	0.5	0.7	
IC_{50} LmDHFR-TS	0.14	0.46	2.1	0.12
Leishmania EC_{50}	0.5[a]	0.1[a]	0.2[a]	13.8[b]

[a]*L. major* promastigote assay. [b]*L. donovani* amastigote assay.

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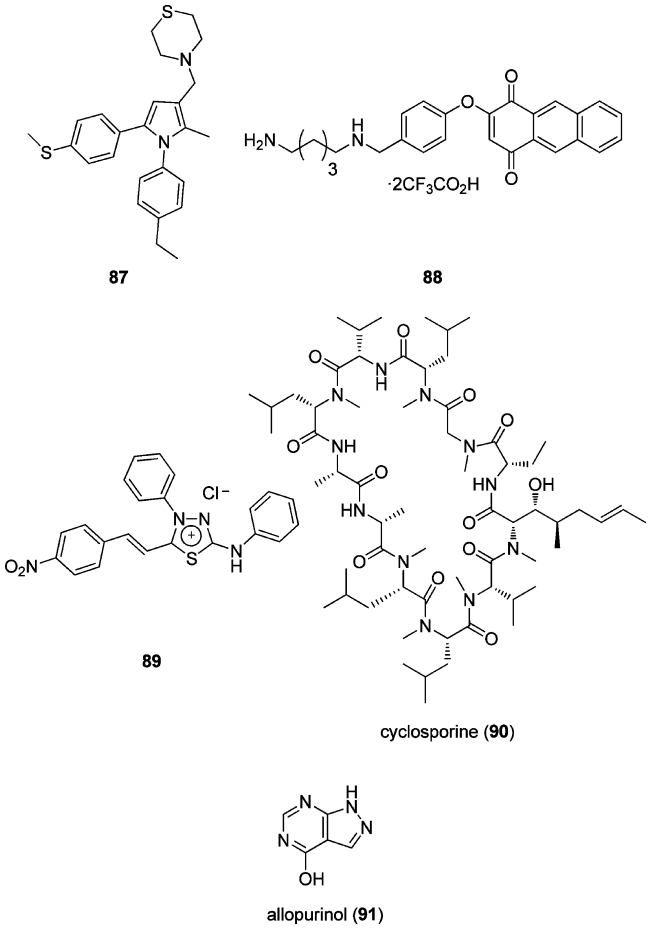

Figure 16. Representative inhibitors of trypanothione, cyclophilin, and purine salvage pathways.

trypanothione reductase activity along with the ability to reduce cytoplasmic ATP and mitochondrial potential. In addition to *T. brucei* activity, the compound showed activity on *L. donovani* amastigotes as well as promastigotes in the 2−3 μM range with a SI index of 2−3 for cytotoxicity on L6 cells.[252]

Mesoionic heterocycles have been linked to a variety of biological activities as a result of their ionic character and high dipole moment. Previous studies have identified this class of compounds having antitrypanocidal activity. Based on this result, mesoionic 1,3,4-thiadiazolium-2-aminide derivatives were studied for trypanothione reductase activity. Among them, the nitro-containing compound **89** exhibited a non-competitive inhibition profile with an IC_{50} of 1.63 μM. Molecular docking studies have indicated that these mesoionic compounds effectively fit into the substrate binding site together with the substrate molecule.[253] Compound **89** was also active on *L. amazonensis* promastigotes with an EC_{50} = 1.5 μM.[254] The compound was used in an *L. infantum* murine model where it exhibited high efficacy upon intraperitoneal dosing at 20 mg/kg/day for 4 weeks. No parasites were detected in the liver or the spleen. In an *L. amazonensis* mouse model, intralesional topical treatment of 20 mg/kg/day led to superior therapeutic efficacy than treatment with meglumine antimoniate.[253]

It has been shown that the enzymes in the trypanothione pathway: trypanothione synthetase (TS), and trypanothione reductase (TR) and tryparedoxin peroxidase (TP) are absent in human hosts and are essential to parasites. While trypanothione reductase has structural similarity with its human homologue glutathione reductase, which could potentially impede the path to design selective analogues, the other two enzymes TS and TP hold the promise of delivering selective inhibitors against them.

3.3.4. Cyclophilins. Cyclophilins are groups of proteins which bind to cyclosporine (**90**) (Figure 16). Proteins in this family share approximately 109 amino acids which are referred to as the cyclophilin-like domain. This domain is responsible for peptidylprolyl isomerase (PPIase) which influences a number of biological processes such as protein folding, assembly of multiprotein complexes, and signal transduction. Cyclosporine (**90**) is known to have antileishmanial activity on intracellular *L. tropica*- and *L. major*-infected mouse macrophages. However, the repurposing of cyclosporine (**90**) is not feasible because of its immunosuppressive effect. Späth and co-workers have proven that cyclosporine acts on *Leishmania* cyclophilins and the structural differences between human and parasite orthologs, potentially enable the design of compounds to selectively act against the parasite.[255]

3.3.5. Purine Salvage Pathway. *Leishmania* species have to utilize purine from the mammalian host to synthesize purine nucleotides. While the protozoan transporters are different from their mammalian counterparts in terms of substrate specificity, there are numerous uptake mechanisms which make targeting of these transporters difficult as the nontargeted transporters provide escape mechanism.[256] The most important enzyme in this pathway is phosphoribosyl transferase (PRT). There are three known homologues of PRT namely, adenine phosphoribosyl transferase, hypoxanthine-guanine phosphoribosyl transferase (HGPRT), and xanthine phosphoribosyl transferase (XPRT).[206b,257] HGPRT converts hypoxanthine to inosine monophosphate and guanine to guanine monophosphate. One of the known inhibitor of HGPRT is allopurinol (**91**) (Figure 16), which is phosphorylated by HGPRT and incorporated into nucleic acids leading to death of the parasite. Allopurinol (**91**) has been shown to be efficacious against both cutaneous and visceral leishmaniasis.[258] Moreover, it was found to be synergistic with other antileishmanial drugs.[113a,259] However, it was found that PRTs are not essential for parasitic survival raising doubts about the validity of this target.[260] Nevertheless given the orthogonal mechanism, a pan-purine transport inhibitor might be able to provide the necessary parasite growth inhibition. This approach has not been reported in the literature.

3.3.6. Topoisomerase. DNA topoisomerases are enzymes that play an important role in numerous biological processes such as DNA replication, transcription, recombination, and repair. While topoisomerases are ubiquitous in all organisms, studies have shown that kinetoplastid topoisomerases have some distinguishing features that differentiate the parasite enzyme from its prokaryotic and eukaryotic counterparts.[261] Broadly, they are classified as type I and type II topoisomerases and cleave single stranded and double stranded DNA, respectively. Both type I and type II topoisomerase have been characterized from *L. donovani*. The type I topoisomerase enzyme was found to be independent of ATP and is present in both the kinetoplast and nucleus. In contrast, type II topoisomerase was found to exhibit both ATP dependent and independent activity. DNA topoisomerase inhibitors have been extensively covered in the literature.[261a,b,262]

Based on the success of camptothecin (**92**) (Figure 17), a known topoisomerase inhibitor in the field of oncology,

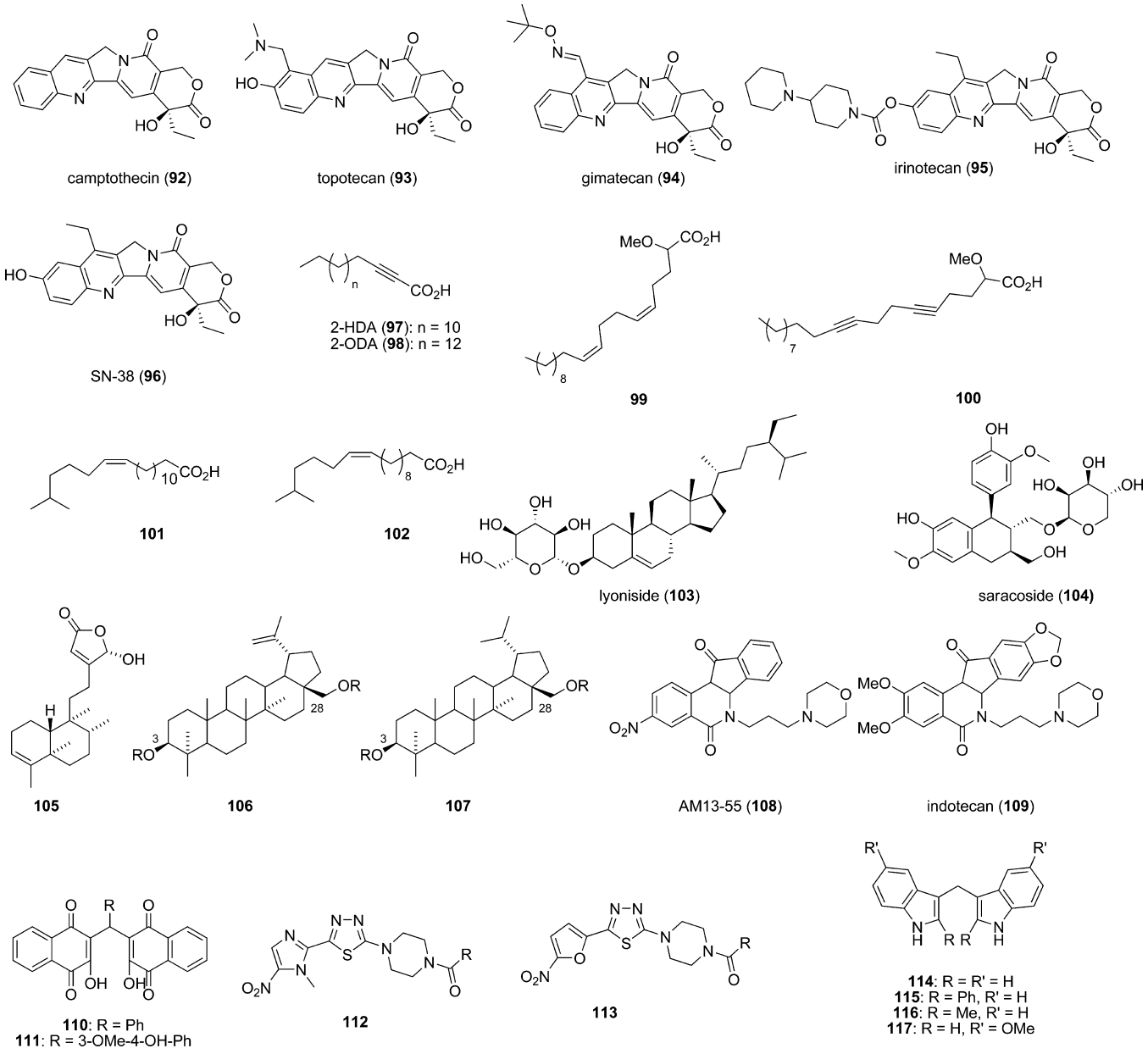

Figure 17. Representative examples of topoisomerase inhibitors.

camptothecin analogues used in therapy were evaluated for antileishmanial activity. Three compounds, namely topotecan (Hycantim, **93**), gimatecan (ST1481, **94**), and the pro-drug irinotecan (Camptosar, **95**) as well as its active metabolite SN-38 (**96**) were evaluated against *L. infantum*. Gimatecan (**94**) and camptothecin (**92**) were most potent on *L. infantum* promastigotes with activity in the micromolar range (Table 9). Moreover, all these compounds except for irinotecan (**95**) inhibited *L. infantum* splenocyte-infecting amastigotes in the nanomolar potency range. The inhibitory potency of campothecin derivatives on recombinant *L. infantum* topoisomerase IB demonstrated that all the compounds affected topoisomerase activity, with gimatecan (**94**) being the most potent compound preventing the relaxation of supercoiled DNA at submicromolar concentration.[263]

2-Alkynoic fatty acids have been described to have broad range of biological activity including antileishmanial, antimycobacterial, antifungal, and anticancer properties. In particular, 2-hexadecynoic acid (2-HDA, **97**) and 2-octadecynoic acid (2-ODA, **98**) (Figure 17) demonstrated activity against *L. donovani* (Table 10). These fatty acids are inhibitors of the *L. donovani* DNA topoisomerase IB enzyme (LdTopIB) and the potency against LdTopIB is dependent on chain length.[264] Also (5Z,9Z)-(±)-2-methoxy-5,9-eicosadienoic acid

Table 9. *L. infantum* Activity on Topoisomerase Inhibitors

	L. infantum EC_{50} (μM)			
compound	promastigote	intracellular amastigote	splenocyte culture EC_{50} (μM)	SI
92	1.12	0.03	0.62	20.7
93	10.86	0.16	4.96	31
94	1.73	0.001	0.21	175
95	>200	>100	>200	ND
96	12.20	0.05	0.54	9.8
6	25.15	8.7	504.1	57.9

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Table 10. Topoisomerase and Antiparasitic Activity on Fatty Acid Derivatives

compound	*Leishmania* EC_{50} (μM)	LdTopIB IC_{50} (μM)	hTopIB IC_{50} (μM)	macrophage CC_{50} (μM)
2-HDA (**97**)	17.8[a]	28.7	>100	>100
2-ODA (**98**)	11.0[a]	5.3	51.9	>100
99	260[b]	31	>100	>100
100	240[b]	22	>100	90
101	19.8[a]	activity at 50 μM		
102	165[a]	62		604

[a]*L. donovani* promastigote assay. [b]*L. infantum* amastigote assay.

(**99**) and its acetylenic analog (±)-2-methoxy-5,9-eicosadiynoic acid (**100**) were shown to be active against the *L. donovani* DNA topoisomerase IB enzyme (LdTopIB). The potency for LdTopIB inhibition correlated with the degree of unsaturation. Unsaturated fatty acids **101** and **102** were isolated from marine sponge Polymastia penicillus and *Dragmaxia undata* respectively and displayed antiprotozoal activity against *L. donovani* through inhibition of *Leishmania* DNA topoisomerase IB enzyme (LdTopIB) as well. All the reported compounds appear to have selectivity over human topoisomerase IB enzyme (hTopIB).[264a] These findings supported the previous hypothesis that monounsaturated iso-methyl-branched fatty acids impart selectivity over human DNA topoisomerase I.[265]

Two lignan glycosides namely, lyoniside (**103**) and saracoside (**104**) (Figure 17) were evaluated for activity against *L. donovani* promastigotes as well as intracellular amastigotes. Both compounds inhibited promastigotes in a sodium antimony gluconate sensitive AG83 strain as well as an antimony resistant GE1 strain in the 2−4 μM range. Moreover, both compounds were more potent on intracellular amastigotes with submicromolar activity against sensitive and resistant strains. These noncompetitive topoisomerase inhibitors stabilize the DNA-LdTopIB cleavage complexes inside *Leishmania* cells and induce apoptosis. Both lyoniside (**103**) and saracoside (**104**) demonstrated impressive antileishmanial efficacies in a BALB/c mice model of leishmaniasis when dosed intraperitoneally at 2.5 and 5 mg/kg with almost complete clearance of the liver and splenic parasite burden at the higher dose.[266]

16α-Hydroxycleroda-3,13(14)Z-dien-15,16-olide (**105**) (Figure 17) was isolated from *Polyalthia longifolia* and showed in vitro activity on intracellular transgenic GFP tagged expressed *L. donovani* amastigotes of 5.8 μg/mL which was equipotent to miltefosine (5.0 μg/mL). The in vivo efficacy was assessed using a dosing regimen of 25, 50, 100, and 250 mg/kg for 5 days against established *L. donovani* infection in hamsters. Dose dependent efficacy was observed, and at 250 mg/kg, a 91% reduction of parasite burden was observed in spleen, 87.5% in liver, and 89.1% in bone marrow. No overt signs of toxicity were observed in animals after 6 months of treatment. The topoisomerase activity was established when the compound was added together with DNA and enzyme leading to an observed inhibition of relaxation activity at various concentrations.[267]

A series of triterpene analogues were synthesized from betulin (**106**, R = H) and dihydrobetulin (**107**, R = H) (Figure 17), isolated from the cork layer of Betula spp. Three analogs (disuccinyl betulin **106**, R = succinate; diglutaryl dihydrobetulin **107**, R = glutarate; disuccinyl dihydrobetulin **107**, R = succinate) inhibited relaxation activity of the enzyme type IB topoisomerase (IC_{50} = 12−23 μM) and were also able to reduce the intracellular parasite burden in macrophages infected with wild-type *L. donovani* and with sodium antimony gluconate resistant parasite (GE1) parasites with EC_{50} values in the range of 6−10 μM. Further mechanistic work indicated that these compounds interact with the enzyme in a reversible manner. The stoichiometry of these compounds binding to LdTOP1LS is 1:1 (mol/mol) with a dissociation constant on the order of $\sim 10^{-6}$ M. In contrast to campothecin (**92**), these compounds do not stabilize the cleavage complex; rather, they destroy the covalent complex formation. These results suggest that betulin derivatives could be exploited for antimony resistant leishmaniasis.[268]

Two known topoisomerase indenenoisoquinoline alkaloids, namely AM13-55 (**108**) and indotecan (**109**) (Figure 17), were investigated for antileishmanial activity. Both these compounds were found to be potent in *L. infantum* cultured in splenocytes with an EC_{50} of 100 nM and with SI index >48 over uninfected splenocytes. The efficacy for these compounds was evaluated in a murine BALB/c model of infected splenocytes with *L. infantum*. Mice were treated intraperitoneally with 0.5 mL solutions of indotecan (**109**) or AM13-55 (**108**) in DMSO-saline at a dose of 2.5 mg/kg every 2 days for 15 days. Mice treated with indotecan (**109**) exhibited drastic reduction in parasite load both in the liver and the spleen. Surprisingly, the same dose of AM-1355 (**108**) led to a greater than 90% reduction in parasites only in spleen and with no change in parasite burden in the liver. Lack of reduction of parasites in the liver could be attributed to metabolism of the parent compound to inactive metabolite(s). The inhibitory potency of both alkaloids on *L. infantum* recombinant TopIB was assessed with results showing that indotecan (**109**) was the most potent compound on topoisomerase IB.[269]

A series of bislawsone analogues were assessed for their activity on *L. donovani* promastigotes as well as on leishmanial DNA topoisomerase I. The best analogs (**110** and **111**) (Figure 17) showed a promastigote activity of 2 μM indicating that these compounds are active at the cellular level. In the topoisomerase I enzymatic assay; the range of activity varied from 51 to 70 μM in a simultaneous assay and 15−16 μM in a preincubation assay indicating that topoisomerase I is one of the targets for these compounds. Given the weak activity, however, additional targets cannot be ruled out.[200]

A series of nitroheteroaryl-1,3,4-thiadiazoles were investigated. The results showed that the nitroimidazole and nitrofuran analogs are active against intracellular amastigotes in the single digit micromolar range with low toxicity against the host cells. An assay against *Leishmania* topoisomerases proved that compounds from series **112** and **113** (Figure 17) acted against both topoisomerase I and II with inhibition in the range of 8−83% at EC_{50} doses of compounds against *Leishmania* (Table 11). These series of compounds were also found to be active against both *L. infantum* and *L. tropica*.[270]

In an interesting study involving known topoisomerase I inhibitor 3,3′-diindolyl methane (DIM, **114**) (Figure 17), resistant parasite strains of *L. donovani* were generated by gradually increasing the concentration of the drug leading to random mutations in the large and small subunits of heterodimeric DNA topoisomerase I (LdTOP1LS). It was discovered that the mutation of the large subunit of LdTOP1LS at F270L is responsible for resistance to DIM. A series of DIM analogues was generated (**115**-**117**) which were not only active

Table 11. Topoisomerase and Antiparasitic Activity on Thiadiazole Derivatives

compound	R	*L. major* Top I (%)	*L. major* Top II (%)	intra am EC_{50} (μM)
112	Ph	73	59	4.2
	5-Cl-2-thiophene	62	57	2.7
113	Ph	64	76	3.7
	2-Cl-Ph	39	55	8
	3-Cl-Ph	49	51	6.8
	5-Cl-2-thiophene	37	83	2.8
	5-Br-2-thiophene	8	58	6.2

on resistant *L. donovani* parasites but were also active against the wild type parasite.[256]

3.3.7. Proteases. There are a total of 154 proteases in the *Leishmania* genome. These proteases are in the cysteine, serine, aspartate, and metalloprotease family. Out of these proteases, cysteine proteases and metalloprotease have proven to be important in the pathogenesis of leishmaniasis.[271]

3.3.8. Cysteine Protease. The cysteine proteases in *Leishmania* exist in the gene families CPA, CPB, and CPC. It has been established that at least two of the families need to be targeted to absolutely block the parasite invasion and replication in host cells.[272] In an effort to find new starting points for cysteine protease inhibitors, *L. mexicana* cysteine protease CPB2.8, which shows significant differences with bovine cathepsin B, was selected as a target. High throughput screening of a compound library against this enzyme and bovine cathepsin B (BtCatB) identified four novel inhibitor classes broadly classified into 3 groups depending on the warhead-types, namely thiosemicarbazones (118, 119), nitriles (**120**), and semicarbazones (**121**) (Figure 18). The thiosemicarbazone 118 showed an IC_{50} on CPB2.8ΔCTE (which is the recombinant form of the amastigote specific isoform CPB2.8 expressed without the C-terminal extension) in the nanomolar range with complete selectivity over bovine Cat B (IC_{50} >30 μM). In contrast, the thiosemicarbazone (119) was equipotent on both CPB2.8ΔCTE and on BtCatB in the nanomolar range. The nitrile 120 was approximately ten times less potent on CPB2.8ΔCTE (K_i = 570 nM) and had some degree of selectivity over bovine protease BtCatB (IC_{50} = 13.8 μM). The most promising hit was **121** with a K_i of 5 nM and an IC_{50} >30 μM for BtCatB. These chemotypes prove that reasonable starting points can be discovered for further optimization of cysteine protease inhibitors.[273] In a separate effort by Augustyns and co-workers, a set of α-ketoheterocycles was designed and synthesized as cysteine protease inhibitors of *L. mexicana*. However, there was no correlation between the enzymatic activity and cellular activity, thus bringing into question the validity of the target.[274]

A series of semisynthetic morelloflavone (**122**) (Figure 18) analogs were evaluated. All compounds exhibited inhibition of *L. amazonensis* promastigotes as well as amastigote activity in nanomolar range with low cytotoxicity. In addition, compounds **123**−**125** were active against recombinant-CPB2.8 of *L. mexicana* and r-CPB3 of *L amazonensis* with IC_{50} values of 0.7−1.5 μM, respectively. These results provide new starting points for lead optimization.[275]

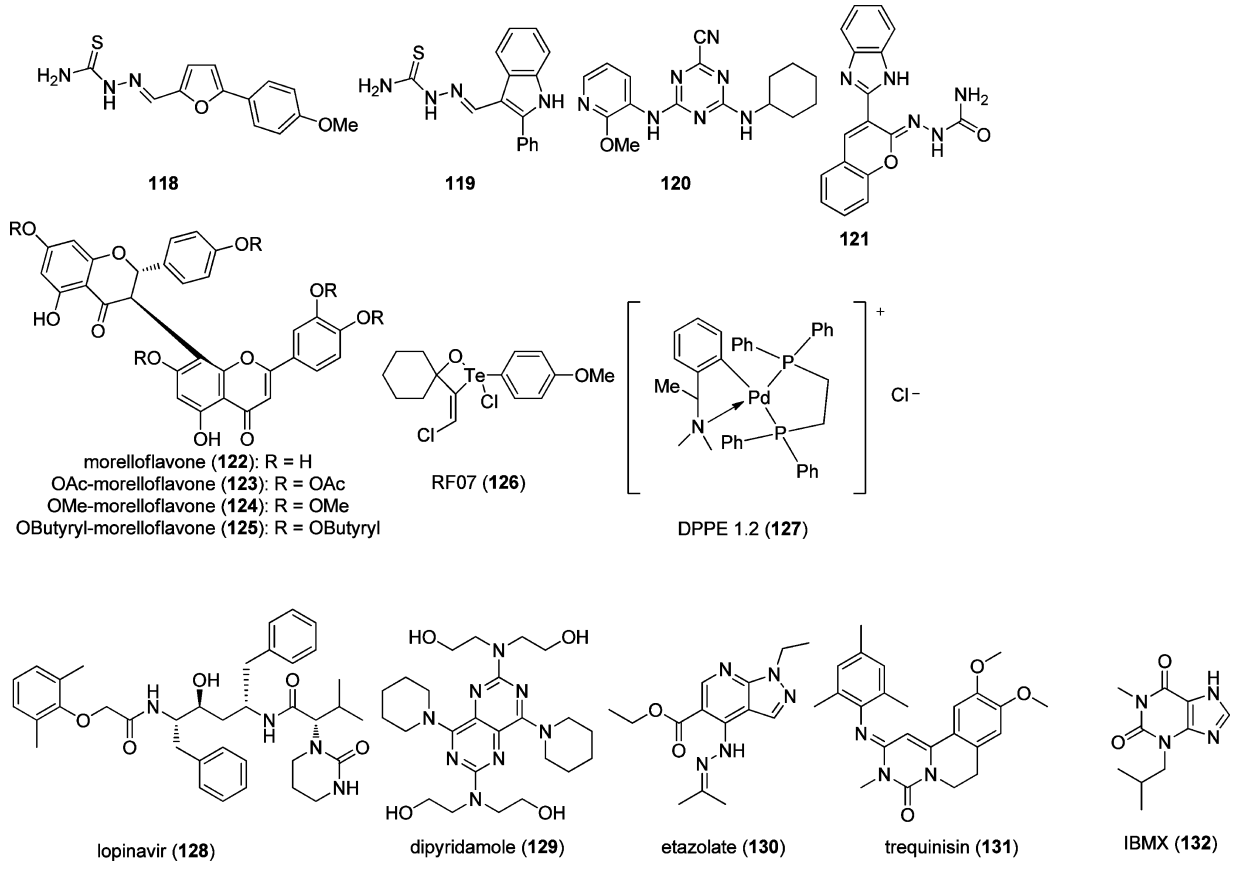

Figure 18. Examples of protease and phosphodiesterase inhibitors.

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Tellurium compounds as chemotherapeutic agents are being investigated for variety of indications. Organic telluranes are also known to be inhibitors of cysteine proteases.[276] Based on the earlier reports of organotellurane compounds being active on promastigote and amastigote forms of *L. amazonensis*,[277] tellurium compound RF07 (**126**) (Figure 18) was evaluated against *L. chagasi*, a causative agent of visceral leishmaniasis in Latin America. In vitro assays indicated that the compound was active on intracellular amastigotes with an EC_{50} of 530 nM and a 10-fold cytotoxic window when compared to noninfected macrophages. Intraperitoneal injection of RF07 (**126**) in *L. chagasi*-infected hamsters exhibited a 99.6% reduction of parasite burden when compared to control animals which received an antimonial drug Glucantime or PBS. The effect of RF07 (**126**) on cathepsin B activity on *L. chagasi* amastigotes was evaluated spectrofluorometrically using fluorogenic substrates and the IC_{50} values were 10-fold higher suggesting the potential involvement of other targets in cells and in vivo.[278] The palladacycle's trypanocidal activity as well as their ability to affect cathepsin B activity has been previously demonstrated.[279] Inspired by this, palladacycle compound DPPE 1.2 (**127**) was evaluated for activity against *L. amazonensis*, which is prevalent in in the Amazon region of Brazil and is responsible for cutaneous leishmaniasis. The compound was found to be active against axenic *L. amazonensis* promastigotes with an EC_{50} of 2.13 nM. It was also found to be active on intracellular parasites with an EC_{50} of 128 nM, and the compound was 10-fold less toxic in macrophages (CC_{50} = 1,267 nM). In an efficacy study, *L. amazonensis*-infected BALB/c mice were injected subcutaneously with DPPE 1.2 (**127**) at 4.8 mg/kg every other day. The treated animals showed a significant decrease in foot lesion size and a 97% reduction of parasite burden when compared to controls that were treated with PBS. DPPE 1.2 (**127**) inhibited the cysteine protease activity of *L. amazonensis* amastigotes and more significantly the cathepsin B activity which was determined by zymography after electrophoresis.[280]

3.3.9. Aspartic Protease. The role of aspartic proteases in *Leishmania* was discovered when HIV aspartyl peptidase inhibitors were profiled for *L. amazonensis* proliferation. The HIV protease inhibitors affected parasite growth in a dose-dependent fashion with nelfinavir (**16**) (Figure 2) and lopinavir (**128**) (Figure 18) exhibiting an EC_{50} of 15.1 μM and 16.5 μM on promastigotes. The protease activity of these compounds was established by measuring proteolytic hydrolysis of the peptide substrate in a dose dependent fashion in *L. amazonensis*. Lopinavir (**128**) was able to reduce the proteolytic hydrolysis of the substrate by approximately 90% at 1 μM, and demonstrated full activity at 10 μM. On the other hand, nelfinavir (**16**) exhibited weak activity with inhibition of 98% at 10 μM and no observable activity at 1 μM.[281]

In a separate effort, an ortholog of the yeast Ddi1 protein was identified as the only member of the aspartic protease family in *Leishmania* parasites and was explored as a potential drug target. An enzymatic assay was developed by incorporating genes encoding Ddi1 orthologs from *L. major* and humans. Nelfinavir (**16**) was active on human as well as *L. major* with an IC_{50} value of 3.4 and 0.44 μM, respectively. These values correlate well with observed cellular activity.[282]

3.3.10. Serine Protease and Metalloprotease. In the serine protease family, oligopeptidase and oligopeptidase B play an important role in the interaction of pathogens with their host and are considered to be important targets. A number of medicinal chemistry efforts have been undertaken in the past which have been described previously.[271a] *Leishmania* metalloprotease GP63 is located on the surface of promastigotes and is thought to be a key player in evasion and survival from lysis prior to internalization by macrophages. However, there are no medicinal chemistry efforts reported for this target.[271a]

3.3.11. Phosphodiesterase. Phosphodiesterases (PDEs) control the cellular concentration of the second messenger's cAMP and cGMP that are key regulators of many important biological processes.[283] The human genome contains twenty-one PDE genes that are categorized into 11 families. In comparison, the genome of the protozoal parasite *L. major* contains five PDE genes encoding LmjPDEA, LmjPDEB1, LmjPDEB2, LmjPDEC, and LmjPDED. Two of these, LmjPDEB1 and LmjPDEB2, are adjacently situated on chromosome 15 and share extensive similarity in their overall architecture.[284] Early studies showed that three human PDE inhibitors (dipyridamole (**129**), etazolate (**130**), and trequinsin (**131**)) (Figure 18) inhibit the proliferation of *L. major* promastigotes and *L. infantum* amastigotes with EC_{50} values in the micromolar range (Table 12).[284] Recently, the cocrystallization of the catalytic domain of LmjPDEB1 in complex with 3-isobutyl-1-methylxanthine (IBMX, **132**) was reported. IBMX (**132**) is a nonspecific PDE inhibitor with an enzymatic activity of 580 nM against that catalytic domain of LmjPDEB1. A comparison between the structures of LmjPDEB1 and human PDEs has identified a novel pocket in the LmjPDEB1 structure, which may thus be useful for the design of parasite selective inhibitors for the treatment of leishmaniasis.[285]

Table 12. Antileishmanial Activity of Phosphodiesterase Inhibitors

compound	*L. major* pro EC_{50} (μM)	*L. infantum* am EC_{50} (μM)
129	45	
130	58	
131	44	10.2
132	1000	

3.3.12. Tubulin. Tubulin, which is highly conserved across all species, is a superfamily of globular proteins with six distinct families, the alpha-, beta-, gamma-, delta-, epsilon-, and a sixth family zeta-tubulin, which is specific to kinetoplastid protozoa. Tubulins have been associated with a variety of cellular functions such as maintenance of cell shape among others. Tubulin has proven to be an attractive target in the field of oncology and there are several successful drugs in the clinic.[286] The comparison of tubulin sequences from mammalian cells and yeast cells reveals a homology of 70−90%. However, specific antitubulins are known and it is suggested that differences in amino acid sequence lead to different conformations of tubulins making the targeting of parasitic tubulin possible.[287]

Interest in antitubulins for antiparasitic therapy was piqued when Chan and co-workers demonstrated that radioactive herbicide trifluralin (**133**) (Figure 19) binds selectively to tubulin extracts from *Leishmania* species.[288] Based on this finding, another dinitro compound (**134**) was found to selectively bind to *L. tarentolae* tubulin (IC_{50} = 7.4 μM). Furthermore, this compound was found to have activity against *L. donovani* axenic amastigotes (EC_{50} = 2.3 μM) with no cytotoxicity on Vero cells. Given the liabilities of the nitro group, the compound was further optimized to **135** which showed an IC_{50} of 6.6 μM against *L. tarentolae* tubulin and

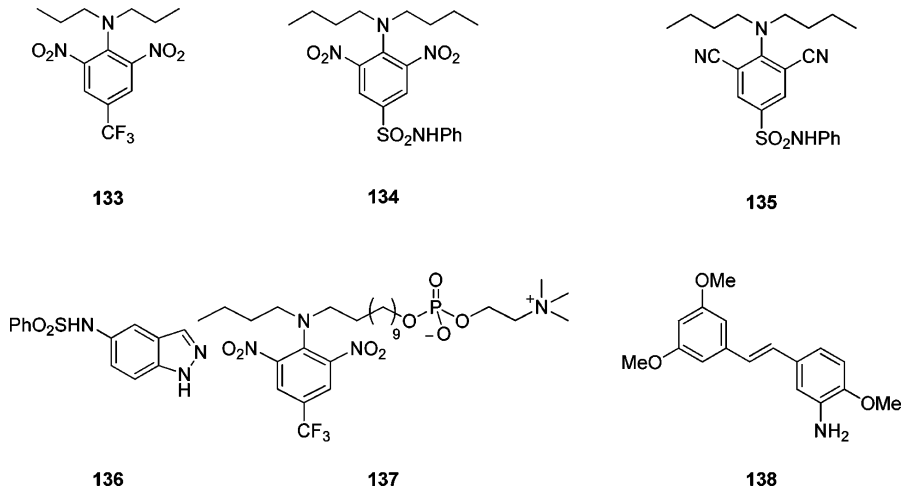

Figure 19. Examples of tubulin inhibitors.

cellular activity against *L. donovani* axenic amastigotes with an EC_{50} of 4.4 μM.[289] In addition, a screen was carried out on 10 000 compounds using *L. tarentolae* tubulin which led to the identification of new chemotypes for future optimization campaigns. Inspired by the success of sulfonamides, a benzopyrazole sulfonamide (**136**) was designed and synthesized.[287a] Compound **136** had an EC_{50} of 37−48 μM against promastigotes of different *Leishmania* subspecies. This cellular activity was in the same range as miltefosine (EC_{50} = 17 μM). Furthermore, compound **136** when dosed via i.p. route was able to reduce the parasite load in the liver and spleen by 96−97% in an acute *L. infantum* mouse model.[290] Rodrigues and co-workers have designed and synthesized a hybrid of dintroaniline and alkyl phosphocholine to attempt to combine the tubulin binding mechanism with that of miltefosine. Compound **137** has an EC_{50} of 2.6 and 1.2 μM against *L. amazonensis* promastigotes and intracellular amastigotes, respectively. Fluorescence microscopy with alpha tubulin antibody in conjunction with scanning electron microscopy show changes in the cytoskeleton and alterations in the shape of the plasma membrane proving that the hybrid molecule is still acting on tubulin.[291]

Stilbene based compounds are widely found in nature and are known for their pharmacological properties.[292] There are previous reports where stilbenes have been reported for their antileishmanial activity.[293] A series of stilbene derivatives were also evaluated for their antileishmanial activity. Based on the SAR, it was observed that *trans*-stilbenes were more potent than cis isomers. *trans*-3,4′,5-Trimethoxy-3′-amino-stilbene (TTAS, **138**) was the most active stilbene, showing a LD_{50} value of 2.6 μg/mL in *L. infantum*. It was observed that TTAS (**138**) had low toxicity when tested on normal hemopoietic cells. TTAS has the ability to block *Leishmania* parasites in G(2)-M phase of cell cycle which is in line with the affinity chromatography results that identified tubulin as the putative target.[294]

4. INTRODUCTION TO HUMAN AFRICAN TRYPANOSOMIASIS (HAT) AND CLINICAL DESCRIPTION

Also known as African sleeping sickness, HAT is caused by the protozoan parasite, *Trypanosoma brucei*. Two forms of the disease exist in humans, the more common caused by the subspecies *Trypanosoma brucei gambiense*, and the less common form caused by *Trypanosoma brucei rhodesiense*. Both forms are transmitted to humans by the painful bite of blood-feeding tsetse flies. Infectious metacyclic trypomastigotes present in the salivary fluid of flies establish a primary lesion in the skin known as a trypanosomal chancre that appears 5−15 days after the initial bite. The parasites proliferate and spread to the blood where they disseminate throughout the body. During the early "hemolymphatic stage," patients experience nonspecific symptoms of intermittent fevers, malaise, arthralgias, and headaches. The acute disease has protean manifestations including gastrointestinal complaints, cardiac features, ophthalmological complications, endocrine dysfunction, to name a few. In the form of HAT caused by *T. brucei gambiense*, the early stage evolves over a time frame of months or even years.[295] In one of the early clinical descriptions of HAT, Thomas Winterbottom in 1803 referred to the swollen lymph nodes along the posterior neck as an important characteristic and mentioned that this finding, now known as Winterbottom's sign, was used by Arab slave traders to exclude potential slaves.[296] In the other form of HAT caused by *T. brucei rhodesiense*, the early stage runs a more rapid course of weeks before evolving into late-stage disease. As a zoonotic infection, the *rhodesiense* form of HAT may be less well adapted to the human host compared to the anthroponotic *gambiense* form. In both forms of HAT, late-stage disease is defined by the entry of trypanosomes into the central nervous system. A patient is judged to have late-stage HAT when trypanosomes (or elevated white blood counts) are detected in cerebral spinal fluid upon doing a spinal tap. In late-stage disease, parasites are also present within parenchymal brain tissue giving rise to the encephalitic picture for which the disease is so feared. Symptoms include psychiatric, motor, and sensory disturbances along with abnormal reflexes. Approximately three-quarters of patients have profound sleep disturbance, including nocturnal insomnia and daytime somnolence,[297] giving rise to the disease name, sleeping sickness. Without treatment, patients inevitably progress to coma and death.

5. BACKGROUND OF HAT

5.1. History and Epidemiology of HAT

Other species of trypanosomes such as *T. congolense, T.vivax,* and *T. brucei brucei* infect animals and have greatly limited man's ability to bring domesticated animals into many regions

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Table 13. Drugs for Treating Human African Trypanosomiasis

Disease	Stage	Drug	Year introduced	Route of administration	Liabilities
Gambiense HAT	Early	Pentamidine	1941	IM or IV	No oral formulation
		Eflornithine	1981	IV	Expensive, every 6 h dosing
		Melarsoprol	1949	IV	Arsenical (toxic encephalopathy)
	Late	NECT[a]	2009	IV + PO	Expensive, IV for eflornithine part
Rhodesiense HAT	Early	Suramin	1922	IV	No oral formulation
	Late	Melarsoprol	1949	IV	Arsenical (toxic encephalopathy)

[a]NECT: nifurtimox/eflornithine combination therapy.

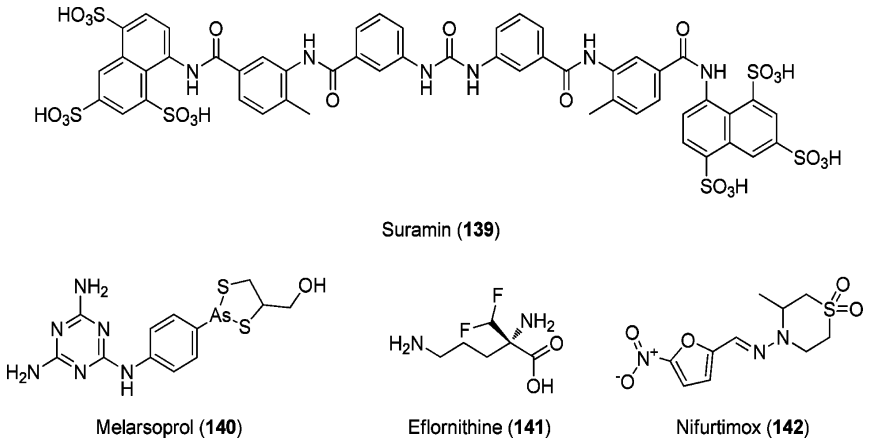

Figure 20. Established drugs to treat HAT.

of Africa. The disease affecting cattle, nagana, has been recognized since antiquity. Interestingly, humans are resistant to these species due to trypanosome lytic factors circulating in their blood,[298] which points to the long evolution of humans in the presence of these parasites in Africa. It is thought that HAT is a relatively recent event in human development.[296] In fact, the infectivity of *T. brucei rhodesiense* to humans is due to a serum-resistance associated gene that arose as a single event and spread through East Africa by genetic exchange.[296,299]

Tsetse flies were recognized to cause nagana 50 years before the Scottish microbiologist, David Bruce, first reported *Trypanosoma brucei* in the blood of cattle in 1895.[296] The first microscopic detection of trypanosomes in human blood was made on a steamboat captain in The Gambia in 1901 by British surgeon R. M. Forde.[296] This was named, *Trypanosoma gambiense.* The second trypanosome species causing infection in humans, *T. rhodesiense*, was identified in 1910.

Transmission of HAT is limited to the range of tsetse flies, thus the disease is confined to the African continent. In the 20th century, three major sleeping sickness epidemics have afflicted the Africa. The first epidemic at the turn of the 20th century, killed about 300 000−500 000 people in the Congo basin, Uganda, and Kenya and led to the introduction of arsenical compounds as the first treatments for HAT. Subsequent work by the German chemical/pharmaceutical company, Bayer, led to the discovery of suramin in 1916, the first truly effective treatment for HAT, and one that is still in use. The second major epidemic occurred between about 1920 and 1940. In response to these epidemics, control measures were introduced including tsetse fly control using traps and brush clearing, host reservoir control, and game destruction.[296] Colonial powers introduced mobile teams to carry out these control measures with positive impacts on prevalence of HAT. The third major HAT epidemic occurred following the departure of colonial powers (1960−70s) with the associated political instability and interruption of control programs (exacerbated by the banning DDT in the 1970s). The most heavily impacted countries were Angola, Congo, Sudan, and Uganda with more than 300 000 cases per year occurring in the late 1990s. The WHO along with partner agencies and governments stepped in with aggressive case detection, treatment, and vector control to bring rates down to 50 000−70 000 by 2006. Reported cases dropped below 10 000 for the first time in 2009, although the factor gap between reported cases and actual cases is probably at least three.[300] Areas with political and social instability, particularly in the Democratic Republic of the Congo and the Central African Republic continue to see high rates of HAT that help sustain the risk of future epidemics to the continent.[301] Thirty-six countries are currently listed as endemic for HAT.[300]

5.2. Biology of HAT

African trypanosomes have fascinated biologists since their discovery. The complex life-cycle of *T. brucei* between the vertebrate and invertebrate hosts provides reservoirs and means of transmission to ensure efficient propagation in nature. Parasites undergo dramatic morphological and biochemical adaptions when cycling between these vastly different hosts. In humans, during the early stage, the trypanosomes spend most of their time in the nutrient-rich environment of the bloodstream where normal glucose levels run about 100 mg/dL. For ATP production, bloodstream trypanosomes are entirely dependent on the conversion of the blood sugar glucose. Oxidative metabolism involving mitochondrial Krebs cycle enzymes and oxidative phosphorylation are essentially shut down.[302] On top of this, the glycolytic pathway in trypanosomatids is organized in a unique manner: the majority of the glycolytic enzymes are sequestered inside peroxisome-like organelles known as glycosomes, presumably concentrating

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the enzymes and their substrates for efficiency. While living in the bloodstream, trypanosomes are continually under attack by the body's immune system, particularly antibodies directed at surface antigens. As a countermeasure, as much as 10% of *T. brucei*'s genome encodes variant surface glycoproteins (VSGs) that coat the outer membrane by attachment to glycosylphosphatidylinositol anchors. Only one VSG is expressed at a time with stochastic switching to provide antigenic variation that allows for evasion of the immune system. Due to myriad VSGs, attempts at making effective vaccines for HAT have been unsuccessful. The *T. brucei* genome of ∼9000 genes has been fully sequenced and has accelerated our understanding of the biology of this sophisticated parasite. Areas of unique biology point to attractive targets for drug discovery, such as the machinery involved in extraordinary process of RNA editing that takes place in the sole mitochondrion known as the kinetoplast.[303] Further discussion of target-based drug discovery follows later.

6. DRUG DISCOVERY FOR HAT

6.1. Current Treatments

The drugs currently recommended for treating HAT are listed in Table 13 and Figure 20. Approximately 98% of cases of HAT are due to *T. brucei gambiense* which predominates in central and western African countries.[304] For early stage *gambiense* HAT, pentamidine (**3**) is considered first line treatment. This diamidine drug was developed in the 1930s by English chemist A. J. Ewins of the pharmaceutical company May and Baker.[296] It is administered by intramuscular injections once daily for 7 days, although it can also be given intravenously. The drug is usually effective and relatively inexpensive, but it is associated with pain at the injection site, hypo- or hyperglycemia, prolonged QT interval on electrocardiogram, leukopenia, nephrotoxicity, hypotension, and gastrointestinal symptoms. Binding to tissue proteins contributes to a large volume of distribution and long terminal half-life. It does not cross the blood-brain barrier, hence its use is limited to patients with early stage HAT. The drug is thought to have a fairly nonspecific mechanism of killing trypanosomes by binding DNA and disrupting mitochondrial functions. It is able to mediate selective toxicity on trypanosomes over mammalian cells by virtue of being concentrated to millimolar levels inside trypanosomes by P2 and other surface transporters.[305] Resistance to pentamidine has been generated in laboratory strains but is not reported to be a widespread problem in the field.[306]

Suramin (**139**) is perhaps the oldest antimicrobial drug in continuous use since its introduction (i.e., 1922). It is the first-line treatment for *rhodesiense* HAT and given by slow intravenous infusion every 3−7 days for a 4-week period, typically.[307] It is highly protein bound and has very long terminal half-life of 41−78 days; it does not cross the blood-brain barrier. It is effective therapy, but is associated with urticarial rash in about 90% of patients that usually resolves without discontinuation of the drug. Other common side effects include pyrexia, nausea, and reversible nephrotoxicity. The mechanism of action for suramin on trypanosomes is unknown. Resistance in the field has been rarely reported.[307]

Melarsoprol (**140**) is an arsenical drug used for late-stage HAT. As discussed above, arsenicals were the first drugs introduced for treating sleeping sickness starting with a drug called atoxyl, an ironic name since clinical studies showed it caused blindness due to optic nerve atrophy.[296] Melarsoprol was the first and still the only effective drug for late-stage HAT due to *T. brucei rhodesiense.* It has largely been replaced by eflornithine-based treatment for management of late stage HAT due to *T. brucei gambiense.* However, due to challenges with distributing and administering eflornithine, melarsoprol was still being used to treat 88% of persons with second stage *T. brucei gambiense* as recently as 2003.[308] Melarsoprol is perhaps one of the most dangerous drugs used for treating an infectious disease with reactive encephalopathy occurring in ∼10% of patients and fatalities occurring in ∼5% of these cases. However, since late-stage HAT is uniformly fatal, medical providers have been forced for decades to accept melarsoprol as the best therapeutic option. On top of this, melarsoprol is associated with agranulocytosis, skin rashes, peripheral neuropathy, cardiac arrhythmias, and multifocal inflammatory disorder.[308] Melarsoprol is administered by intravenous injection in a 10 day regimen.[309] Like pentamidine, it is concentrated in trypanosome cells via uptake by P2 transporters; it then disrupts the redox environment within the cell by disrupting the protein, trypanothione.[310] Treatment failures occur with increasing frequency in many regions although the direct responsibility of drug-resistant parasites has not been firmly established.[307]

Eflornithine (**141**), introduced in 1981, was an important breakthrough for HAT as it provided a safer alternative to melarsoprol for late-stage disease caused by *T. brucei gambiense.* Eflornithine (difluoromethylornithine, DFMO) was repurposed from investigations as an anticancer agent. It blocks the enzyme, ornithine decarboxylase (ODC), which is integral to polyamine biosynthesis. It acts on the mammalian enzyme as well as the trypanosomal ODC, but owing to the rapid turnover of the mammalian ODC, the drug exerts much less toxicity on host cells compared to bloodstream trypanosomes. It is less effective on *T. brucei rhodesiense* so its use is restricted to cases of late stage *gambiense* HAT. Eflornithine by itself is given at a dose of 100 mg/kg intravenously every 6 h for 14 days. For a typical-sized individual, this demanding regime translates to nearly a half-kilogram of drug administered while the patient is confined to a hospital. Through support from WHO, eflornithine kits for two weighing 40 kg and costing US$1420 were made available for distribution in disease endemic countries.[300] The frequent administration schedule of eflornithine is necessary due to its short plasma half-life of 3 h. It is associated with side effects of fever, headache, alopecia, hypertension, rash, peripheral neuropathy, tremor, and diarrhea.[307] Resistance, due to mutations in a putative amino acid transporter, has been shown in vitro.[311]

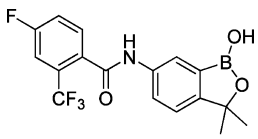
An important recent advancement in HAT chemotherapy was the introduction of nifurtimox eflornithine combination therapy (NECT), which is currently the first line of treatment for HAT.[312] Nifurtimox (**142**) was repurposed as a drug for treating American trypanosomiasis (Chagas disease) caused by *Trypanosoma cruzi.* For HAT, nifurtimox is orally administered three times a day for 10 days in combination with intravenous eflornithine. The advantage is that eflornithine is given every 12 h for 7 days at 200 mg/kg rather than every 6 h for 14 days that is used in monotherapy. Although the burden of intravenous therapy is still a factor, it is considerably reduced by the longer dosing frequency and shorter total duration. Compared to eflornithine alone, NECT was associated with a higher incidence of tremors, anorexia, and nausea.[312a] NECT was added to the WHO Essential Medicines List in 2009. Kits for four full treatment courses weigh 36 kg and cost US$1440, and

are being widely adopted in disease endemic countries.[300] Despite this positive advancement, the need for intravenous treatment coupled with the high costs of distribution, makes NECT a far cry from optimal chemotherapy for treating late stage HAT. A target product profile (TPP) for a better drug for HAT has been proposed by the Drugs for Neglected Diseases initiative.[141] The ideal drug would be effective against both early- and late-stage disease, orally administered over a relatively short course (i.e., 7 days), safe for all persons including children and pregnant women, and cost less than 30 euros per course. By being effective in both early and late-stage disease, the drug would obviate the need to perform lumbar punctures for staging purposes, a major advantage. Due to the large gap between the profiles of currently used HAT drugs and the ideal HAT drug, there is much work to be done in the field of drug discovery. Recent discoveries and advancements to be discussed below give us optimism that these goals are achievable in the coming decade.

6.2. Drug Candidates in Clinical Trials for HAT: Fexinidazole and Oxaborole SCYX-7158

Fexinidazole (**13**) was identified in a phenotypic screen of >700 nitroheterocyclic compounds against *T. brucei* cultures.[313] It was originally synthesized by Hoechst in the 1970s and shown to have antitrypanosomal activity.[307] The compound is active against *T. b. rhodesiense* and *T. b. gambiense* and cures both the acute and chronic mouse models of HAT infection.[314] Fexinidazole is metabolized by P450 enzymes to sulfoxide and sulfone derivatives that have similar antitrypanosomal activity as the parent compound (range: 0.4−0.8 μg/mL). Oral bioavailability in mice was 41%, and the parent compound and metabolites achieved brain concentrations above IC_{50} values.[313] Fexinidazole was mutagenic in the Ames test due to bacterial specific metabolism, but not genotoxic on mammalian cells. Four week repeat-dose toxicokinetic studies in rats and dogs demonstrated a no observed adverse event at 200 mg/kg/day in both species. The drug entered phase I human studies in 2009[315] and progressed to phase II/III safety and efficacy studies in October 2012 where it is being compared to NECT. The API is produced by Sanofi. The phase II/III studies are taking place in the Democratic Republic of the Congo and Central African Republic under direction by the Drugs for Neglected Diseases Initiative in collaboration with the Swiss TPH.

SCYX-7158 (**143**) (Figure 21) is the second compound for HAT that has recently entered human clinical trials. It was



(**143**)

Figure 21. Structure of SCYX-7158.

derived from screening a library of boron-based compounds from Anacor Pharmaceuticals against *T. brucei* cultures.[316] A lead-optimization program conducted at Scynexis led to the benzoxaborole compound, SCYX-7158, with an IC_{50} of 0.29 μg/mL against *T. brucei* 427 strain. It cures both the acute and chronic mouse models of HAT infection.[317] Oral bioavailability in mice was 55%; it is CNS permeable and highly metabolically stable in rodents. SCYX-7158 was negative in Ames and hERG channel assays. It was well tolerated in mice at doses up to 100 mg/kg twice per day.[317] DNDi is directing the first-in human studies in France which started in March, 2012, to assess the safety, tolerability, and pharmacokinetics in healthy volunteers of sub-Saharan origin.

6.3. Amidines and Diamidines

Tidwell and co-workers have extensively developed bisamidines patterned after pentamidine (**3**) for treatment of HAT. One compound entered into clinical trials but the trial was halted due to the occurrence of nephrotoxicity. These compounds have been reviewed previously.[305] It has been previously reported that these dicationic compounds are selectively cytotoxic to *T. brucei* over mammalian cells due to the differences in the active transport mechanism, which aids in the accumulation of drug into parasites at levels ∼1000-fold higher than in mammalian cells.[305]

More recently, Alp et al. reported a series of amidinobis-benzimidazoles including compound **144** (Figure 22).[318] The best compound in the series (compound **144**) blocked the growth of *T. b. rhodesiense* in vitro with an IC_{50} of 0.036 μg/mL and displayed cytotoxicity on mammalian cells at 29.4 μg/mL. No pharmacokinetic or efficacy studies were reported.

Dicationic flexible triaryl guanidines and imidamides were evaluated as antiprotozoal agents by Arafa et al.[319] The most potent compound in the series **145** had an in vitro IC_{50} of 151 nM against *T. b. rhodesiense* with cytotoxicity of 11.6 μM. Although molecular modeling and DNA binding studies were reported, the detailed mode of action and animal data were not available.

Huang et al. reported the SAR of alkanediamide linked bisbenzamidines as antitrypanosomal agents.[320] Compound **146** (Figure 22) in this series had an IC_{50} of 0.003 μM against *T. b. brucei* and an IC_{50} of 0.002 μM against *T. b. rhodesiense*. Compound **146** was found to be less cytotoxic to the A549 human lung carcinoma cell line with cytotoxicity of 1193 μM. Although the mechanism of action of bisbenzamidines is credited due to the binding to DNA, the antitrypanosomal activity of the bisbenzamidines reported did not directly correlate with the corresponding binding affinity to DNA. No animal data were provided.

Dicationic substituted bis(phenoxymethyl)arene analogues of pentamidine were evaluated for antiprotozoal activities by Bakunova et al.[321] The most active compound against *T. brucei rhodesiense* was 1,3-bis(4-amidinophenoxymethyl)benzene **147** (Figure 22) with an IC_{50} of 2.1 nM. Compound **148**, the *N*-isopropyl derivative of **147**, was identified to be active in the acute mouse model of HAT following i.p. dosing (4 × 5 mg/kg), but none of the compounds exhibited significant oral activity.

Patrick et al. reported the SAR on cationic benzyl phenyl ether derivatives for activities in vitro and in vivo against *T. b. rhodesiense* (STIB900).[322] Several of the dicationic benzyl phenyl ether derivatives displayed good in vitro and in vivo activity against *T. b. rhodesiense*. In particular, methamidoxime derivative **149** achieved 4 out of 4 cures by oral administration (4 × 25 mg/kg) in a murine model.

The SAR on pentamidine derivatives bearing the benzofuran residue was reported by Bakunov et al.[323] The authors reported that the potency of these compounds against *T. b. rhodesiense* depended upon the nature of the cationic motif, the orientation of the benzofuran residue and the length of the carbon linker. The most active compound in this series **150** (Figure 22) had

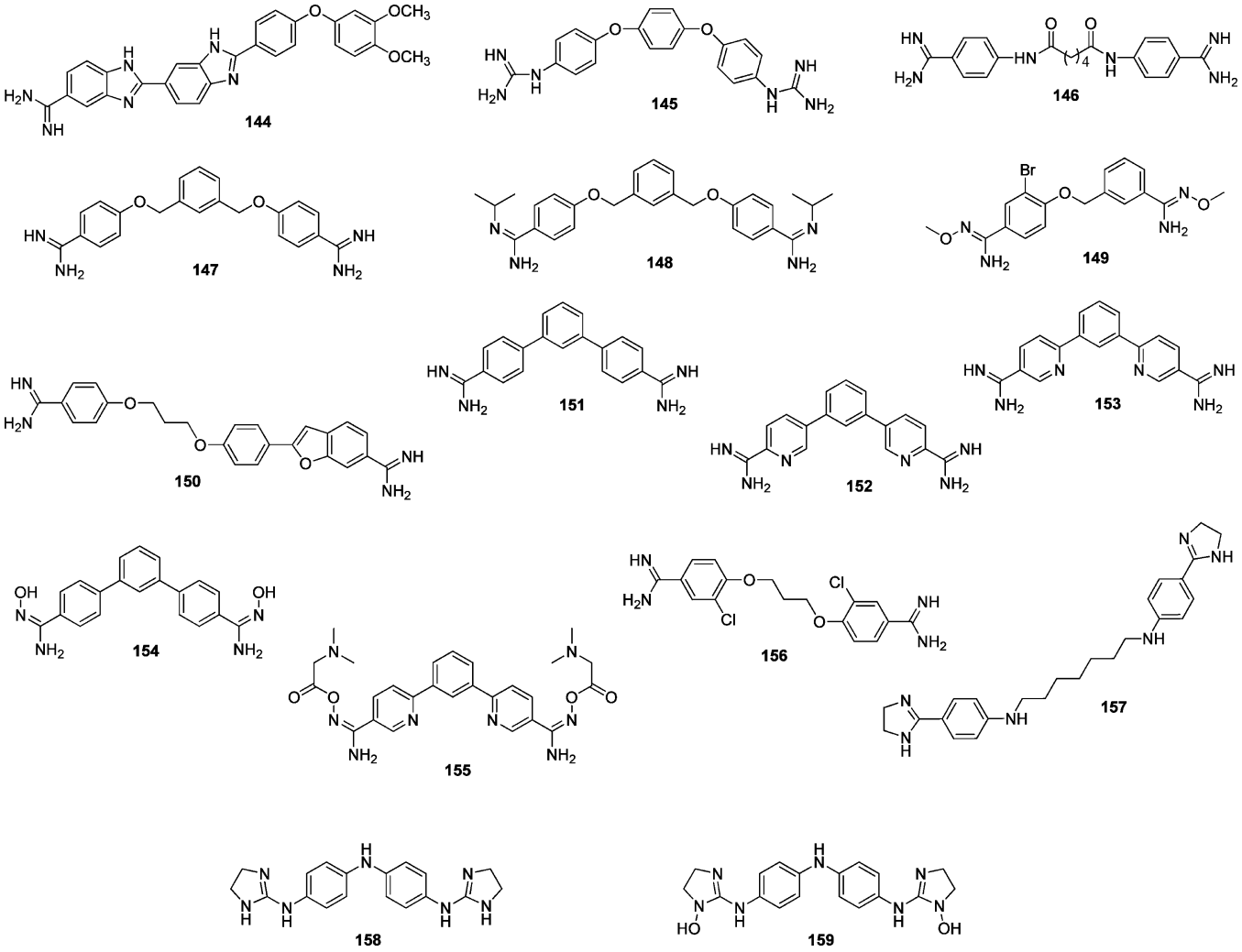


Figure 22. Amidine and diamidine compounds active against strains of *T. brucei*.

an in vitro IC_{50} of 0.025 μM against *T. b. rhodesiense* with cytotoxicity of 8.6 μM against L6 cells. The target of these compounds and in vivo data were not reported.

Patrick et al. reported the antiprotozoal activity of dicationic *m*-terphenyl and 1,3-dipyridylbenzene derivatives.[324] Herein several diamidine derivatives displayed good in vitro activity against *T. b. rhodesiense* and proved to be curative in mouse model of early stage HAT. In particular, compounds **151**, **152**, and **153** (Figure 22) achieved 4/4 cure rate in mice infected with *T. b. rhodesiense* (STIB900) with four daily 5 mg/kg i.p. doses and also by a single i.p. dose of 10 mg/kg. Furthermore, prodrugs **154** and **155** attained a cure rate of 3/4 with four daily oral doses of 25 mg/kg. Mechanism of action and pharmacokinetic studies were not reported.

Structure activity and cytotoxicity analysis of pentamidine derivatives as antiprotozoal agents was reported by Bakunova et al.[321] Herein they have identified several derivatives of pentamidine with potent in vitro activity and decreased cytotoxicity to mammalian cells by varying the aliphatic chain lengths, replacing the oxygen atom in the aliphatic linker with sulfur and sulfone moieties and through *N*-substitutions. Compounds **156** and **157** produced good in vivo activity in an acute mouse model of trypanosomiasis by attaining a cure rate of 4/4 with four daily i.p. doses of 5 mg/kg. Mode of action and the pharmacokinetic studies were not reported.

Nieto et al. reported the synthesis and evaluation of *N*-alkoxy analogues of 4,4′-bis(imidazolinylamino)diphenylamine **158** to improve the blood-brain barrier penetration of the parent compound.[325] Compound **159**, the *N*-hydroxy analogue of **158**, displayed 3 times increase in blood-brain barrier permeability compared to lucifer yellow as determined by in vitro transport assays through the hCMEC/D3 human brain endothelial cell line. While the parent compound **158** showed a 4/4 cure rate (i.p. dose of 4 × 20 mg/kg) in the STIB900 mouse model that mimics the stage-I of the disease, the *N*-hydroxy derivative **159** was only moderately active through i.p. administration.

6.4. Natural Product Derived Compounds

2-Arylpaullones as antitrypanosomal agents was reported by Ryczak et al.[326] The initial set of 2-arylpaullones tested possessed good activity against *T. b. rhodesiense* bloodstream parasites, but they were also cytotoxic against human THP-1 macrophages. Further SAR studies on the 2-arylpaullones led to compounds with good potency against *T. b. rhodesiense* and selectivity over THP-1 macrophages. The most active compound in this series **160** (Figure 23) displayed an activity of 0.51 μM against *T. b. rhodesiense* with a selectivity index of 157 fold over human THP-1 cells. Animal studies were not carried out, and the mode of action of these compounds is unknown.

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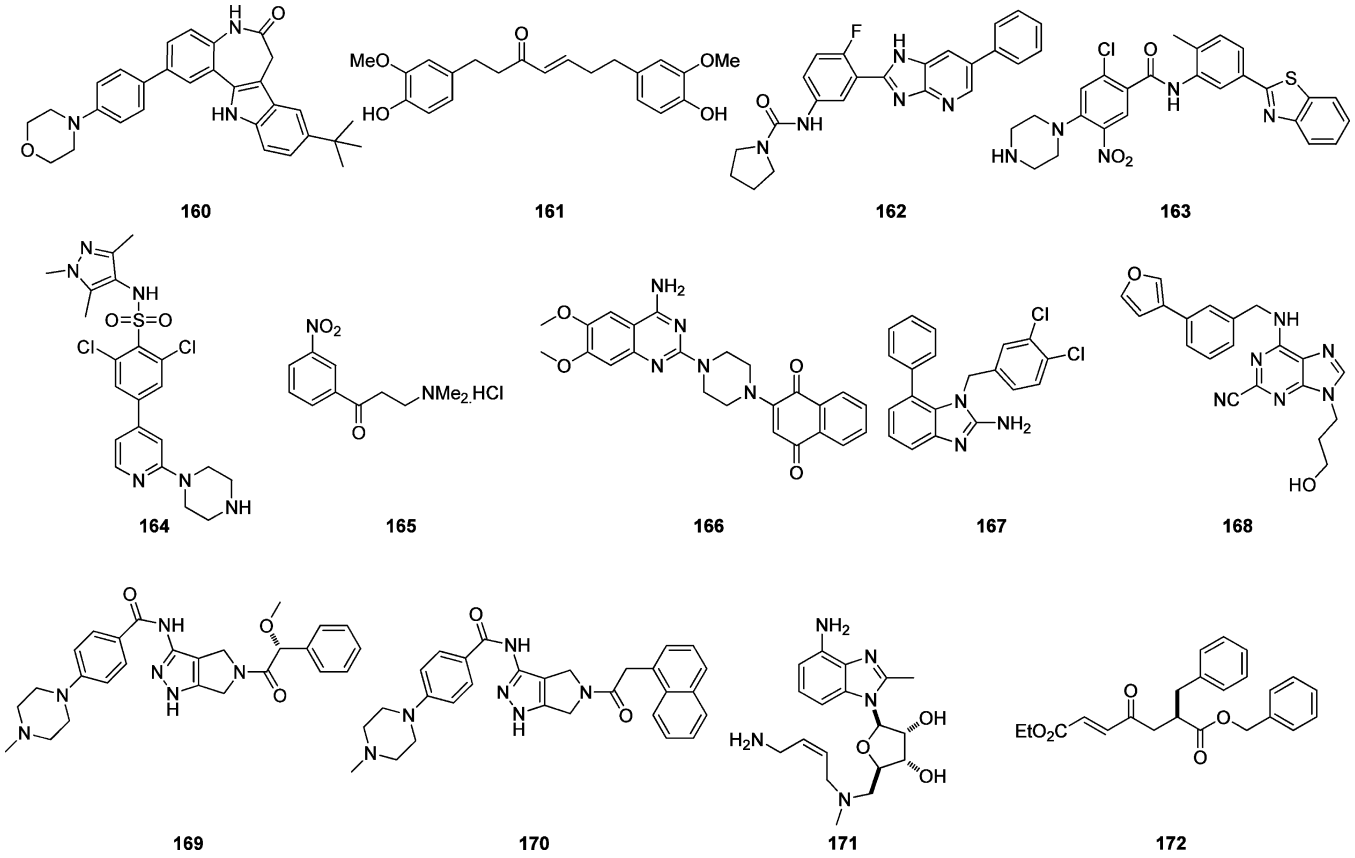

Figure 23. Miscellaneous anti-HAT compounds.

Inhibition of *T. brucei* by curcuminoid analogs was reported by Changtam et al.[327] The naturally occurring curcuminoids exhibited low potency against *T. brucei*. To enhance the activity the authors made several structural modifications to these curcuminoids to get 43 different analogs. Thirteen compounds from this library displayed submicromolar activity, notably compound **161** with an IC_{50} of 0.053 μM against *T. b. brucei*. Compound **161** was equally potent against *T. b. brucei* strains resistant to diamidines and melaminophenyl arsenical drugs. In addition the compound exhibited a selectivity index of 453-fold over the human embryonic kidney (HEK) cell line.

6.5. Lead Structures Resulting from Phenotypic Screens

A high throughput screen was carried out at the Genomics Institute of the Novartis Research Foundation designed to identify new small molecules with antiparasitic activity toward *T. brucei* within a library of 700 000 compounds.[328] Substituted 2-phenyl-imidazopyrines from this screen were studied in detail. Several compounds in this series including compound **162** (Figure 23) blocked *T. b. brucei* growth with an IC_{50} in the 2−4 nM range. Compound **162** showed good penetration into the brain which may translate into a drug candidate for stage-II infection. This compound displayed excellent oral pharmacokinetics in mice and cured mice of stage-I *T. brucei* infection when dosed twice a day at 5 mg/kg orally for 5 days.[328] A similar lead was found independently by Ferrins et al. by a high throughput phenotypic screen of 87 00 compounds for growth arrest of *T. brucei*.[329] The target for these compounds is not yet known.

Hwang et al. reported the optimization of chloronitrobenzamides found in a phenotypic screen against *T. brucei*.[330] Compound **163** blocked *T. brucei* growth in vitro with an IC_{50} in the 2−10 nM range and did not inhibit mammalian cell growth at micromolar concentrations. This compound showed excellent stability to liver microsomes in vitro. No in vivo data of antiparasite efficacy was reported, and the mechanism of action of these compounds is not known.

6.6. Target Based Approaches for HAT

RNA interference knockdown studies suggested that *T. brucei* *N*-myristoyltransferase is a valid drug target as a decrease in this enzyme lead to parasite growth arrest in vitro and a negation of infectivity of parasites in mice.[331] Brand et al. reported the optimization of an *N*-myristoyltransferase inhibitor discovered via high throughput screening.[332] Compound **164** with an in vitro IC_{50} of 2 nM against *T. brucei* was identified. The compound cured rodents of stage-I infection with *T. b. rhodesiense* and *T. b. brucei* after oral dosing. Overexpression of *N*-myristoyltransferase in parasites leads to a shift of IC_{50} to higher concentration thus providing strong evidence that this enzyme is the target of these compounds. Also, compound **164** blocked incorporation of radiolabeled myristic acid into parasite proteins. Unfortunately, these compounds do not enter the brain and thus cannot be developed as stage-II drug candidates.

Gelb, Hamilton, Buckner, Van Voorhis, and their co-workers reported extensive work on *T. brucei* farnesyltransferase inhibitors as antiparasite agents.[333] This enzyme attaches 15-carbon farnesyl groups to the C-terminus of a specific set of parasite proteins (human cells contain a similar enzyme). Farnesyltransferase inhibitors have been extensively developed by pharma as anticancer drug candidates, and thus a wealth of farnesyltransferase inhibitors are available for repurposing to treat HAT. Unfortunately, after extensive studies, inhibitors that are potent on the parasite enzyme could not be modified

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to improve pharmacokinetic properties. Furthermore, farnesyltransferase inhibitors with good pharmacokinetic properties in humans and that entered anticancer clinical trials were not potent on the *T. brucei* ortholog. The reasons for this interspecies inhibitor specificity is not apparent since almost all of the residues in the parasite enzyme seem to be conserved with those in the active site of human farnesyltransferase, for which a crystal structure is available.

Trypanothione reductase has been extensively studied as a drug target for HAT. Recent work in this area by Martyn et al. involved a high throughput screen against a library of 134,500 compounds.[334] One compound from this work is **165** with an IC_{50} of 0.68 μM against *T. brucei* and a 59-fold selectivity for trypanothione reductase over human glutathione reductase. No in vivo studies for these compounds were reported.

Cavalli et al. reported the antitrypanosomal activity of quinazoline derivatives that target trypanothione reductase, a flavoenzyme essential for the parasite survival.[335] The authors reported several low micromolar quinazoline based inhibitors for *T. b. rhodesiense* which also inhibited the enzyme in vitro. The best compound in the series **166** had a potency of 0.12 μM against bloodstream *T. brucei rhodesiense* and a 23-fold selectivity over mammalian L6 cells.

Using virtual screening, Mpamhanga et al. identified two scaffolds for the inhibition of the *T. brucei* pteridine reductase 1 (*Tb*PTR1), an enzyme essential for parasite survival.[336] On the basis of the crystal structure of one of these compounds bound to the enzyme, further analogs were designed to increase the potency, selectivity and favorable physicochemical properties. To fill the hydrophobic pocket near the binding site, a phenyl group was added to the parent structure to get compound **167**. This compound displays an apparent K_I value of K_i^{app} 0.007 μM on the pteridine reductase 1 and was 100-fold more active than the parent compound and displayed good selectivity over human versus parasite enzyme. However, this compound displayed poor inhibition of *T. brucei* cell growth in culture with IC_{50} of 10 μM and no animal data was reported.

Mallari et al. reported purine-derived nitriles as antitrypanosomal agents by targeting the trypanosomal cathepsin B.[337] Through a structure guided lead development, inhibitors of this enzyme with good selectivity for the parasite enzyme over human cathepsins B and L was reported. The most potent compound in the series compound **168** had an in vitro IC_{50} of 0.46 μM against *T. brucei* cathepsin B and 0.03 μM against rhodesain, a trypanosomal cathepsin L-type protease. Further it also possesses selective trypanocidal activity with an IC_{50} of 0.56 μM against *T. brucei*. No animal data was reported.

Ochiana et al. reported the repurposing of a human Aurora kinase inhibitor scaffold for specifically targeting trypanosomal Aurora kinase 1.[338] An SAR investigation was done on an established human Aurora kinase inhibitor **169** by focusing on decreasing the activity against the acute myelogenous leukemia cell line (MOLT-4) and maintaining the activity against *T. brucei rhodesiense*. The study yielded compounds with selectivity indices ranging from 2- to 23-fold. Compound **170** was the most selective with a potency of 0.61 μM against *T. brucei rhodesiense* and a selectivity ratio of 23 against MOLT-4. No animal studies were reported.

Hirth et al. reported the antitrypanosomal activity of the base modified adenosine derivatives that target S-adenosylmethionine decarboxylase (AdoMetDC), an enzyme that is essential in the synthesis of polyamines critical for trypanosomes survival.[339] The 8-methyl adenosyl derivative **171** was the most active compound in this series with an IC_{50} of 0.001 μM against *T. brucei rhodesiense* and 0.027 μM against *T. brucei brucei*. This compound was observed to possess good blood brain barrier penetration based on an intraperitoneal administration study on mice.

Peptidic Michael acceptor-based inhibitors of trypanosomal cysteine proteases, called rhodesain, exhibiting antitrypanosomal activity were reported by Breuning et al.[340] A library of 45 fumaric acid-based peptidic analogs containing Asn, Gln, or Phe residues were synthesized and tested against rhodesain from *T. b. rhodesiense*. In general it was observed that the E isomers were more potent than the corresponding Z isomers, and most of the compounds in this series were nontoxic to mammalian macrophages. The most active compound in this series against *T. brucei brucei* was **172** with IC_{50} of 0.25 μM against *T. b. brucei* and a K_I of 7.6 μM against rhodesain. No animal studies were reported.

7. CONCLUDING REMARKS

In summary, there has been significant progress in the treatment of both leishmaniasis and HAT during the past decade. Newly introduced VL treatments, which include paromomycin, miltefosine, geographic extensions of liposomal amphotericin B, and various drug combinations, have substantially improved options for patients affected by VL. This has been especially critical for treating VL cases in the state of Bihar, India, where resistance toward pentavalent antimonials is widely spread. Similarly, the treatment of stage II HAT patients dramatically improved in recent years as the result of the introduction of nifurtimox-eflornithine combination therapy (NECT). There is insufficient data to firmly establish the clinical efficacy of various regimens used for treatment of CL. Many of these infections are self-healing and the decision to initiate treatment is typically determined by the nature of lesions and risk of developing MCL.

However, in spite of this recent progress, new drugs for both leishmaniasis and HAT are still urgently needed. Treatment options for patients with VL in East Africa, HIV-VL coinfections, and those with PKDL diagnosis are still inadequate, and new drugs that are inexpensive, orally bioavailable, short acting, and do not require hospitalization, would dramatically improve the treatment of VL patients in endemic areas. For HAT, the current treatment options are even more limited, thus making the situation dire. The current target profile necessitates for drug candidates to be effective against both stage I and stage II disease. This makes the task scientifically challenging as only a small percentage of chemical leads have the potential to penetrate the BBB. Currently, the number of infected individuals is uncertain and probably lower than during other times because of public health campaigns. However, even with the low numbers, HAT disease figures in the top 10 of diseases responsible for loss of life and productivity in the African continent.[341]

The drug pipelines for both diseases are very thin: very few compounds are in development and drug discovery efforts are limited. There are only two compounds in clinical trials for HAT (nifurtimox, SCYX-7158) and one for VL (nifurtimox) making the need for enriching the pipeline with novel chemical entities of critical importance.

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Notes

The authors declare no competing financial interest.

Biographies

Advait Nagle immigrated from Mumbai, India to the University of South Florida where he earned his Ph.D. under Professor Kyung Woon Jung. Upon graduation, Adi completed his postdoctoral studies under the mentorship of Nathanael Gray and Peter Schultz, where he made contributions in the field of kinase drug discovery. His current focus in medicinal chemistry has been in the arena of infectious diseases and oncology. He was a team member for the GNF malaria program which led to the discovery of KAF156, a novel small molecule being evaluated in clinical trials. Adi leads a chemistry effort to discover new molecules to treat leishmaniasis. His research interests include phenotypic drug discovery in the field of infectious diseases.

Shilpi Khare studied Biochemistry and Molecular Biology (B.A.) at the University of California at Berkeley. She then received her Ph.D. in Biochemistry and Molecular Biology from the University of California at Los Angeles (UCLA, Class of 2011) where she worked under the direction of Dr. Steven G. Clarke to characterize the interplay between insulin signaling and protein repair in longevity and stress resistance in the soil nematode *Caenorhabditis elegans* (*C. elegans*). She was nationally acknowledged for her collaborative work exploring the mechanisms by which small amounts of ethanol extend lifespan in *C. elegans*. Currently, Shilpi is working as a postdoctoral fellow at the Genomics Institute of the Novartis Research Foundation (GNF), where she has applied her knowledge in metabolism and signal transduction and experience in pharmaceutical drug discovery to uncover novel drug targets for the treatment of neglected parasitic diseases. Shilpi enjoys exploring the world of chemistry as a member of the American Chemical Society (ACS) and Association for Women in Science (AWIS).

Arun Babu Kumar was born in Chennai (India), where he received his bachelor's and master's degree in Chemistry from the University of Madras and Anna University, respectively. Upon graduation, he worked as a research associate at the Unilever research center in India. In 2006, he moved to the U.S. to pursue his graduate studies at the University of South Florida under the guidance of Dr. Roman Manetsch, where he worked on developing ambient light stable diazirine photolabels and received his Ph.D. in 2012. In 2013, he joined Dr. Michael Gelb's lab at the University of Washington as a postdoctoral researcher, where his research focuses on developing molecular probes for the early detection of lysosomal storage disorders (LSD) in newborns.

Frantisek Supek is currently a Senior Research Investigator at the Genomics Institute of the Novartis Research Foundation in San Diego. Early in his scientific career Frantisek studied structure and function of vacuolar ATPases and metal ion transporters in the laboratory of Nathan Nelson (Roche Institute of Molecular Biology, Nutley, NJ) and vesicular protein trafficking in the budding yeast, *Saccharomyces cerevisiae* (the laboratory of Randy Schekman, Howard Hughes Medical Institute, UC Berkeley, CA). During the past 7 years his research has focused on new drug discovery for infectious diseases caused by kinetoplastid parasites *Leishmania donovani* and *Trypanosoma cruzi* and on determination of mechanism of action of new antiparasitics.

Andriy Buchynskyy obtained his Master's degree in Chemistry from Lviv National University (Ukraine) in 1995. In 2001 he received a Ph.D. in Organic/Medicinal Chemistry from Leipzig University (Germany) under the supervision of Prof. Peter Welzel for the work on synthesis of biochemical tools based on antibiotic moenomycin A. He then conducted postdoctoral work in the Prof. Chi-Huey Wong's laboratory at The Scripps Research Institute (2002−2004). In 2004 he joined ChemDiv Inc. (San Diego, USA) as a research scientist and worked on combinatorial libraries synthesis. Since 2011 he is a postdoctoral researcher in the group of Prof. Michael H. Gelb at University of Washington, working on the antitrypanosomal drugs discovery.

Casey J. N. Mathison was born and raised on the island of Maui, Hawaii and went on to receive his B.Sc. degree in Chemistry from the Massachusetts Institute of Technology in 2002. He received his Ph.D. from The Scripps Research Institute in 2007 under the mentorship of Prof. K. C. Nicolaou and was the recipient of a Bristol-Myers Squibb Graduate Fellowship in Synthetic Organic Chemistry. His dissertation examined the synthesis of β-tricarbonyl natural products and methodologies pertaining to hypervalent iodine(V) reagents. Upon graduation, he subsequently joined the Medicinal Chemistry department at Exelixis, and in 2010, he then went on to join the Medicinal Chemistry department at GNF. In his professional career, he has conducted research in a range of therapeutic areas, including autoimmune and inflammatory diseases, cardiovascular and metabolic disorders, infectious diseases, and oncology.

Naveen Kumar Chennamaneni was born in Telangana State, India. He received his B.Sc. (Biology and Chemistry) and M.Sc. in Chemistry from Osmania University, Hyderabad, India. In 2006, he earned his Ph.D. in Organic Chemistry under the supervision of Dr. Sadagopan Raghavan at Indian Institute of Chemical Technology (Hyderabad), during which he developed new synthetic methodologies for the synthesis of bromosulfonamides from unsaturated sulfilimines. He worked as a Postdoctoral fellow in the research group of Professor Yong Sup Lee at Kyung Hee University, Seoul, South Korea. He began his current appointment as a Research Associate at University of Washington, under Professor Michael H. Gelb in 2008. His research work involves antiparasite drug discovery and developing newborn screening methods for lysosomal storage diseases.

Nagendar Pendem is a native of Miryalguda, State of Telangana, India. He obtained his B. Sc. (1997) and M. Sc. (1999) from Osmania University, Hyderabad. He received a Ph.D. (2008) as a CSIR Research Fellow at the Indian Institute of Chemical Technology (IICT), Hyderabad, supervised by Dr. G. V. M. Sharma. During his Ph.D. studies, he worked on the design and synthesis of non-natural carbo-β-amino acids with novel helical structures. Then he worked for few months as a Research Scientist on drug development in Ogene Systems (I) Pvt. Ltd, Hyderabad, India. He started his postdoctoral research career at the IBMC, University of Strasbourg, France (2009) and IECB, University of Bordeaux, France (2009−2010) with Dr. Gilles Guichard, where he worked on the synthesis of novel urea oligomers. From 2011 onwards he has been working as a Postdoctoral Research Associate on the development of antiparasite drugs at the University of Washington, Seattle with Prof. Michael H. Gelb.

Fred Buckner, MD, is an infectious disease specialist and molecular parasitologist. His research concentrates on drug discovery for diseases caused by pathogenic protozoa. These include *Trypanosoma cruzi* (the cause of Chagas disease), *Trypanosoma brucei* (the cause of African sleeping sickness), *Leishmania* species (the cause of leishmaniasis), and *Plasmodium falciparum* (the cause of malignant malaria). His lab focuses mainly on several biochemical targets for developing antiparasitic drugs including sterol biosynthesis, protein prenylation, protein synthesis, and protein kinases.

Michael H. Gelb received his Ph.D. from Yale University working on cytochrome P450 with Stephen G. Sligar. He was a postdoctoral fellow with Robert H. Abeles (Brandeis University) working on inhibitors of proteases. In 1985 he started his own lab at the University of Washington where he works on medicinal enzymology. His major research accomplishments include: (1) Development of methods to evaluate interfacial enzymes, mainly phospholipases A2, at the lipid–water interface; (2) Discovery of protein prenylation; (3) Co-developer of the ICAT reagents for quantitative proteomics; (4) Development of several advanced drug leads for the treatment of parasitic infections; (5) Developed tandem mass spectrometry for newborn screening of lysosomal storage diseases (used worldwide).

Valentina Molteni is currently a Director of Chemistry at the Genomics Institute of the Novartis Research Foundation (GNF) in San Diego. She received her Ph.D. in Organic Chemistry in 1997 from the University of Milan in Italy and was a postdoctoral scientist at the University of California San Diego working on the identification of HIV integrase inhibitors. In her medicinal chemistry career first at Dupont Pharmaceutical and then at GNF since 2002, Valentina has been involved in many drug discovery programs delivering development candidates for a variety of indications including infectious, respiratory, cardiovascular, liver diseases and oncology.

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