

New Approaches to the Development of Anti-Protozoan Drug Candidates: a Review of Patents

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As doenças causadas por protozoários afetam hoje em dia uma grande parcela da população mundial, provocando muitas mortes e exercendo grande influência na qualidade de vida e no desenvolvimento de muitos países. Essas doenças afetam principalmente países pobres e por isso, a pesquisa e o desenvolvimento de novos fármacos são negligenciados. De fato, a maioria das drogas usadas no tratamento dessas doenças data de décadas passadas e apresentam muitas limitações, incluindo o aparecimento da resistência às drogas. Este artigo tem como foco os mais recentes desenvolvimentos publicados no campo de patentes, entre 2001-2008, com especial atenção a promissores compostos atuando contra tripanossomiase, leishmaniose, malária, toxoplasmose, amebíase, giardíase, balantidíase e pneumocistose.

Protozoan infections are parasitic diseases that affect hundreds of millions of people worldwide, but have been largely neglected for drug development because they affect poor people in poor regions of the world. Most of the current drugs used to treat these diseases are decades old and have many limitations, including the emergence of drug resistance. This review will focus on the most recent developments, from 2001 to 2008, published in the field of patents and publications, paying particular attention to promising compounds acting against trypanosomiasis, leishmaniasis, malaria, toxoplasmosis, amebiasis, giardiasis, balantidiasis and pneumocystosis, their chemistry and biological evaluation, and to new chemical and pharmaceutical processes.

Keywords: neglected disease, potential antiprotozoan compounds, patents

1. Introduction

Neglected parasitic diseases are a group of tropical infections which are especially endemic in low-income populations in developing regions of many countries. Despite affecting millions of people around the world, causing many deaths and having a great and limiting influence on the quality of life, the selection of new molecular targets and the development of more efficient drugs against neglected diseases are scarce (Table 1).

Nowadays, among them, zoonotic diseases attract much attention. Those are defined as diseases shared by animals and humans. In fact, wildlife serves as a reservoir for many diseases common to domestic animals and humans. Generally, disease is more easily prevented than treated. This discussion reviews common zoonotic diseases, including those ailments that are often erroneously cited as being closely linked to wildlife.

Several species of protozoans infect humans and inhabit the body as commensals or parasites. Protozoa have traditionally been divided based on their means of locomotion, although this character is no longer believed to represent genuine relationships:^{2,3} (i) Flagellates (Zoomastigophora): Flagellates are characterized by having one or more flagella. Parasitic species generally have more flagella than those that are free living. Pathogens: *Giardia intestinalis*, *Trichomonas vaginalis*, *Trypanosoma cruzi*, *Leishmania donovani*; (ii) Amoebae (actinopods, rhizopoda): Amoebae may be divided into several morphological categories based on the form and structure of the pseudopods. It can live in a number of places within the human body, but most are found in the intestine. Those in which the pseudopods are supported by regular arrays of microtubules are called actinopods, and forms in which they are not, are called rhizopods, further divided into lobose, filose, and reticulose amoebae. Pathogens: *Entamoeba histolytica*; (iii) Sporozoans (Apicomplexa, myxozoa, Microsporidia): The members of this group share

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Table 1. The main neglected parasitic diseases¹

Disease	Organism	Scope	Therapy needs
Malaria	<i>Plasmodium</i> spp.	500 million infections annually	Circumventing drug resistance
Leishmaniasis	<i>Leishmania</i> spp.	2 million infections annually	Safe, orally bioavailable drugs, especially for the visceral form of the disease
Rypanosomiasis (sleeping sickness, Chagas' disease)	<i>T. brucei</i> (sleeping sickness); <i>T. cruzi</i> (Chagas' disease)	HAT: 300,000 cases annually Chagas: 16 million existing infections	Safe, orally bioavailable drugs, especially for the chronic phases of disease
Schistosomiasis	<i>Schistosoma</i> spp	> 200 million existing infections	Backup drug should resistance to praziquantel arise
Giardiasis	<i>Giardia lamblia</i> ;	Millions of cases of diarrhea annually	Well-tolerated drugs
Amebiasis	<i>Entamoeba histolytica</i>	Millions of cases of diarrhea annually	Well-tolerated drugs
Pneumocystosis	<i>Pneumocystis carinii</i> or <i>Pneumocystis jiroveci</i>	-	Trimethoprim-sulfamethoxazole often in conjunction with corticosteroids

HAT: Human African trypanosomiasis.

an “apical complex” of microtubules at one end of the cell that many prefer to the old name of sporozoans. All the members of the phylum are parasites. They do not have a set body plan like the other parasitic protozoans, although they are characterized by having complex life-cycles with an alternation of sexual and asexual generations. The most well known of all the sporozoans are the organisms which cause the malaria disease -*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*- and pneumocystosis; (iv) Ciliates (ciliophora): These are larger protozoans, growing to over 100 µm. They have hundreds of tiny cilia which beat in unison to propel it through the water. Often cilia are fused together in rows or tufts (called cirri) and are used for special functions such as food gathering. In addition to locomotion, the Paramecium and other ciliates like the Stentor use cilia to sweep food down into their central channel or gullet. There is only one species of pathogenic ciliate known to parasitise humans: *Balantidium coli*; (v) Microsporidia: They are so difficult to diagnose, very little work has been done into their importance in human disease, although they are known to be a major cause of productivity loss in aquaculture facilities such as prawn farms. Microsporidia have also been implicated in causing disease in immunocompromised hosts.

In this paper, we focused on some diseases, such as trypanosomiasis, leishmaniasis, toxoplasmosis, giardiasis, amoebiasis, balantidiasis and malaria. In this context, we believe that a review of patents on the highlighted inventions and innovative ideas in this field could be of importance for scientists, managers and decision-makers in the pharmaceutical industry.

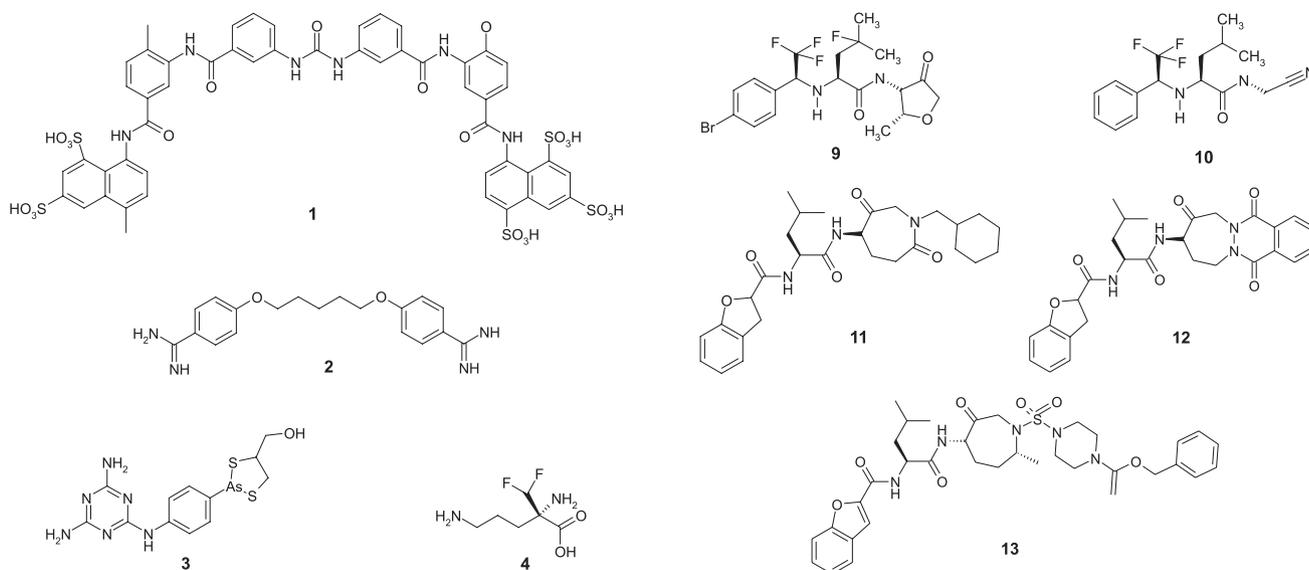
2. Targeting New Anti-Protozoan Drug Candidates

2.1. Treatment of African trypanosomiasis

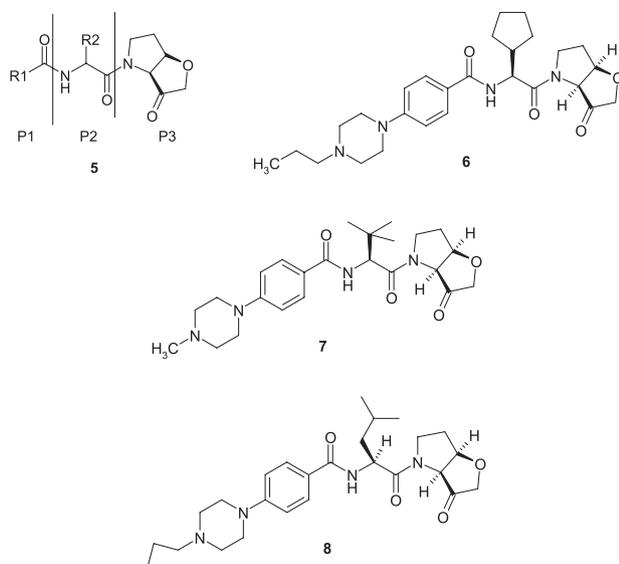
African trypanosomiasis is a parasitic disease of people and animals, caused by protozoa of the species *Trypanosoma brucei gambiense* or *T. brucei rhodesiense* and transmitted by the tsetse fly bite. The fly is infected when it bites an infected host and the trypanosome develops in the vector, culminating in infection transmission by its saliva.

The chemotherapy of Human African trypanosomiasis (HAT) is restricted to the clinically approved drugs: suramin (1), pentamidine (2), melarsoprol (3) and eflornithine (4). Suramin and pentamidine are restricted to the treatment of early-stage HAT prior to central nervous system (CNS) infection, while melarsoprol is used once the parasites have penetrated the CNS. Eflornithine is not effective against rhodesiense infections. These drug regimens can cause toxicity and poor efficacy. Thus, the development of novel compounds to provide novel treatment against HAT is necessary.

The major lysosomal cysteine proteinase of HAT is a candidate target for novel chemotherapy for sleeping sickness. Lysosomal cysteine proteases, generally known as the cathepsins, were discovered in the first half of the 20th century. Quibell and Watts,⁴⁻¹⁰ at the Amura Therapeutic Ltda, described a series of tetrahydrofuro [3,2-*b*]pyrrol-3-one analogues as cathepsin K inhibitor. Cathepsin K exhibits the highest capability to degrade components of the extracellular matrix.¹¹ Compounds of general formula 5 may be conveniently considered as a combination of

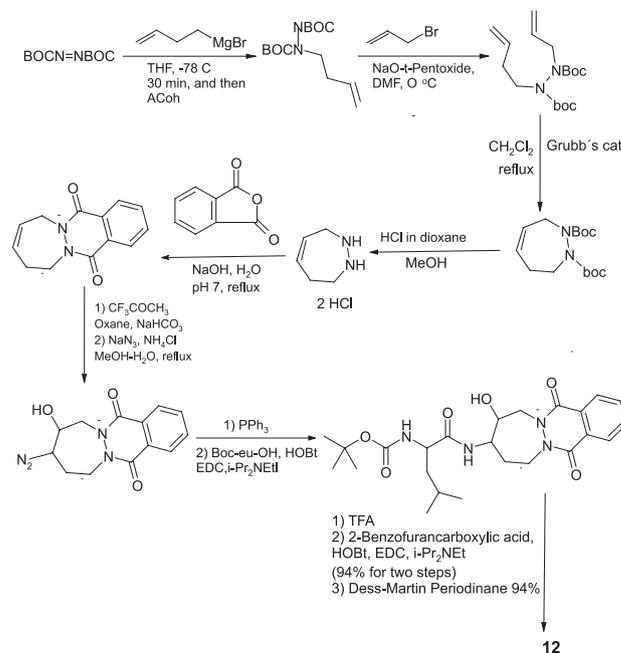


three building blocks (P1, P2, and P3) that respectively occupy the S1, S2 and S3 binding sites of the protease. The compounds **6**,⁹ **7**¹⁰ and **8**⁸ exhibited more than 80% inhibition at a concentration of $1 \mu\text{mol L}^{-1}$.



Cysteine protease inhibitors were also described by Merck Frosst Canada Ltd. for the treatment of trypanosomiasis.¹²⁻¹⁶ Compounds such as **9** and **10** were claimed in the patent, however no biological data were presented. In addition, SmithKline Beecham Corporation described a novel substituted 3,7-dioxo-azepan-4-ylamide,¹⁷ diazepino[1,2-*b*]phthalazine **11**,¹⁸ oxo-amino-sulfonylazapanes **12**,¹⁹ 5-substituted-6-oxo-[1,2]diazepane, **13**,²⁰ their pharmaceutical compositions, processes for their preparation and methods of their use for inhibiting a protease, particularly a serine protease and a cysteine protease such as cathepsin K and falcipain, and for the

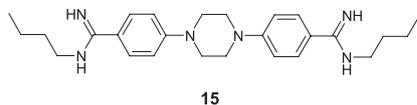
treatment of parasitic infections including trypanosomiasis. For instance, the compound **12** is obtained according to the scheme 1.



Scheme 1.

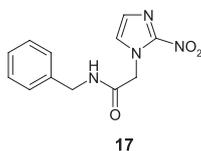
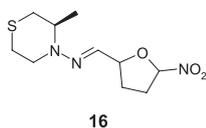
Based on the **2** structure, Xavier, University of Louisiana, disclosed bisbenzamides and bisbenzamidoximes analogs as compounds effective against infection by parasitic hemoflagellates, in particular *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*.²¹ It has been postulated that aromatic bisbenzamides are selectively taken up by transporters into trypanosomes, where they bind strongly to DNA in the nucleus and mitochondria, inhibiting the topoisomerases II, thus the synthesis of DNA, RNA, and proteins by the parasites will

be inhibited.²² In mice infected with *T. brucei*, treatment with the compound **15** at a dose of 5 mg kg⁻¹ per day resulted in a mean survival in days, of 18 (excluding cured animals) and 5 of the 6 treated animals were cured.



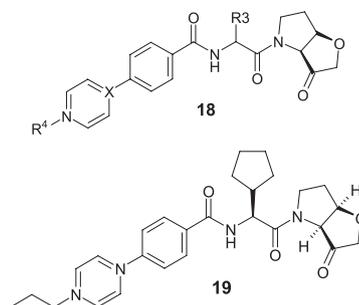
2.2. Treatment of American trypanosomiasis

American Trypanosomiasis is a tropical parasitic disease caused by the flagellate protozoan *Trypanosoma cruzi*. The disease may also be spread through an insect vector, blood transfusion and organ transplantation, parasite contaminated food ingestion, and from a mother to her fetus. Nifurtimox (**16**) or benznidazole (**17**) is used in the acute Chagas disease phase and in reactivation in immunosuppressed patients. Another irrefutable sign refers to the treatment of laboratory accident victims, which demands a very early start for the administration. The action mechanism of **17** may depend on the formation of nitro anion radicals, but precise details are not known. Little is also known regarding mode of action of benznidazole. Because of the adverse events associated with these medications, patients being treated require careful monitoring. In a patent application, Reed and co-workers,^{23,24} at the Infectious Disease Research Institute, claimed a fusion protein useful for the diagnosis of *Trypanosoma cruzi* infection within a biological sample. More specifically, the invention is related to the use of *T. cruzi* antigenic polypeptides and fusion polypeptides (TcF and ITC-6) in methods for screening individual and blood supplies for *T. cruzi* infection. Also included are details of a kit which uses the invention to detect *T. cruzi* involving the fusion protein. It is a protein created through the joining of two or more genes which originally coded for separate proteins. The reactivity of sera from *T. cruzi*-infected individuals and control sera from non-infected individuals against the fusion polypeptides TcF and ITC-6 was determined by ELISA. The data show that the invention is capable of recognizing sera that are negative or low with TcF.^{23,24}

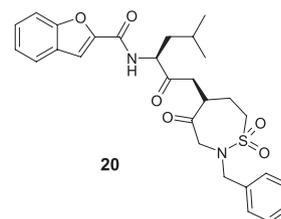


Quibell and Watts, at Amura Therapeutics Limited, described novel tetrahydrofuro[3,2-*b*]pyrrol-3-one compounds (**18**), their salts, hydrates, complexes or

prodrugs. A process for their preparation, compositions comprising them and their use as inhibitors of CAC1 cysteine proteases, particularly cathepsin K, in the treatment of Chagas disease were also described.⁴⁻¹⁰ Compound **18** exhibited good primary DMPK (dystrophia myotonica-protein kinase) properties along with promising activity in an *in vitro* cell-based human osteoclast assay of bone resorption. Compound **19** exhibited >80% inhibition at a concentration of 1 μmol L⁻¹. In addition, hexahydrofuro[3,2-*b*]pyridine-3-one, hexahydro-2-oxa-1,4-diazapentalene and hexahydropyrrolo[3,2-*c*]pyrazole were also synthesized and pharmacologically tested.⁴⁻¹⁰ *In vitro* activity was observed against a range of CAC1 cysteinyl proteinases, however tetrahydrofuro[3,2-*b*]pyrrol analogs showed a better *in vitro* activity value.²⁵



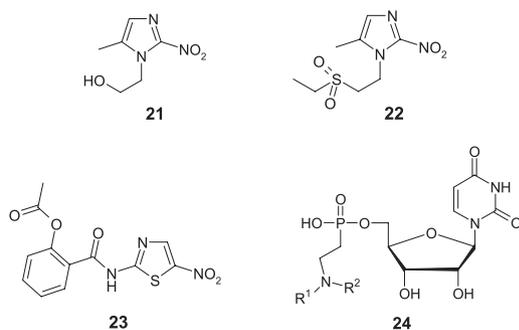
In a further patent application with cathepsin K inhibitors against Chagas disease or American trypanosomiasis, SmithKline Beecham Corporation reported new promising trioxo-[1,2]thiazepanylamide derivatives.²⁶ These compounds are substituted asymmetrical imidodicarbonimide diamides derived from hydroxylamines. They include the structure **20** as one of several compounds specifically claimed, but biological data are not presented in this patent.



2.3. Treatment of giardiasis

Giardiasis is a disease caused by *Giardia lamblia* and only the cyst form is infectious by the oral route. People catch *Giardia* by eating food or drinking water which has been contaminated by the organism -usually from feces. When there is a lot of *Giardia* present, this generates inflammation, which causes nausea, stomachache and diarrhea.

Giardiasis has been recently included in the WHO Neglected Diseases Initiative.²⁷ *Giardia lamblia* infection in humans is frequently misdiagnosed. Multiple stool examinations are recommended, since the cysts and trophozoites are not consistently shed. Given the difficult nature of testing to find the infection, including many false negatives, some patients should be treated on the basis of empirical evidence; treating based on symptoms. Human infection is conventionally treated with metronidazole (**21**), tinidazole (**22**) or nitazoxanide (**23**).²⁸ Nitroimidazoles are the most effective drugs available and no drug was reported to be unsafe, causing only mild to moderate and transient side effects. Suk *et al.*²⁹ reported the design, synthesis, and activity of a potent and non-toxic second generation anti-giardial agent that was designed as an inhibitor of cyst wall synthesis. In the formation process of the *Giardia* cyst wall, the UDP-GalNAc is polymerized by cyst wall synthase into a polysaccharide, which, in conjunction with polypeptides, forms the filamentous outer cyst wall of *Giardia*.³⁰ The compound **24**, a phosphonoxin, was the more potent inhibitor of *Giardia* cyst formation and should have clinical potential as a new anti-giardia drug.

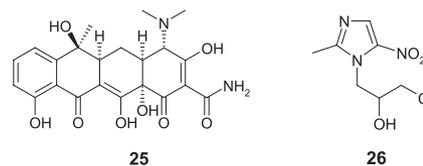


Currently (2001-2008), there are no patents of novel anti-giardiasis compounds. However, oligonucleotide molecules for detection of *G. lamblia* were disclosed by Macquarie Research Limited.³¹ Preferably, the oligonucleotide molecule hybridises specifically unique to 18S rDNA/rRNA sequences of *G. lamblia* under medium to high stringency conditions. The sample can be environmental, from water sources, from waste materials, from medical and body fluids.

2.4. Treatment of balantidiasis

Balantidiasis is a disease caused by the large ciliated protozoan, *Balantidium coli* and tetracycline or iodoquinol is usually used in the treatment. Human infection occurs when human contact with pigs is particularly close. Ingestion of cysts or trophozoites from the infected animal or human feces is the infectious form.

Balantidiasis disease is treated with metronidazole (**21**) or tetracyclin (**25**) for 5-10 days. It is usually effective, but without antibiotic mortality levels. In the treatment of acute balantidiasis, tetracycline is the drug of choice to eliminate *B. coli* trophozoites in humans. The disease is especially dangerous in immunocompromised patients as pulmonary parenchyma involvement is possible. *B. coli* is rare in Western Europe and North America and alternative drugs for the treatment of balantidiasis may be 5-nitroimidazole drugs such as tinidazole (**22**) and ornidazole (**26**).³² As well as in the case of *G. lamblia*, currently (2001-2008), there are no new patents for anti-balantidiasis drugs.

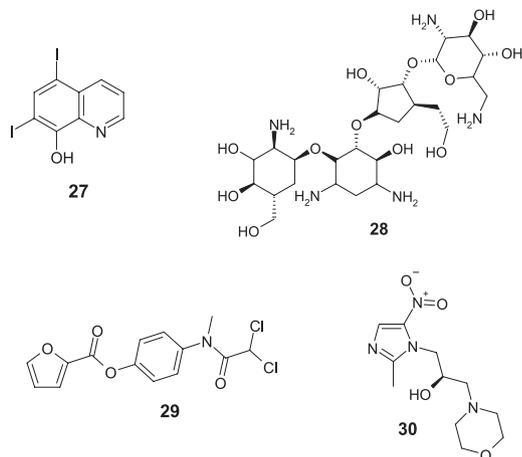


2.5. Treatment of amoebiasis

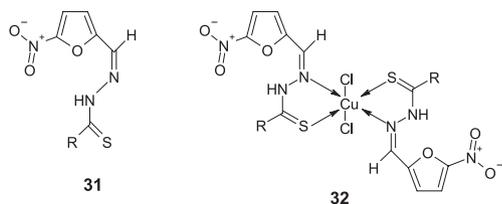
Amoebiasis is an infection of the intestine (gut) caused by an amoeba called *Entamoeba histolytica*, that are found in contaminated food or drink. Virulent strains such as *Entamoeba coli* and *Entamoeba hartmanni*, can produce mild diarrhea and dysentery. Only a few cysts are needed to cause infection. Amoebic cysts resist iodine and chlorine if concentration of these chemicals is too low.

Different drugs are available to treat amoebiasis. Oral antiparasitic medication is the standard treatment. For asymptomatic infections, iodoquinol (**27**) or paromomycin (**28**) are drugs of choice. For mild, moderate, or severe intestinal disease, and for extraintestinal infections (*e.g.*, hepatic abscess) the drugs of choice are metronidazole (**21**) or tinidazole (**22**), immediately followed by treatment with iodoquinol (**27**), paromomycin (**28**), or diloxanide furoate (**29**). Cen and Lv, Jiangsu Hansoh Pharmaceutical Co Limited, described novel optically pure α -substituted 2-methyl-5-nitroimidazole-1-ethanol derivatives and their use for the treatment of amoebiasis.³³ In an assay testing resistance activity in rats, compound **30** showed ED₅₀ value 7 mg kg⁻¹ against *Amoeba dysenteriae*, whereas metronidazole showed 25 mg kg⁻¹, and ornidazole showed 10 mg kg⁻¹.

Thiosemicarbazones and their metal complexes have been extensively studied during recent years due to their wide variety of biological activities.³⁴ Certain drugs even show enhanced activity when administered as their metal chelates. Copper(II) is a biologically active essential metal ion; its chelating ability and positive redox potential allow participation in biological transport reactions. Sharma *et al.*³⁴



reported the synthesis of 5-nitrofuran 2-carboxaldehyde thiosemicarbazones (**31**) and their subsequent bidentate Cu^{II} complexes (**32**). These compounds were screened for their anti-amoebic activities against *HK-9* strain of *Entamoeba histolytica* in *in vitro* experiments and found that the chelation induced significant changes in the biological activity of the ligands and some copper showed better IC_{50} value than metronidazole *in vitro*.

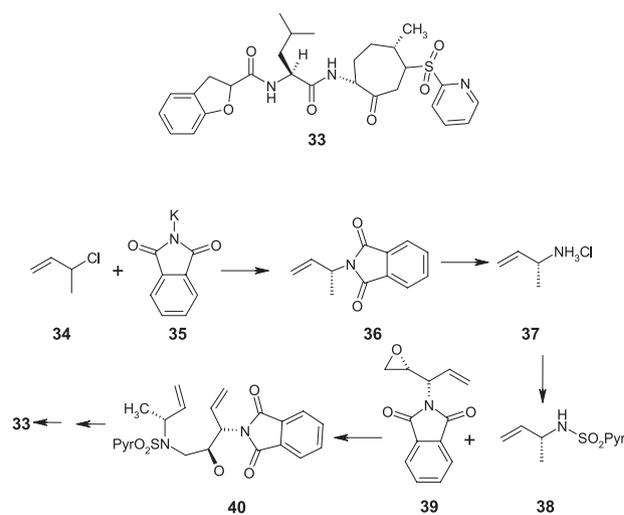


2.6. Treatment of *Plasmodium falciparum* infection

Malaria is a mosquito-borne disease caused by small, one-celled parasites called *Plasmodium* that infect and destroy red blood cells. Four different plasmodia can cause disease in humans: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax*. People get malaria from the bite of an infected mosquito. A mosquito can become infected when it bites a person who has malaria organisms in the blood.

The effectiveness of antimalarial drugs differs with different species of the parasite and with different stages of the parasite's life cycle. Drugs include chloroquine, mefloquine, primaquine, quinine, pyrimethamine-sulfadoxine (Fansidar), and doxycycline. Two aspects have stimulated new efforts regarding the development of chemotherapy, vaccines and sanitary studies on malaria: the resistance to currently available antimalarial drugs and the inefficacy of antimalarial vaccines.³⁵ Clark *et al.*,³⁶ at the SmithKline Beecham Corporation, described a process able to prepare commercial quantities of benzofuran-2-carboxylic

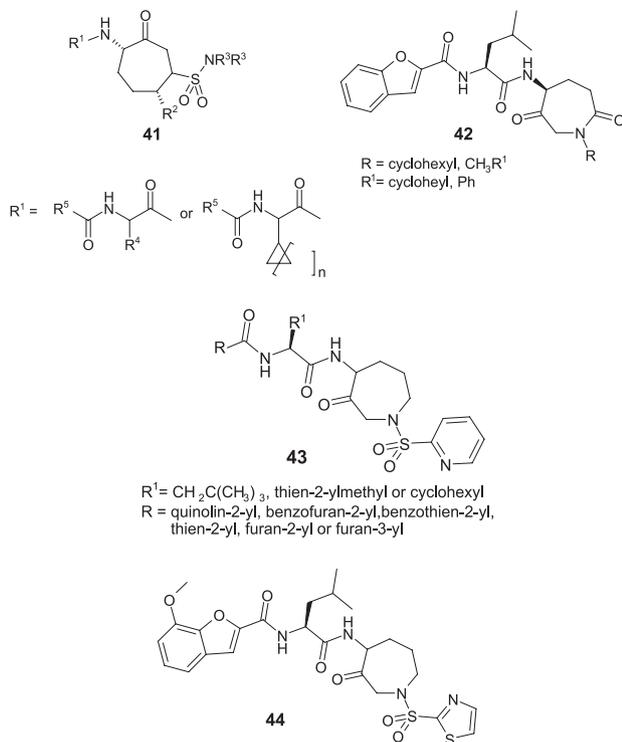
acid {(*S*)-3-methyl-1-[(4*S*,7*R*)-7-methyl-3-oxo-1-(pyridine-2-sulfonyl)-azepan-4-yl-carbamoyl]-butyl}-amide, **33**, and analogs. The method starting from the cycloaddition of 3-chloro-1-butene with potassium phthalimide in the presence of an alkali metal carbonate to form compound **36** *N*-(α -methylallyl) phthalimide. The compound **36** reacts with alkyldiamine followed by azeotropic distillation with ethanol and gaseous HCl treatment to give (*R*)-3-amino-1-butene hydrochloride, **37**. 2-Chlorosulfonyl pyridine is coupled with **37** in the presence of a thialkylamine base to form the pyridine sulfonamide **38**. Mitsunobu reactions of (2*S*,3*R*)-1,2-epoxy-4-penten-3-ol lead to the nitrogen-protected epoxide fragment **40**. The reaction of sulfonamide fragment **39** and epoxide fragment **40** provides **33**. There are twelve oxidation reaction steps to yield the final product (Scheme 2). The compound **33** is the Relacatib used for treatment of malaria.



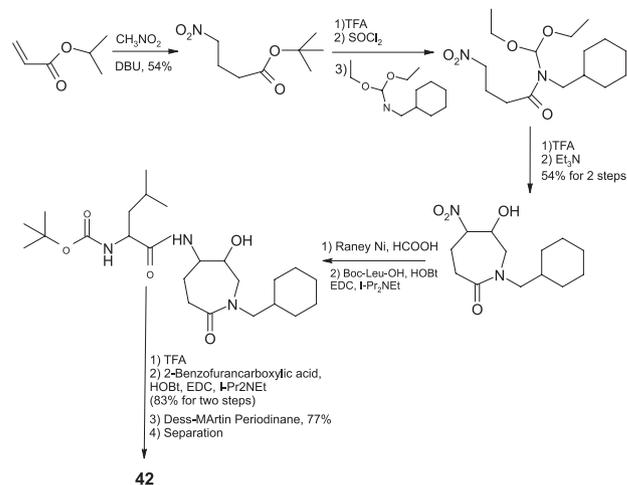
Scheme 2.

SmithKline Beecham Corporation has also patented new promising dihydrotriazine derivatives.³⁷ These compounds derived from oxo-amino-sulfonyl-azapanes **41** also are cathepsin K inhibitors and useful for the treatment of *P. falciparum* infection. Each R^2 is H, C_{1-8} alkenyl, C_{2-8} alkynyl, or substituted C_{1-4} alkyl. R^3 is independently H, C_{1-8} alkyl, C_{2-8} alkenyl, C_{2-8} alkynyl or substituted C_{1-6} alkyl. R^4 is H, optionally substituted C_{1-7} alkyl, C_{2-7} alkenyl, C_{2-7} alkynyl, C_{3-7} cycloalkyl, or C_{3-7} cycloalkenyl. R^5 is optionally substituted C_{3-7} cycloalkyl, C_{3-7} cycloalkenyl, aryl, heterocycloalkyl. Additionally, SmithKline Beecham Corp claimed novel dioxo-azepan (**42**)³⁷ and amino-azepan (**43**)³⁸⁻⁴³ derivatives and their salts, a process for their preparation, compositions containing them and their use in methods for inhibiting proteases, particularly cathepsin K and falcipain.³⁷⁻⁴⁴ For instance, the scheme for preparation of

the compound **42** with substituent CH_2 -cyclohexyl is shown in Scheme 3. Those compounds were tested for treating diseases characterized by bone loss such as osteoporosis, periodontitis and gingivitis, and diseases characterized by parasitic infections particularly malaria, trypanosomiasis and leishmaniasis. In the patent,⁴⁵ the Corporation related a method of treatment of parasitic diseases, especially malaria. The compound **44** exhibited a K_i value of 1.9 nmol L^{-1} against the falcipain cysteine protease.

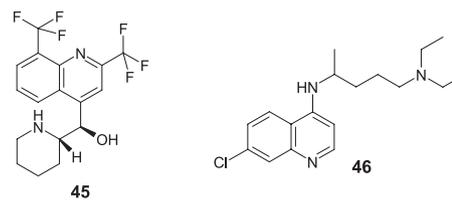


US Army Medical Research Institute of Infectious Diseases patented novel tricyclic compounds that reduced side effects and restore the clinical efficacy of antimalarial drugs, including mefloquine (**45**) and chloroquine (**46**).⁴⁶ Their salts and prodrugs, and their use as chemosensitizing agents and for modulating resistance to a drug, particularly an antimalarial drug is also claimed. IC_{50} values were determined for each candidate compound alone and in combination with chloroquine. The fractional inhibitory concentration indices (FIC, actual IC_{50} of one compound in the presence of a second compound but is expressed as a fraction of its IC_{50} when used alone) for 1:1 combinations were determined using a chloroquine-resistant *P. falciparum* W2 clone (Table 2). The combination of the compounds **47b** and **47e** and chloroquine showed the best MDR (multidrug resistance)-reversing activity. Chloroquine with compounds containing saturated (**47i** and **47m**) and unsaturated seven-membered central rings (**47q** and **47r**) possessed similar activity. The compounds **47s** and **47t** were

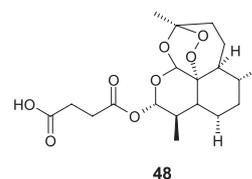


Scheme 3.

not as potent as their tricyclic ring counterparts. A FIC index of less than 1.0 represents synergy or potentiation and a FIC index greater than 1.0 represents antagonism (Table 2).



Mepha Pharma AG studied the administration of the combination of artesunate (**48**) and mefloquine (**30**) on humans with acute, uncomplicated *P. falciparum* malaria. Artemisinin and its derivatives are the most rapidly acting antimalarial drugs, however the duration of antimalarial activity is short.⁴⁷ Thus, in those studies, patients were randomized to receive accordingly in: Group A - artesunate 200 mg *per day* and mefloquine 250 mg *per day* simultaneously once daily for 3 days; Group B - artesunate 200 mg *per day* and mefloquine 750 mg (no dose on the 1st day, 250 mg on the 2nd day and 500 mg on the 3rd day) once daily for 3 days. The cure rate after 14 days and 28 days was monitored. The results show that group A had a 100% cure rate and group B had a cure rate of 98% after 14 days. Also in group A, the low incidence rate of early vomiting and overall vomiting was observed.

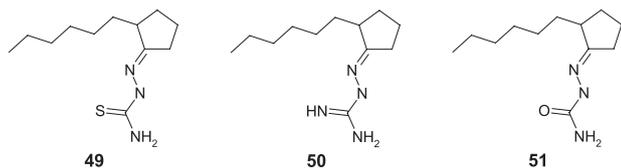


Three companies, Universidade de Lisboa, Cenix BioScience GmbH and Instituto De Medicina Molecular, reported the use of an inhibitor of a scavenger receptor

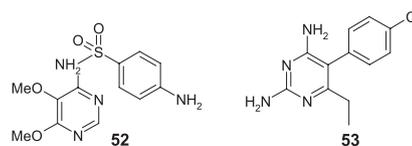
Table 2. FIC (fractional inhibitory concentration - 1:1 combination of drug and chloroquine) indices of new modulators in *P. falciparum* W2 clone

compound	X	n	Y	R ¹	R ²	FIC
47a	S	4	-	CH ₃	CH ₃	0.23
47b	S	4	-	C ₂ H ₅	C ₂ H ₅	0.23
47c	S	4	Pyrrolidinyl	-	-	0.21
47d	S	4	Piperidinyl	-	-	0.49
47e	S	4	Morpholinyl	-	-	0.39
47f	S	4	4-Methylpiperazinyl	-	-	0.40
47g	C ₂ H ₄	4	-	CH ₃	CH ₃	0.23
47h	C ₂ H ₄	4	-	C ₂ H ₅	C ₂ H ₅	0.39
47i	C ₂ H ₄	4	Pyrrolidinyl	-	-	0.32
47j	C ₂ H ₄	4	Piperidinyl	-	-	0.45
47k	C ₂ H ₄	4	Morpholinyl	-	-	0.31
47l	C ₂ H ₄	4	4-Methylpiperazinyl	-	-	0.52
47m	C ₂ H ₄	4	-	C ₂ H ₅	C ₂ H ₅	0.53
47n	C ₂ H ₄	4	Pyrrolidinyl	-	-	0.33
47o		4	-	C ₂ H ₅	C ₂ H ₅	0.48
47p		4	Pyrrolidinyl	-	-	0.45
47q	C ₂ H ₄	5	-	C ₂ H ₅	C ₂ H ₅	0.44
47r	C ₂ H ₄	5	Pyrrolidinyl	-	-	0.48
47s	C ₂ H ₄	6	-	C ₂ H ₅	C ₂ H ₅	0.48
47t	C ₂ H ₄	6	Pyrrolidinyl	-	-	0.57

class protein (ScarB1) for the therapy and/or prophylaxis of a malaria infection.⁴⁸ ScarB1 appears to mediate HDL-transfer and uptake of cholesterol. It was observed that inhibitors of ScarB1 function inhibit the growth of protozoa in liver cells, thus, inhibitors of ScarB1 can be used to treat infectious diseases involving liver cells and because the ScarB1 is expressed in erythrocytes, in hematopoietic cells. Human hepatoma cells were treated and the influence of the compounds on proliferation and infection with *Plasmodium* sporozoites was calculated as percentage of the plate mean for all samples, with the mean set to 100%. In each compound a score was assigned between 0 and 4 for inhibition of infection. The compounds **49**, **50** and **51** showed score 4 (corresponding to an IC₅₀ of 1 μmol L⁻¹ or lower), 1 (IC₅₀ between 3 and 4 μmol L⁻¹) and 0 (IC₅₀ between 4 μmol L⁻¹ or large), respectively.



Fansidar is a combination of sulfadoxin (**52**) and pyrimethamine (**53**) used for the treatment of chloroquine (**47**) resistant *P. falciparum*.⁴⁹ Council of Scientific and Industrial Research patented a kit of α,β-arteether (an artemisinin derivative), for increasing the sites of action on the parasites and thus will be more effective in controlling them. The result shows that intramuscular α,β-arteether (7.5 mg kg⁻¹) and the combination of sulfadoxin and pyrimethamine (5 mg kg⁻¹) produced 100% curative efficacy compared to the individual compounds alone.⁴⁹



The Holding Company For Biological Products And Vaccines developed a scorpion venom (*Pandinus imperator*) drug that has the ability to stop the development of asexual life cycle from ring to schizont stage.⁵⁰ Doses of the venom stimulated the immune system producing specific immune response against malaria parasite. The

venom is free of side effects and does not cause histamine release. Curiously, the scorpion venom, is used as a carrier to deliver radioactive iodine into tumour cells left behind after surgery, has removed the bulk of the tumour.

The *P. falciparum* erythrocytic stage causes several million deaths yearly, primarily in Africa. Thus, National Institutes of Health developed a novel vaccine formulation comprising a *P. falciparum* erythrocyte binding protein, BAEBL.⁵¹ BAEBL polynucleotide is used to induce an immune response to a *Plasmodium* parasite, because it has homology to EBA-175, a *P. falciparum* receptor that binds specifically to glycophorin A on erythrocytes. Like EBA-175, the erythrocyte receptor for BAEBL is destroyed by neuraminidase and trypsin, however BAEBL can bind to erythrocytes that lack glycophorin A and Gerbich erythrocytes (preent glycophorin C and absent glycophorin D) bind BAEBL much more weakly than normal erythrocytes. The compound of this invention can be employed in admixture with conventional excipients and can be combined with other active agents, *e.g.*, vitamins. In addition, Jensen *et al.*⁵² isolated VAR4, VAR5 or VAR6 polypeptide (from *var* genes) and its nucleic acid for use as vaccines for the diagnosis and treatment of malaria. The proteins are responsible for inducing the immunoglobulin G (IgG) antibody with specificity to *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) on infected red blood cells. Thus, severe malaria is caused by parasites expressing a subset of PfEMP1 molecules and antibodies directed against these will be responsible for the protection against severe disease acquired early in life.

Yet in vaccine preparation, Claudianos *et al.*,⁵³ at the Imperial College Innovations Limited, claimed novel secreted scavenger receptor (PfSR) proteins from the *P. falciparum* genome database, and their use in the preparation of vaccines against malaria and in diagnosis. PfSR protein was shown to have 64% homology to proteins found in the mouse malarial parasites *P. berghei* (PbSR) and *P. yoelii* (PySR), whilst nucleic acid comparisons showed 74% homology. It contains Limulus clotting factor C, lipid binding, cysteine rich scavenger receptor and pentraxin domains. The protein causes disruption of the normal expression of the proteins, so the malaria parasite dies at an early stage in its life cycle. Parasite disruption *via* drugs or vaccines is therefore more likely to be effective when parasite numbers are low, *i.e.*, at the early infective stages: sporozoites in human and ookinete in mosquito.

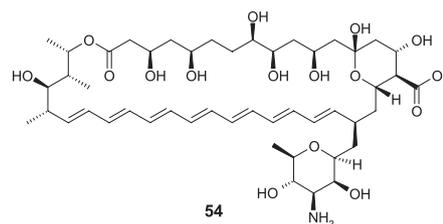
Walter and Eliza Hall Institute of Medical Research developed a vaccine for inducing an immune response to *P. falciparum* using a glycosylphosphatidylinositol (GPI) inositolglycan domain or its derivative or equivalent.⁵⁴ GPI has been identified as a candidate toxin of parasite origin.

Experiments using GPI in mice with cerebral malaria showed relatively low parasitemia levels between days 5 to 12 post-infection.

2.7. Treatment of Leishmaniasis

Cutaneous leishmaniasis is caused by *Leishmania mexicana* and *L. brasiliensis* in the Americas, and *L. tropica* in the Old World; and by visceral leishmaniasis *L. donovani*, *L. infantum* and *L. chagasi*. Sandfly vectors transmit cutaneous leishmaniasis. Treatment remains inadequate because of drug toxicity (sodium antimony gluconate), long courses required, and frequent need for hospitalization.

For the treatment of Leishmaniasis the currently used drugs are limited to four. The first line compounds are the two pentavalent antimonials, sodium stibogluconate and meglumine antimoniate. They were used for the first time in 1947 and 1950, respectively. Failures and relapses occur in all forms of leishmaniasis and constitute approximately 10-25% of cases. If these drugs are not effective, the second line compounds of pentamidine (**2**) and amphotericin B (**54**) are used, which were introduced in 1940 and 1959, respectively.

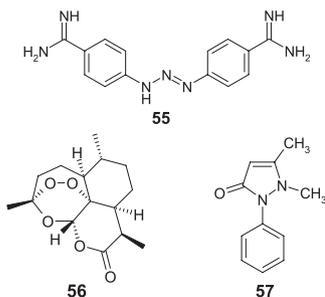


Fuertes and Jimenez⁵⁵ at the Mologen Holding AG Dana-Farber Cancer Institute, claimed to have discovered a new DNA-expression construct for treatment of infections with leishmaniasis and a corresponding vaccine. The DNA expression constructs may code for one or more *Leishmania* antigens, including the p36 LACK antigen. p36 Antibodies were determined in mice following immunization.

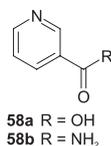
Duran *et al.*⁵⁶ (Universidad Complutense de Madrid) described a novel method of obtaining water-dispersible albumin microspheres containing amphotericin B. The invention is based on the atomisation of the albumin with the drug that is microencapsulated for treatment of leishmaniasis and not requiring the pre-formation of an emulsion, avoiding the use of oils and organic solvents. This composition is less toxic, has fewer side effects than current amphotericin B formulations, such as Fungizone®, and is easy to produce on an industrial scale. The product is suitable for parenteral administration or intravenously.

A preparation containing diaminazen-diacetate (**55**) and/or pentamidine and chloroquine (**47**) and/or

artemisinin (**56**) or a derivative for treating leishmaniasis is related by Tropmed GmbH.⁵⁷⁻⁵⁹ The inclusion of procaine or lidocaine in the formulation reduces the pain of injection. A representative tablet composition comprises the following compounds: 350 mg of diminazene diacetate (**55**), 250 mg of chloroquine (**47**) and 400 mg of antipyrine (**57**).



The Institut de Recherche pour le Developpement reported the use of niacin (vitamin B₃, **58a**, R = OH) or nicotinamide (**58b**, R = NH₂) for treating parasitic diseases. Nicotinamide is currently in trials as therapy to prevent cancer recurrence and insulin-dependent (type I) diabetes. In particular, they can be used to treat protozoan parasitic diseases such as leishmaniasis in immunodepressed patients.⁶⁰ It has been recently demonstrated that nicotinamide is a substrate of SIR2-like enzymes *in vitro*.⁶¹ The data presented that nicotinamide strongly inhibited the proliferation of both promastigotes and amastigotes with promastigote forms showing less sensitivity to nicotinamide than amstigotes. Also the patent reported the composition and administration in combination with another active agent selected from, *e.g.*, amphotericin B, paromomycin, melarsopol and antimonials, using oral, intravenous, topical or intralesional routes. In addition, the Institut described in a patent, the nucleic acid constructs, which comprise nucleic acid encoding an immunogenic protein from a promastigote or amastigote of *Leishmania*.^{62,63}



Amine-borane compounds exhibit high similarity to organic compounds mainly due to the atomic radii and the characteristics of the B–N bond, which resemble those of carbon-carbon bonds. Thus, novel families of amine-borane compounds, including fluorinated aminoboranes, novel bis-aminoboranes and aminoboranes having saturated and unsaturated alkyl chains are provided by Soreq Nuclear Research Center Israel Atomic Energy

Commission and Yissum Research Development Co.⁶⁴ Processes for preparing, pharmaceutical compositions and methods utilizing these novel compounds are also provided. Radiolabeled aminoboranes and uses thereof in radioimaging (*e.g.*, PET) and radiotherapy are further provided. They are used for the treatment of leishmaniasis and demonstrated excellent antimicrobial activity with reduced adverse side effects and improved pharmacokinetic profile. The *in vitro* anti-leishmanial effect of compound **59** (dimethyl-undecyl-amine cyanoborane) was determined on promastigotes and amastigotes. According to Table 3, the measured radioactivity, which is indicative of the parasite, diminishes rapidly with the increased dosage of amine-borane, indicating a strong anti-leishmanial effect.

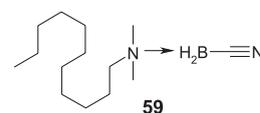
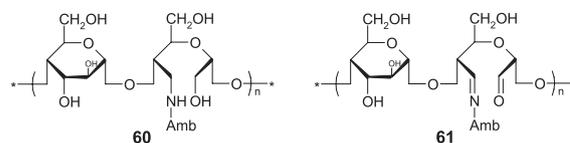


Table 3. Concentration of the amine-borane is given in $\mu\text{g mL}^{-1}$ versus the counts per minute (cpm) recorded for the parasite sample

mg mL ⁻¹	cpm
0	8699
3.6	8003
11	7898
33	537
100	138

In a further patent, Soreq Nuclear Research Center Israel Atomic Energy Commission and Yissum Research Development Co. disclose modified polymer conjugates. The conjugate of a polymer (*e.g.*, polysaccharide) and a drug (*e.g.* amphotericin B, **54**) reduced the toxicity relative to the unmodified parent compound, increased the solubility and retained substantially the same degree of therapeutic activity of the unmodified parent compound.⁶⁵ It is an antiparasitic composition against *L. donovani* and formulated in the form of a nanoparticle, micellar dispersion and a liposome. The test was performed in dextran-AmB imine (**60**) conjugate with or without ethanolamine (Table 4). The result shows that the conjugate had an IC₅₀ value of 0.25 $\mu\text{g mL}^{-1}$ compared to the Dextran-AmB imine alone (1.2 $\mu\text{g mL}^{-1}$).

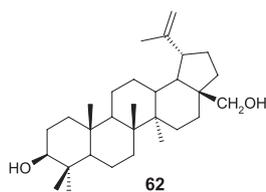


Valtion Teknillinen Tutkimuskeskus reported the use of betulin (**62**) derivatives or their salts against leishmaniasis

Table 4. *In vitro* activity against *Leishmania donovani*, cytotoxicity and hemolysis of conjugates

Compound	Antiparasitic activity ^a IC ₅₀ / (µg AmB mL ⁻¹)	Toxicity ^b IC ₅₀ / (µg AmB mL ⁻¹)	Hemolysis ^c / (µg AmB mL ⁻¹)
Free AmB (54)	0.05	9	16
Dextran-AmB amine (60)	1.2	1400	>500
Dextran-AmB imine (61)	0.3	200	250
Dextran-AmB-ethanolamine imine	0.25	400	>500

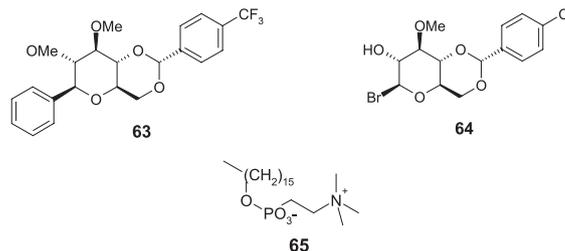
^aIC₅₀ values were derived from the activity test of AmB and different dextran-AmB conjugates against *L. donovani*. ^bIC₅₀ values were derived from cytotoxicity test AmB and different dextran-AmB conjugates against the murine RAW 264.7 cell line. ^cHemolysis was evaluated visually after 1 h incubation at 37 °C with sheep erythrocytes.⁶⁵



in pharmaceutical industry applications.⁶⁶ Betulin is the most abundant triterpenoid of the lupane series and is found in the bark of some tree species, particularly in birch bark (*Betula*). It is a component of various food additives and cosmetic products. The activity of the betulin derivatives was tested for both *L. donovani* and *L. tropica* species (Table 5).

Kemin Pharma Europe patented the use of bicyclic carbohydrates for the treatment of leishmaniasis.⁶⁷ Compound **63** and **64**, bicyclic carbohydrates with

halogen containing aryl groups, possessed significant activity against *L. donovani*, with IC₅₀ values of 1.01 and < 0.98 µmol L⁻¹, respectively, compared with 0.47 µmol L⁻¹ for miltefosine **65**. The use of **65** in leishmaniasis therapy should be carefully considered.⁶⁸



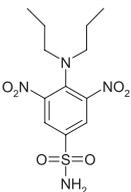
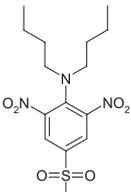
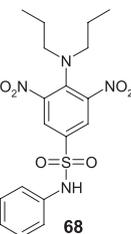
National Institutes of Health reported a novel dinitroaniline sulfonamide based on the herbicide

Table 5. Activity *in vivo* of the compounds tested for *L. donovani*. Initially, the concentration of the compounds was 50 µmol L⁻¹. Amphotericin B (1 µmol L⁻¹) was included as positive control. Broth containing DMSO was used as negative control

Compound	Name	Inhibition of <i>L. donovani</i> / (%)
62	Betulin	35.0
62a	28-Acetate of betulonic alcohol	40.6
62b	28-Methylester of betulonic acid	40.1
62c	Betulonic aldehyde	65.0
62d	Betulin 3,28-dioxime	72.4
62e	Betulin 28-oxime	66.8
62f	Betulonic alcohol	44.0
62g	Betulin 3-acetoxoxime-28-nitrile	66.4
62h	Betulin 28-acetic acid methylester	95.3
62i	20,29-Hydrobetulonic acid	73.4
62j	2,3-Didehydro-3-deoxybetulin	13.2
62k	Betulonic acid	97.6
62l	Betulonic acid	39.8
62m	28-Aspartateamide dimethylester of betulonic acid	69.3
62n	Betulonic aldehyde	46.2
62o	Betulin 28- <i>N</i> -acetylthranilic acid ester	59.2
62p	betulin 28-chrysanthemate	13.4
62q	Betulin 28-carboxymethoxy mentholester	16.6
	positive control: amphotericin B (1 µmol L ⁻¹)	55.4
	negative control: broth + DMSO	0.0

oryzalin **66**⁶⁹ preferably less cytotoxic to normal cells than oryzalin.⁷⁰ Tubulin, a critical eukaryotic protein responsible for formation of the mitotic spindle has been implicated as the target of dinitroaniline analogs. Compounds **67** and **68** are significantly more potent than compound **66** in blocking leishmanial tubulin assembly (Table 6). The compound **68** had K_d (dissociation constant) values of $57 \mu\text{mol L}^{-1}$ leishmanial tubulin, and the corresponding value for oryzalin were $170 \mu\text{mol L}^{-1}$. These data are consistent with the hypothesis that tubulin is the target of the dinitroanilines in *Leishmania*.

Table 6. Activity *in vivo* of Oryzalin and new dinitroaniline compounds against *L. donovani* and leishmanial tubulin *in vitro*

Compound	IC ₅₀ vs. <i>L. donovani</i> promastigotes / ($\mu\text{mol L}^{-1}$)	IC ₅₀ vs. <i>L. donovani</i> amastigotes / ($\mu\text{mol L}^{-1}$)	Inhibition % of leishmanial tubulin assembly at $20 \mu\text{mol L}^{-1}$ compound
 66	44.1	72.5	54
 67	17.8	20.1	89
 68	14.7	5.41	100

In 2006, De Souza and Chequer Bou-Habib⁷¹ related a prophylaxis vaccine for blocking transmission of *Leishmania* infections. It is based on *L. donovani* saponin and promastigotes or amastigotes fractions called Fucose Mannose ligand (FML, an isolated glycoprotein complex). Leishmune® vaccine (FML vaccine) developed 92-95% protection against canine visceral leishmaniasis and showed immunotherapy potential. In 2008, due to high production cost of Leishmune®, Gazzinelli and co-workers⁷² developed a vaccine formulation comprising amastigote A2 antigen.

The antibodies produced by the dogs in the vaccination process with this antigen are non-reactive to serologic diagnostic infection tests. The formulation is composed of 50 to 200.00 mg mL⁻¹ of recombinant A2-HIS (rA2) protein of *Leishmania*, produced in *E. coli*, 0.125 to 0.500 mg mL⁻¹ of Saponin, 1.00 mL of q.s.p buffered saline solution and 0.01 mL of thimerosal. Therefore, the main innovation of this vaccine formulation is the production of antibodies specific against the vaccine antigen. The main innovation of this formulation is that the animals vaccinated present serologic reaction against the antigen, but remain seronegative in the reaction with the total parasite extract, *e.g.*, the antibodies do not react with the extract of the promastigote forms of *Leishmania* in the ELISA tests, for example.

Rosalind Franklin University of Medicine & Science disclosed the use of a live mutant *Leishmania* as a suicidal vaccine.⁷³ The mutant can be selected from natural *Leishmania* species or constructed by genetic engineering. The invention also exploits the virtual absence of heme biosynthesis pathway in trypanosomes to identify or construct the mutant porphyrinic *Leishmania* as suicidal vaccines. The vaccinated hamsters showed *ca.*10 fold reduction of parasite loads than the control group.

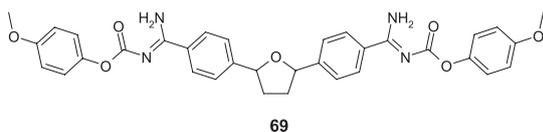
2.8. Treatment of pneumocystosis

Pneumocystosis is a pneumonia that is characterized by the accumulation of very large numbers of a eucaryotic single-celled organism called *Pneumocystis carinii*, which has not been cultured. Pneumocystosis also occurs in many other mammalian species. It is not yet established whether *Pneumocystis carinii* is a fungus or a protozoan.

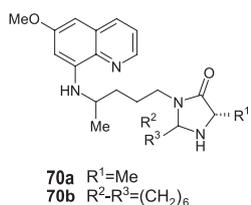
Pneumocystis carinii causes *P. carinii* pneumonia (PCP) in people with depressed immune systems such as AIDS patients, patients undergoing chemotherapy, or transplant patients being treated with immunosuppressants. The infection is treated with pentamidine (**2**) and the combination of trimethoprim (**77**) and sulfamethoxazole, however there are side effects and the mortality rate remains high. An alternative treatment is the preparation containing methioninase in combination with an antibiotic, either pentamidine (**2**) or combination of trimethoprim (**77**) and sulfamethoxazole related by AntiCancer Inc.⁷⁴ Infection by *P. carinii* can be treated by administering methioninase optionally in combination with additional therapeutic agents, such as antibiotics, either pentamidine or combination of trimethoprim and sulfamethoxazole. The use of methioninase depletes plasma levels of methionine and deprives the parasites of *S*-adenosylmethionine.

In a further patent application and development, the preparation of ten carbamate and carbonate prodrugs of

bisamidinophenyl furans was patented by The University of North Carolina at Chapel Hill.⁷⁵ Compounds were tested against *P. carinii* infected immunosuppressed rats. The more potent compound, **69**, inhibited cyst formation in the lung by 100 and 97.9% inhibition at doses of 22 and 33 $\mu\text{mol kg}^{-1}$ per day, respectively.



A series of primaquine-derived imidazolidin-4-ones were screened for their *in vitro* activity against *P. carinii* by Vale *et al.*⁷⁶ One of the tested imidazolidin-4-ones, **70**, was slightly more active ($\text{IC}_{50} = 1.9 \mu\text{mol L}^{-1}$) than the parent primaquine ($\text{IC}_{50} = 2.5 \mu\text{mol L}^{-1}$).



Dihydrofolate reductase (DHFR) is a key enzyme in the treatment of Pneumocystosis. Its role in thymidine biosynthesis (Figure 1) is the reduction of 7,8-dihydrofolate (DHF) to 5,6,7,8-tetrahydrofolate (THF) using the cofactor nicotinamide adenine phosphate (NADPH). After reduction, serine hydroxymethyltransferase (SHMT) catalyses the regeneration of 5,10-methylenetetrahydrofolate; and deoxyuridine monophosphate (dUMP) is methylated to give deoxythymidylate (dTMP) in a reaction catalysed by thymidylate synthase (TS). This reaction converts methylene-tetrahydrofolate back to dihydrofolate, completing the cycle. Therefore, the inhibition of

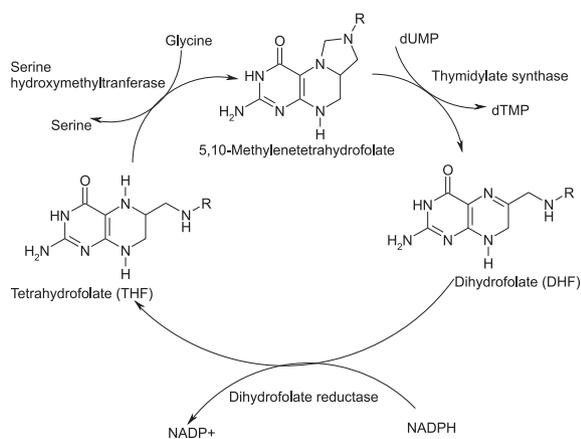


Figure 1. Role of DHFR in the synthesis of thymine.

DHFR prevents the biosynthesis of thymidine and, as a consequence, DNA biosynthesis.⁷⁷ Novel substituted 2,4-diamino-5-benzylpyrimidine tested as inhibitors of *P. carinii* DHFR, was claimed by Forsch *et al.*⁷⁸ In summary (Table 7), the results indicated that, 5'-(5-carboxy-1-pentynyl) substitution is more favorable when the benzyl ring is 3',4',5'-trisubstituted than when it is 2',5'-disubstituted. Compound **73** has the highest species selectivity for DHFR *P. carinii*. For this reason this novel analogue may be viewed as a novel lead for further structure-activity optimization of DHFR binding. Rosowsky *et al.*⁷⁹ reported a concise new route allowing easy access to five 2,4-diaminopyrido[2,3-*d*]pyrimidine

Table 7. Inhibition of *P. carinii* DHFR by 2,4-diamino-5-(3',4'-dimethoxy-5'-substituted benzyl)pyrimidines with a carboxyalkynyl or carboxyphenylalkynyl group in the side chain

Compound	IC_{50} / (nmol L^{-1})
	28
	1300
	1.0
	1200
	1.0

analogues containing a substituted phenyl or other aromatic ring attached to C6 *via* by a short bridge. The derivatives were tested against *P. carinii* DHFR, however none of the quinazolines or pyridopyrimidines tested (IC_{50} 0.087–72 $\mu\text{mol L}^{-1}$) were more potent against the *P. carinii* enzyme than the structurally related reference compound piritrexim (**77**, IC_{50} = 0.013 $\mu\text{mol L}^{-1}$). In the patent application, Forsch and Rosowsky,⁸⁰ at the Dana-Farber Cancer Institute Inc., synthesized several lipophilic DHFR inhibitors having an aromatic group and a heteroaromatic group linked by a methylene group in the treatment of *P. carinii*. Trimethoprim (**77**) and piritrexim (**76**) are lipid-soluble antifolates that have been used clinically for the prophylaxis and treatment of *P. carinii* infections in patients with AIDS. In addition, Rosowsky and Forsch,⁸¹ synthesized novel **77** derivatives (Table 8), compositions comprising them and their use as DHFR inhibitors. The most potent of the *O*-alkyl derivatives against *P. carinii* DHFR was compound **78c** ($n = 6$). Moreover there was an increase in potency as the length of the 5'-*o*-alkyl group increased from $n = 4$ to $n = 6$, followed by a decrease in potency as this length increased from $n = 6$ to $n = 8$. The most potent of the 5'-*o*-(ω -carboxyalkyl) analogs was **60g** ($n = 4$), 245 times more potent than **77**.

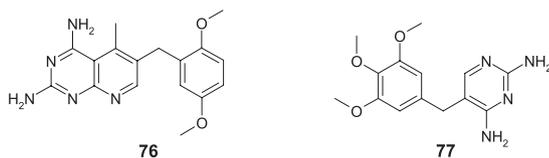


Table 8. Inhibition of *P. carinii* DHFR by trimethoprim derivatives

Chemical structure of the trimethoprim derivative core (**77**), showing a pyrimidopyrimidine ring system with a 4-methoxyphenyl group at the 6-position and a variable *O*-alkyl group at the 5-position.

Compounds	R	IC_{50} / ($\mu\text{mol L}^{-1}$)
78a	Me, $n = 4$	19
78b	Me, $n = 5$	14
78c	Me, $n = 6$	5.6
78d	Me, $n = 7$	44
78e	Me, $n = 8$	51
78f	CO_2H , $n = 3$	0.25
78g	CO_2H , $n = 4$	0.049
78h	CO_2H , $n = 5$	0.80
78i	CO_2H , $n = 6$	2.6
78j	CO_2H , $n = 7$	7.1
78k	CO_2H , $n = 8$	4.8
77 - trimethoprim		12
76 - piritrexim		0.031

The activity of four triazolyl analogues (**79a-d**) was evaluated as inhibitors of *P. carinii* DHFR by Chan *et al.*⁸² The two most potent compounds are **80a** and **80b**, against the *P. carinii* DHFR. Four compounds with a nitrogen bridge (benzanilides and benzylamines) were also tested for activity. Compounds in the benzanilide series (**80a,b**) are less potent than the benzylamines (**81a,b**; Table 9). With an IC_{50} value of 0.12 mmol L^{-1} , compound (**81a**) is the most potent member of the group as an inhibitor of *P. carinii* DHFR. Interestingly, methylation of the amino bridge (**81b**) showed a 10-fold reduction of activity towards the *P. carinii* enzyme and nearly a 7-fold gain of potency towards the rat liver DHFR.

Table 9. Inhibition of *P. carinii* DHFR by benzanilides and benzylamines derivatives

Chemical structures of benzanilide derivatives (**79a-d**) and benzylamine derivatives (**81a,b**). Benzanilide (79) has a 1,2,4-triazole ring with an amino group and an ethyl group, linked via a methylene bridge to a 4-chlorophenyl ring with a substituent R². Benzylamine (81) has a 1,2,4-triazole ring with an amino group and an ethyl group, linked via a methylene bridge to a 4-chlorophenyl ring with a substituent R¹.

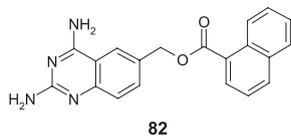
Compound	R ¹	R ²	IC_{50} / ($\mu\text{mol L}^{-1}$)
79a	H	CH_2Ph	5.18
79b	H	Ph	3.53
79c	H	SiMe_3	10.5
79d	H	H	24.8

Compound	R	IC_{50} / ($\mu\text{mol L}^{-1}$)
80a	Ph	10.8
80b	$\text{C}_6\text{H}_2(\text{OMe})_3$	1.3

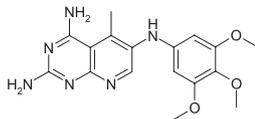
Compound	R	R ¹	IC_{50} / ($\mu\text{mol L}^{-1}$)
81a	H	CH_2Ph	0.12
81b	Me	CH_2Ph	1.07

Hallberg *et al.*⁸³ reported a novel series of DHFR inhibitors, where the methylenamino-bridge of non-classical inhibitors was replaced with an ester function. The most potent of the new ester-based DHFR inhibitors, the 1-naphthyl derivative (**82**), exhibits an IC_{50} -value of 110 nmol L^{-1} , and is less active than trimetrexate (**83**) (IC_{50} = 42 nmol L^{-1}).

Cushion and co-workers⁸⁴ synthesized a series of pentamidine (**2**) congeners and screened for their *in vitro* activity against *P. carinii*. The ATP assay was used to evaluate the effects of this pentamidine on the viability of



82



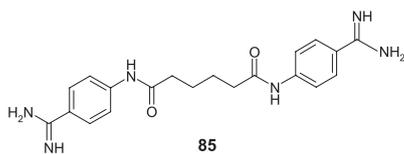
83

A549 epithelial lung cell monolayers derived from a human carcinoma. All diamide derivatives (**84a-e**, Table 10), including the most potent agents **84c** and **84e**, showed no cytotoxicity at 100 times the IC_{50} concentration against *P. carinii*. This study identified the simple small molecules **84c** and **84e** as two very promising therapeutic leads, which deserve further pre-clinical and clinical testing.

Table 10. Inhibition of *P. carinii* DHFR by diamide derivatives

Compound	Linker	IC_{50} ($\mu\text{mol L}^{-1}$); ($\mu\text{g mL}^{-1}$)
84a		5.3; 2.3
84b		1.2; 0.6
84c		0.003; 0.0013
84d		22.8; 0.0013
84e		0.002; 0.0009

In a patent application, Walzer *et al.*,⁸⁵ University of Cincinnati, disclosed a method of combating infectious agents, such as *Pneumocystis pneumonia*, and a method of treating a subject in need of such treatment. The method comprises administering a *bis*-benzamidoxime to the subject. The most active compound of the series, compound **85**, had an IC_{50} value of $0.00087 \mu\text{g mL}^{-1}$ against *P. carinii*. In comparison, pentamidine (**2**) had an IC_{50} value of $0.300 \mu\text{g mL}^{-1}$.

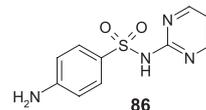


85

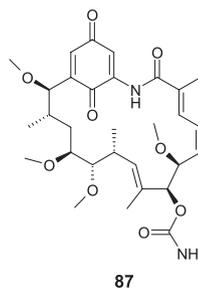
2.9. Treatment of toxoplasmosis

Toxoplasmosis is a disease caused by protozoan *Toxoplasma gondii*. Transmission to man occurs *via* ingestion of meat containing cysts or tachyzoites, ingestion of oocysts become infective after sporulation or transplacentally.

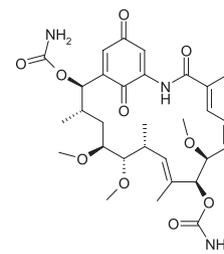
Toxoplasma gondii (*T. gondii*) is an ubiquitous parasitic protozoan that infects up to one-third of the US population and up to 90% of certain European populations.⁸⁶ Risk factors for infection include exposure to infected cats, consumption of rare meats and contact with contaminated soil. Medications that are prescribed for toxoplasmosis are pyrimethamine (**53**) (an antimalarial medication) and sulfadiazine (**86**) (an antibiotic). Thus, the need for new chemotherapeutics active against *T. gondii* is therefore acute. Sanofi-Aventis developed herbimycin (**87**) derivatives, a benzoquinone ansamycin antibiotic, as heat shock protein 90 (Hsp90) inhibitors, useful for the treatment of toxoplasmosis.⁸⁷ Hsp90 is a molecular chaperone and is one of the most abundant proteins expressed in cells. It is highly conserved and expressed in a variety of different organisms from bacteria to mammals. In an *in vitro* assay, **88** had an IC_{50} of $0.928 \mu\text{mol L}^{-1}$ for the inhibition of the ATPase activity of Hsp82.



86



87



88

One feature that distinguishes *T. gondii* from its human host is its inability to synthesize purine “salvage”.⁸⁸ Differently from its human host, the *T. gondii* recovers purine precursors from the adenosine kinase, enzyme which converts adenosine in adenosine monophosphate (AMP) (Figure 2). Through it, all other purine nucleotides can be synthesized. Then, the inhibition of the adenosine kinase activity interrupts the purine recovery route (“salvage”), offering a number of potential targets for the antiparasite chemotherapy. It is known in the literature that benzylthioinosine analogues are substrates for the parasite adenosine kinase, but not for human

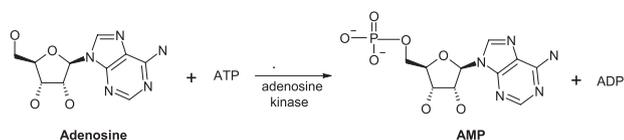


Figure 2. Reaction catalyzed by the adenosine kinase enzyme.

adenosine kinase.⁸⁹ Rais *et al.*^{90,91} described a series of 6-benzyladenosine analogues that act as potent and selective substrates for *T. gondii* adenosine kinase. 6-Benzylthioinosine analogs (Table 11) were identified as excellent subversive substrates of *T. gondii* adenosine kinase.

Table 11. Inhibition of *T. gondii* AK by 6-benzyladenosine derivatives

The structure shows a ribose sugar attached to an inosine base. A benzylthio group (-S-CH2-C6H5) is attached to the 6' carbon of the ribose sugar. The benzene ring has substituents 'p' and 'm'.

Compound	Structure	IC ₅₀ / (μmol L ⁻¹)
89a	<i>o</i> -Methyl-Benzylthioinosine	7.7
89b	<i>o</i> -Chloro-Benzylthioinosine	6.7
89c	<i>m</i> -Methyl-Benzylthioinosine	8.2
89d	<i>m</i> -Trifluoromethyl-Benzylthioinosine	8.7
89e	<i>m</i> -Nitro-Benzylthioinosine	6.2
89f	<i>p</i> -Methyl-Benzylthioinosine	7.8
89g	<i>p</i> -Bromo-Benzylthioinosine	14.3
89h	<i>p</i> -Methoxy-Benzylthioinosine	3.5
89i	<i>p</i> -tert-Butyl-Benzylthioinosine	23.3
89j	<i>p</i> -Acetoxy-Benzylthioinosine	15.0
89k	<i>p</i> -Chloro-Benzylthioinosine	8.11
89l	<i>p</i> -Nitro-Benzylthioinosine	12.0
89m	<i>p</i> -Cyano-Benzylthioinosine	4.3
89n	<i>p</i> -Fluoro-Benzylthioinosine	10.3
89o	2,4-Dichloro-Benzylthioinosine	7.3
89p	2-Chloro-6-fluoro-Benzylthioinosine	8.7
89q	<i>m,p</i> -Dichloro-Benzylthioinosine	10.4
89r	<i>o,m,p</i> -Trimethyl-Benzylthioinosine	31.1

Currently, there are no tests which can differentiate between oocyst ingestion versus tissue cyst ingestion as the infection route.⁹³ Recombinant proteins have been developed by US Department of Agriculture for the detection of *T. gondii* oocyst proteins for example in biological fluids. Isolates and recombinant *T. gondii*-specific proteins

(rDGP5p) can be adsorbed to the surface of microtiter plates or to immunoblotting membranes and used in an ELISA format for detection of antibodies. Recombinant antigens also can be used to prepare monoclonal antibodies which selectively identify *T. gondii* oocyst proteins. In addition, primers directed to *T. gondii*-specific regions of the DNA sequences can be produced for sensitive detection of the parasite by polymerase chain reaction. Using ELISA and MAT, (microscopic agglutination test) assays were performed on 127 human sera known to have resulted from oocyst exposure, five sera from infections resulting from congenital infection, and 76 sera from MAT negative individuals. From the 76 uninfected sera, all were negative using the recombinant ELISA and from the 127 oocyst induced infections, the recombinant antigen ELISA detected 119, and did not detect 8. Sera from 40 oocyst infected pigs and 45 tissue cyst infected pigs also were tested using ELISA and MAT. From the 40 pigs infected with oocysts, 39 were detected using ELISA assay.

In the development of vaccine patents, GlaxoSmithKline Biologicals patented a vaccine composition comprising the toxoplasma protein, SAG3, the major glycosylphosphatidylinositol (GPI)-anchored surface protein of *T. gondii*.⁹⁴ SAG1 mediates the host-cell invasion and mono/polyclonal antibodies directed to SAG1 inhibited invasion of cells by tachyzoites, probably by interfering at the parasite attachment level.⁹⁵ The binding of SAG1 and SAG3 to CHO K1 cells (Chinese hamster ovary cells) was examined. Just SAG3 displays affinity for sulfated proteoglycans. The presence of alternative cellular receptors used by *T. gondii* was already suggested, as inhibition of parasite attachment by soluble GAGs or by heparinase treatments of host cells was not complete. The studies show that soluble heparin (glycosaminoglycan) inhibited SAG3 binding by up to 90%. Dermatan sulfate, a glycosaminoglycan that is a structural component of certain body tissues also inhibited the binding of SAG3, but chondroitin sulfate A, a chondrin derivative, did not. In a further patent application on vaccines, Kyushu TLO Co Ltd [Kyushu University] developed a novel fused DNA vaccine produced by constructing a fused gene comprising a gene from an intracellular protozoal parasite, such as toxoplasma, with a ubiquitin gene.⁹⁶ This vaccine is said to form an ubiquitinated pathogen antigen which is processed with a proteasome which strongly induces the production of CD8⁺ T cells.

3. Current and Future Developments

Neglected tropical diseases are widely related to poverty and disadvantage. The poorest populations often living in

remote, rural areas, urban slums or in conflict zones are the most affected. Actually, the neglected tropical diseases show a clear link between health and development. Adding HIV,⁹⁷ tuberculosis,⁹⁸⁻¹⁰⁰ dengue,¹⁰¹⁻¹⁰³ Chagas disease,¹⁰⁴⁻¹⁰⁶ leishmaniasis¹⁰⁷ and malaria¹⁰⁸⁻¹¹¹ to the mix, the diseases kill several million each year, shorten lives and reduce productivity.

Biologists have identified more than 50,000 species of protozoa, of which a fifth are parasitic and some can reach humans by food and water. The majority of food and waterborne infections of parasitic origin are related to poverty, low sanitation, and old food habits. Parasitic protozoa do not multiply in foods, but they may survive in or on moist foods for months in damp environments.

Diseases caused by intestinal protozoa are increasing and new treatments are very important because of their association with acute and chronic diarrhea in immunocompromised patients as well as those with a normal immune system.

For African trypanosomiasis, cysteine protease inhibitors were described by Merck for the treatment of trypanosomiasis¹²⁻¹⁶ and for American Trypanosomiasis, inhibitors of CAC1 cysteine proteases cathepsin K are arising as promising compounds. It is important to mention those compounds were also described in the treatment of Chagas disease.⁴⁻¹⁰ SmithKline Beecham Corporation reported a new promising trioxo-[1,2] thiazepanylamide derivative.²⁶

Turning to Giardiasis, Suk *et al.*²⁹ reported the design, synthesis, and activity of a potent and non-toxic second generation anti-giardial agent that was designed as an inhibitor of cyst wall synthase.

However, as in the case of *G. lamblia*, currently (2001-2008), there are no new patents for anti-balantidiasis drugs. Balantidiasis disease is especially dangerous in immunocompromised patients as pulmonary parenchyma involvement is possible. Alternative drugs for the treatment of balantidiasis may be 5-nitroimidazole drugs, such as tinidazole (**22**) and ornidazole (**26**).³²

In this scenario, 5-nitrofuran 2-carboxaldehyde thiosemicarbazones (**31**) and their subsequent bidentate Cu^{II} complexes (**32**) showed promising results (IC₅₀ value *in vitro*) when compared to the metronidazole (conventional compound used for the Treatment of Amebiasis).³⁴

Regarding malaria, two points call special attention: the resistance to currently available antimalarial drugs and the inefficacy of antimalarial vaccines.³⁵ Thus, new promising cathepsin K inhibitors (dihydrotriazine derivatives) and inhibitors of a scavenger receptor class protein (ScarB1) were proposed.^{37,48} However, in this scenario, a promising way is to use different vaccine formulations against malaria.⁵¹⁻⁵⁴

It is interesting to mention that Dihydrofolate reductase (DHFR) is key in the treatment of Pneumocystosis. Novel series of DHFR inhibitors may be viewed as a novel lead for further structure-activity optimization of DHFR binding. In this line, Hallberg and co-workers⁸³ reported a serie of DHFR inhibitors, where the methylenamino-bridge of non-classical inhibitors was replaced with an ester function leading to interesting results.

The need for new chemotherapeutics active against *T. gondii* is also acute. On this point, herbimycin (**87**) derivatives, protein 90 (Hsp90) inhibitors, and 6-benzylthioinosine analogs, which are subversive substrates of *T. gondii* adenosine kinase, were identified as promising compounds in the treatment of toxoplasmosis.

Nowadays, ultraviolet (UV) irradiation is regarded as being widely effective against all pathogens, bacteria, protozoa and viruses that can be transmitted through drinking water.⁹⁴ However, more research is needed to understand the extent to which particles in water may hinder the UV treatment efficacy by interacting with microbial pathogens. UV irradiation is a primary disinfection technology for use in water and wastewater effluents. It has been demonstrated that UV radiation is very effective against (oo)cysts of giardia, a pathogenic microorganism of major importance for the safety of drinking water.¹¹²

In Europe, UV has been widely applied for drinking water disinfection since the 1980s, for the control of incidental contamination of vulnerable groundwater and for reduction of Heterotrophic Plate Counts. Protozoan infections are especially endemic and affect millions of people around the world. In spite of their importance, the development of more efficient drugs against those diseases is still scarce. Furthermore, surprisingly few patents for new drug candidates acting on these diseases have appeared. Looking at that scenario, we believe that some advances in anti-protozoan drug candidate research can be expected from the recently published X-ray structures of enzymes, such as *Leishmania trypanothione* synthetase, *Entamoeba histolytica* glyceraldehyde-3 phosphate dehydrogenase, glycerol kinase in *Plasmodium falciparum*, and substrates of *Toxoplasma gondii* adenosine kinase.¹¹³⁻¹¹⁷

Therefore, we strongly feel that molecular modeling studies, combining docking and molecular dynamic simulations, can improve our understanding of the inhibitor-protein interactions. Furthermore, using these methodologies it is feasible to investigate the structural factors responsible for selectivity of some target enzymes with their inhibitors, and therefore hasten much needed research.¹¹⁸⁻¹²¹

However, it should be kept in mind that computers are an essential tool in modern medicinal chemistry. Currently,

computational approaches and 3D visualization are not used simply to depict pretty pictures of molecules in biological systems; these powerful computational tools allow one to obtain insights on the interaction between enzyme-substrate, mechanism reaction, statistical behavior of molecules and much more, at the molecular level, contributing significantly to the problem solving in biological systems.

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