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Review Article

Human African trypanosomiasis: future prospects for chemotherapy

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Abstract

Chemotherapy for human African trypanosomiasis (sleeping sickness) currently relies on four approved drugs: pentamidine, suramin, melarsoprol and eflornithine. This review presents an overview of the current status of the drugs used to treat this neglected disease, new treatments under development and perspectives for the future.

Introduction

Human African trypanosomiasis, or sleeping sickness, is a disease that occurs in approximately 250 foci in 36 sub-Saharan countries. It is transmitted to humans via bites from blood-sucking tsetse flies (genus *Glossina*), which inject protozoan parasites into the body (1) (Fig. 1). Although exact figures are not available, the WHO estimates that 300,000-500,000 people are infected, with 60,000 new cases and 40,000 mortalities per year (2). Only 3-4 million people at risk are under surveillance, with regular examinations and access to healthcare centers providing screening. Because of the lack of resources, most people with sleeping sickness die before they can ever be diagnosed. This disease contributes to poverty by limiting the introduction of new livestock that are not tolerant to the disease. It also affects meat and dairy production and influences the use of draught animals across Africa. It is estimated that sleeping sickness causes 3 million cattle deaths and significant agricultural losses in terms of production (estimated at $4.75 billion/year) and human health (3, 4).

Disease status and epidemiological trends

According to the WHO, countries can be placed in four categories in terms of prevalence. First, countries where the disease is epidemic with a very high cumulative prevalence (20-50%) and high transmission rates: Angola, Democratic Republic of Congo (DRC), Uganda (3) and Sudan. Sleeping sickness is the first or second greatest cause of mortality, ahead of HIV/AIDS, in these territories. Second, highly endemic countries, where the prevalence is moderate but an increase is certain: Cameroon, Central African Republic, Chad, Congo, Côte d'Ivoire, Guinea, Mozambique and United Republic of Tanzania. Third, countries where the endemic level is low: Benin, Burkina Faso, Equatorial Guinea, Gabon, Kenya, Mali, Nigeria, Togo and Zambia. Fourth, countries where the present status is unclear: Botswana, Burundi, Ethiopia, Liberia, Namibia, Rwanda, Senegal and Sierra Leone.

Two morphologically indistinguishable subspecies of the *Trypanosoma brucei* complex, which differ in their clinical presentation and epidemiology, cause human

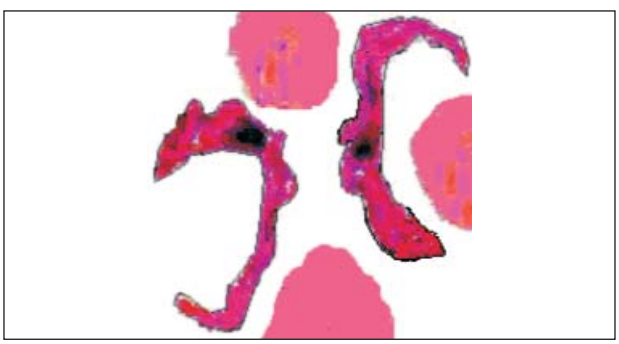


Fig. 1. *Trypanosoma brucei* in blood smear from a patient with African sleeping sickness.

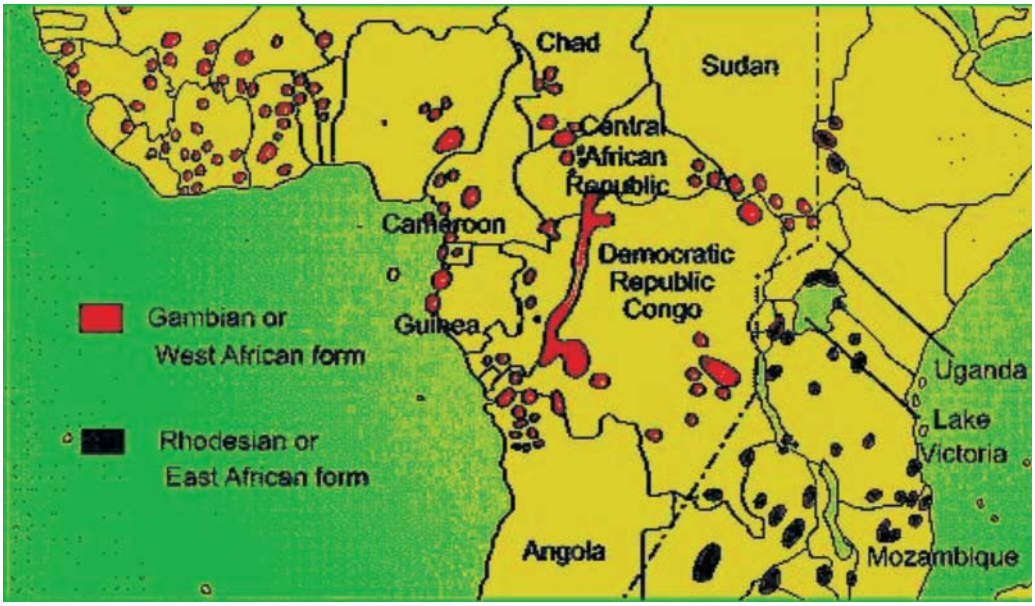


Fig. 2. Distribution of the main foci of the two forms of human African trypanosomiasis, or sleeping sickness, in sub-Saharan Africa.

African trypanosomiasis (Fig. 2). The trypanosome is also transmitted by different species of tsetse flies with different breeding habits and preferences. West and Central Africa are areas abundant in rivers, vegetation and tropical rain forests. The *Glossina palpalis* species of tsetse fly prefers this type of habitat with permanent bodies of water. This species transmits a chronic form of the disease (*T. brucei gambiense*). Apart from the initial manifestation, this disease can last for years without obvious symptoms, which only emerge when the disease has reached the late acute stage. It is a continuous and re-emerging risk to the people living in these areas. The risk increases with the number of bites from a tsetse fly, as transmission is primarily human-to-testse fly and then back to humans. Tourists are not at great risk from this form of trypanosomiasis unless they spend long periods of time traveling in rural areas.

Eastern and Southern Africa are mainly comprised of grassland or savannah, with some rural woodland. The *Glossina morsitans, Glossina pallidipes* and *Glossina swynnertoni* species of tsetse flies prefer this habitat and are responsible for the transmission of the more virulent acute form of the disease (*T. brucei rhodesiense*). As the disease emerges only after a few weeks to months before death occurs, it is vital that patients be diagnosed and treated immediately. This disease is a zoonosis, residing in the extensive animal reservoirs of domestic and wild ungulates and the game animals of the savannah. Transmission is primarily human-to-animal and then back to humans. Human cases occur as a result of the intrusion of people into habitats containing tsetse-infected animal reservoirs or larger outbreaks due to population displacement “into the bush” as a result of wars and civil unrest, thus introducing the disease into areas where it was formerly under control. Displacement also increases the danger of the two forms of sleeping sickness converging (5). Uganda is currently the front line where this could occur (see Fig. 2), raising concerns that both forms of the disease could eventually be transmitted by the same *Glossina* species (6). This would create major problems in disease control due to differences in symptoms, diagnosis and treatment regimens.

In Europe, 84 imported cases of *T. brucei gambiense* were reported before 1963 and 12 cases in France from 1969 to 1979 (7). Intensive control activities in trypanosomiasis-endemic regions decreased the incidence during this period. Since 1985, there have been 8 imported cases of *T. brucei gambiense* in Europe (7-14), 2 additional cases in France, and for the first time, 2 cases in Italy (15).

T. brucei rhodesiense has been contracted by European tourists visiting national parks in Tanzania, which alerted the medical community to the need for vigilance with regard to travel in Africa. In the U.K., there have been 8 reported cases of *T. brucei rhodesiense* in the last 18 years (16) and an additional 9 in Europe from visiting Tanzania (16-18). In America, there have been 21 documented cases of *T. brucei rhodesiense* since 1967 as a result of travel to Africa (19, 20), and cases have recently appeared in Australia and Mexico (21).

Clinical presentation

For practical therapeutic purposes, human African sleeping sickness can be divided into two clinically different stages, which, if left untreated, result in death. The first stage is the hemolymphatic systemic stage and the second is an encephalitic stage resulting in parasites and/or an increased number of white blood cells (WBCs) in the cerebrospinal fluid (CSF).

Infection and symptoms

The bite of a tsetse fly can be painful and the first symptoms begin at the site of infection within a few days (minimum 5 days). The first signs are the manifestation of a red sore called a trypanosomal chancre, which is caused by localized proliferation of pathogens within the subcutaneous tissue. This is followed by an incubation period which lasts a few weeks (2-3) for *T. brucei rhodesiense* and several weeks to months for *T. brucei gambiense*.

First stage of the disease

The dissemination of the organism into the bloodstream leads to the emergence of bouts of fever due to increases in parasite numbers. This is accompanied by regional lymphadenopathy (the swelling of lymph nodes), especially on the back of the neck. This is known as Winterbottom's sign. It is more common in East African than West African trypanosomiasis (22), and in the former case, the symptoms may already be severe enough to cause myocardial involvement and death, as is the case for approximately one-tenth of the patients who lack immediate access to treatment. Other nonspecific signs in the case of West African trypanosomiasis include hepatosplenomegaly (enlarged spleen and liver) and a faint rash. Other general symptoms include aching muscles and joints, swelling and fatigue.

Second stage of the disease

In the second stage, which occurs after weeks in East African and after months in West African trypanosomiasis, the parasite crosses the blood-brain barrier and invades the central nervous system (CNS) (23). The second-stage symptoms include severe headaches, stiff neck, sleep disturbance and depression, followed by weight loss, progressive mental confusion, personality changes, slurred speech, irritability, loss of concentration, seizures and tremors. Finally, the patient eventually enters a terminal somnolent state, which is where the disease derives the name of sleeping sickness (maladie de sommeil). In rare cases, an infected pregnant woman can pass the infection to her baby, although this may result in miscarriage and perinatal death. Accidental infections can occur in laboratories handling infected blood, blood transfusions or organ transplants, but cases are rare.

Diagnosis

Exposure and a history of travel within an endemic region, a memory of a tsetse fly bite or a scar from a 'trypanosomal chancre' (Fig. 3) are key to the diagnosis of African sleeping sickness. A definitive diagnosis is made by identifying the trypanosome in blood films by microscopy or aspirates of the lymph nodes, especially during fever. More elaborate techniques involving centrifugal concentration of parasites in the blood (24), all of which require a high degree of specialized training and expertise, can also be used.

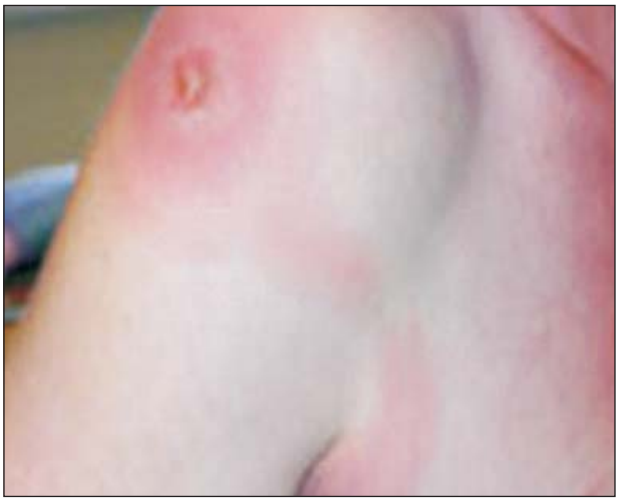


Fig. 3. Trypanosomal chancre on the shoulder of a patient, with lymphangitis toward the axilla (18).

Molecular tools such as ELISA may be used to identify antigens. However, in the field the serodiagnostic Card Agglutination Test for Trypanosomiasis (CATT) has high sensitivity and specificity and the Card Indirect Agglutination Trypanosomiasis Test (CIATT) has been shown to distinguish between the two species of trypanosomes, although there are concerns about its specificity (25).

Identification of trypanosomes in the blood or lymph nodes requires the determination of their stage by lumbar puncture and analysis of the CSF. The presence of trypanosomes or increased lymphocyte counts (> 20 cells/μl) or increased protein levels (> 35 mg/dl) confirms invasion of the CNS. The correct choice of treatment depends on differentiating the extent of infection, whether stage 1 (hemolymphatic stage) or stage 2 (encephalitic stage).

Drugs and treatment

Vaccination is not an option in the case of these extracellular parasites because they evade immune destruction due to antigenic variations in their variable surface glycoprotein (VSG) coat proteins (26). Therefore, chemotherapy remains the principal means of treatment and control of these diseases, despite setbacks due to resistance (26-28). Effective treatment of the disease is possible if diagnosis and treatment occur in the early stage, prior to progression to stage 2, when the disease invades the CNS. This is because the most effective drugs do no cross the blood-brain barrier to kill the parasite. With the exception of eflornithine (DFMO), the drugs used to treat human African trypanosomiasis (Fig. 4) are over 40 years old and would not pass current safety standards. The majority produce severe toxicity and adverse side effects (29), and currently none of them, except nifurtimox, can be given orally and have to be administered

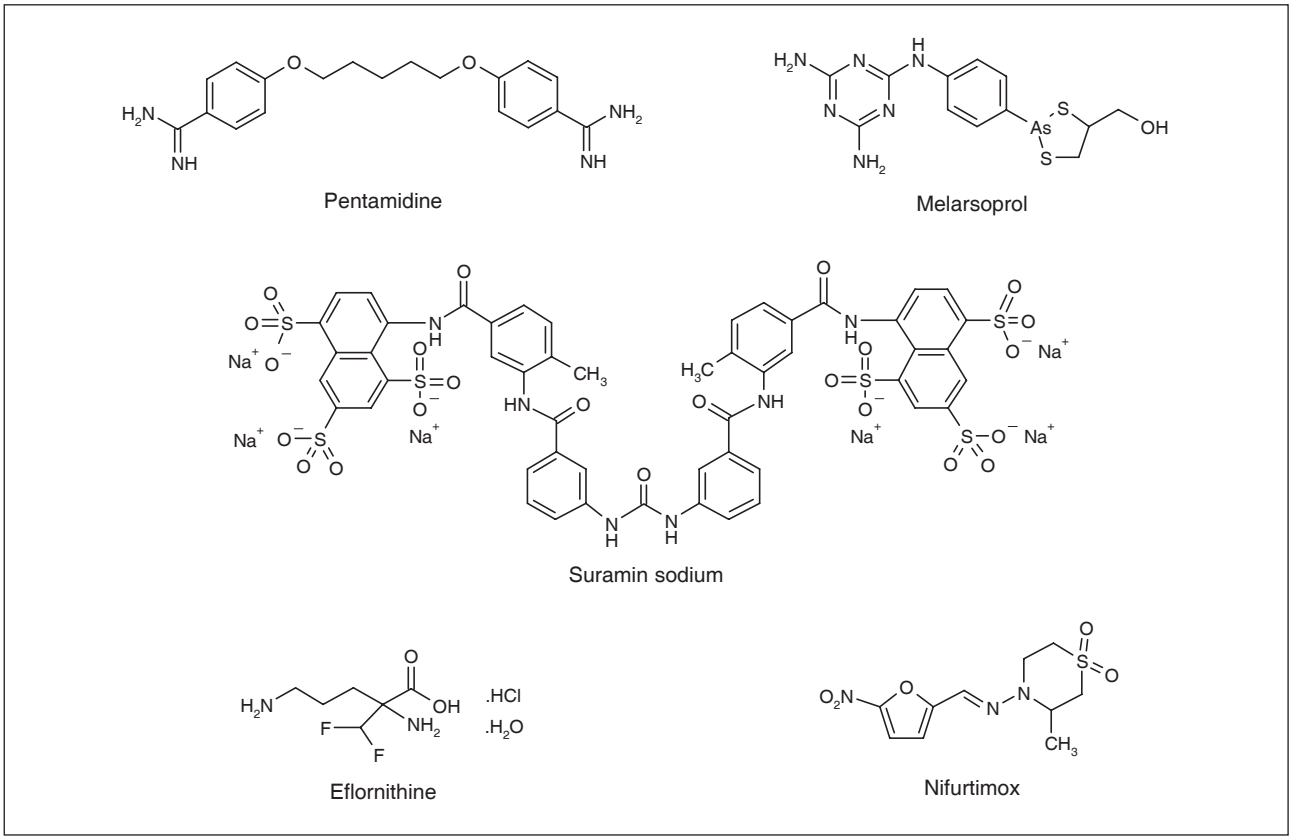


Fig. 4. Structures of currently used drugs to treat human African trypanosomiasis.

intravenously. The drugs used can be divided into two types: those that work against stage 1 disease and those that cross the blood-brain barrier and are suitable for use against stage 2 disease (Fig. 5). Prompt treatment is essential.

For stage 1 *T. brucei gambiense*, pentamidine isethionate (Lomidine) is given intramuscularly (3-4 mg/kg/day for 7-10 days). Side effects include hypotension and hypoglycemia. For stage 1 *T. brucei rhodesiense,* the drug suramin is given by slow intravenous injection. A typical protocol is 5 mg/kg on day 1, 10 mg/kg on day 3 and 20 mg/kg on days 5, 11, 23 and 30. Severe side effects include anaphylactic shock, severe cutaneous reactions, neurotoxic effects and renal failure.

For stage 2 human African trypanosomiasis, when the CNS is compromised the only drug effective for both types of disease is the toxic trivalent arsenical melarsoprol (Mel B, Arsobal). This drug is usually given as 2-4 courses in three injections. For example, if the WBC count is > 100/μl in the CSF, 3 courses of three injections are typically given with an interval of 1 week between each course (30). If the WBC count is 20-100/μl in the CSF, then just 2 courses of three injections are given with an interval of 1 week between courses. The usual dose is 3.6 mg/kg. However, a shorter 10-day continuous dose regimen has been devised, *i.e.*, daily doses of 2.2 mg/kg for just 10 days (31). Melarsoprol kills 5% of those who receive it due to post-treatment encephalopathy (PTRE)

West African trypanosomiasis

Stage 1
First-line: Pentamidine isethionate
Second-line: Melarsoprol or eflornithine

Stage 2
First-line: Melarsoprol
Second-line: Eflornithine

East African trypanosomiasis

Stage 1
First-line: Suramin
Second-line: Melarsoprol

Stage 2
First-line: Melarsoprol
Second-line: Eflornithine combined with nifurtimox

Fig. 5. Drug regimens used to treat human African trypanosomiasis (22).

(32, 33). PTRE usually occurs between the first and second course of melarsoprol treatment in a 3-4-week treatment regimen (30), and between the eighth and ninth injection in the case of the new 10-day regimen (34). To prevent PTRE, prednisolone (35), a corticosteroid, is co-

administered with melarsoprol. Treatment lasts a minimum of 10 days and for stage 2 treatment it is necessary to have specialist hospital nursing care and intensive monitoring (36).

In melarsoprol-refractory *T. brucei gambiense* disease, eflornithine can be given as an alternative, but it is ineffective against *T. brucei rhodesiense* infections. Eflornithine is administered as four daily infusions of 400 mg/kg/day for 7-14 days. The potential side effects of this drug include bone marrow toxicity, diarrhea and seizures, although it is much safer than melarsoprol.

Nifurtimox is used to treat American trypanosomiasis (Chagas' disease) (37). Although it has not been registered to treat human African trypanosomiasis, it has been the subject of small-scale studies (38-42) against melarsoprol-resistant *T. brucei gambiense*. A typical protocol is 15 mg/kg/day in three divided doses for 2 weeks. Side effects include anorexia and neurological side effects.

Follow-up

Ideally, both stage 1 and 2 patients should have clinical and laboratory evaluations of blood and CSF analysis (for CNS cases) every 6 months for a period of 2 years. If all the screening tests are negative or normal, the patient is considered cured. However, relapses of the disease may occur and treatment with melarsoprol should be repeated if the CSF is active (by WHO criteria) even if the patient has no symptoms.

Combination treatments

High therapeutic failure rates due to the development of parasitic resistance to existing drugs have been reported in epidemic areas such as Angola (25% within 30 days) (43), Sudan (18% at 6 months) (44) and northwest Uganda (30.4% within 2 years) (45), but not yet in the DRC (46). The use of combinations of existing drugs aims to delay the widespread occurrence of resistance. A few combinations have been used on compassionate grounds in patients when existing therapies failed, but information about their efficacy and safety is too limited to recommend their systematic use. Combinations investigated for *T. brucei gambiense* include pentamidine and suramin (1983-1992) (47), eflornithine and melarsoprol (stage 2) (1996) (48-50), melarsoprol and nifurtimox (1998, 1999-2000) (44, 46) and diminazene (Berenil®, **1**) (51). Combinations investigated for stage 2 *T. brucei rhodesiense* include suramin and eflornithine (52), and suramin and metronidazole (**2**) (53, 54).

Treatments in development

New drugs

The increase in resistance to existing drugs for human African trypanosomiasis has resulted in interest in the development of new therapies. However, there are few candidate drugs on the horizon. The most promising new medication for the last 50 years is the diamidine derivative pafuramidine maleate (DB-289) (55, 56) (Fig. 6), being developed by Immtech for use against *Pneumocystis carinii* (57). Unlike pentamidine, this compound is a prodrug with the major advantage that it shows promise as an oral therapy for early stage 1 disease. The Bill and Melinda Gates Foundation has supported the development of DB-289 based on promising phase II clinical trials. The aim is to complete a preliminary phase III trial before seeking regulatory approval from the FDA (58).

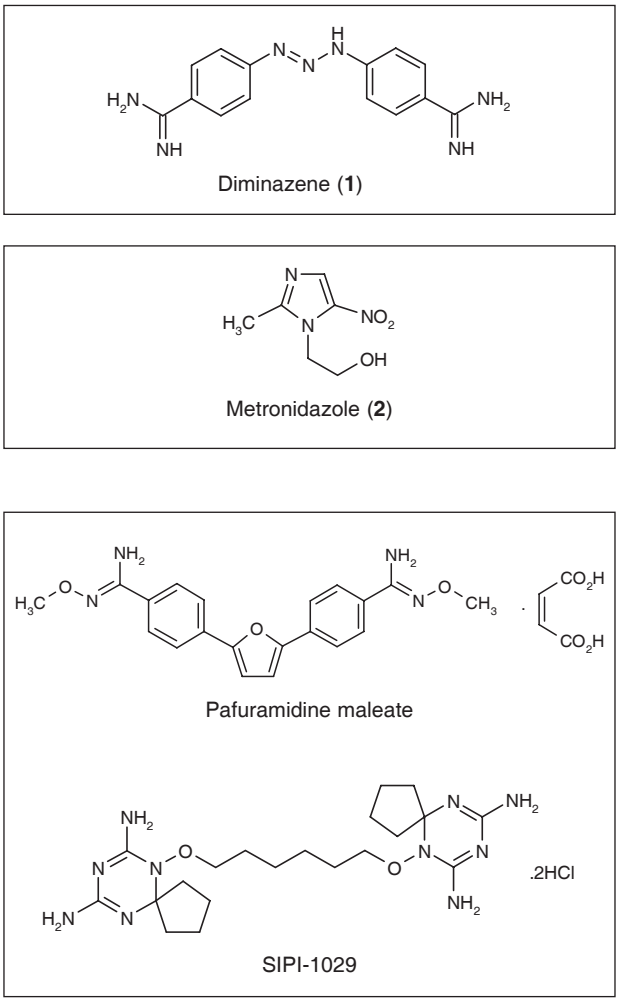


Fig. 6. Structures of new drug candidates under development.

Other compounds of interest are the triazines. The lead compound SIPI-1029 (59) (Fig. 6) has been used in China against *Trypanosoma evansi* in buffaloes (29) and shows activity against early-stage human African trypanosomiasis *in vitro* and *in vivo*. However, pharmacokinetic analysis of the compound in the CSF has shown low efficacy against multidrug-resistant *T. brucei brucei* and late-stage disease. This is due to limited blood-brain barrier transport and thus further development has been halted (60). Despite these early setbacks, work on analogues with improved CNS penetration continues and combination treatment with SIPI-1029 and eflornithine has been shown

to cure CNS infections in animal models (61). The mode of action of this compound is unclear, but it shows activity against *S*-adenosylmethionine decarboxylase (62).

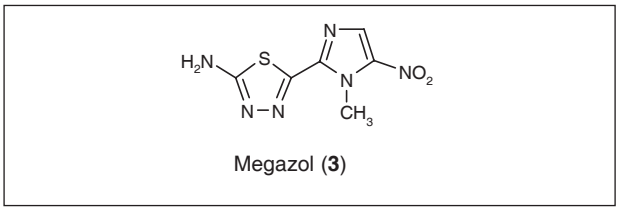
New regimens and formulation approaches

In its Seventeenth Program Report, TDR 2005, the United Nations indicated that it has a program in Angola involving 400 human African trypanosomiasis-positive patients where the efficacy of a short-course (3-day) pentamidine treatment for early-stage disease is being assessed in comparison with the standard 5-day treatment regimen. Other treatment options in the pipeline for late-stage disease include an oral formulation of eflornithine, with phase II studies on efficacy and safety intended to evaluate its clinical utility currently in progress in France. The U.N. has also indicated that it is reviewing studies on the efficacy of nifurtimox (Fig. 4), a drug used against Chagas' disease (American sleeping sickness) that might be effective against human African trypanosomiasis, including the late-stage disease caused by either *T. brucei rhodesiense* or *T. brucei gambiense.* A combination of nifurtimox and intravenous eflornithine is currently being assessed in a clinical study. Megazol (**3**), a nitroheterocyclic compound, was considered for development but genotoxicity issues led to discontinuation of its development (63). Other compounds of this class, however, may prove useful (64).

Perspectives for the future

Potential leads against African trypanosomiasis have been the subject of several recent reviews (65-71). Existing targets include glycolytic pathways (66, 70), trypanothione metabolism (66, 68, 69, 71, 72), polyamine pathways (66, 68, 69, 71), glycosylphospatidylinositols (GPIs) (66), topoisomerases (66) and folate pathways (70).

The major development in the field has been the rational design of irreversible inhibitors of trypanothione reductase (TR), a key enzyme in trypanothione metabolism and one of the proposed targets of melarsoprol (73) (see Fig. 4), the only drug effective against late-stage *T. brucei rhodesiense* infections. Compounds **4-6** (Fig. 7) are analogues of the naturally occurring spermidine alkaloid lunarine, derived from the European garden plant *Lunaria biennis*, reported to be a slow-binding inhibitor of TR with an apparent K_i of 144 ± 30.5 μM (74, 75). These compounds, like lunarine, are irreversible inhibitors of TR (76), which in the case of compound **5** involves a conjugate addition reaction of the redox active-site thiol group of the enzyme with the double bond attached to the tricyclic nucleus. Only compound **6** and the racemic form of the naturally occurring lunarine alkaloid show *in vitro* activity against the bloodstream form of *T. brucei rhodesiense,* with IC_{50} values for **6** of 29-56 μM compared to 65 μM for racemic lunarine. Major support for this work has come from the Wellcome Foundation and future work may lead to the development of a new rationally designed antitrypanosomal compound.



Megazol (**3**)

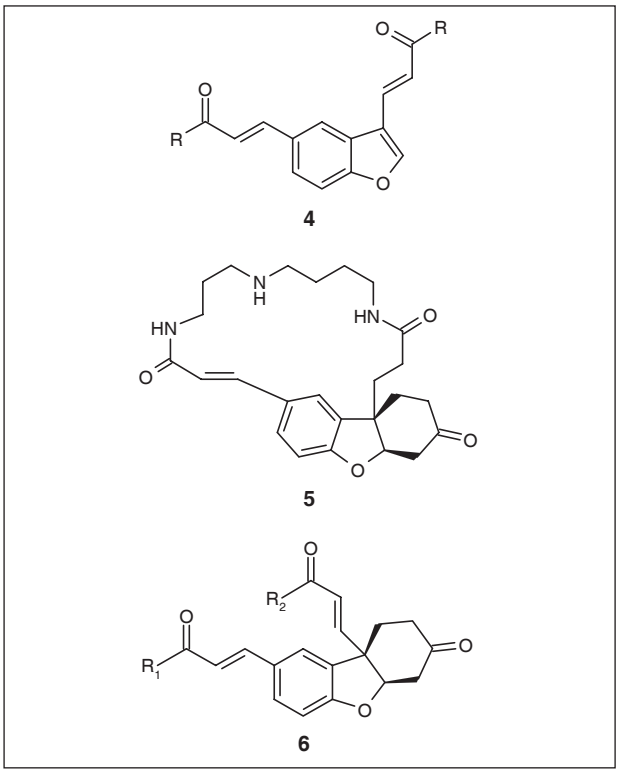


Fig. 7. Structures of irreversible inhibitors of trypanothione reductase.

Farnesyl pyrophosphate synthase

Farnesyl pyrophosphate synthase has been shown to be an essential enzyme in *T. brucei* (77) and to be involved in the formation of farnesyl pyrophosphate, a compound involved in the mevalonate/isoprene biosynthesis pathway. Bisphosphonates such as compounds **7-9** (Fig. 8) are inhibitors of farnesyl pyrophosphate (FPP) synthase in the range 0.007-0.010 μM. These compounds have been shown to be *in vitro* inhibitors of the bloodstream form of *T. brucei rhodesiense* trypomastigotes (78, 79), with IC_{50} values of 0.61, 0.7 and 8.6 μM, respectively, for compounds **7**, **8** and **9** (risedronate). *In vivo* studies of these compounds in *T. brucei*-infected mice showed risedronate to have some activity when used in a split-dose regime (2 x 5 mg/kg/day) for 5 days, with a 60% survival rate (3 of 5 mice survived while 1 died on day 7 and another on day 14), whereas untreated mice died at 5-6 days (77). This enzyme therefore represents a promising target for the design of inhibitors active against stage 1 human African trypanosomiasis. However, the survival rate of < 100% for infected mice

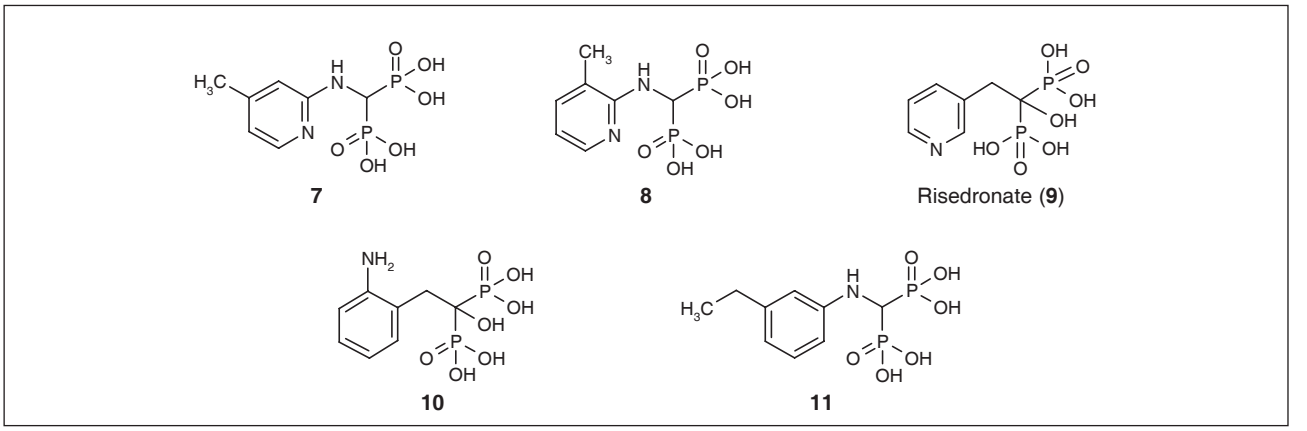


Fig. 8. Structures of bisphosphonate inhibitors of farnesyl pyrophosphate (FPP) synthase and pyrophosphatase (TbVSP1).

justifies concerns that the high water solubility of bisphosphonates reduces their ability to penetrate the CNS, rendering them ineffective against stage 2 disease. These studies indicate that patients taking risedronate for bone resorption diseases may be afforded a small degree of protection against this disease.

Pyrophosphatase

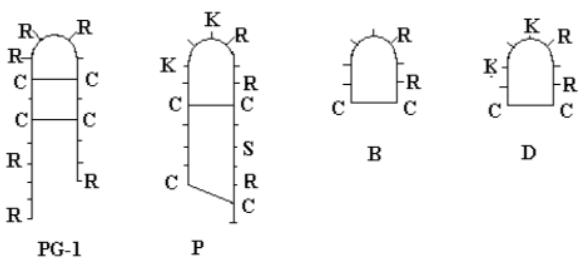
Other possible targets for bisphosphonates are *T. brucei* pyrophosphatase (TbVSP1) and the PPX1 exopolyphosphatase (80). A study of some 81 bisphosphonate compounds against TbVSP1 identified compounds **10** and **11** (Fig. 8) as the most active against TbVSP1, with IC_{50} values of 2.1 and 2.4 μM , respectively. *In vivo* studies on compounds **10** and **11** in *T. brucei brucei*-infected mice showed moderate increases in their long-term survival, but only compound **11** provided a 40% survival rate at 30 days postinfection. The low survival rate is probably due to a lack of efficacy of these compounds when the CNS is compromised, for the reasons mentioned above.

Antimicrobial peptides (AMPs)

Antimicrobial peptides (AMPs) are essential factors of innate immunity that have been conserved throughout evolution (81). In mammals, two broad classes of AMPs have been identified, the defensins and the cathelicidins, both of which kill organisms by inserting into their cell membranes and disrupting membrane integrity (82). Both classes have been shown to have varying degrees of antibacterial and antifungal activity. The cathelicidin-derived peptides SMAP-29 and protegin-1 (PG-1; see Table I) have been shown to decrease parasitemia and prolong the survival of *T. brucei*-infected mice (83).

Table I: Sequence, structure and inhibitory properties of truncated and variant analogues of mussel defensins and cathelicidins, with special mention of disulfide bond(s) and lysine substitutions.

Peptide identity	Amino acid sequence	ID_{50} *T. brucei* (μM)	*T. brucei* killed (50 μM)
SMAP-29 (83)	RGLRRLGRKIAHGVKKYGPTVLRIIRIAG	-	71.5%
Protegrin-1 (PG-1) (83)	RGGRLCYCRRRFCVCVGR(NH_2)	-	39.4%
MGD1(1-39) (84, 85)	GFGCPNNYQCHRHCKSIPGRCGGYCGGWHRLRCTCYRCG	-	-
P (MGD1[21-39]) (85)	CGGYCGKWKRLRCTSYRCG	12	-
B (MGD1[25-33]) (85)	CGGWHRLRC	-	3.3%
D (85)	CGKWKRLRC	4	-
BMAP-27 (86)	GRFKRFRKKFKKLFKKLSPVIPLLHLG(NH_2)	2.42-4.84	-



Amino acids are expressed as one-letter codes: C, cysteine; S, serine; K, lysine; R, arginine.

The defensins have been isolated from mammals, arthropods, plants and, more recently, molluscs. These are cationic molecules belonging to the cysteine-rich family of antimicrobial peptides. Mammalian and other vertebrate defensins are quite different from the arthropod/mollusc defensins in terms of both sequence and structure. MGD1 is a defensin of 39 residues (see Table I), isolated from the plasma and hemocytes of the edible Mediterranean mussel *Mytilus galloprovincialis*. Its three-dimensional structure has been solved using ^{1}H-NMR and it contains three loops within its structure (84). The nonapeptide corresponding to residues 25-33 of MGD1 (CGGWHRLRC; B) loop 3 (Table I), once cyclized to form a non-naturally occurring disulfide bridge, exhibits trypanosomal activity against the bloodstream form of *T. brucei,* as does a variant analogue (CGKWKRLRC; D) (Table I) and peptide P corresponding to residues 21-39 of MGD1 (loops 2 and 3), but containing two extra positive charges. The ID_{50} values of fragments D and P against *T. brucei in vitro* are 4 and 12 μM, respectively (85). BMAP-27 is another cationic antimicrobial peptide proven to be highly effective in killing the bloodstream form of the parasites, with limited hemolytic activity (86). A truncated version of this peptide, BMAP-18-mer, which contains the *N*-terminal end but lacks the *C*-terminal tail, is currently being investigated in transgenic Misra potatoes, with the hope that it may be used either as an edible treatment for trypanosomiasis, or that the purified peptide may yield a concentrated pharmaceutical (87). Interestingly, the most active peptides identified against *T. brucei* (PG-1, P, B and D) contain a loop region of amino acids incorporating both cationic and hydrophobic groups to facilitate membrane penetration.

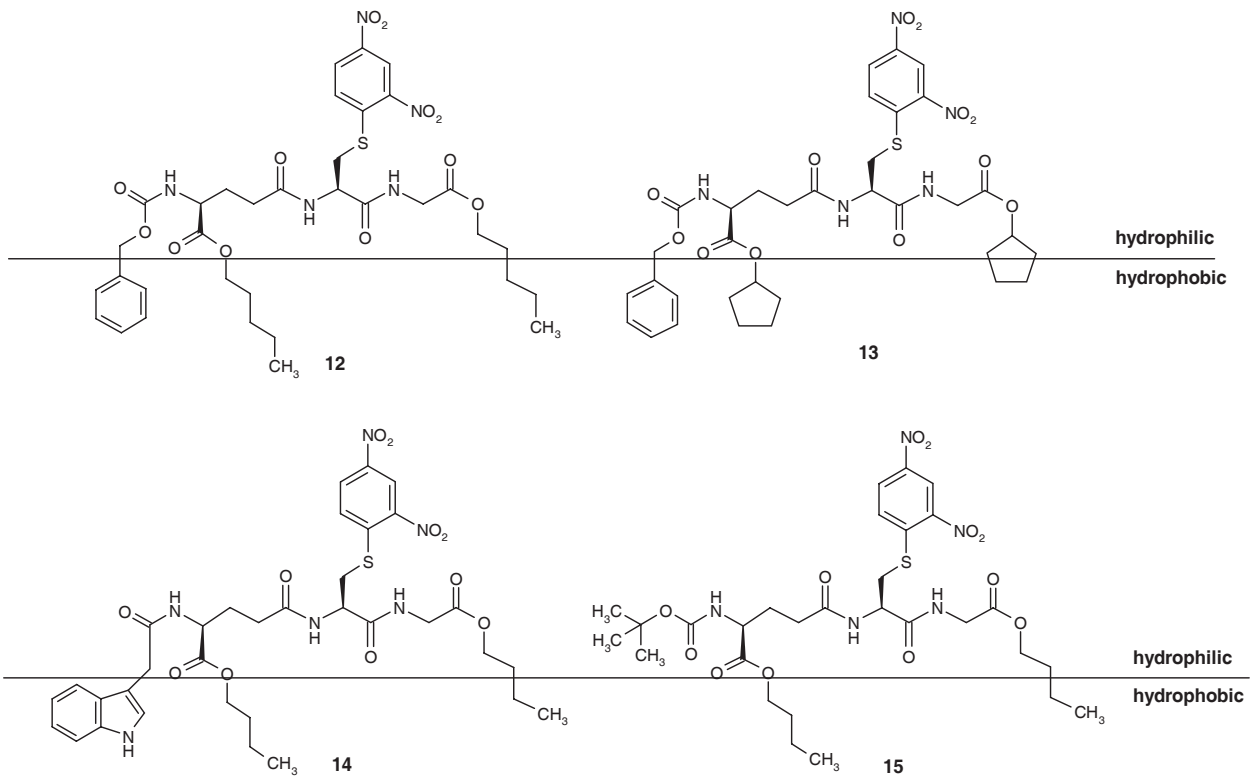
Antiparasitic glutathione peptides (AGPs)

The activity of these peptides, determined based on QSAR (88, 89) and HPLC studies, was found to be dependent on membrane binding, with a small amount of the peptide found to be de-esterified to the free acid form. This

Table II: Structure and inhibitory properties of glutathione-based antimicrobial peptides (AGPs).

Peptide number	ED_{50} *T. brucei brucei* (μM)	ED_{50} *T. brucei rhodesiense* (μM)	Relative toxicity*	ED_{50} *L. donovani* (μM)	ED_{50} *T. cruzi* (μM)
12 (88, 89)	0.38	0.55	> 545	> 30	6.7
13 (88, 89)	0.18	0.28	180	29.6	> 30 (30%)
14	0.91	0.30	67		35.0
15	0.14	0.22	360	9.9	16.5

*Ratio of toxicity in KB cells to that in *T. brucei rhodesiense*.



observation led to the conclusion that, based on their small size, these peptides may act as prodrugs which, upon de-esterification, produced peptides that were inhibitors of enzymes of the trypanothione cycle (88). The absence of an inhibitory effect of these peptides against enzymes of the trypanothione cycle (trypanothione reductase, trypanothione peroxidase and tryparadoxin) (90) led us to re-evaluate the mode of action. The inhibitory activity of these peptides is now considered, based on similarities of parasitic cell death to the AMPs, to be due to esterified peptide binding to the parasitic membrane, thereby disrupting its integrity. These peptides, unlike the AMPs, are uncharged molecules and rely on their amphiphilic properties, a result of the tripodal distribution of hydrophobic groups relative to the hydrophilic backbone, to cause membrane disruption and cell death. The absence of activity of these compounds against *Leishmania donovani* amastigotes indicates that the highly active endocytosis mechanism present in trypanosomes but absent in related parasites is responsible for the specificity of these compounds. The *in vitro* activity of some of these peptides against *T. brucei rhodesiense* is shown in Table II. These compounds also show activity against *L. donovani* (leishmaniasis) and *T. cruzi* (Chagas' disease) when tested against infected macrophages, but not amastigotes. The observed antiparasitic activity against *L. donovani* is therefore considered to be due to the action of these compounds on the macrophage (host) rather than on the parasite, as may be the case for *T. cruzi,* although this has yet to be confirmed. *In vivo* testing of the compounds against *T. brucei brucei*- and *T. brucei rhodesiense* STIB900-infected mice at 10 and 40 mg/kg i.p. x 4 days, respectively, failed to show a significant increase in survival *versus* untreated control samples. This was attributed to the short half-life of these diesters due to hydrolysis by human serum (71). Work aimed at increasing the *in vivo* stability of these compounds continues.

Conclusions

The increase in resistance to frontline drugs used against human African trypanosomiasis has resulted in the development of combination drug therapies. The majority of new and potential lead drug compounds currently being investigated can potentially only act against stage 1 human African trypanosomiasis, as they are unable to cross the blood-brain barrier to address stage 2 disease. In view of these observations, the need for surveillance technology in the monitoring of human African trypanosomiasis and the treatment of stage 1 disease has become more of an issue to prevent the disease developing further into the stage 2 form, to control the high levels of mortality in human African trypanosomiasis-endemic regions.

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