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Monthly Focus: Anti-infectives

Novel antitrypanosomal agents

Dietmar Steverding† & Kevin M Tyler
†*School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 TJ7, UK*

Trypanosomes are the causative agents of Chagas' disease in Central and South America and sleeping sickness in sub-Saharan Africa. The current chemotherapy of the human trypanosomiases relies on only six drugs, five of which were developed > 30 years ago. In addition, these drugs display undesirable toxic side effects and the emergence of drug-resistant trypanosomes has been reported. Therefore, the development of new drugs in the treatment of Chagas' disease and sleeping sickness is urgently required. This article summarises the recent progress in identifying novel lead compounds for antitrypanosomal chemotherapy. Particular emphasis is placed on those agents showing promising, selective antitrypanosomal activity.

Keywords: anti-trypanosomal agents, anti-tubulin agents, Chagas' disease, cysteine protease inhibitors, DNA topoisomerase inhibitors, proteasome inhibitors, sleeping sickness, sterol biosynthesis inhibitors, *Trypanosoma brucei*, *Trypanosoma cruzi*

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1. Introduction

Trypanosomes are parasitic protozoa that cause morbidity and mortality in millions of people in Africa and America [1]. In sub-Saharan Africa, *Trypanosoma brucei gambiense* and *T. b. rhodesiense* are the aetiological agents of sleeping sickness in humans. Over 60 million people living in 36 countries are threatened with sleeping sickness and the estimated number of cases is thought to be between 300,000 and 500,000 [1,2]. African trypanosomes live and multiply extracellularly in the blood and tissue fluids of humans and are transmitted by the bite of infected tsetse flies (*Glossina* spp.). In Latin America, infection with *T. cruzi* is responsible for Chagas' disease, which is the leading cause of heart disease [3]. Nearly 90 million people in 19 endemic countries are at risk of contracting Chagas' disease and ~ 16 million people are already infected [1,4]. American trypanosomes are found both extracellularly and intracellularly in the blood, lymph and tissue of the human host and are normally transmitted by contamination of mucosal membranes or bite wounds with excretions from infected reduviid bugs (*Triatoma* spp., *Rhodnius* spp., *Panstrongylus* spp.).

Chemotherapy of both African and American trypanosomiasis is unsatisfactory [1,5,6]. For the treatment of sleeping sickness only four drugs, of which three were developed > 50 years ago, are available (**Figure 1**). Suramin, a polyanionic sulfated naphthylamine introduced in 1922, and pentamidine, an aromatic diamidine first used in 1937, are effective against the early stage of *T. b. rhodesiense* and *T. b. gambiense* infections, respectively. The early stage of African trypanosome infection is defined by the restriction of the parasites to the blood and lymph system. Melarsoprol is a trivalent arsenical and was introduced in 1949 for the treatment of late-stage sleeping sickness caused by *T. brucei* spp. The late stage of sleeping sickness is characterised by the presence of the parasite in the cerebrospinal fluid. Eflornithine (DL-α-difluoromethylornithine, DFMO), a selective inhibitor of ornithine decarboxylase, is the only new drug for chemotherapy of sleeping sickness. It was first used in 1990 and is only effective against *T. b. gambiense*. Only two drugs are available for the treatment of Chagas' disease, which were developed in the 1970s: nifurtimox, a nitrofuran, and benznidazole, a nitroimidazole (**Figure 1**). They are

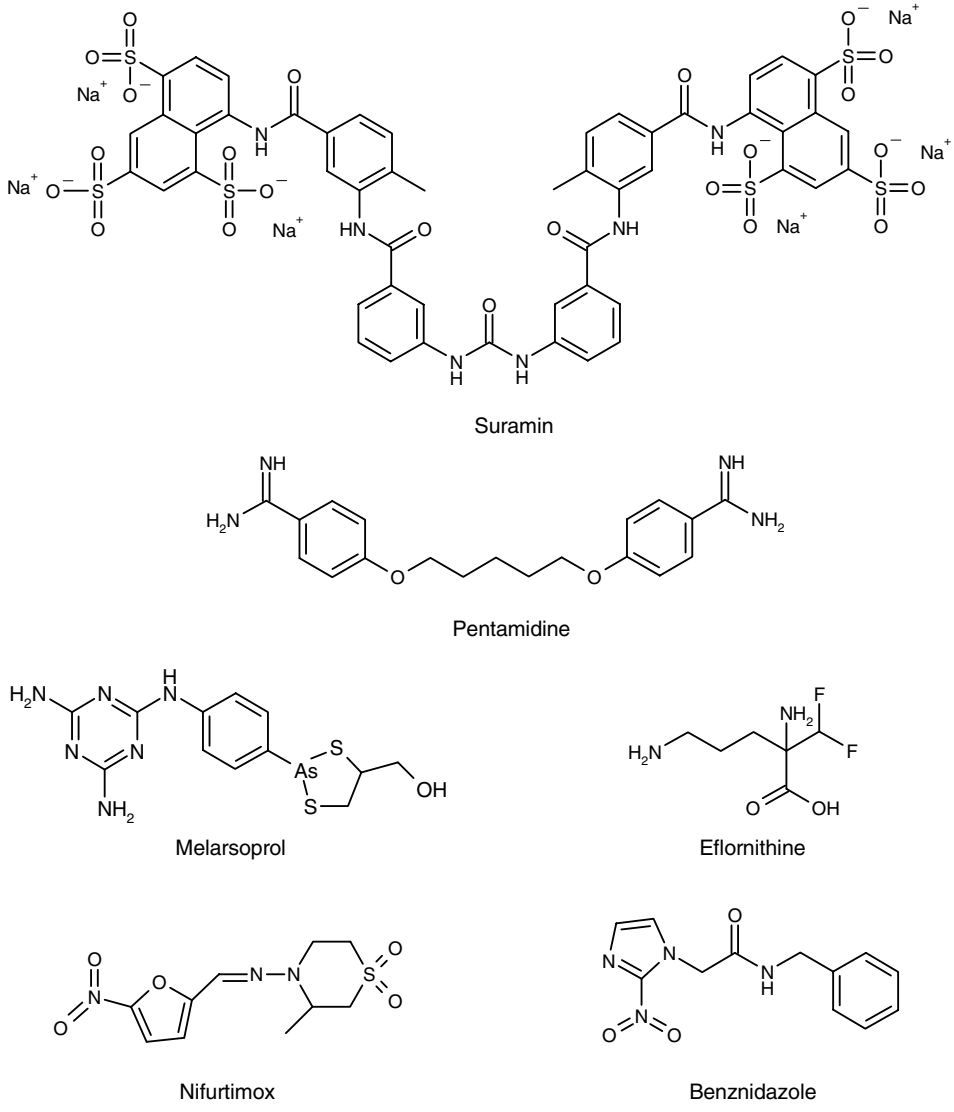

Figure 1. Structures of current antitrypanosomal drugs.

most active against the extracellular forms of *T. cruzi* during the acute phase of the infection. Both drugs seem to be ineffective against the intracellular forms of the parasite that cause chronic disease [7]. All drugs currently used to treat human trypanosomaises have significant side effects ranging from nausea to life-threatening complications.

With the exception of the prodrug DB-289 (**Figure 2**), an orally bioavailable diamidine for the treatment of early-stage sleeping sickness that has recently entered Phase IIb clinical trials [8], and the antifungal drug posaconazole (**Figure 2**), a triazole derivative for the treatment of Chagas' disease and about to be evaluated in early clinical trials [6], no other compounds are in clinical development. In addition, the production of the currently available drugs for therapy of human trypanosomiasis was recently under threat [9]. Thus, new drugs for chemotherapy of sleeping sickness and Chagas' disease are required. This article focuses on recent progress in the identification of promising agents with selective, trypanocidal activity that can serve as lead compounds for future antitrypanosomal drug development.

2. Cysteine protease inhibitors

T. brucei spp. and *T. cruzi* contain a cathepsin L-like cysteine protease termed brucipain (trypanopain-Tb) and cruzipain (cruzain), respectively. These enzymes are responsible for the major proteolytic activity of all life-cycle stages of these parasites [10]. Cruzipain is found on the cell surface of intracellular amastigotes and in the endosomal/lysosomal system of insect epimastigotes of *T. cruzi* [11,12], whereas brucipain is localised in lysosomes of bloodstream forms of *T. brucei* [13,14]. Research over the past few years with cultured parasites and experimentally infected mice has shown that trypanosome

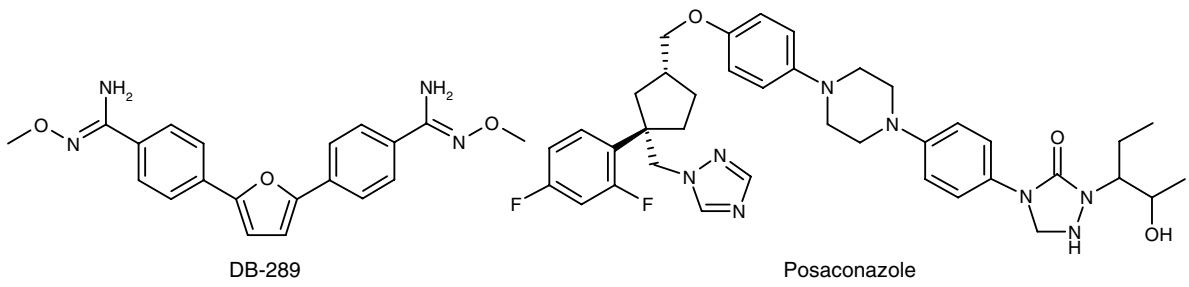

Figure 2. Structures of antitrypanosomal drugs in development.

cysteine proteases are valid targets for the development of new drugs. In particular, peptidyl and peptidomimetic compounds have proved trypanocidal.

2.1 Peptidyl inhibitors

Dipeptidyl substrate analogues linked to the reactive fluoromethylketone (CH_2F) were the first cysteine protease inhibitors reported to lyse trypanosomes, albeit at high concentrations (> 100 µM) [15]. Halomethylketones are irreversible inhibitors and react covalently with the active site cysteinyl-SH group of cysteine proteases [16]. Later, a number of carbobenzoxy (Cbz) fluoromethylketones, chloromethylketones (CH_2Cl) and diazomethylketones (CHN_2) were tested for *in vitro* killing of *T. brucei* bloodstream forms, and it was shown that Cbz-Phe-Ala-CHN_2 (Figure 3) was one of the most potent inhibitors with a 50% growth inhibition (GI_{50}) value in the low micromolar range [17,18]. Moreover, it was also demonstrated that Cbz-Phe-Ala-CHN_2 had an effect on the growth of *T. brucei in vivo* [18]. Parasitaemia of mice infected with *T. brucei* decreased to undetectable levels for 3 days following Cbz-Phe-Ala-CHN_2 250 mg/kg i.p. on days 3 – 6 after infection. Although, after discontinuation of treatment, parasitaemias returned to similar levels as those in control animals, the average survival time of mice was doubled. This probably indicates that parasites that had evaded killing by the inhibitor activated an immune response capable of controlling the infection.

To decrease toxicity *in vivo*, new generation inhibitors with a peptidomimetic, rather than a peptidyl, backbone coupled to the reactive phenyl vinyl sulfone (VSΦ) group have been developed. The vinyl sulfone moiety is less reactive towards nucleophiles than halo- and diazomethylketones and thus sufficiently inert in the absence of the target [19]. Like the halo- and diazomethylketones, vinyl sulfones are irreversible inhibitors that covalently react with the functional cysteinyl-SH group of cysteine proteases [19]. To increase *in vivo* activity and bioavailability, morpholinyl urea (Mu) and *N*-methylpiperazinyl urea (MePip) residues were incorporated at the terminal P_3-position of VSΦ peptides. On incubation with Mu-Phe-hPhe-VSΦ (**Figure 3**) at concentrations > 10 µM, *T. cruzi* epimastigotes stopped dividing and died after 5 days [20]. The peptide also inhibited the growth of bloodstream forms of *T. brucei* with a GI_{50} value of 5.6 µM [17]. The most potent vinyl sulfone inhibitor, however, was MePip-Phe-hPhe-VSΦ (Figure 3), which was reported to kill bloodstream forms of *T. brucei in vitro* with a GI_{50} value of 0.1 – 0.4 µM [10,17]. The compound also inhibited the growth of *T. brucei* and *T. cruzi in vivo*. Treatment of *T. brucei*-infected mice with MePip-Phe-hPhe-VSΦ 50 mg/kg i.p. b.i.d. from days 3 to 7 post-infection reduced parasitaemia to undetectable levels for the following 3 days [10]. As for Cbz-Phe-Ala-CHN_2, the effect was not permanent on cessation of treatment and parasitaemia soon reached control levels [10]. More encouraging were the results of experimental infections of mice with *T. cruzi*. Mice were rescued from lethal infection with *T. cruzi* if treated three times-daily with MePip-Phe-hPhe-VSΦ 35 mg/kg i.p. for 24 days [21]. Furthermore, using the same dosing regimen over 21 days cured mice that were chronically infected with *T. cruzi* for 3 months [21].

The latest development of dipeptidyl inhibitors of cysteine proteases is the synthesis of vinyl sulfonamide compounds [22]. Whereas VSΦ have second inactivation constants of 181,000 – 420,000 s^{-1} M^{-1} against cruzipain [19,22], vinyl sulfonamides exhibit values of 1,260,000 – 6,480,000 s^{-1} M^{-1} [22]. Although very potent cruzipain inhibitors, only the vinyl *N*-sulfonyl hydroxylamine (VSNHOΦ) derivatives Mu-Phe-hPhe-VSNHOΦ and MePip-Phe-hPhe-VSNHOΦ (**Figure 3**) exhibited significant activity in cell culture assays. Like their parent VSΦ, they inhibited the growth of *T. cruzi* in macrophages at a concentration of 10 µM [22]. However, in contrast to the parent compounds, parasites reappeared in the macrophage cultures 4 days after treatment with Mu-Phe-hPhe-VSNHOΦ, and MePip-Phe-hPhe-VSNHOΦ was terminated [22].

2.2 Non-peptidyl inhibitors

Recently, new lead scaffolds for inhibitors of trypanosome cysteine proteases with potent trypanocidal activities against cultured parasites have been identified. These include acyl hydrazides, ureas, aryl thioureas and thiosemicarbazones, all of which are non-peptidyl reversible inhibitors [23-27]. Many of these compounds were active in the low to submicromolar concentration range against pure brucipain and cruzipain [23-27]. Interestingly, structure–activity relationship studies for these compounds revealed that the most trypanocidal inhibitors have recurring patterns. For

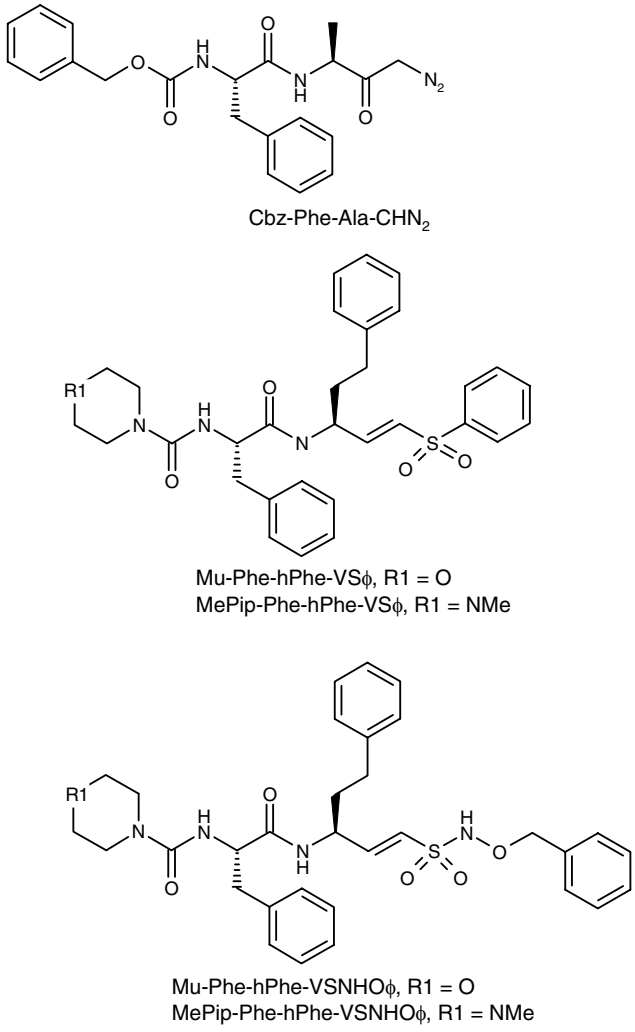

Figure 3. Structures of peptidyl cysteine protease inhibitors with promising trypanocidal activities.

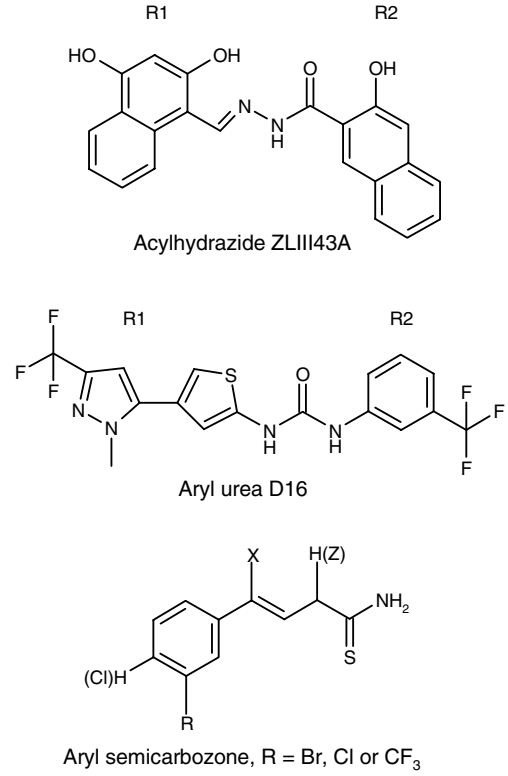

Figure 4. Structures of non-peptidyl cysteine protease inhibitors with encouraging antitrypanosomal activities.

instance, acyl hydrazides with GI_{50} values < 2 µM for *in vitro* growth inhibition of *T. brucei* have on their R2 side an unsubstituted or substituted naphthyl group [23,24]. One of the compounds, ZLIII43A (**Figure 4**), was shown to combat an experimental acute infection of *T. brucei* in mice, if given at the time of infection [23]. However, if the administration of the inhibitor was delayed, the treatment was ineffective [23]. Another example is the urea compound D16 (**Figure 4**), which has been shown to prolong the survival of *T. cruzi*-infected macrophages by 22 days [25], that shares the same substituent (1-methyl-3-trifluoromethylpyrazol-5-yl) on the R1 side with several acyl hydrazides recently identified to be active against bloodstream forms of *T. brucei* [24]. Finally, *N*-aryl semiarbazones that were effective at protecting macrophages against *T. cruzi* have trifluoromethyl, bromine or chlorine substituents at the C3-position of their single phenyl rings (**Figure 4**) [26].

3. Proteasome inhibitors

The eukaryotic proteasome is a multicatalytic protease complex that plays a critical role in intracellular protein degradation. The 20S core of the proteasome is a barrel-shaped structure made up of four rings [28]. The two inner rings are composed of seven distinct β-protein subunits. In each ring, three of these subunits contain the three major proteolytic activities of the proteasome [28]. These three catalytic activities are commonly referred to as the peptidyl-glutamyl peptide hydrolysing activity, the trypsin-like activity and the chymotrypsin-like activity located on the β1, β2 and β5 subunits, respectively [28]. By using RNA interference to selectively block the expression of β-subunits, it has been shown that the catalytic proteins are vital for trypanosomes [29], thus indicating that the proteasome is a valid target for antitrypanosomal drug development. In addition, biochemical analysis has revealed that the trypanosomal proteasome differs in terms of substrate specificity from the mammalian proteasome; the trypanosomal proteasome exhibiting high trypsin-like but low chymotrypsin-like activities, whereas the converse is true for the mammalian proteasome [30,31].

Proteasome inhibitors are usually short peptidyl substrate analogues modified at the C terminus by a functional group. Although the peptide portion directs the association of the

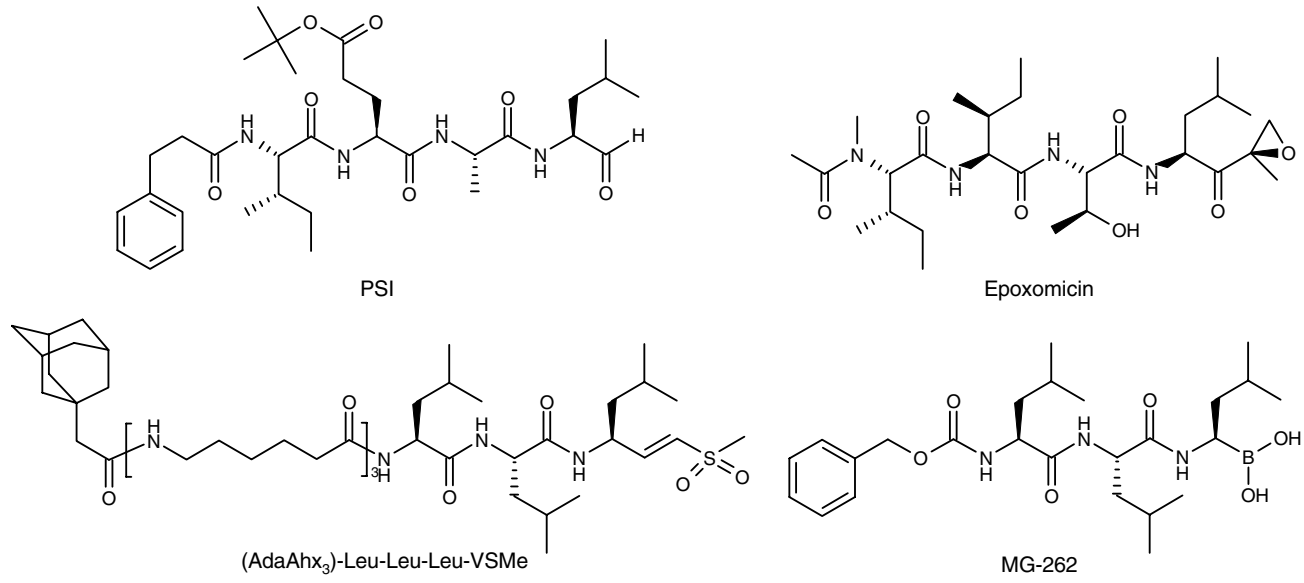

Figure 5. Structures of proteasome inhibitors with promising antitrypanosomal activities.

inhibitor to the enzyme's substrate binding site, the functional group interacts with the catalytic threonine residue to form reversible or irreversible covalent adducts [32].

Peptide aldehydes were the first proteasome inhibitors to be developed. They are well-known reversible inhibitors of cysteine and serine proteases. Recently, it has been shown that peptide aldehydes inhibit the growth of bloodstream forms of *T. brucei in vitro* [33]. The most trypanocidal peptide aldehyde proteasome inhibitor was PSI (**Figure 5**), with a GI_{50} value of 0.086 nM [33]. However, peptide aldehydes are not very specific and can inhibit other proteases such as lysosomal cathepsins and calpains [32,34]. In addition, the highly reactive aldehyde group can form Schiff's bases with circulating free amines, leading to possible side effects. These drawbacks are overcome by peptides linked to the vinyl sulfone moiety (see Section 2.2).

Previous studies have shown that vinyl methyl sulfones (VSMes) with a trileucine core sequence are very effective inhibitors of the proteasome [34,35]. Depending on the N-terminal substituent, trileucine VSMes also displayed promising trypanocidal activity [36]. For instance, adamantanylacetate ($AdaAhx_3$)-Leu-Leu-Leu-VSMe (**Figure 5**) [37], which has an incorporated extended N-terminal substituent of three 6-aminohexanoyl residues capped with an $AdaAhx_3$ group, killed bloodstream forms of *T. brucei in vitro* with a GI_{50} value of 0.4 μM [36]. Moreover, it was found that higher trypanocidal activity of trileucine VSMes generally correlates to a higher observed rate constant (k_{obs})/concentration of the inhibitor [I] value for the inhibition of the proteasomal trypsin-like activity but not the inhibition of the proteasomal chymotrypsin-like activity [36]. These findings indicate that trypanosomes are particularly sensitive to the inhibition of the trypsin-like activity.

A similar observation has been recently reported for α′,β′-epoxyketone proteasome inhibitors [38]. Peptide epoxyketones are the most selective inhibitors of the proteasome currently known. The reason for the high specificity of epoxyketones lies in their unique reaction mechanism by forming a cyclical morpholino ring with a N-terminal catalytic threonine residue of the proteasome. With serine or cysteine proteases, epoxyketones cannot form such a ring [39]. The peptide epoxyketone epoxomicin (**Figure 5**) has been recently shown to exhibit a selectivity index (ratio of cytotoxic to trypanocidal activities) of > 1000 [38]. As this selectivity index approaches those of commercially available drugs used for the treatment of human African sleeping sickness [40], epoxomicin is a promising lead compound for antitrypanosomal drug development.

Boronic acids are a new class of proteasome inhibitors displaying promising antitrypanosomal activity. Peptide boronates are much more potent and selective inhibitors than their corresponding aldehydes [41]. The boronate analogue of Cbz-Leu-Leu-Leu-CHO, MG-262 (**Figure 5**), is 400-fold more trypanocidal than its aldehyde with an impressive minimum inhibitory concentration (MIC) value of 10 nM [33]. MG-262 has also been shown to have a significant effect on the growth of *T. brucei in vivo* [42]. Treatment with MG-262 10 μg/day i.p. from days 4 to 6 after infection markedly slowed down the growth of the parasites in the blood of infected mice compared with infected control animals [42]. These results suggest that boronic acid proteasome inhibitors provide novel leads for the development of antitrypanosomal agents.

4. DNA topoisomerase inhibitors

DNA topoisomerases are essential enzymes that catalyse topological changes in the DNA molecule. They play a key role

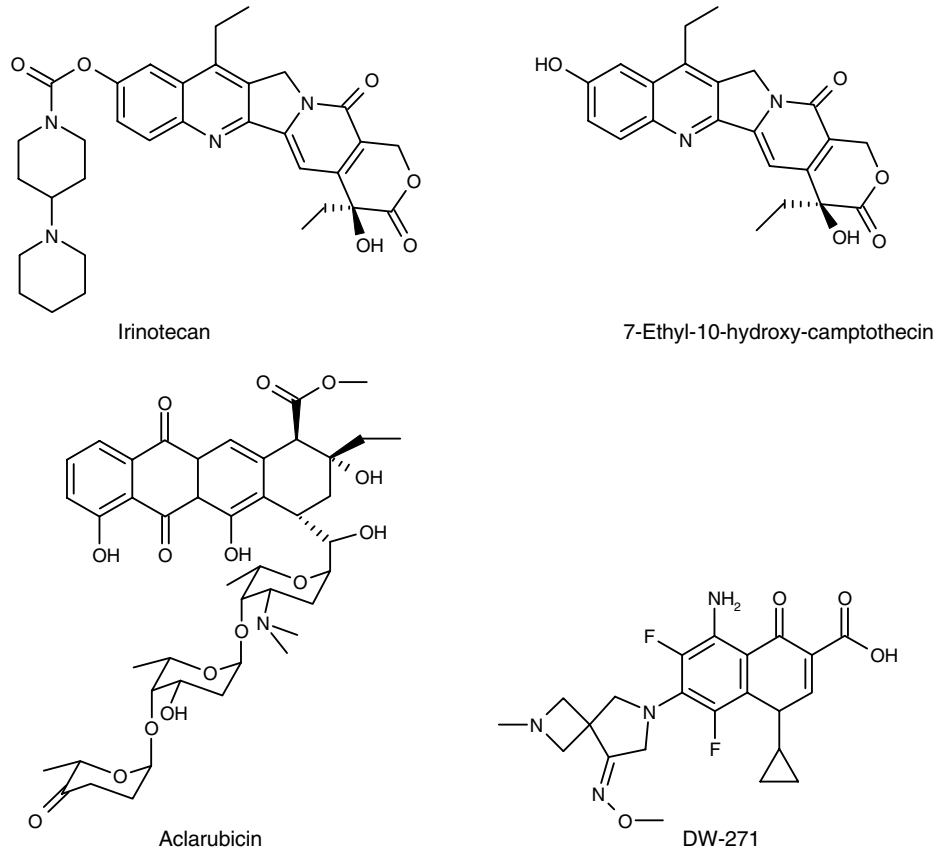

Figure 6. Structures of DNA topoisomerase inhibitors with encouraging trypanocidal activities.

in DNA metabolism such as replication, transcription, recombination and condensation. Two types of DNA topoisomerases have been characterised [43]. Topoisomerase I introduces transient single-strand breaks whereas topoisomerase II initiates transient double-strand breaks [44,45]. Both topoisomerases I and II have been purified from trypanosomes and their corresponding genes sequenced [46]. In contrast to other eukaryotic type I topoisomerases, the trypanosomal enzyme is a heterodimer whose subunits are encoded by different genes [47]. Both topoisomerases probably have dual localisations in the nucleus and the mitochondrion of trypanosomes [46]. By using RNA interference, it was shown that both topoisomerase I and II are vital for *T. brucei* [48,49], thus indicating that these enzymes are valid drug targets for antitrypanosomal chemotherapy. In addition, topoisomerases have an essential role in the replication of kinetoplast DNA in trypanosomes [46].

DNA topoisomerase inhibitors represent a major group of anticancer drugs [50]. Based on the mechanisms of action, DNA topoisomerase drugs can be divided into two classes. Class I drugs act by stabilising covalent topoisomerase–DNA complexes and are referred to as topoisomerase poisons [50]. The main class I drugs are camptothecins, anthracyclines, epipodophyllotoxins and quinolones. Class II drugs interfere with the catalytic functions of the enzyme, are referred to as topoisomerase inhibitors and include couramin antibiotics and fostriecin analogues [50].

4.1 Camptothecins

Camptothecin is a topoisomerase I inhibitor with powerful antitumour properties. A series of camptothecin derivatives have been shown to display trypanocidal activities with GI_{50} values in the submicromolar range [51]. Meanwhile, two camptothecin analogues, irinotecan and topotecan, have been developed into drugs for cancer chemotherapy. The trypanocidal activities of these two drugs have been recently investigated but neither drug was found to be very active against bloodstream forms of *T. brucei* [52]. However, irinotecan is a prodrug that has to be converted into its active metabolite 7-ethyl-10-hydroxy-camptothecin (**Figure 6**) by a carboxylesterase [53]. As the active metabolite exhibited substantial trypanocidal activity with a GI_{50} value of 0.12 μM [52], the low efficacy of irinotecan may be attributed to a lack of carboxylesterase in bloodstream forms of *T. brucei*. Therefore, it may be that irinotecan will display antitrypanosomal activity *in vivo*, the host carboxylesterases acting to convert the prodrug to its active form.

4.2 Anthracyclines and mitoxantrone

Anthracyclines and mitoxantrone are topoisomerase II inhibitors that are currently used in cancer chemotherapy. Daunomycin and its hydroxyl derivative doxorubicin have long been known to be active on bloodstream forms of *T. rhodesiense* affecting parasite motility and infectivity to mice [54].

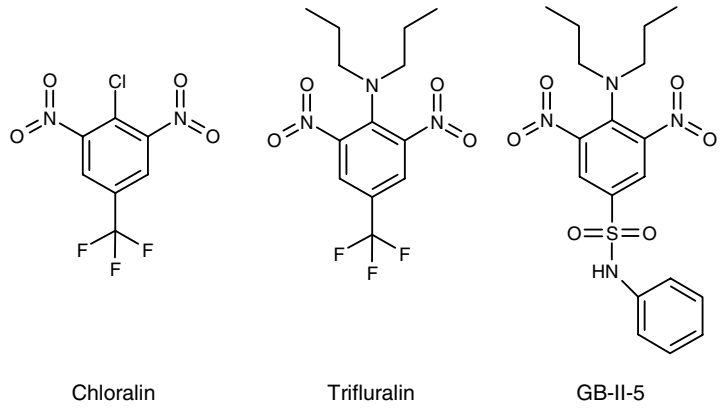

Figure 7. Structures of dinitroaniline compounds with trypanocidal activities.

Recently, it was shown that doxorubicin and aclarubicin and the related anthracenedione mitoxantrone exhibited promising activities against *in vitro*-cultured bloodstream forms of *T. brucei* with GI_{50} values in the low nanomolar range [52]. Based on the MIC values, aclarubicin (**Figure 6**) exhibited a selectivity index of 1000 [52]. If these compounds and other members of the anthracycline family prove to be active against trypanosomes *in vivo*, licensing and time-consuming drug development could be avoided. In addition, as the *in vivo* toxicities of these compounds are well described, a more rapid application for the treatment of human trypanosomiasis with less extensive clinical trials might be possible.

4.3 Fluoroquinolones

Fluoroquinolones are specific inhibitors of prokaryotic topoisomerase II, some of which are important drugs in antibacterial chemotherapy. In a number of recent studies, ~ 190 fluoroquinolones and their derivatives were tested for activity against bloodstream-form trypanosomes [55-57]. The most potent compounds exhibited GI_{50} values in the low micromolar range. However, selectivity indices were modest for most quinolones [55-57]. The most promising fluoroquinolone was DW-271 (**Figure 6**) with a selectivity index of 428 [56]. In contrast to its *in vitro* activity, DW-271 and the other fluoroquinolones tested showed no curative effect *in vivo* in dose-escalation experiments with a maximum dose of 100 mg/kg b.i.d. and failed to affect the murine parasitaemias [56]. Nevertheless, fluoroquinolones remain of interest as they display low toxicity [56] and can cross the blood–brain barrier [58].

5. Tubulin directed drugs

Tubulin is a molecule that is highly conserved among eukaryotes and is essential for cellular replication. Chemicals directed against tubulins are widely used for applications as diverse as cancer chemotherapy, gout treatment, weedkillers and antihelminthics. Trypanosomes are heavily dependent on microtubules for most of the aspects of cellular morphology, motility and intracellular transport [59]. Trypanosome tubulins display particular diversity in biochemistry and may be modified post-translationally by acylation, tyrosination and polyglutamylation. In addition to a tandem array of paired α- and β-tubulin genes, trypanosomes are unusual in possessing four other tubulin genes; -γ, -δ, -ε and -ζ [60,61]. The novel aspects of the trypanosome microtubule cytoskeleton, tubulin repertoire and biochemistry make it an attractive target for therapeutics. In addition, trypanosome tubulins differ markedly from mammalian tubulins with respect to their drug sensitivities. They are relatively insensitive to colchicine for which they have reduced affinity and to the vinca alkaloids vinblastine and vincristine [62]. However, trypanosomes are sensitive to some antitubulin compounds such as taxol [63] and benzimidazoles [64]. In particular, rhizoxin induces growth defects in trypanosomes at concentrations as low as 5 nM in culture [65].

The dinitroanilines chloralin and trifluralin (**Figure 7**) are microtubule-disrupting herbicides that have shown activity against trypanosomes in culture [66]. Chloralin in particular was active against *T. cruzi* epimastigotes with GI_{50} values of 6.8 – 17.6 μM [66]. In a mouse model of Chagas' disease, trifluralin-treated animals survived significantly longer than untreated controls [67]. However, the trypanocidal activity of trifluralin is thought to be due to the presence of the contaminant chloralin. From studies with analogues of the herbicide oryzalin, N^1-phenyl-3,5-dinitro-N^4,N^4-di-*n*-propylsulfanilamide (GB-II-5; **Figure 7**) has emerged as the most active antimitotic agent [68]. This compound has GI_{50} values of 0.41 and 0.73 μM *in vitro* against two strains of *T. brucei* with a selectivity index of 40 – 80 and is, therefore, a promising lead. However, initial results in animal models were disappointing [69].

6. Inhibition of lipid synthesis

The composition of the membrane systems of trypanosomes is distinct from those of the host cell. In common with fungi, trypanosomes synthesise ergosterol (provitamin D2) but not cholesterol [70,71] and synthesis of phosphitidylcholine appears to be via the Greenberg (transmethylation) pathway

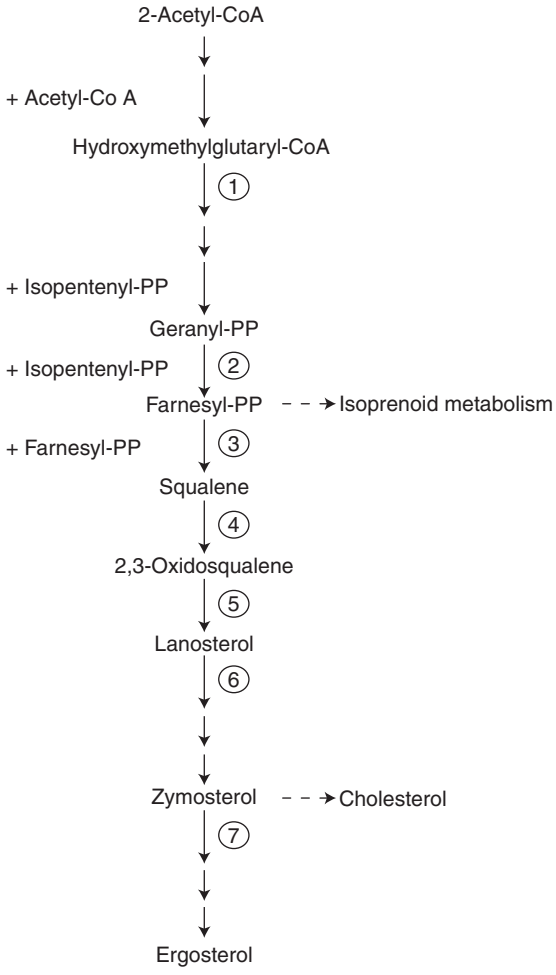

Figure 8. Simplified scheme of ergosterol biosynthesis. Target enzymes for chemotherapy of *Trypanosoma cruzi* are (**1**) hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34), (**2**) farnesyl pyrophosphate synthase (EC 2.5.1.10), (**3**) squalene synthase (EC 2.5.1.21), (**4**) squalene epoxidase (EC 1.14.99.7), (**5**) lanosterol synthase (EC 2.4.99.7), (**6**) C14α sterol demethylase (EC 1.14.13.70), and (**7**) sterol 24-C-methyltransferase (EC 2.1.1.41). Branched pathways for isoprenoid and cholesterol metabolism are indicated by dashed arrows.
CoA: Coenzyme A; PP: Pyrophosphate.

[72]. In bloodstream forms of *T. brucei* that contain cholesterol but not ergosterol, cholesterol is scavenged from lipoprotein complexes of the blood utilising an LDL receptor [73,74]. However, *T. cruzi* appears to maintain a requirement for ergosterol [6]. One existing drug, amphotericin B, which has an increased affinity for ergosterol over cholesterol [75], is a drug of choice against the related disease leishmaniasis and has shown good efficacy against *T. cruzi* [76]. Similarly, miltefosine, a lysophospholipid inhibitor now in widespread use against leishmaniasis that is active against *T. cruzi* [77], is believed to selectively inhibit phosphatidylcholine synthesis via the Greenberg pathway [72]. These findings provide proof-of-principle for targeting the lipid biosynthesis pathway in trypanosomes.

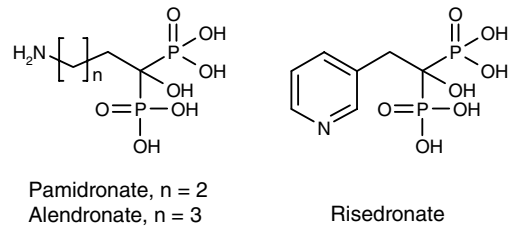

Figure 9. Structures of bisphosphonates with promising anti-*Trypanosoma cruzi* activities.

6.1 Inhibition of sterol synthesis

Ergosterol synthesis (**Figure 8**) is an obvious target for the chemotherapy of *T. cruzi*, an essential metabolic pathway in this parasite in which the latter reaction steps are divergent from cholesterol synthesis in mammals. In fact, even those enzymes shared with sterol synthesis in mammals represent valid targets as the inhibition of cholesterol synthesis is well tolerated in humans. This is because the dietary intake of cholesterol is normally sufficient to maintain adequate lipoprotein levels and inhibition of endogenous sterol synthesis may even be desirable in its own right for many [78].

6.1.1 Hydroxymethylglutaryl coenzyme A reductase inhibitors

Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) catalyses the rate-limiting reaction in sterol biosynthesis (**Figure 8**), the NADPH-dependent reduction of HMG-CoA to mevalonate. Statins, which are some of the best-selling drugs in the world (e.g., mevastatin, lovastatin, simvastatin, pravastatin and fluvastatin), target this enzyme in order to lower low-denisty lipoprotein cholesterol levels. It has been clear for some time that lovastatin is trypanocidal against *T. cruzi in vitro* [79] and when supplied as part of a cocktail of other sterol synthesis inhibitors *in vivo*, but is much less effective when adminsitered alone [80]. More recently, fluvastatin and cerivastatin were shown to have good activity against the *T. cruzi* enzyme [81], which appears to localise to the mitochondrion in the parasite unlike host cells [82].

6.1.2 Farnesyl pyrophosphate synthase inhibitors

The nitrogen-containing drugs etidronate, pamidronate, alendronat, and risedronate are metabolically inert inorganic pyrophosphate analogues and potent inhibitors of bone resorption. Ergosterol deficiency is associated with defects in calcium resorption in humans and the inhibition of ergosterol synthesis in trypanosomes may be linked to poor calcium resorption from the parasite's intracellular store of pyrophosphate, the acidocalcisomes.

Farnesyl pyrophosphate synthase (EC 2.5.1.10) is the branching point for the synthesis of polyisoprenoids and sterols (**Figure 8**). The recombinant enzyme of *T. cruzi* was shown to be inhibited by bisphosphonates [83]. In trypanosomes, these inhibitors block sterol synthesis at a presqualene level [84]. In

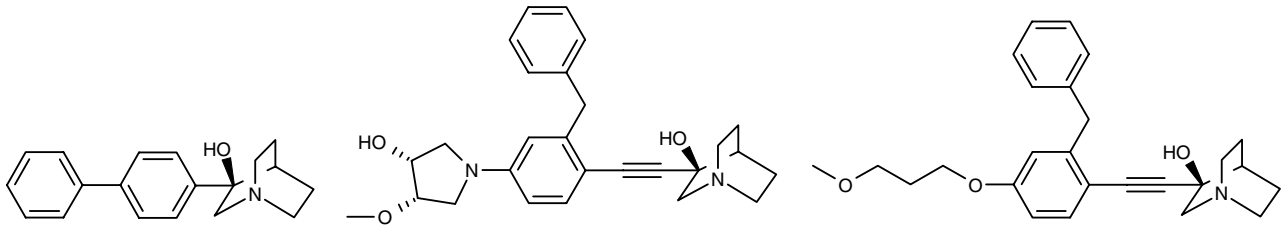

Figure 10. Structures of quinuclidines with encouraging anti-*Trypanosoma cruzi* activities.

addition, bisphosphonates have been shown to be active against *T. cruzi* without apparent toxicity to the host cells. Pamidronate and alendronate (**Figure 9**) were selectively active against amastigotes in culture [84] and pamidronate was also effective in ameliorating parasitaemia in mouse models [85]. Recently, risedronate (**Figure 9**) was shown to be able to cure cultures of vero cells infected with *T. cruzi* amastigotes and prevent infestation of the heart by the parasite in acute-phase animal models [86,87]. The selective activity that was observed may be explained by the preferential accumulation of these inhibitors in the calcium- and pyrophosphate-rich acidocalcisome, which could be considered as being compositionally analogous to hydroxyapatite in bone with which bisphosphonates are known to bind with high affinity [88].

6.1.3 Squalene synthase inhibitors

Squalene synthase (EC 2.5.1.21) catalyses a head-to-head reductive dimerisation of two molecules of farnesyl pyrophosphate in a two-step reaction to form squalene. It is the first committed step in sterol biosynthesis (**Figure 8**). 3-(Biphenyl-4-yl)-3-hydroxyquinuclidine (BPQ-OH; **Figure 10**), a quinuclidine-based inhibitor of this enzyme, was shown to have activity against the *T. cruzi* enzyme and has selective antitrypanosomal activity *in vitro* [89,90]. E5700 and ER-119884 (**Figure 10**), two other quinuclidine-based inhibitors of mammalian squalene synthase that are currently in development as cholesterol- and triglyceride-lowering agents in humans and orally available, are an order of magnitude more effective as inhibitors of the *T. cruzi* squalene synthase [91]. Moreover, the GI_{50} values of both inhibitors against different life-cycle stages of *T. cruzi* were in the nanomolar to subnanomolar range [91]. In animal models, E5700 was clearly more potent than ER-119884, preventing mortality at 50 mg/kg/day but not resulting in a complete cure [91]. Non-quinuclidine, aryloxyethyl thiocyanate derivatives have also yielded a potent lead directed against this enzyme. 4-Phenoxyphenoxyethyl thiocyanate (WC-9) has been reported to be selectively trypanocidal but tests in animal models have not yet been published [92].

6.1.4 Squalene epoxidase inhibitors

Squalene epoxidase (EC 1.14.99.7) catalyses the second committed step in sterol biosynthesis (**Figure 8**), thus making it an attractive target for antitrypanosomal chemotherapy. The alkylamine terbinafine, a potent inhibitor of fungal squalene epoxidase, has been reported to possess activity against epimastigotes and amastigotes of *T. cruzi* [93]. A series of *N,N*-dimethyl-2-propen-1-amine derivatives has been shown to be trypanocidal against *T. cruzi in vitro* [94-96] and a recent report describes 3-(4′-bromo-[1,1′-biphenyl]-yl)-3-(4-bromo-phenyl)-*N,N*-dimethyl-2-propen-1-amine as being more effective than benznidazole in treating a murine model of acute Chagas' disease [97]. The report suggests that the mode of action of this class of compounds is the inhibition of squalene epoxidase as the treatment of trypanosomes leads to reduced levels of ergosterol but raised levels of squalene [97].

6.1.5 Lanosterol synthase inhibitors

Lanosterol synthase (EC 5.4.99.7) is a key enzyme in sterol biosynthesis as it catalyses the cyclisation of 2,3-oxidosqualene to lanosterol (**Figure 8**), the initial precursor for sterols. A series of electron-poor aromatic mimics of a monocyclised transition state or high-energy intermediate formed from 2,3-oxidosqualene has been reported to show strong *in vitro* activities against several *T. cruzi* strains of which 12 compounds had GI_{50} values of ≤ 25 nM (**Figure 11**) [98].

6.1.6 C14α sterol demethylase inhibitors

Ketoconazole was the first orally available antifungal. Subsequently, a variety of new azole drugs (imidazoles and triazoles) have been introduced for the treatment of fungal infections. All of these target the cytochrome P450-dependent C14α sterol demethylase (EC1.14.13.70) leading to the accumulation of lanosterol and other sterol intermediates (**Figure 8**), which adversely affects normal membrane function [99]. These drugs have been used to treat Chagas' disease with mixed results. In animal models, ketoconazole treatment alone was able to cure some animals in acute (but not chronic) phase models [100,101]. Usage seems to be most effective when used in combination with other sterol synthesis inhibitors or with benznidazole [80,102]. Published reports that utilised ketoconazole or itraconazole to treat chagasic patients record a mixture of outcomes, with improvement for some but not all of those who were treated [103,104]. The induction of resistance to azoles in *T. cruzi* and cross-resistance to other azoles observed in *in vitro* experiments point to difficulties in the use of these compounds as chemotherapeutic agents [105].

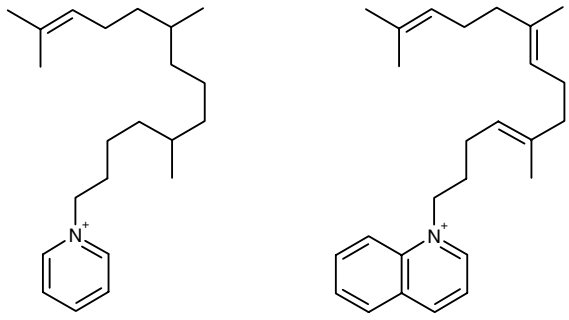

Figure 11. Structures of lanostrol synthase inhibitors mimicking the monocyclised cationic intermediate formed in the cyclisation of oxidosqualene to lanosterol.

In the past decade, new triazole derivatives with potent *in vitro* and *in vivo* activities have been synthesised. D0870 (**Figure 12**) shows efficacy in both acute and chronic mice models, with 30 – 50 times higher activity than ketoconazole and leading to 60 – 70% of parasitological cure [106]. Although very promising for the treatment of Chagas' disease, AstraZeneca terminated the development of D0870 in 1995 due to an adverse event in a patient receiving the drug and some unpredictable pharmacokinetic properties in humans [107]. Posaconazole (SCH-56592; **Figure 2**) inhibits epimastigote proliferation and ergosterol synthesis much more effectively than ketoconazole and D0870, and produces an apparent cure rate of 50% in animals infected with strains resistant to nifurtimox, benznidazole and ketoconazole [108]. Posaconazole is currently in Phase III clinical trials as a systemic antifungal and is about to be evaluated in clinical trials in chagasic patients [6]. Albaconazole (UR-9825; **Figure 12**) is very active *in vitro* [109] and was shown to be effective in the treatment of *T. cruzi* of dogs although a significant emergence of resistance was observed during the course of treatment [110]. TAK-187 (**Figure 12**) is an experimental antifugal triazole with potent trypanocidal activity. It has a MIC value of 1 nM against clinically relevant intracellular amastigotes of *T. cruzi* and induces complete protection against lethal infection and high levels of parasitological cures at a dose of 20 mg/kg p.o. [111]. Ravuconazole (BSM-207147; **Figure 12**) is an investigational triazole derivative currently in development as a systemic antifungal that shows very potent *in vitro* activity against *T. cruzi* amastigotes with a MIC value of 1 nM [112]. However, ravuconazole treatment did not lead to a parasitological cure in a chronic mouse model of Chagas' disease [112]. A new class of C14α sterol demethylase inhibitors, disubstituted imidazoles, with potent anti-*T. cruzi* activity was recently identified [113]. The compounds have GI_{50} values in the mid-nanomolar range, caused a dramatic decrease in parasitaemia when administered orally and led to 100% survival in mice with acute *T. cruzi* infection [113].

6.1.7 Sterol 24-C-methyltransferase inhibitors

Sterol 24-*C*-methyltransferase (EC 2.1.1.41) is an enzyme that methenylates a range of sterols with a double bond in the side chain although zymosterol is the preferred substrate (**Figure 8**). As there is no equivalent enzyme in the biosynthesis of cholesterol, this enzyme should be a selective target for anti-*T. cruzi* chemotherapy. A series of azasterols have been reported to exhibit activity at micro- to nanomolar concentrations against *T. cruzi* (**Figure 13**) [114,115]. The compounds were also active against bloodstream forms of *T. b. rhodesiense* [115] and, therefore, represent interesting lead compounds for antitrypanosomal chemotherapy.

6.2 Lysophospholipid analogues

The archetypal examples of lysophospholipid analogues were originally derived for cancer chemotherapy and include the alkylglycerophosphocholine edelfosine, the thioether substituted phosphatidylcholine ilmofosine and the alkylphosphocholine miltefosine (hexadecylphosphocholine). Miltefosine has recently emerged from clinical trials as the treatment of choice for several forms of leishmaniasis [116]. Some of the lysophospholipid analogues that have been tested so far have been found to be active against epimastigotes, intracellular amastigotes and trypomastigotes of *T. cruzi* by damaging the surface membranes and inhibiting metacyclogenesis, and were shown to reduce the parasitaemia in *T. cruzi*-infected mice [72,77,117,118]. Although none of the lysophospholipid analogues have yet been reported to achieve cure, they appear to be more effective in combination with sterol synthesis inhibitors such as ketoconazole [72,119].

7. Lipoylation inhibitors

Trypanosome membranes are rich in peripheral membrane proteins, in particular on the external face where glycosylphosphatidylinositol (GPI)-anchored proteins, such as the variable-surface glycoprotein (VSG) in *T. brucei* and transsialidases in *T. cruzi*, are abundant. Intracellular proteins are frequently prenylated or acylated. These modifications are often a key to the localisation and function of the lipoprotein; thus, the disruption of lipoylation can give dramatic and terminal phenotypes. Agents have not yet emerged that specifically disrupt the acylation and GPI anchor modification of proteins in trypanosomes. In contrast, the protein farnesyltransferase inhibitors used in cancer therapy have been shown to express trypanocidal activity [120]. In particular, a series of isothiazole dioxides and imidazole-containing peptidomimetic compounds have shown good activity against the purified trypanosome enzyme and trypanocidal activity in culture [121,122].

8. Other compounds

Several other compounds have been identified as potential leads for the development of antitrypanosomal agents. By using

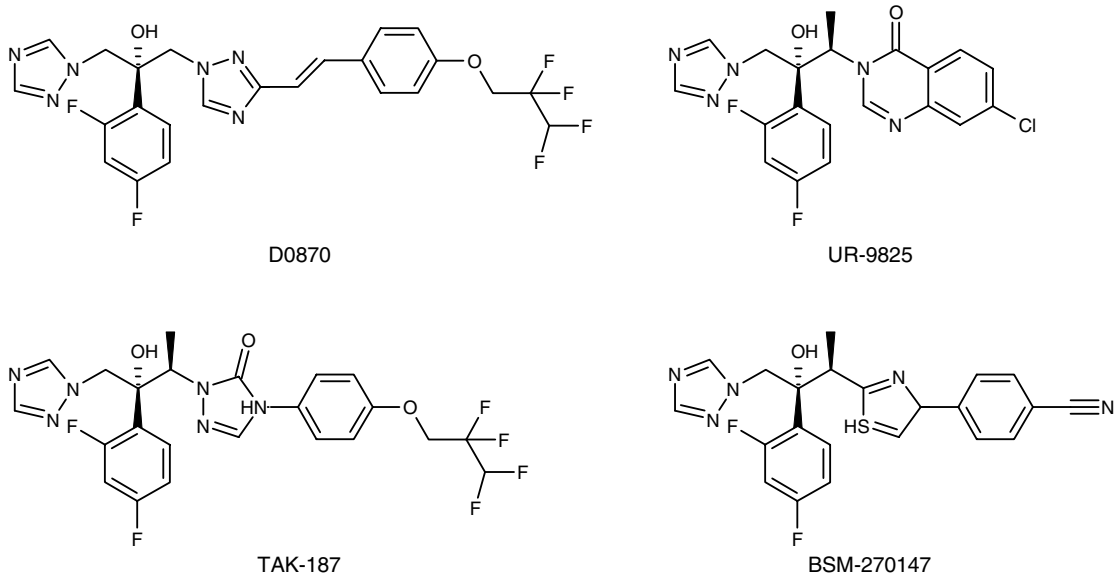

Figure 12. Structures of triazoles with potent and selective anti-*Trypanosoma cruzi* activities.

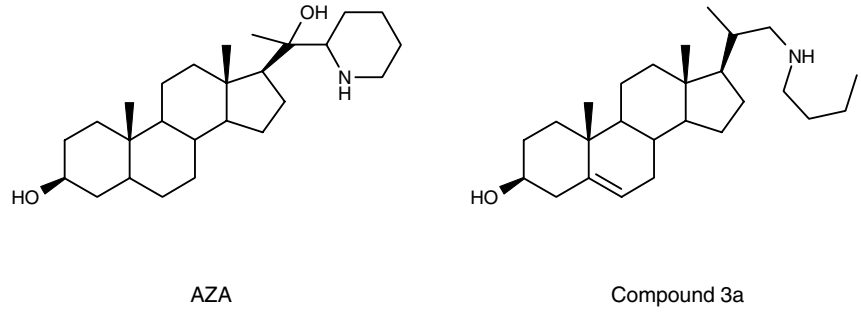

Figure 13. Structures of azasterols with potent trypanocidal activities.

structure–activity relationships to identify inhibitors of the trypanothione cycle, a biochemical pathway unique to trypanosomatids, quinoxaline *N*,*N*′-dioxide derivatives and diesters based on Cbz-*S*-2,4-dinitrophenylgluthathione have been reported to be active against trypanosomes *in vitro* [123,124]. As trypanothione is a spermidine-bridged bis-glutathione, polyamine analogues have been synthesised and evaluated for their trypanocidal activities, some of which display GI_{50} values in the nanomolar range [125-127]. The inhibition of *S*-adenosylmethionine decarboxylase, another key enzyme involved in the biosynthesis of polyamines, has led to the development of the agent *cis*-5′-deoxy-5′-(4-amino-2-butenyl)methylaminoadenosine (MDL-73811). This compound possesses both antitumour and antiparasitic activity and was recently shown to inhibit the growth of *T. b. rhodesiense* and *T. b. gambiense* both *in vitro* and *in vivo* [128]. Other agents with promising antitrypanosomal activity include benzo-δ-carbolines and cryptolepines [129], aminoadamatane and aminoalkylcyclohexane derivatives [130], benzo[1,2-c]1,2,5-oxadiazole *N*-oxide derivatives [131], prolylisoxazoles [132] and bicycle[2.2.2]octan derivatives [133,134]. In addition to the development of new agents for the chemotherapy of human trypanosomiases, the derivatives and analogues of existing antitrypanosomal drugs have been synthesised and tested for their biological activities. In particular, derivatives of the anti-chagasic drug nifurtimox [135-140] and analogues of the anti-sleeping sickness drug pentamidine [127,141-144] have been investigated.

9. Natural products

Many of the drugs that are used today are natural products or natural product-derived compounds. Hence, it is not surprising that some natural substances display trypanocidal activities. Several natural compounds including alkaloids, phenolic derivatives, quinones and terpenes have been shown to inhibit the growth of trypanosomes *in vitro* with GI_{50} values in the submicromolar range [145]. One interesting lead compound is ascofuranone, a prenylated phenol antibiotic produced by the phytopathogenic fungus *Ascochyta visiae*. Ascofuranone is an inhibitor of the trypanosome alternative oxidase, a unique

mitochondrial electron transport system in these parasites. A recent study has shown that ascufuranone can cure *T. brucei*-infected mice if the compound is administered intraperitoneally for four consecutive days at 100 mg/kg or orally for eight consecutive days at 400 mg/kg [146]. For information about other natural substances with antitrypanosomal activities, the interested reader is referred to the recent review by Hoet *et al.* [145].

10. Expert opinion and conclusion

The combination of undesirable toxicity and poor efficacy of the current drugs result in an urgent and unmet need to develop novel, cheap and effective chemotherapies for the treatment of Chagas' disease and African sleeping sickness. Unfortunately, pharmaceutical companies have drastically reduced their investment in drug development for tropical diseases, and the rational design of new antitrypanosomal agents is primarily undertaken from within academic research. Over the last decade, several new lead compounds for the treatment of human trypanosomiases have been identified. Inhibitors of ergosterol synthesis are encouraging anti-*T. cruzi* compounds as they target an essential metabolic pathway of this parasite. Peptidyl and non-peptidyl cysteine protease inhibitors are very promising antitrypanosomal agents as these compounds are small in size and inexpensive to produce. Other promising compounds are the DNA topoisomerase and proteasome inhibitors currently used in cancer chemotherapy. If these anticancer drugs prove to be useful against trypanosomes, a more rapid application for the treatment of Chagas' disease and sleeping sickness with less extensive clinical trials might be possible as their *in vivo* toxicities are already well established. As the drug discovery and development process is expensive in terms of both time (~ 10 – 12 years) and money (~ US $400 million), the cross-application of existing drugs with selective trypanocidal activity may be the best prospect to new antitrypanosomal drugs in the short term.

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Affiliation

Dietmar Steverding† & Kevin M Tyler
†Author for correspondence
School of Medicine, Health Policy and Practice, University of East Anglia, Norwich NR4 TJ7, UK
Tel: +44 (0)160 359 1291;
Fax: +44 (0)160 359 3752;
E-mail: dsteverding@hotmail.com