New drugs are urgently needed for the treatment of tropical and subtropical parasitic diseases, such as African sleeping sickness, Chagas’ disease, leishmaniasis and malaria. Enzymes in polyamine biosynthesis and thiol metabolism, as well as polyamine transporters, are potential drug targets within these organisms. In the present review, the current knowledge of unique properties of polyamine metabolism in these parasites is outlined. These properties include prozyme regulation of AdoMetDC (S-adenosylmethionine decarboxylase) activity in trypanosomatids, co-expression of ODC (ornithine decarboxylase) and AdoMetDC activities in a single protein in plasmodia, and formation of trypanothione, a unique compound linking polyamine and thiol metabolism in trypanosomatids. Particularly interesting features within polyamine metabolism in these parasites are highlighted for their potential in selective therapeutic strategies.

Key words: α-difluoromethylornithine, Leishmania, malaria, ornithine decarboxylase (ODC), polyamine, S-adenosylmethionine decarboxylase (AdoMetDC), spermidine synthase, Trypanosoma.
Figure 1  Polyamine biosynthetic pathways and polyamine transporters of trypanosomatid and apicomplexan parasites compared with those of their human host

The parasites as well as their vectors are depicted. *T. brucei* is transmitted by tsetse flies and *T. cruzi* is transmitted by kissing bugs. These trypanosomatids cause sleeping sickness and Chagas’ disease respectively. *Leishmania* spp. are transmitted by sandflies, whereas malaria-causing *Plasmodium* parasites are transmitted by *Anopheles* mosquitoes. Cell membrane transporters (cylinders) are sized approximately in proportion to their efficiencies. All images are in the public domain with photo images credited to the CDC Public Health Image Library (http://phil.cdc.gov/phil/) and the human body image to Wikimedia commons (http://commons.wikimedia.org/). Cad, cadaverine; homoT(SH)₂, homotrypanothione; Orn, ornithine; Put, putrescine; ROS, reactive oxygen species; Spd, spermidine; Spm, spermine.

the Lys⁶⁹ residue in the N-terminal domain. In the C-terminal domain of the second subunit, the Cys⁶⁰⁰ residue plays an essential role in ensuring correct protonation of an intermediate in the decarboxylation reaction (Figure 2A).

Human AdoMetDC is synthesized as a pro-enzyme, which undergoes an autocatalytic internal serinolysis reaction [7,8]. This reaction generates the two subunits (α and β), as well as the active site pyruvoyl group, which is involved in the decarboxylation process. Human AdoMetDC is a homodimer with two individual active sites and two putrescine-binding sites (Figure 2B).

Both of the human aminopropyltransferases SpdS and SpmS exist as dimers of two identical subunits. Neither of the enzymes requires a cofactor for catalytic activity. SpdS and SpmS are highly specific for putrescine and spermidine respectively, as the amine acceptor for an aminopropyl group from dcAdoMet [15,16]. The active sites of the two enzymes are similar, but differ in the sizes of their amine substrate-binding pockets [15,16]. In SpdS (Figure 2C) [15], as well as in SpmS [16], a flexible gate-keeping loop keeps the substrate (putrescine or spermidine) in the correct position for subsequent catalysis and opens to release the product (Figure 2C).

African Sleeping Sickness

African sleeping sickness (African trypanosomiasis) is a fatal disease caused by infection with two of three subspecies of *T. brucei*. The disease is endemic in sub-Saharan Africa in areas populated by the tsetse fly (genus *Glossina*), which transmit the parasites with their bite. It is estimated that 70,000–80,000 people are infected and that approximately 30,000 deaths from the disease occur annually [17]. The disease progresses from a haemolymphatic stage to a meningoencephalitic stage. More than 90% of the reported cases of African sleeping sickness are caused by *T. b. gambiense* [18]. This subspecies is responsible for the more chronic disease, occurring in the central and western regions, whereas *T. b. rhodesiense* causes the acute disease and is encountered in the eastern and southern regions of Africa. A third subspecies, *T. b. brucei*, and several other species (*Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma suis* and *Trypanosoma simiae*) cause nagana, a wasting disease among domestic animals. *T. b. brucei* is non-pathogenic to humans, and serves as a model organism for drug discovery.

Despite the death toll and the heavy burden of illness, there is an acute lack of satisfactory drugs against African sleeping sickness since all of the drugs in use suffer from serious limitations. These include a lack of selectivity against the parasite, excessive host toxicity, emerging drug resistance, requirement for parenteral administration, limited availability and high cost of treatment.

Polyamine metabolism as a drug target in *T. brucei*

Putrescine and spermidine are synthesized in *T. brucei* parasites by ODC, AdoMetDC and SpdS (Figure 1). However, SpmS is missing in *T. brucei* as well as in the other parasites...
Polyamine homeostasis as a drug target in pathogenic protozoa

Inhibition of ODC in T. brucei

A large number of ODC inhibitors have been developed and characterized. DFMO (α-difluoromethylornithine or efornithine), which functions as an enzyme-activated irreversible inhibitor [19], proved to be very effective against T. b. brucei [20] and T. b. gambiense [21]. Merrell Dow in fact filed its New Drug Application for DFMO (called Ornidylium) in 1988, noting only one indication: T. b. gambiense. Eventually, DFMO has become the first-line drug against late-stage disease, i.e. when the parasites have invaded the CNS (central nervous system). It has been nicknamed ‘the resurrection drug’ because of its striking effects on comatose patients [22].

The effectiveness of DFMO against African sleeping sickness is probably attributable to the following facts: (i) the parasite ODC remains irreversibly inhibited due to its slow turnover rate [23], whereas the inactivated human host ODC is rapidly replaced by newly synthesized active enzyme; (ii) the extracellular parasite cannot obtain polyamines from the blood of the host because of its negligible polyamine uptake capability [24]; (iii) in the absence of spermidine, the parasites cannot make trypanothione, and are therefore seriously compromised in their antioxidant capability (see below); (iv) the polyamine-depleted parasites exhibit a marked decrease in protein synthesis, in particular the variant surface glycoprotein coat [25], which is essential for evasion of the host immune response; (v) AdoMet and deAdoMet accumulate dramatically in the parasites and may cause aberrant methylation [26]; (vi) the polyamine-depleted parasites differentiate irreversibly into short stumpy forms, which are incapable of binary fission and have lost their infectivity for the vertebrate host [27]; and (vii) since these parasites spend their entire life-cycle extracellularly they are more directly exposed to DFMO than are parasites that replicate intracellularly (e.g. T. cruzi, Leishmania spp. and Plasmodium spp.).
Crystal structures have been solved for the native T. brucei ODC, as well as for the enzyme in complex with PLP, DFMO or putrescine [28,29] (Figure 2A). T. brucei ODC, like human ODC, is an obligate homodimer with two identical active sites formed at the dimer interface by interactions between the Cys660 residue in the C-terminal domain of one subunit and the Lys60 residue in the N-terminal domain of the other subunit [14,30]. The contacts at the dimer interface are formed primarily by the C-terminal domains, which interact through six aromatic rings that form a stacking interaction across the domain boundary [28]. In the absence of the substrate, the PLP cofactor forms a Schiff base with the Lys60 residue in the boundary between the two ODC subunits. Similarly to the corresponding Cys660 residue in the human enzyme [31], the conserved Cys660 residue in T. brucei ODC plays an essential role in the formation of a covalent adduct with DFMO. Within the dimer interface, DFMO forms a Schiff base with PLP on one of the monomers and a covalent bond to the Cys660 residue in the other monomer. The binding of DFMO induces a 145° rotation of the Cys660 residue toward the active site, compared with the native T. brucei ODC structure (Figure 2A) [28]. This mechanism of action explains the success of DFMO as a suicide inhibitor of ODC, causing polyamine depletion and subsequent inhibition of cell growth and proliferation.

Besides its remarkable success against T. brucei, DFMO unfortunately has poor pharmacokinetic characteristics. Recent clinical trials, using DFMO in combination with the trypanocidal agent nifurtimox, demonstrated that the course of DFMO treatment could be shortened and simplified (with infusion every 12 h for 7 days, instead of every 6 h for 14 days) without compromising the antiparasitic effect of DFMO [32]. This combination therapy is presently considered suitable for first-line use in the treatment of second-stage West African sleeping sickness.

The active sites of the human and T. brucei ODCs are very similar, which suggests that rational design of inhibitors selectively targeting the enzyme of the parasite may prove challenging. However, the prolonged half-life of T. brucei ODC compared with that of the human counterpart is obviously a beneficial characteristic for targeting this protein in the treatment of African sleeping sickness. Four novel inhibitor classes that are selective and specific for T. brucei ODC have recently been identified in a high-throughput screen [33]. The most active compounds, dithioamidines, were shown to be able to inhibit ODC activity by binding to allosteric sites and thereby controlling enzyme activity.

Inhibition of AdoMetDC in T. brucei

Trypanosomatid genomes are unique in that they contain two types of AdoMetDC genes: (i) an orthologue to the gene that encodes a functional AdoMetDC in other species; and (ii) a paralogous gene, encoding a protein called prozyme, that is catalytically dead but allosterically active [34]. By forming a high-affinity heterodimer with AdoMetDC, prozyme can up-regulate the enzyme activity 1200-fold. It has been suggested that the prozyme has evolved through duplication of the ancestral AdoMetDC gene and subsequent mutational drift, losing catalytic activity in the process, but gaining allosteric regulatory function. Prozyme shares only approximately a 30% amino acid sequence identity with the catalytically active T. brucei AdoMetDC. In addition, it is missing several critical residues required for processing and catalytic activity. In this respect, AdoMetDC from T. brucei and other trypanosomatids represent a novel structural subclass of eukaryotic AdoMetDCs, that otherwise exist as monomers (e.g. plant AdoMetDC) or homodimers (e.g. mammalian AdoMetDC) [8].

Although not a substrate of AdoMetDC, putrescine was found to bind and strongly activate the homodimeric mammalian AdoMetDC, but not the monomeric plant enzyme [8]. There is positive co-operativity in putrescine and substrate (AdoMet) binding to the homodimeric form of human AdoMetDC [35]. Interestingly, putrescine was also found to stimulate the activity of homodimeric T. brucei AdoMetDC, but only had a negligible effect on the heterodimeric complex with prozyme [34]. It is conceivable that the allosteric activation of T. brucei AdoMetDC by prozyme and the co-operativity seen in the human enzyme may somehow be related [8].

The discovery of prozyme opens up new prospects for inhibiting AdoMetDC in trypanosomatids, either by blocking formation of the active AdoMetDC-prozyme complex or by stabilizing the inactive conformation of AdoMetDC. Inhibitors developed against the heterodimeric active site may block the AdoMetDC activity more effectively than inhibitors of the homodimeric active site [34]. It is anticipated that the crystal structures of the heterodimeric and the homodimeric forms of T. brucei AdoMetDC will provide valuable information for structure-based drug design, as well as for understanding of the evolution of the AdoMetDC gene.

AdoMetDC has been chemically validated as a drug target in T. brucei. MDL73811 (5′-{[(Z)-4-amino-2-butyl]methylamino}-5′-deoxyadenosine), a structural analogue of deAdoMet and an enzyme-activated irreversible inhibitor of AdoMetDC, was shown to be at least 100-fold more potent than DFMO against murine T. b. brucei infections [36]. MDL73811 treatment could even cure mice infected with a multidrug-resistant strain [36], as well as clinical isolates of T. b. rhodesiense [37]. In combination, DFMO and MDL73811 acted synergistically, curing T. b. rhodesiense-infected mice, thus permitting lower doses to be used [37].

The AdoMet analogue AdoMao [5′-{(5′-deoxy-5′-adenosyl)-1-aminoxy-4′-(methylsulfonyl)-2-cyclopentene}] inhibits AdoMetDC irreversibly [38]. Of several diastereomeric forms synthesized, trans-15,45-AdoMao proved to be the most promising in all respects. It inhibited in vitro growth of T. b. brucei bloodstream forms with an IC50 of 0.9 μM, and was also effective against two clinical isolates of T. b. rhodesiense. Interestingly, growth inhibition was configuration-dependent, indicating that the unique trypanosomal AdoMet transporter [39] and/or the trypanosomal AdoMetDC prefer substrates in the S-configuration. Because of its minimal effects on human cells, trans-15,45-AdoMao may be considered parasite-specific. It is possible that the T. brucei parasites exhibit a facilitated uptake of AdoMet and its analogues, whereas only small quantities of these enter mammalian cells [39].

Lead optimization around MDL73811 resulted in an analogue (Genz-644131) that was 5-fold more potent than MDL73811 against the T. b. brucei AdoMetDC–prozyme complex [40]. This analogue exhibited a longer half-life than MDL73811 as well as improved potency and CNS penetration in T. b. brucei-infected mice. The mechanism by which AdoMetDC inhibitors kill the parasites is believed to be associated not only with depletion of spermidine and related thiol derivatives [41], but with the accumulation of AdoMet, which may cause aberrant methylation [42].

Inhibition of SpdS in T. brucei

Several SpdS inhibitors have been tested against T. brucei in vitro. These include a nucleoside bisubstrate (transition state) analogue, namely, AdoDATO (5′-adenosyl-1,8-diamino-3-thioctane) [43], a relatively large compound (423.5 Da) that fills both the deAdoMet- and putrescine-binding sites [15]. Two other SpdS inhibitors, CHA (cyclohexylamine) and dicyclohexylamine, compete with putrescine binding [43], but...
off-target effects have also been suggested. Administration of dicyclohexylamine, the most potent of the SpdS inhibitors, to mice with trypanosomiasis did not increase their survival time. Nevertheless, RNA interference-mediated silencing of the SpdS gene in bloodstream form T. brucei led to depletion of polyamine pools and thiol derivatives, which resulted in cell growth cessation and ultimately cell death [44, 45]. Thus SpdS may be considered a genetically validated drug target in African trypanosomes, but chemical validation will have to await the development of inhibitors that selectively and irreversibly inactivate the enzyme.

Polyamine-dependent redox metabolism as a drug target in T. brucei

Trypanosomatids have a unique thiol-based redox metabolism, depending on trypanothione as their principal thiol, in contrast with their human host, which utilizes GSH [46]. Trypanothione provides reducing equivalents to a variety of essential reactions in the parasites through oxidation of the dithiol form of trypanothione [T(SH)2 (dihydrotypantnothione/reduced trypanothione)] into the disulfide form [TS2 (trypanothione disulfide/oxidized trypanothione)]. This is followed by regeneration of T(SH)2 by TryR (trypanothione reductase), an NADPH-dependent flavoenzyme (Figure 3). The transfer of the reducing equivalents from trypanothione is mediated by tryaredoxins, which are small highly abundant proteins with oxidoreductase activity. A major function of the trypanosomatid redox system is to overcome the oxidative stress induced by the host defence mechanisms. The hydroperoxides produced by the host are detoxified to their respective alcohols by a family of tryaredoxin-dependent peroxidases (Figure 3). For a comprehensive review of thiol redox homoeostasis see Krauth-Siegel and Comini [47].

Trypanothione is synthesized from GSH and spermidine by an ATP-dependent monomeric C–N ligase [TryS (trypanothione synthetase)], in a two-step reaction with glutathionylspermidine as an intermediate (Figures 3 and 4). In addition to a TryS domain, the enzyme contains an amidase domain, which catalyses the hydrolysis of T(SH)2 into GSH and spermidine [48]. This bifunctional TSA (TryS-amiadase) is a key enzyme in trypanothione homoeostasis, catalysing both synthesis and degradation of T(SH)2 (Figure 4). Experiments with a tetracycline-dependent conditional double-knockout of TSA in T. brucei, revealed a marked reduction of T(SH)2, together with an accumulation of GSH, when the parasites were grown in vitro without tetracycline [48]. These effects were followed by inhibition of growth and eventually cell death. In the absence of tetracycline, the parasites were unable to infect mice, further validating TSA as a drug target in T. brucei. If the amidase activity of TSA was knocked out alone, the inhibitory effects on parasite growth in vitro and virulence in vivo were less pronounced, indicating that the TryS function is a better drug target than the amidase function. TryS has also been chemically validated as a drug target [49].

In addition, TryR has been genetically validated as a drug target in T. b. brucei [50]. Trypanosomes lacking TryR are unable to infect mice, and show increased sensitivity to oxidative stress [50]. In cell cultures, TryR activity has to be reduced by > 90% to considerably reduce the growth rate. Therefore any drug designed to target this enzyme would have to be exceptionally effective to be pharmaceutically relevant. Studies of small-molecule inhibitors of T. brucei TryR identified several competitive inhibitors, the most promising being clomipramine (Ki = 3 μM), a CNS-active drug [51]. However, this compound failed to extend survival...
time in an acute mouse model of African trypanosomiasis. Since reversible TryR inhibitors may be displaced by accumulation of TS₂, occurring as a consequence of further cellular metabolism of T(SH)₂ (Figure 3), irreversible active-site-directed TryR inhibitors may be required in a clinical setting. Another major challenge with druggability of the TryR target is the large size of its active site, which must accommodate the substrates, TS₂ (721 Da) or glutathionylspermidine disulfide (867 Da) [51]. It has been estimated that, for an orally bioavailable drug, the molecular mass should ideally be under 500 Da [52]. Nevertheless, the trypanothione pathway and the linked redox pathways (Figure 3) appear to be essential for T. brucei function, which makes the enzymes in these pathways suitable drug targets [47,53].

Polyamine transport as a drug target in T. brucei

T. brucei exhibits very low rates of putrescine uptake and no evidence of any polyamine transporter has been found in the genome [24]. The parasite is therefore unlikely to circumvent the effect of DFMO by obtaining putrescine from the host. On the other hand, T. brucei transfectants expressing LmPOT1, a high-affinity transporter for both putrescine and spermidine derived from L. major (see below), were markedly stimulated in their putrescine uptake [24], indicating that there is a major difference between these two organisms in their capacity to use extracellular putrescine.

CHAGAS’ DISEASE

Chagas’ disease (American trypanosomiasis) is a devastating and potentially life-threatening illness caused by infection with T. cruzi. It is endemic and widespread in Latin America (19 countries), with 8–11 million chronically infected individuals mainly residing in rural areas with abject poverty. It is estimated that 100 million people are at risk and that 14,000 deaths occur annually [17]. Recently, the disease has spread to other continents because of population mobility and failure to cure the chronic disease. The parasite is most often transmitted to humans by the infected faeces of ‘kissing bugs’ (mainly by species belonging to the Triatoma, Rhodnius and Panstrongylus genera). The bugs hide in cracked walls of adobe houses and come out at night to suck blood. Reservoirs of parasites are present both in wild and domestic animals. An individual can also become infected with T. cruzi through contaminated food, blood transfusion, organ transplantation, vertical transmission (passed from an infected mother to her child during pregnancy or birth) or accidental injection.

Benzimidazole and nifurtimox are the only drugs available against Chagas’ disease. Although almost 100% effective if given soon after infection, these drugs cause adverse reactions in up to 40% of treated patients, and are ineffective against the chronic form of the disease. Therefore there is a pressing need for new and better drugs. One problem to consider when designing new drugs against T. cruzi, is that these parasites circulate in the blood in high numbers only during the initial acute phase. During the chronic phase, which is thus far incurable, the parasites reside intracellularly, mainly in the heart muscle and in the smooth muscle of the digestive tract.

Polyamine metabolism as a drug target in T. cruzi

The polyamine requirement of T. cruzi is met in a strikingly different way than in T. brucei. The former trypanosomatid lacks ODC, the first enzyme in polyamine biosynthesis, and is therefore utterly dependent on uptake of putrescine from the host (Figure 1). Although T. cruzi does not possess a complete polyamine biosynthetic pathway, the parasite does express both AdoMetDC [54,55] and SpdS, implying that once putrescine is transported into the parasite, it can be converted into spermidine (and spermine) [56]. Although other trypanosomatids do not contain spermine, it appears as if T. cruzi SpdS may be somewhat promiscuous like the Thermotoga maritima SpdS [15] and able to convert some spermidine into spermine [56].

Inhibition of AdoMetDC in T. cruzi

The auxotrophic nature of T. cruzi for putrescine makes the polyamine pathway an especially attractive drug target. Obviously the lack of ODC in T. cruzi excludes the possibility of using any of the ODC inhibitors, but the AdoMetDC inhibitor MDL73811 has been shown to decrease the capability of T. cruzi to infect and multiply in rat heart myoblasts [57]. Since the inhibition of infectivity was not bypassed by the addition of spermidine or spermine, it is more likely that the adverse effect of MDL73811 on infectivity is due to the intracellular accumulation of AdoMet than to polyamine depletion.

As in T. brucei, AdoMetDC is allosterically activated by prozyme (a catalytically dead homologue), but the T. cruzi AdoMetDC—prozyme heterodimer also requires putrescine for activation to reach a similar efficiency as that of the fully activated T. brucei heterodimeric complex [58]. It is interesting to note that heterodimers formed between prozyme from T. brucei and AdoMetDC from T. cruzi, and prozyme from T. cruzi and AdoMetDC from T. brucei, are functional. It has been suggested that the stimulation of the T. cruzi AdoMetDC—prozyme heterodimer by putrescine, may represent a regulatory mechanism related to the lack of ODC in T. cruzi and its need for putrescine import from the host cell [58]. Human blood, in which T. brucei parasites reside, is a poor source of putrescine, whereas the cells in which T. cruzi parasites reside are rich in putrescine. The fact that the AdoMetDC—prozyme heterodimer interaction is unique to the trypanosomatids offers a species-selective mechanism that can be targeted in development of new types of drugs against these parasites.

Another group of compounds, MGBG [methylglyoxalbis(guanylhydrazone)] and a series of its aromatic derivatives, are much less potent inhibitors of T. cruzi AdoMetDC than of the human enzyme [54]. Although one such derivative (CGP40215A) had an IC₅₀ of 4.5 nM for T. brucei AdoMetDC, and prozyme from T. brucei parasites, is a poor source of putrescine, whereas the cells in which T. cruzi parasites reside are rich in putrescine. The fact that the AdoMetDC—prozyme heterodimer interaction is unique to the trypanosomatids offers a species-selective mechanism that can be targeted in development of new types of drugs against these parasites.

Inhibition of SpdS in T. cruzi

In addition to AdoMetDC, SpdS may provide an essential targeting point in T. cruzi polyamine metabolism. However, very limited information is available regarding the specific inhibition of the T. cruzi SpdS. Therefore structure-based inhibitor discovery may provide novel inhibitors to target SpdS and this may have particular application to the inhibition of T. cruzi. The previously
published crystal structures of \textit{T. cruzi} SpdS could provide important insights into inhibitor identification, as the co-inhibition of AdoMetDC and SpdS would have severe consequences on the availability of polyamines for these parasites. To date structures of the apo form of SpdS as well as the protein with bound AdoMet have been solved (Structural Genomics of Pathogenic Protozoa Consortium, http://www.sgpp.org/description.shtml) [60a]. The structures are highly similar to that of the mammalian enzyme but, for both of these structures, the gate-keeping loops were not defined, which could be due to the absence of bound dAdoMet. The relatively large size of the SpdS active site [10–15 Å (1 Å = 0.1 nm), Figure 2C] could again prove problematic for the design of inhibitors that conform to Lipinski’s rule [52]. Innovative strategies therefore need to be considered in order to effectively inhibit the enzyme, and at the same time achieve high selectivity.

**Inhibition of polyamine function in \textit{T. cruzi} with polyamine analogues**

In addition to biosynthesis inhibition through the targeting of specific enzyme active sites, polyamine analogues have been investigated in various organisms to perturb polyamine homoeostasis. Ample evidence is available to substantiate that counteraction of polyamine function by these analogues is a rational approach for anticancer drug development [2]. Although the mechanisms of action of polyamine analogues differ between human cells and parasites, some analogues clearly exhibit antiparasitic effects. Ideally the analogue should (i) use a polyamine transporter to gain entry into the parasite, thus competing with the natural polyamines for uptake; (ii) down-regulate the polyamine biosynthetic enzymes; (iii) reduce the pools of the natural polyamines; (iv) counteract the natural polyamines in their infection- and growth-related functions; and (v) exhibit parasite-selective activity (reviewed in [2,61,62]).

Among the first compounds that were synthesized to meet several of these criteria were the \(N,N\)-bis(benzyl)polyamine analogues [63]. Two of these (MDL27695 and MDL27699) [64] and two \(N,N\)-bis(ethylthiophene)polyamine analogues (MDL28302 and MDL29431) [65] were found to inhibit host cell invasion and intracellular amastigote multiplication of \textit{T. cruzi}. These compounds are all analogues of spermine, suggesting that they may interfere with the intracellular binding and thus the normal functions of spermine and the other polyamines. In fact MDL27695 binds to DNA and may compete for spermine- or spermidine-binding sites on DNA [63].

**Polyamine-dependent redox metabolism as a drug target in \textit{T. cruzi}**

\textit{T. cruzi}, like other trypanosomatids, has a bifunctional TSA with separate active sites for the synthesis of TSH\(_1\), and for the amidase reaction, hydrolysing TSH\(_1\) into GSH and spermidine (Figure 4) [66,67]. However, \textit{T. cruzi} is uniquely capable of converting the alternative polyamine cadaverine (1,5-diaminopentane) into aminopropylcadaverine, bis(aminopropyl)cadaverine and \(N^3,N^7\)-bis(glutathionyl)spermidine (homotryptophane) [56]. Thus SpdS and TryS in \textit{T. cruzi} accept cadaverine and aminopropylcadaverine as substrates, in addition to putrescine and spermidine respectively. A source of cadaverine may be the cadaverine-containing actinomycetes [68], which are known to reside in the gut of the \textit{Rhodinia} vector [69]. The fact that homotryptophane disulfide is readily reduced by \textit{T. cruzi} TryR, with similar kinetic parameters to those of TS\(_2\), suggests that it is a physiological substrate. Another novel thiol, \(N^1,N^{12}\)-bis(glutathionyl)spermine has also been identified in \textit{T. cruzi} [66], indicating that the \textit{T. cruzi} TryS site in TSA can use spermine as a substrate. Interestingly, \(N^3,N^7\)-bis(glutathionyl)spermine disulfide is also a physiological substrate of TryR [66]. The crystal structure of \textit{T. cruzi} TryR has been solved and is nearly identical with the structure of the \textit{T. b. brucei} enzyme [51].

**Polyamine transport as a drug target in \textit{T. cruzi}**

As mentioned above, \textit{T. cruzi} is a diamine (putrescine/cadaverine) auxotroph. The rate of putrescine uptake is approximately 40 times higher in \textit{T. cruzi} than in other trypanosomatids [70], and exceeds that of spermidine by about as much. Biochemical characterization of polyamine transport in \textit{T. cruzi} epimastigotes showed that the transporter exhibits a high specificity for putrescine and cadaverine, but low specificity for spermidine and spermine [71]. The parasite can obtain putrescine, but not cadaverine, from the human host, which lacks this polyamine. However, as mentioned above, a source of cadaverine may be the actinomycetes [68] residing in the gut of the vector [69].

The first cell-surface polyamine transporter to be molecularly identified in eukaryotic cells was a high-affinity putrescine/spermidine transporter (LmPOT1) from \textit{L. major} [24] (see below). Results from the \textit{T. cruzi} genome-sequencing project was screened for amino acid/auxin permeases and 60 PAT (putative amino acid transporter) genes clustered in 12 groups (\textit{TcPAT1–12}) were identified [72]. Further investigation of \textit{TcPAT12}, the most divergent member in terms of amino acid sequence, revealed 55% amino acid identity with \textit{LmPOT1} [73]. Functional expression of \textit{TcPAT12} in \textit{Xenopus laevis} oocytes indicated that it is a high-affinity spermidine transporter \((K_m = 14–26 \mu M)\). It also transports putrescine (and L-arginine), but at a 7-fold lower rate than spermidine [73].

Two orthologues (\textit{TcPOT1.1} and \textit{TcPOT1.2}) of \textit{LmPOT1} were recently identified within the \textit{T. cruzi} genome [74]. Both genes are heterozygous alleles located on chromosome 11, and their open reading frames predict polypeptides of 613 and 627 amino acids with a 95% identity. Hydropathy profiles indicate that both proteins have 12 transmembrane domains with intracellular N- and C-termini [74]. One of these orthologues, \textit{TcPOT1.2}, is the \textit{TcPAT12} gene previously characterized by Carrillo et al. [73]. In contrast with the earlier findings, \textit{TcPOT1.2} and its parologue \textit{TcPOT1.1} recognize putrescine and cadaverine (but not spermidine or spermine) as ligands [74]. Interestingly, transporter function and localization is regulated by the polyamine content of the extracellular milieu [74]. \textit{TcPOT1.1}, and to some extent \textit{TcPOT1.2}, are able to cover the entire cell surface and thus increase the cell’s putrescine uptake when putrescine becomes scarce. This suggests that \textit{T. cruzi} uses a signalling system that can sense putrescine availability. Collectively, the signalling and transport systems seem to enable the parasite to overcome its inability to synthesize polyamines \textit{de novo}. Given the auxotrophy for putrescine, these systems are very promising targets [74].

DAB (1,4-diamino-2-butane) is a putrescine analogue that inhibits axenic growth of \textit{T. cruzi} epimastigotes in a dose-dependent manner, leading to oxidative stress and subsequent mitochondrial damage (Table 1) [75]. Co-incubation with putrescine reversed the inhibitory effects of DAB. Since \textit{T. cruzi} is dependent on polyamine uptake for survival, it is postulated that DAB competes for or inhibits the uptake of putrescine by the cell-surface putrescine transporter, which consequently leads to depletion of putrescine, spermidine and trypanothione. It is therefore conceivable that the oxidative stress observed is due to lack of trypanothione.
Table 1  Selected inhibitors of ODC, AdoMetDC, SpdS and polyamine functions that have shown efficacy against parasites

Inhibitory concentrations (IC\textsubscript{50}, \textmu M) of these drugs against the parasites in vitro are indicated. Values are from references as indicated. The promastigote [(p)] and amastigote [(a)] forms of Leishmania are indicated.

| Inhibitor          | T. brucei IC\textsubscript{50} | Reference(s) | T. cruzi IC\textsubscript{50} | Reference | L. donovani IC\textsubscript{50} | Reference(s) | P. falciparum IC\textsubscript{50} | Reference(s) |
|--------------------|-------------------------------|--------------|-------------------------------|-----------|---------------------------------|--------------|---------------------------------|--------------|
| **ODC inhibitors** |                               |              |                               |           |                                 |              |                                 |              |
| DFMO               |                               |              |                               |           |                                 |              |                                 |              |
| APA                |                               |              |                               |           |                                 |              |                                 |              |
| **AdoMetDC inhibitors** |                           |              |                               |           |                                 |              |                                 |              |
| MDL73811           |                               |              |                               |           |                                 |              |                                 |              |
| MGBG               |                               |              |                               |           |                                 |              |                                 |              |
| CGP48664A          |                               |              |                               |           |                                 |              |                                 |              |
| CGP40215A          |                               |              |                               |           |                                 |              |                                 |              |
| **SpdS inhibitors** |                               |              |                               |           |                                 |              |                                 |              |
| CHA                |                               |              |                               |           |                                 |              |                                 |              |
| 4-MCHA             |                               |              |                               |           |                                 |              |                                 |              |
| Dicyclohexylamine  |                               |              |                               |           |                                 |              |                                 |              |
| **Polyamine analogues/ transport inhibitors** | |              |                               |           |                                 |              |                                 |              |
| MDL27695           |                               |              |                               |           |                                 |              |                                 |              |
| DAB                |                               |              |                               |           |                                 |              |                                 |              |

© 2011 The Author(s)
The author(s) has paid for this article to be freely available under the terms of the Creative Commons Attribution Non-Commercial Licence (http://creativecommons.org/licenses/by-nc/2.5/) which permits unrestricted non-commercial use, distribution and reproduction in any medium, provided the original work is properly cited.
LEISHMANIASIS

Leishmaniasis is a complex of diseases caused by protozoan parasites of the genus Leishmania [76]. The disease is transmitted through the bite of infected sand flies of the genera Phlebotomus and Lutzomyia, which are the most common vectors in the Old and New World respectively. Leishmaniasis is mainly a zoonotic disease (with reservoirs in rodents and canines). However, in Africa and the Indian subcontinent, visceral leishmaniasis caused by Leishmania donovani is an anthropoontic disease transmitted from human-to-human by sandflies [77].

The main forms of the disease are cutaneous, mucocutaneous and visceral leishmaniasis, with clinical spectra ranging from skin ulcers to liver damage and anaemia. The most severe form is visceral leishmaniasis (also known as kala azar or black fever), in which the parasites cause hepatosplenomegaly, fever, weight loss and anaemia [76]. Visceral leishmaniasis is lethal unless promptly diagnosed and treated. Leishmaniasis is considered endemic in 88 countries with 350 million people at risk [17]. The number of people infected is estimated to be more than 12 million with 1–2 million new cases of the cutaneous form and 0.5 million new cases of the visceral form occurring each year. It is estimated that visceral leishmaniasis causes more than 50,000 deaths worldwide annually.

There are approximately 20 species of Leishmania that infect humans. Visceral leishmaniasis is mainly caused by three species of the L. donovani complex (L. donovani, Leishmania infantum and Leishmania chagasi) and is endemic in approximately 70 countries, although the majority of new cases are confined to only relatively few countries (India, Bangladesh, Nepal, Brazil, Kenya, Sudan and Ethiopia) [76]. Leishmania parasites occur in two different forms, extracellular promastigotes (infectious form) in the sandfly vector, and intracellular amastigotes (replicative form) within the phagolysosomes of the human host macrophages.

Current drug treatments of visceral leishmaniasis suffer from various drawbacks such as host toxicity, high cost, difficulty to administer and drug-resistance development. Pentavalent antimonials have been the first-line drugs in the treatment of visceral leishmaniasis for more than 70 years, except in India due to the extensive development of resistance [78–80]. The antifungal drug amphotericin B has become the second-line treatment of visceral leishmaniasis, in spite of its severe and potentially lethal side effects [79,80]. Lipid formulations of amphotericin B with reduced toxicity have been developed and are now the choice of treatment for visceral leishmaniasis in developed countries [79,80]. However, due to high cost, this treatment is not an option in most of the endemic countries. There are drugs such as miltefosine and paromomycin in the pipeline with promising therapeutic effects [80]. Unfortunately, they also suffer from limitations such as high cost, toxicity, difficult administration route, long treatment schedule as well as the generation of drug resistance.

Polyamine metabolism as a drug target in Leishmania spp.

Similarly to T. brucei, Leishmania parasites are able to synthesize putrescine and spermidine since ODC, AdoMetDC and SpdS activities are present in the parasite (Figure 1) and trypanothione is synthesized for redox control.

Systematic experiments by Ullman and colleagues [81–83] genetically validated the polyamine biosynthetic enzymes in L. donovani as drug targets. Knockout strains of L. donovani promastigotes, deficient in ODC, AdoMetDC or SpdS, were all demonstrated to be auxotrophic for polyamines. The ODC-null mutants were dependent on either putrescine or spermidine for their growth in vitro [83], whereas the AdoMetDC- and SpdS-null mutants were shown to be auxotrophic for spermidine only [81,82]. Taken together, these results indicate that spermidine, not putrescine (unless converted into spermidine), is the essential polyamine for growth of L. donovani promastigotes.

Inhibition of ODC in Leishmania

The potential of polyamine biosynthesis as a drug target in Leishmania was indicated by the findings that DFMO effectively inhibited the growth of L. donovani and Leishmania infantum promastigotes in culture [84,85]. In addition, APA (1-amino-oxy-3-aminopropane), a putrescine analogue that inhibits ODC in the nanomolar range, also reduced growth of L. donovani promastigotes (and amastigotes) (Table 1) [86,87]. The growth-inhibitory effect of APA was completely reversed by the addition of putrescine or spermidine. Interestingly, parasites overexpressing ODC showed an increased resistance to the antimonial drug Pentostam, concomitantly with resistance to APA, indicating a role for ODC in the development of Leishmania drug resistance (see below) [86].

Although in vivo treatment with DFMO suppressed the number of parasites in the liver and spleen of mice and hamsters infected with L. infantum and L. donovani, the effect was limited. These results suggested at the time that ODC, and possibly the other enzymes in the polyamine biosynthetic pathway, was not a useful drug target for treatment of visceral leishmaniasis [88,89]. Since the parasites live and replicate within the macrophages of the human host, it seemed conceivable that they might escape polyamine depletion by obtaining polyamines from the host cell [90].

To determine whether ODC deficiency also affects the growth of the intracellular amastigote form, Ullman and colleagues [91] investigated the capacity of ODC-knockout strains of virulent L. donovani to infect mice. The number of parasites in the liver and spleen of mice infected with the ODC-null mutants were found to be 6–3 orders of magnitude lower respectively than the number found in mice infected with the wild-type L. donovani. The infectivity was restored by episomal complementation of the ODC knockouts with an ODC expression plasmid, demonstrating the importance of ODC for growth and survival of L. donovani amastigotes in the mammalian host [91]. Recently, it was also shown that provision of oral putrescine could restore the virulence of the ODC-deficient parasites [92]. Thus despite earlier contrary indications, targeting ODC and the other polyamine biosynthetic enzymes of L. donovani could be a viable approach for treatment of visceral leishmaniasis. Unfortunately, structural information of any of the leishmanial polyamine biosynthetic enzymes is not currently available.

Inhibition of AdoMetDC in Leishmania

Potent inhibitors of AdoMetDC, such as CGP40215A and MDL73811, have been shown to inhibit growth of L. donovani promastigotes (Table 1) [82,93]. The effects of the enzyme inhibitors could be reversed by supplementation of putrescine or spermidine to the growth medium. In addition, it was demonstrated that overexpression of AdoMetDC in L. donovani promastigotes increased their resistance to the growth-inhibitory effect of MDL73811 [82]. However, no effect on the growth of L. donovani amastigotes in mouse macrophages was observed using CGP40215A at concentrations up to 90 μM (Table 1) [59].

Genomic analysis of the trypanosomatid AdoMetDC family indicates that Leishmania spp. also express prozyme [34], although a functional role for prozyme as an activator of AdoMetDC has yet to be demonstrated for this parasite [34,58].
Inhibition of SpdS in Leishmania

Very limited information is available regarding effects of SpdS inhibitors on Leishmania growth and survival. The only SpdS inhibitor tested is n-butylamine, which is as potent as the methylated CHA derivative 4MCHA (trans-4-methylcyclohexylamine), against the mammalian enzyme [94]. When the effect of n-butylamine was tested against L. donovani strains overproducing ODC, AdoMetDC or SpdS, it was shown that not only the SpdS overproducer exhibited resistance to n-butylamine, but also the ODC overproducer [95]. This may suggest that n-butylamine is not a specific inhibitor of SpdS, but may also act on ODC. A more probable explanation is that the increased levels of putrescine found in the ODC overproducers make these cells resistant to the inhibitor, which competes with putrescine for the same binding site of SpdS. This suggestion is also supported by the finding that putrescine supplementation protected wild-type parasites from n-butylamine toxicity [95].

Inhibition of polyamine function in Leishmania with polyamine analogues

The N,N′-bis(benzyl)polyamine analogue MDL27695, was shown to have potent antileishmanial activity, eliminating 77–100% of L. donovani amastigotes from mouse macrophages at a 1 μM concentration [63]. In mice and hamsters, the parasite burden was effectively suppressed by MDL27695, and in hamsters this analogue was equally effective against antimony-resistant L. donovani. The mechanism of action of MDL27695 was thought to involve interference with DNA and RNA synthesis [63].

Several novel types of polyamine analogues, synthesized by Cellgate, have been shown to inhibit L. donovani growth in vitro [95]. The oligoamines CGC-11211 and CGC-11226, and the macrocyclic polyamine analogue CGC-11235, all exhibited IC₅₀ values of less than 1 μM. By screening various antileishmanial compounds against L. donovani strains overproducing ODC, AdoMetDC or SpdS, it was possible to identify those compounds that exert their effect predominantly by interfering with the polyamine biosynthetic enzymes [95].

Polyamine-dependent redox metabolism as a drug target in Leishmania

Structural analysis of L. major TSA revealed two catalytic domains: a C-terminal synthetase domain that generates T(SH)₂ and an N-terminal amidase domain that can hydrolyse T(SH)₂ [96]. The C-terminal active site of TryS is a triangular-shaped cavity that accommodates each of the three substrates (ATP, GSH and spermidine). The N-terminal amidase active site catalyses the cleavage of T(SH)₂ and glutathionylspermidine, releasing GSH and spermidine. The four reactions catalysed by TSA are shown in Figure 4. The TryS and amidase activities of this bifunctional TSA obviously must be balanced to achieve the optimal concentrations of GSH, spermidine, glutathionylspermidine and T(SH)₂. By restricting access to the amidase active site, the C-terminus may provide a mechanism by which the levels of these metabolites can be controlled [96]. This indicates an additional target for interference with polyamine and related metabolic processes in this parasite.

The use of pentavalent antimonials as first-line drugs in the treatment of visceral leishmaniasis is threatened by rapid development of drug resistance. The mechanism by which Leishmania spp. develop resistance to antimonials has not yet been fully clarified [97]. It is generally believed that pentavalent antimonials have to be reduced to the trivalent form Sb(III) before being biologically active. The mechanism(s) by which this occurs is still unknown. However, treatment of L. donovani with Sb(III) causes a rapid depletion of intracellular T(SH)₂ and GSH as well as inhibition of TryR, resulting in a concomitant accumulation of the disulfide forms. This imbalance in thiol homeostasis is believed to be lethal to the parasite. Experimentally selected antimonial-resistant strains of Leishmania spp. often show increased levels of T(SH)₂ and GSH. These are caused by overexpression of γ-glutamylcysteine synthase and ODC, the rate-limiting enzymes in the biosynthesis of the two precursors to trypanothione, GSH and putrescine [98,99]. It is conceivable that increased synthesis of these thiols renders the cells more resistant to the perturbing effects of Sb(III) on thiol homeostasis. Overexpression of ODC has been found in antimony-resistant field isolates of L. donovani [86]. Antimony-resistant field isolates have also been shown to exhibit elevated levels of tryparedoxin and tryparedoxin peroxidase, which are involved in hydroperoxide detoxification (Figure 3), strongly indicating that amplified antioxidative defences play a major role in clinical resistance to antimonials [100]. Interestingly, tryparedoxin peroxidase has been genetically validated as a drug target in T. b. brucei [53].

Polyamine transport as a drug target in Leishmania

Leishmania promastigotes as well as amastigotes are able to take up polyamines from their environment [101,102]. In Leishmania spp., the polyamine transport systems are often stage-specific, with different polyamine transport systems for the promastigote and amastigote stages [102]. In promastigotes of L. donovani and Leishmania mexicana and in amastigotes of L. mexicana, polyamine transport was found to be temperature- and pH-dependent [102]. It was also saturable and independent of the Na⁺ concentration, but dependent on the protomotive force. Results from kinetic analyses of polyamine transport indicate the existence of separate transport systems for putrescine and spermidine in Leishmania. Putrescine transport in L. infantum promastigotes has been shown to occur via a specific energy-dependent and saturable carrier [85].

The cloning of the gene LmPOT1 encoding a high-affinity putrescine/spermidine transporter from L. major by Hasne and Ullman [24] was the first molecular identification of a eukaryotic cell-surface polyamine transporter. Sequence analysis predicted a protein of 803 amino acids with 9–12 transmembrane domains and also indicated that LmPOT1 belongs to the APC family of membrane transporters [24]. Expression of LmPOT1 in X. laevis oocytes induced high-affinity saturable transport of both putrescine and spermidine. However, when expressed in T. brucei (which normally transport polyamines poorly) LmPOT1 appeared to mainly transport putrescine with a Kₘ value similar to that obtained in the Xenopus oocytes. Immunocytochemical analysis revealed a high expression of LmPOT1, which was localized to the cell surface and flagellum of L. major promastigotes [24].

DAB has been shown to be active against L. major promastigotes by targeting ODC as well as putrescine uptake into these parasites (Table 1). Similarly to the effect of DFMO treatment in other organisms, pretreatment of the parasites with DAB results in an increased uptake of putrescine [103].

MALARIA

Malaria is recognised by the WHO (World Health Organization) as one of the three most important infectious killers in the world, with around 350–500 million clinical malaria episodes occurring annually [104,105]. More than 80% of malaria-associated deaths occur in Africa south of the Sahara. In endemic African countries,
malaria accounts for 25–35% of all outpatient visits, half of all hospitalizations and 35% of all hospital deaths. In some African countries, this disease constitutes 40% of the public health expenditures. Malaria is also prevalent in many other subtropical and tropical countries, e.g. in Southeast Asia and Latin America. This disease therefore rates as one of the major health, socioeconomic and developmental challenges facing many of the world’s poorest countries.

Malaria is caused in humans by four major species of plasmodia that are transferred by the bite of a blood-feeding female mosquito of the Anopheles genus. Plasmodium vivax, Plasmodium ovale and Plasmodium malariae are less virulent than Plasmodium falciparum, which causes the most lethal form of this disease. The recent discovery of the zoonotic transfer of Plasmodium knowlesi (simian malaria) to humans indicates the evolving nature of the plasmodia and highlights our need for tight control of the spread of these parasites to humans [106]. Malaria parasites mature intracellularly in hepatocytes before infecting erythrocytes to commence the asexual life cycle. Treatment of severe malaria is negated by the alarming increase in resistance of the malaria parasite to all known antimalarials. Traditionally, malaria prophylaxis relied on antifolates (e.g. pyrimethamine and sulfadoxine) and chloroquine or its derivatives (e.g. mefloquine). Current treatment regimes advocated by the WHO include new antimalarials such as artesinin in combination with conventional drugs to prolong the useful lifetime of the limited antimalarial dispensary. Ideally an antimalarial should be selective and essentially non-toxic to the human host. It should also have good oral bioavailability and be curative in a few days to limit the development of resistance.

Polyamine metabolism as a drug target in P. falciparum

The polyamines are present as major metabolites within the parasite and, with a total concentration of 10 mM, the polyamines make up 14% of the P. falciparum metabolome [107]. P. falciparum-infected erythrocytes contain high levels of putrescine and spermidine, but only low levels of spermine (Figure 1) [108]. Like in T. cruzi, spermine is synthesized by a somewhat promiscuous P. falciparum SpdS, which cannot only use putrescine, but also spermidine as a substrate (Figure 1) [109]. There is no evidence of trypanothione synthesis in these apicomplexan parasites.

Inhibition of the bifunctional AdoMetDC/ODC of P. falciparum

Possibly the most distinctive feature of polyamine biosynthesis in plasmodia is that a single open reading frame encodes both ODC and AdoMetDC in a unique bifunctional protein of ∼330 kDa molecular mass (AdoMetDC/ODC) (Figure 5) [110]. The advantage of this bifunctional AdoMetDC/ODC protein probably is not a metabolic channelling of substrates to optimize downstream synthesis, because AdoMetDC and ODC do not catalyse consecutive reactions in the polyamine biosynthetic pathway. Instead, the bifunctionality may be important for the regulation of polyamine pools in the parasite [111]. In contrast with their single-enzyme orthologues in other organisms, ODC activity is strongly feedback-inhibited by putrescine, and AdoMetDC activity is not inhibited by putrescine in the bifunctional enzyme [112,113]. Protein–protein interactions, as well as long-range communication, are proposed to modulate the activities of ODC and AdoMetDC within the bifunctional complex [111], although each decarboxylase active site in the respective domains is able to function independently of the other [112]. The ODC activity seems to be allosterically stimulated by the presence of the AdoMetDC domain [114] and conversely AdoMetDC activity is inhibited by the presence of the ODC domain [112]. In this manner, it seems that the production of equimolar levels of putrescine and dcAdoMet is achieved.

The potential of AdoMetDC/ODC as an antimalarial target is largely based on the efficacy of DFMO to prevent malaria parasite proliferation in vitro [115]. However, DFMO does not appear to be as therapeutically useful as against African trypanosomiasis, because it is not curative of Plasmodium berghei infections in vivo [116]. Despite the therapeutic failure of DFMO against malaria infections, the dependency of the parasite on polyamines for survival has been well established [108,117–119].

New generation ODC inhibitors including APA are far more effective than DFMO against P. falciparum and exhibit an IC50 value of ∼1 μM (Table 1). However, the effects of these inhibitors are also reversed by putrescine [108]. The apparent recovery of parasites treated with ODC inhibitors is troublesome since it implies that the parasite can overcome ODC inhibition through, for example, efficient polyamine uptake mechanisms (see below), and that additional activities in the biosynthesis or uptake of polyamines may need to be targeted.

Inhibition of AdoMetDC activity of the bifunctional protein with MLD.73811 (1000-fold more active than DFMO) also arrests P. falciparum growth in vitro [120], but is not curative in in vivo malaria models (Table 1). In contrast with the trypanosomes [37,42], inhibition of AdoMetDC activity in P. falciparum does not result in accumulation of AdoMet levels and the methylation status of the parasite is unaffected [117].

Co-inhibition of both activities of plasmodial AdoMetDC/ODC results in an additive growth-inhibitory effect and a total block in polyamine biosynthesis, indicating possible applications for therapeutic interventions [117]. The decarboxylase active sites of plasmodial AdoMetDC/ODC are structurally conserved compared with the mammalian orthologues, but the bifunctional nature of AdoMetDC/ODC makes it attractive to target protein–protein interacting sites instead, a strategy that is gaining popularity for selective inhibition of multi-subunit proteins [121,122].

A structural description of the complete AdoMetDC/ODC bifunctional complex is lacking, mostly due to the refractory expression of this large protein complex in heterologous systems. However, homology models have been described for the individual AdoMetDC and ODC domains and were used to predict interaction sites between these domains (Figure 5) [113,123]. The P. falciparum AdoMetDC is predicted to fall within its own structural class [35] due to its presence within the bifunctional protein complex (Figure 5). The homology model of ODC showed a similar topology to those of other ODCs, but also identified important differences in the residues involved in its active site. These differences may be exploited for selective inhibitor design [113,123]. Moreover, the ODC domain is more refractory to change and is dependent on the presence of the so-called linker or hinge region (Figure 5) of the bifunctional complex for activity [111,114].

Inhibition of SpdS in P. falciparum

Investigations of the potential of SpdS as a drug target have not been as extensive as for AdoMetDC/ODC, but global analyses of the functional consequence of its inhibition suggest that it is an important flux-controlling point within the polyamine biosynthetic pathway [119]. Treatment with the SpdS inhibitors CHA, 4MCHA and dicyclohexylamine (Table 1) resulted in a marked reduction in P. falciparum cell growth in vitro [109].
Figure 5  The unique bifunctional AdoMetDC/ODC of malaria parasites

(A) AdoMetDC (N-terminus) is associated via a hinge region to the C-terminal ODC domain. Two of these ~170 kDa AdoMetDC/ODC polypeptides associate to form the functional ~330 kDa AdoMetDC/ODC complex. aa, amino acid. (B) Predicted structural description of the bifunctional arrangement of AdoMetDC/ODC (middle panel) [143]. An ODC monomer (grey ribbon diagram in the right-hand panel) associates with another ODC monomer in a head-to-tail fashion to form the two shared active sites of the obligate ODC dimer (right-hand panel). To this, the areas that would be occupied by two AdoMetDC domains are presented as spheres indicating preferred centre of mass of AdoMetDC docked to ODC. Each individual AdoMetDC domain is formed by α- and β-subunits depicted in pink and yellow respectively in the ribbon diagram (left-hand panel).

Crystal structures of *P. falciparum* SpdS containing MTA (5′-methylthioadenosine), spermidine, spermine, AdoDATO, dcAdoMet or 4MCHA have been obtained [124]. These structures explained, among other phenomena, the active-site promiscuity in terms of spermidine binding, enabling the enzyme to synthesize spermine [124,125]. Moreover, these structures have been used in the further development of novel and potent inhibitors of SpdS, identifying several active-site binders with the use of structure-based virtual screening [126]. Clearly, the essential nature of SpdS to the survival of *P. falciparum* parasites warrants further investigation into the design of new inhibitors.

Inhibition of polyamine function in *P. falciparum* with polyamine analogues

The treatment of murine malaria (*P. berghei*) with a combination of DFMO and the *N,N*-bis(benzyl)polyamine analogue MDL27695 is curative of the disease. Additionally, these cured mice were resistant to re-infection with the same parasite strain [127]. MDL27695 rapidly inhibits growth of both chloroquine-sensitive and -resistant *P. falciparum* strains in vitro (Table 1) [127]. A combination of MDL27695 and chloroquine proved to cause an additive effect [128]. This therefore provides evidence of the therapeutic potential of polyamine analogues in perturbing *P. falciparum* growth.

Several of a series of 1,3,5-triazine-substituted polyamines have shown growth-inhibitory activity against *P. falciparum* in the low micromolar range, especially against chloroquine-resistant parasites [129]. Chemical library screening of diamine derivatives identified compounds with some activity against plasmodia with IC₅₀ values as low as 0.7 μM, provided these derivatives contained a benzyloxy group [130]. There was no indication of selectivity, but antiparasitic activity was confirmed in *Leishmania*.

Polyamine-related metabolic pathways as drug targets in *P. falciparum*

Ornithine, a substrate of ODC, seems to be homoeostatically controlled in *P. falciparum*. Inhibition of the ODC activity of AdoMetDC/ODC increases production of OAT (ornithine aminotransferase) to catabolize potentially toxic ornithine to glutamate-5′-semialdehyde and ultimately to proline [117]. The three-dimensional structure of *P. falciparum* OAT was recently solved and it was shown that, even though the overall structure is similar to that of the human protein, there are parasite-specific differences that can be exploited for chemotherapeutic intervention. Specifically, two cysteine residues within the loop involved in substrate binding were found only in the parasite OAT, and were predicted to mediate a unique interaction with thioredoxin [131]. Thioredoxin plays a crucial role in redox regulation in *P. falciparum*.

The control of ornithine levels links polyamine metabolism to redox control in the parasite. Although there is no evidence for the existence of trypanothione in *P. falciparum*, a clear relationship between polyamine metabolism and the redox status of the parasites is implied since the transcript levels of 1-Cys peroxiredoxin, thioredoxin, glutathione transferase and glutathione reductase are decreased in polyamine-depleted parasites [117].

In the absence of polyamines, *P. falciparum* produces an inducible LDC1 (lysine decarboxylase 1), which is able to form cadaverine from lysine [117,119,132]. In addition, it seems as if
Polyamine transport as a drug target in *P. falciparum*

Putrescine transport has been characterized in rhesus monkey erythrocytes infected with *P. knowlesi* and was shown to be dependent on temperature and competed for by spermidine and spermine [134]. *P. falciparum*-infected erythrocytes exhibited similar putrescine uptake characteristics as did *P. knowlesi*-infected erythrocytes [135].

Both putrescine and spermidine are taken up across the plasma membrane of isolated viable *P. falciparum* parasites via a saturable temperature-dependent process that showed competition between different polyamines, as well as the polyamine precursor ornithine and other basic amino acids (J. Niemand, unpublished work). Inhibition of polyamine biosynthesis in the isolated parasites led to increased total uptake of both putrescine and spermidine and the rate of uptake was independent of extracellular Na⁺ or K⁺ concentrations, but increased with increasing extracellular pH. Putrescine and spermidine uptake decreased with membrane depolarization and increased with membrane hyperpolarization. Unlike in *L. major* and *T. cruzi*, a polyamine transporter has as yet not been identified molecularly in *Plasmodium* spp. The putrescine and spermidine uptake systems may provide a mechanism for selective delivery of antimalarials by conjugating them to polyamines [136, 137]. This approach may prove to be effective in improving the inhibitory capacity of the antimalarials, as well as to decrease their non-specific side-effects.

**CONCLUSIONS AND FUTURE PERSPECTIVES**

The inhibition of polyamine biosynthesis by DFMO has demonstrated impressive cure rates of *T. b. gambiense* infections, validating this pathway as an antiparasitic drug target. However, the poor pharmacokinetic properties of DFMO necessitate the discovery of alternative inhibitors targeting polyamine metabolism. The trypanosomatids as well as malaria parasites have various unique properties that can be exploited in future drug-development efforts. As such, within the trypanosomatids, the synthesis of trypanothione and particularly the bifunctional TSA may be targeted. Targeting the polyamine uptake mechanism to either block polyamine uptake or exploit this transport mechanism to deliver cytotoxic cargo [11, 136] may be highly effective against the parasites. Such an approach may be particularly useful in the case of *T. cruzi*, being a polyamine auxotroph, with a highly efficient putrescine/cadaverine transporter. A unique and selective tool to inhibit AdoMetDC in trypanosomatids may be to prevent its essential interaction with prozyme. Such targeting of protein–protein interactions has led to the development of small-molecule inhibitors of interactions that are currently in clinical trials in other systems [121]. The formation of the bifunctional AdoMetDC/ODC in plasmodia may be similarly interfered with. Furthermore, a global understanding of the importance of the polyamines to these parasites may highlight additional activities that can be targeted in antiparasitic strategies [138].

**ACKNOWLEDGEMENTS:**

We thank Gordon Wells for his help in creating Figure 5(B).

**FUNDING**

This work was supported by a collaborative research exchange grant between the South African National Research Foundation (NRF) and the Swedish International Development Cooperation Agency (SIDA, Swedish Research Links Programme). J.N., M.W., A.J.L., and L.B. are members of the South African Malaria Initiative (http://www.sami.org.za). Support was also obtained from the European Cooperation on Science and Technology [grant number COST-CM0801] (to L.P. and O.H.) and the Royal Physiological Society in Lund (to O.H.).

**REFERENCES**

1. Gerber, E. W. and Meyskens, Jr, F. L. (2004) Polyamines and cancer: old molecules, new understanding. Nat. Rev. Cancer 4, 781–792
2. Casero, R. A. and Marton, L. J. (2007) Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. Nat. Rev. Drug Discovery 6, 373–390
3. Pegg, A. E. (2009) Mammalian polyamine metabolism and function. IUBMB Life 61, 480–494
4. Wallace, H. M. (2009) The polyamines: past, present and future. Essays Biochem. 46, 1–9
5. Heby, O., Persson, L. and Rentala, M. (2007) Targeting the polyamine biosynthetic enzymes: a promising approach to therapy of African sleeping sickness, Chagas’ disease, and leishmaniasis. Amino Acids 33, 359–366
6. Pegg, A. E. (2006) Regulation of ornithine decarboxylase. J. Biol. Chem. 281, 14529–14532
7. Pegg, A. E. (2009) S-Adenosylmethionine decarboxylase. Essays Biochem. 46, 25–45
8. Bale, S. and Ealick, S. E. (2010) Structural biology of S-adenosylmethionine decarboxylase. Amino Acids 38, 451–460
9. Ikeguchi, Y., Bowley, M. C. and Pegg, A. E. (2006) Aminopropyltransferases: function, structure and genetics. J. Biochem. 139, 1–9
10. Casero, R. A. and Pegg, A. E. (2009) Polyamine catalysis and disease. Biochem. J. 421, 323–338
11. Palmer, A. J. and Wallace, H. M. (2010) The polyamine transport system as a target for anticancer drug development. Amino Acids 38, 415–422
12. Ahmrad, J. J., Oliveira, M. A., Kern, A. D., Grishin, N. V., Phillips, M. A. and Hackert, M. L. (2006) Crystal structure of human ornithine decarboxylase at 2.1 Å resolution: structural insights to antizyme binding. J. Mol. Biol. 295, 7–16
13. Kern, A. D., Oliveira, M. A., Coffino, P. and Hackert, M. L. (1999) Structure of mammalian ornithine decarboxylase at 1.6 Å resolution: stereochemical implications of PLP-dependent amino acid decarboxylases. Structure 7, 567–581
14. Tobias, K. E. and Kanaa, C. (1993) Intersubunit location of the active site of mammalian ornithine decarboxylase as determined by hybridization of site-directed mutants. Biochemistry 32, 5842–5847
15. Wu, H., Min, J., Ikeguchi, Y., Zeng, H., Dong, A., Loppnau, P., Pegg, A. E. and Plotnikov, A. N. (2007) Structure and mechanism of spermidine synthases. Biochemistry 46, 8331–8339
16. Wu, H., Min, J., Zeng, H., McCloskey, D. E., Ikeguchi, Y., Loppnau, P., Michael, A. J., Pegg, A. E. and Plotnikov, A. N. (2008) Crystal structure of human spermine synthase: implications of substrate binding and catalytic mechanism. J. Biol. Chem. 283, 16135–16146
17. Stuart, K., Brun, R., Croft, S., Fairlamb, A., Gurrler, R. E., McKerrow, J., Reed, S. and Tarleton, R. (2008) Kinetoplastid: related protozoan pathogens, different diseases. J. Clin. Invest. 118, 1301–1310
18. Simarro, P. P., Janin, J. and Caltand, P. (2008) Eliminating human African trypanosomiasis: where do we stand and what comes next? PLoS Med. 5, e55
19. Metcalfe, B. W., Bey, P., Danzin, C., Jung, M. J., Casara, P. L. and Vevert, J. P. (1978) Catalytic irreversible inhibition of mammalian ornithine decarboxylase by substrate and product analogues. J. Am. Chem. Soc. 100, 2551–2550
20. Bacchi, C. J., Nathan, H. C., Hutner, S. H., McCann, P. and Sjoerdsma, A. (1980) Polyamine metabolism: a potential therapeutic target in trypanosomes. Science 210, 332–334
21. Van Nieuwenhove, S., Schechter, P. J., Declercq, J., Bone, G., Burke, J. and Sjoerdsma, A. (1985) Treatment of gambiense sleeping sickness in the Sudan with oral DFMO (U-β-difluoromethylornithine), an inhibitor of ornithine decarboxylase: first field trial. Trans. R. Soc. Trop. Med. Hyg. 79, 692–698
22. Wickware, P. (2002) Resurrecting the resurrection drug. Nat. Med. 8, 908–909
23. Phillips, M. A., Coffino, P. and Wang, C. C. (1987) Cloning and sequencing of the ornithine decarboxylase gene from Trypanosoma brucei. J. Biol. Chem. 262, 8721–8727
24. Hasne, M. P. and Ullman, B. (2005) Identification and characterization of a pyrimidine permease from the protozoan parasite Leishmania major. J. Biol. Chem. 280, 15188–15194

The content is related to the research on polyamine transport as a drug target in pathogenic protozoa.
25 Bitonti, A. J., Cross-Doersen, D. E. and McCann, P. P. (1988) Effects of α-difluoromethylornithine on protein synthesis and synthesis of the variant-specific glycoprotein (VSG) in Trypanosoma brucei brucei. Biochem. J. 250, 295–298
26 Yarlett, N. and Bacchi, C. J. (1988) Effect of α-difluoromethylornithine on methionine cycle intermediates in Trypanosoma brucei brucei. Mol. Biochem. Parasitol. 27, 1–10
27 Griffin, B. F., McCann, P. P., Bitonti, A. J. and Bacchi, C. J. (1986) Polyamine depletion following exposure to α-difluoromethylornithine both in vivo and in vitro initiates morphological alterations and mitochondrial activation in a monomeric strain of Trypanosoma brucei brucei. J. Protozool. 33, 238–243
28 Grishin, N. V., Osterman, A. L., Brooks, H. B., Phillips, M. A. and Goldsmith, E. J. (1999) X-ray structure of ornithine decarboxylase from Trypanosoma brucei: the native structure and the structure in complex with α-difluoromethylornithine. Biochemistry 38, 15174–15184
29 Jackson, L. K., Brooks, H. B., Osterman, A. L., Goldsmith, E. J. and Phillips, M. A. (2000) Altering the reaction specificity of eukaryotic ornithine decarboxylase. Biochemistry 39, 11247–11257
30 Osterman, A., Grishin, N. V., Kinch, L. N. and Phillips, M. A. (1994) Formation of functional cross-species heterodimers of ornithine decarboxylase. Biochemistry 33, 13662–13667
31 Poulin, R., Lu, L., Ackermann, B., Bey, P. and Pegg, A. E. (1992) Mechanism of the irreversible inactivation of mouse ornithine decarboxylase by α-difluoromethylornithine. Characterization of sequences at the inhibitor and coenzyme binding sites. J. Biol. Chem. 267, 150–158
32 Priotto, G., Kasparian, S., Mutombo, W., Ngouama, D., Ghorashian, S., Arnold, U., Ghafari, S., Baudin, E., Buard, V., Kazadi-Kyanza, S. et al. (2009) Nifurtimox-eflornithine combination therapy for second-stage African Trypanosoma brucei gambiense trypanosomiasis: a multicentre, randomised, phase III, non-inferiority trial. Lancet 374, 56–64
33 Smithson, D. C., Lee, J., Shelat, A. A., Phillips, M. A. and Guy, R. K. (2010) Discovery of the capacities of Trypanosoma cruzi to infect and multiply within a mammalian host cell. J. Biol. Chem. 285, 104–110
34 Willert, E. K. and Phillips, M. A. (1993) Inhibition of S-adenosylmethionine decarboxylase by the regulatory subunit prozyme. Mol. Biochem. Parasitol. 56, 68–74
35 Hunter, K. J., Le Quere, S. A. and Fairlamb, A. H. (1994) Identification and biosynthesis of N² nitrogen-containing bis(glutathionyl)spermine in Trypanosoma brucei. Eur. J. Biochem. 228, 1019–1027
36 Yarlett, N. and Bacchi, C. J. (1988) Effect of DL-difluoromethylornithine on protein synthesis and synthesis of the variant-specific glycoprotein (VSG) in Trypanosoma cruzi. J. Biol. Chem. 263, 527–537
37 Bacchi, C. J., Nathan, H. C., Yarlett, N., Goldber, B., McCann, P. P., Bitonti, A. J. and Sjøreda, A. (1992) Cure of murine Trypanosoma brucei rhodesiense infections with an irreversible inhibitor of S-adenosylmethionine decarboxylase. Antimicrob. Agents Chemother. 34, 1485–1490
38 Casero, Jr, R. A. and Woster, P. M. (2009) Recent advances in the development of polyamine analogs as antitumor agents. J. Med. Chem. 52, 4551–4573
39 Faun, E., Baker, D., Fields, S., Gelb, M. H., Buckner, F. S., Van Voorhis, W. C., Phizicky, E., Dumont, M., Mehlin, C., Grayhack, E. et al. (2008) Structural genomics of pathogenic protozoa: an overview. Methods Mol. Biol. 426, 497–513
40 Hunter, K. J., Le Quere, S. A. and Fairlamb, A. H. (1994) Identification and biosynthesis of N² nitrogen-containing bis(glutathionyl)spermine in Trypanosoma brucei. Eur. J. Biochem. 228, 1019–1027
41 Hunter, K. J., Le Quere, S. A. and Fairlamb, A. H. (1994) Identification and biosynthesis of N² nitrogen-containing bis(glutathionyl)spermine in Trypanosoma brucei. Eur. J. Biochem. 228, 1019–1027
42 Byers, T. L., Bush, T. L., Casara, J. P., Bacchi, C. J., Clarkson, Jr, J. B., McCann, P. P. and Sjöreda, A. (1990) Cure of Trypanosoma brucei rhodesiense infections in mice with an irreversible inhibitor of S-adenosylmethionine decarboxylase. Antimicrob. Agents Chemother. 34, 1485–1490
43 Kinch, L. N., Scott, J. R., Ullman, B. and Phillips, M. A. (1999) Cloning and kinetic characterization of the Trypanosoma cruzi S-adenosylmethionine decarboxylase. Mol. Biochem. Parasitol. 104, 205–210
44 Jones, D. C., Ariza, A., Chow, W. H., Oza, S. L. and Fairlamb, A. H. (2010) Comparative structural, kinetic and inhibitor studies of Trypanosoma brucei/ Trypanosoma rhodesiense decarboxylases with C. Cruzi. Mol. Biochem. Parasitol. 169, 121–133
45 Kinch, L. N., Scott, J. R., Ullman, B. and Phillips, M. A. (1999) Cloning and kinetic characterization of the Trypanosoma cruzi S-adenosylmethionine decarboxylase. Mol. Biochem. Parasitol. 104, 205–210
Polyamine homeostasis as a drug target in pathogenic protozoa

69 Brocher, G. and Wigglesworth, V. B. (1944) The transmission of Actinomyces rhodni
Erikson in Rhodnius prolixus stål (hemiptera) and its influence on the growth of the host. Parasitology 35, 223–224
70 González, N. S., Ceriani, C. and Algranati, I. D. (1992) Differential regulation of putrescine uptake in Trypanosoma cruzi and other trypanosomatids. Biochem. Biophys. Res. Commun. 186, 120–128
71 Le quesne, S. A. and Fairlamb, A. H. (1996) Regulation of a high-affinity diamine transport system in Trypanosoma cruzi epimastigotes. Biochem. J. 316, 481–486
72 Bouvier, L. A., Silber, A. M., Galavo Lopes, C., Canepa, G. E., Miranda, R. M., Tonelli, R. R., Colli, W., Alves, M. J. and Pereira, C. A. (2004) Post genomic analysis of permeases from the amino acid/auxin family in protozoan parasites. Biochem. Biophys. Res. Commun. 321, 547–556
73 Carillo, C., Canepa, G. E., Algranati, I. D. and Pereira, C. A. (2006) Molecular and functional characterization of a spermine transporter (EPA112) from Trypanosoma cruzi. Biochem. Biophys. Res. Commun. 344, 396–390
74 Hasme, M. P., Coppins, I., Soya, R. and Ullman, B. (2010) A high-affinity putrescine-cadaverine transporter from Trypanosoma cruzi. Mol. Microbiol. 76, 78–91
75 Menzes, D., Valentin, C., Oliveira, M. F. and Vannier-Santos, M. A. (2006) Putrescine analogue cytotoxicity against Trypanosoma cruzi. Parasitol. Res. 98, 99–105
76 Chappuis, F., Sundar, S., Hallu, A., Ghaib, H., Rijal, S., Peeling, R. W., Alvar, J. and Boulatt, M. (2007) Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat. Rev. Microbiol. 5, 873–882
77 Berman, J. (2005) Visceral leishmaniasis in the New World & Africa. Indian J. Med. Res. 123, 289–294
78 Croft, S. L., Sundar, S. and Fairlamb, A. H. (2006) Drug resistance in leishmaniasis. Clin. Microbiol. Rev. 19, 111–126
79 Kedzierski, L., Sakhantadenswaren, A., Curtis, J. M., Andrews, P. C., Junk, P. C. and Kedzierski, K. (2009) Leishmaniasis: current treatment and prospects for new drugs and vaccines. Curr. Med. Chem. 16, 599–614
80 Moore, E. M. and Lockwood, D. N. (2010) Treatment of visceral leishmaniasis. J. Glob. Infect. Dis. 2, 151–158
81 Roberts, S. C., Jiang, Y., Jardim, A., Carter, N. S., Heby, O. and Ullman, B. (2001) Genetic analysis of spermidine synthesis from Leishmania donovani. Mol. Biochem. Parasitol. 115, 217–226
82 Roberts, S. C., Scott, J., Gasteier, J. E., Jiang, Y., Brooks, B., Jardim, A., Carter, N. S., Heby, O. and Ullman, B. (2002) S-Adenosylmethionine decarboxylase from Leishmania donovani. Molecular, genetic, and biochemical characterization of null mutants and overproducers. J. Biol. Chem. 277, 5902–5909
83 Jiang, Y., Roberts, S. C., Jardim, A., Carter, N. S., Shi, S., Aryanagayasam, M., Fairlamb, A. H. and Ullman, B. (1999) Ornithine decarboxylase gene deletion mutants of Leishmania donovani. J. Biol. Chem. 274, 3781–3788
84 Kaur, K., Emmett, K., McCann, P. P., Sjoerdsma, A. and Ullman, B. (1986) Effects of α-difluoromethylornithine on parasite suppression and cellular polyamine levels. Antimicrob. Agents Chemother. 51, 528–534
85 Balana-Fouco, R., Ordonez, D. and Alunada, J. M. (1989) Putrescine transport system in Trypanosoma cruzi epimastigotes. Mol. Biochem. Parasitol. 35, 43–50
86 Singh, S., Mukherjee, A., Khoromut, A. R., Persson, L., Heby, O., Chatterjee, M., and Madhubala, R. (2007) Antileishmanial effect of 3-aminooxy-1-aminopropane is due to polyamine synthesis, transport, ultrastructure and intracellular survival in Leishmania amazonensis. Microbiology 154, 3104–3111
87 Leedham, G. A., Birkholtz, L., Joubert, F. and Louw, A. I. (2006) Novel metabolite profiling of the intraerythrocytic malaria parasite Plasmodium falciparum by (1)H NMR spectroscopy. NMR Biomed. 22, 292–302
88 Teng, R., Junankar, P. R., Bob, W. A., Rae, C., Mercier, P. and Kirk, K. (2008) Metabolite profiling of the intraerythrocytic malaria parasite Plasmodium falciparum by (1)H NMR spectroscopy. NMR Biomed. 22, 292–302
89 Das Gupta, R., Krause-Ilie, T., Bergmann, B., Müller, I. B., Khotoum, A., Müller, W., Walter, R. D. and Lüersen, K. (2005) S-Amino-1-aminopropan-3-amine and derivatives have an antiprofibrillar effect on cultured Plasmodium falciparum by decreasing intracellular polyamine concentrations. Antimicrob. Agents Chemother. 49, 2857–2864
90 Haider, N., Eschbach, M. L., Diaz-Sed, S., Gilberger, T. W., Walter, R. D. and Lüersen, K. (2005) The spermidine synthase gene of Plasmodium falciparum contains molecular and biochemical characterisation of the polyamine synthesis enzyme. Mol. Biochem. Parasitol. 142, 224–236
91 Müller, S., Da’dara, A., Lüersen, K., Wrenger, C., Das Gupta, R., Madhubala, R. and Walter, R. D. (2000) In the human malaria parasite Plasmodium falciparum, polyamines are synthesized by a bifunctional ornithine decarboxylase. S-Adenosylmethionine decarboxylase. J. Biol. Chem. 275, 8097–8102
92 Birkholtz, L., Wrenger, C., Joubert, F., Wells, G. A., Walter, R. D. and Louw, A. I. (2004) Parasite-specific inhibitors in the bifunctional S-adenosylmethionine decarboxylase/ornithine decarboxylase of Plasmodium falciparum modulate catalytic activities and domain interactions. Biochem. J. 377, 439–448
93 Wrenger, C., Lüersen, K., Krause, T., Müller, S. and Walter, R. D. (2001) The Plasmodium falciparum bifunctional ornithine decarboxylase, S-adenosyl-L-methionine decarboxylase, enables a well balanced polyamine synthesis without domain–domain interaction. J. Biol. Chem. 276, 29561–29565
94 Wells, G. A., Birkholtz, L., Joubert, F., Walter, R. D. and Louw, A. I. (2006) Novel properties of malarial S-adenosylmethionine decarboxylase as revealed by structural modelling. J. Mol. Graph. Model. 24, 307–318
95 Krause, T., Lüersen, K., Wrenger, C., Gilberger, T., Müller, S. and Walter, R. D. (2000) The ornithine decarboxylase domain of the bifunctional ornithine decarboxylase/S-adenosylmethionine decarboxylase of Plasmodium falciparum: recombinant expression of two different constructs. Biochem. J. 352, 287–292
96 Assaf, Y. G., Golenser, J., Spira, D. T., Messer, G. and Barrach, U. (1987) Cysticotic effect of α-α-difluoromethylornithine against Plasmodium falciparum and its reversal by diamines and spermidine. Parasitol. Res. 73, 313–318
