ANTIOXIDANTS & REDOX SIGNALING
Volume 17, Number 4, 2012

DOI: 10.1089/ars.2011.4436

FORUM REVIEW ARTICLE

Redox-Active Antiparasitic Drugs

Chinmay Pal and Uday Bandyopadhyay

Abstract

Significance: Parasitic diseases affect hundreds of millions of people worldwide and represent major health problems. Treatment is becoming extremely difficult due to the emergence of drug resistance, the absence of effective vaccines, and the spread of insecticide-resistant vectors. Thus, identification of affordable and readily available drugs against resistant parasites is of global demand. ***Recent Advances:*** Susceptibility of many parasites to oxidative stress is a well-known phenomenon. Therefore, generation of reactive oxygen species (ROS) or inhibition of endogenous antioxidant enzymes would be a novel therapeutic approach to develop antiparasitic drugs. This article highlights the unique metabolic pathways along with redox enzymes of unicellular (*Plasmodium falciparum*, *Trypanosoma cruzi*, *Trypanosoma brucei*, *Leishmania donovani*, *Entamoeba histolytica*, and *Trichomonas vaginalis*) and multicellular parasites (*Schistosoma mansoni*), which could be utilized to promote ROS-mediated toxicity. ***Critical Issues:*** Enzymes involved in various vital redox reactions could be potential targets for drug development. ***Future Directions:*** The identification of redox-active antiparasitic drugs along with their mode of action will help researchers around the world in designing novel drugs in the future. *Antioxid. Redox Signal.* 17, 555–582.

Introduction

Approximately 1 billion people of the world's population suffer from neglected tropical diseases, including the vector-borne parasitic diseases (52). Parasitic diseases such as malaria, trypanosomiasis, leishmaniasis, amoebiasis, trichomoniasis, and schistosomiasis are major health problems, particularly in poverty-stricken areas (7, 31, 90, 133, 149). Unlike most antibiotics there are no "broad spectrum" antiparasitic drugs. The selection of antiparasitic drugs varies between different organisms (87). The intrinsic biological factors such as the relative similarity between human and protozoan cells (compared with bacteria) and the pharmacoeconomic condition of developing countries act as major hindrances to the development of novel antiparasitic drugs. The situation is further complicated due to the development of resistance to the commonly available drugs, especially against malaria (94, 141). Therefore, we are now in urgent need of new antiparasitic drugs that will be effective against resistant parasites.

The redox system plays an important role in the survival of the parasite in the host (150). All aerobic organisms are exposed to reactive oxygen species (ROS) such as superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) generated by their metabolism (150). Parasitic protozoa not only have to eliminate their endogenous toxic metabolites but they should also cope with the oxidative (or respiratory) burst of the host immune system. Redox imbalance occurs in the parasite when the endogenous antioxidants fail to cope with the excessive ROS (both endogenous and exogenous), and this leads to the development of oxidative stress (111). In general, antiparasitic drugs, which have the ability to inhibit vital redox reactions or promote oxidative stress in parasites, are considered redox-active antiparasitic drugs (135). In this article, we have focused on the redox-active antiparasitic drugs used against diseases caused by unicellular parasites such as malaria, trypanosomiasis, leishmaniasis, amoebiasis, and trichomoniasis, as well as diseases produced by multicellular parasites such as schistosomiasis. Here, for a better understanding, we first discuss the role of important redox-active enzymes and the redox reactions involved in different metabolic pathways in the parasites. Next, we concentrate on various redox-active antiparasitic drugs along with their mode of action. In this context, we believe that a review on highlighted inventions and innovative ideas in this field could be of importance to scientists, managers, and decision makers for developing novel antiparasitic drugs.

Redox Reactions and Redox Enzymes in Parasites

Redox systems have a variety of important functions in parasitic protozoa, and the key enzymes involved are vital for growth and development of the organisms. In this section, we discuss the reactions carried out by different redox-active

Department of Infectious Diseases and Immunology, Indian Institute of Chemical Biology, Kolkata, India.

enzymes in the parasite. We have summarized the important redox enzymes present in parasites that could be potential drug targets (Table 1).

Trypanothione [$T(SH)_2$] exclusively occurs in trypanosomatid protozoa (91) and carries out several redox reactions. $T(SH)_2$ is synthesized by the conjugation of glutathione (GSH) with spermidine. This reaction is catalyzed by glutathionylspermidine synthetase (GspS) and $T(SH)_2$ synthetase (75, 117). GSH is synthesized in two steps. First, the reaction of glutamate with cysteine is catalyzed by γ-glutamylcysteine synthetase, forming the intermediate γ-glutamylcysteine, which then reacts with glycine in the presence of glutathione synthetase yielding GSH. In African trypanosomes, spermidine is also synthesized in two steps. In the first step, decarboxylation of ornithine by ornithine decarboxylase gives putrescine, which in the presence of spermidine synthase yields spermidine (Fig. 1A). GspS catalyzes the reaction of GSH with spermidine to produce glutathionylspermidine, which reacts with another molecule of GSH catalyzed by $T(SH)_2$ synthetase to produce $T(SH)_2$. $T(SH)_2$ is also synthesized by the reduction of trypanothione disulfide (TS_2) (Fig. 1A). TS_2 is reduced to $T(SH)_2$ by the flavoenzyme TR at the expense of nicotinamide adeninine dinucleotide phosphate (NADPH) (Fig. 1A). $T(SH)_2$ carries out numerous reactions in trypanosomatid protozoa (Fig. 1B) and is a spontaneous reductant of dehydroascorbate as well as of the disulfides of GSH, ovothiol, and the parasitic thioredoxin. $T(SH)_2$ is involved in the detoxification of metals and drugs and in ketoaldehyde reduction. $T(SH)_2$ also reduces nucleoside diphosphate (NDP) in the presence of tryparedoxin (TXN)/ribonucleotide reductase (RiboR) (Fig. 1B). Methionine sulfoxide reductase (MSR), a TXN-dependent enzyme, is a known antioxidant protein for maintaining the redox system in trypanosomes. $T(SH)_2$ is the specific reductant of TXN, a multipurpose redox protein belonging to the TRX superfamily. MSR detoxifies methionine sulfoxide (MetSO) in trypanosomes by using the $T(SH)_2$/TXN couple as a reducing system (Fig. 1B) (10).

Table 1. Enzymes in the Redox System of Selected Parasites as Potential Drug Targets

Diseases	*Parasite*	*Target*
Malaria	*Plasmodium falciparum*	Glutathione reductase (GR) Glutathione-S-transferase (GST) Thioredoxin reductase (TrxR) Peroxiredoxins Glutaredoxin GSH peroxidase
African trypanosomiasis (sleeping sickness) American trypanosomiasis (Chagas disease)	*Trypanosoma brucei* *Trypanosoma cruzi*	Trypanothione reductase (TR)
Leishmaniasis	*Leishmania donovani*	Trypanothione reductase (TR)
Amoebiasis	*Entamoeba histolytica*	Pyruvate: ferredoxin/flavodoxin oxidoreductases (PFORs)
Trichomoniasis	*Trichomonas vaginalis*	Pyruvate: ferredoxin/flavodoxin oxidoreductases (PFORs)
Schistosomiasis	*Schistosoma mansoni*	Thioredoxin-glutathione reductase (TGR) Methionine sulfoxide reductase (MSR)

GSH, glutathione.

The biological reactions based on GSH and thioredoxin (Trx) in *Plasmodium falciparum* are shown in Fig. 2A–D. Two systems interact to protect malarial parasites against ROS. The GSH system comprises GSH, glutathione reductase (GR), glutathione S-transferase (GST), and different glutaredoxin-like proteins. The Trx system includes Trx, thioredoxin reductase (TrxR), and Trx-dependent peroxidases (73, 81–83, 92–93, 123, 160). The newly discovered redox protein plasmoredoxin is one of the links between the two systems (19). TrxR reduces thiredoxin-S_2 in the presence of NADPH (Fig. 2A). In order to maintain adequate levels of GSH, GR converts glutathione disulfide into GSH in the presence of NADPH (Fig. 2B) (155). GSH, which is known to safeguard *P. falciparum* from oxidative damage, also has an additional protective role through the promotion of heme catabolism (20, 39, 82, 90). GSH-specific reactions in the parasite involve the removal of 2-ketoaldehydes such as methylglyoxal by the glyoxalase (GLX) system (GLX I and II) (69, 102) as well as the detoxification of drugs/xenobiotics by GST (Fig. 2C) (147). A well-known function of both Trx and GSH is the reduction of NDPs to deoxynucleoside diphosphates catalyzed by RiboR. Reduced thioredoxin [$Trx(SH)_2$] directly interacts with the enzyme, whereas GSH spontaneously reduces glutaredoxins, which subsequently reduce RiboR in *P. falciparum* (Fig. 2D) (122). In *Schistosoma mansoni*, methionine residue is oxidized by ROS to form the MetSO. The product is then converted back to its previous form by the action of the MSR system in the presence of $Trx(SH)_2$ (Fig. 2E) (115).

Redox-Active Drugs Against Unicellular Parasites

The redox system is the backbone for the survival of parasites (150). Targeting this system would be a parasite-killing strategy (135). Compounds having a redox center and/or affecting redox biology and, hence, causing death of parasites are collectively called redox-active antiparasitic drugs. In this section, we have discussed the redox-active drugs against unicellular parasites based on our literature survey. *P. falciparum*, *Trypanosoma cruzi*, *Trypanosoma brucei*, *Leishmania donovani*, and *Entamoeba histolytica* are important parasites, and we have selected these protozoa, because they are the major infectious parasites of the world.

Redox-Active Antimalarial Drugs

The erythrocyte is the safest place for the malaria parasite to hide from its host's immune system, and the erythrocytic stages of *Plasmodium* spp. are responsible for clinical manifestation. The parasite is becoming increasingly resistant to conventional antimalarial drugs, and this has contributed to increasing morbidity and mortality (158). Malaria infection damages several major organs such as the liver, kidney, brain,

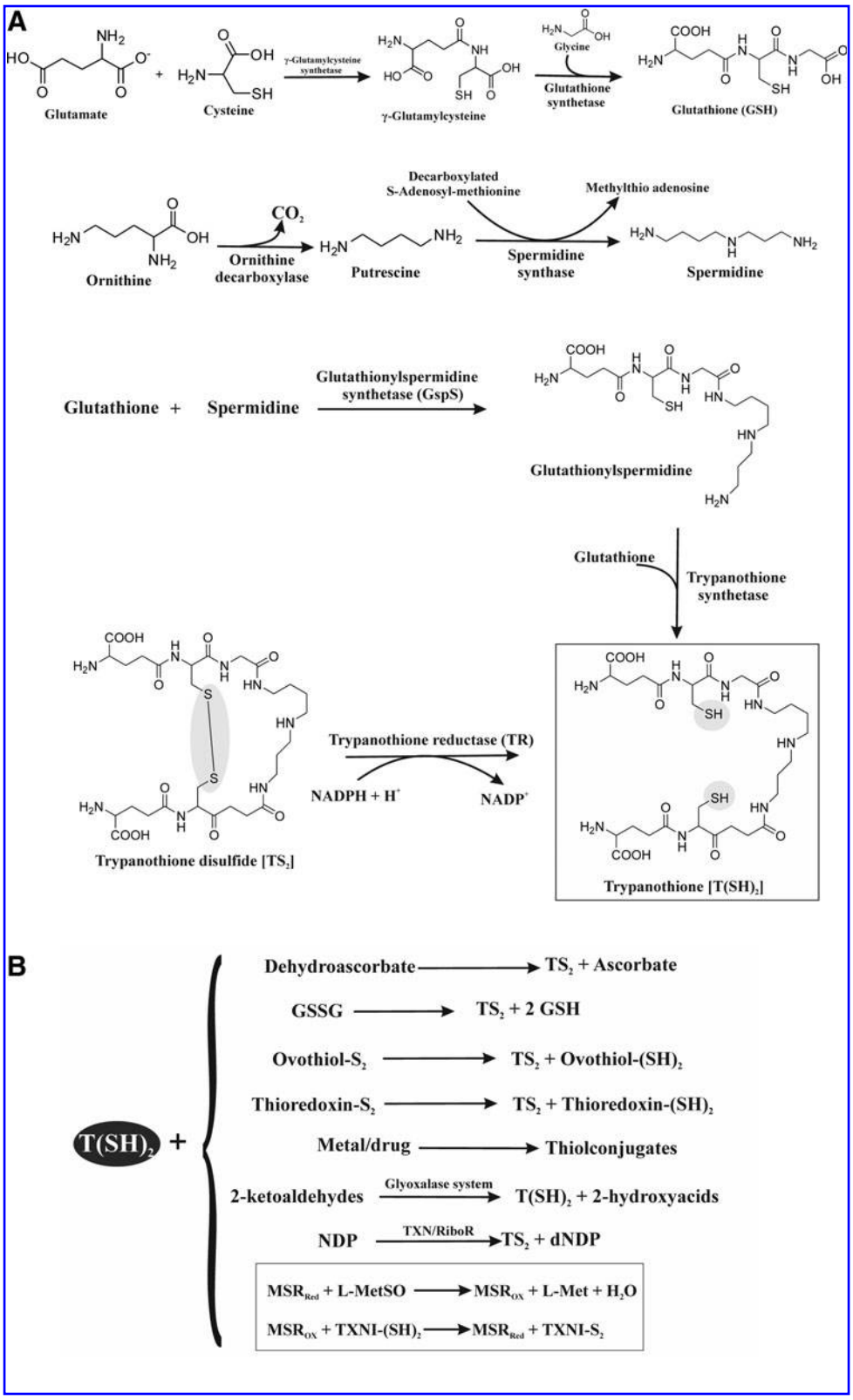


FIG. 1. Biosynthesis and redox reactions carried out by $T(SH)_2$ in trypanosomatids. (A) Biosynthesis of $T(SH)_2$. **(B)** $T(SH)_2$-mediated reactions in trypanosomatids. $T(SH)_2$ is a spontaneous reductant of dehydroascorbate as well as of the disulfides of glutathione, ovothiol, and the parasitic thioredoxin. $T(SH)_2$ is also involved in the detoxification of metals and drugs and in ketoaldehyde reduction. $T(SH)_2$ also reduces NDP in the presence of TXN/RiboR. MSR reduces L-MetSO to L-Met using TXN1-$(SH)_2$/$T(SH)_2$ couple [$T(SH)_2$ keeps TXN1 in reduced state]. $T(SH)_2$, trypanothione; TXN, tryparedoxin; RiboR, ribonucleotide reductase; NDP, nucleoside diphosphate; dNDP, deoxynucleoside diphosphate; MSR, methionine sulfoxide reductase; MetSO, methionine sulfoxide; Met, methionine.

spleen, heart, and lungs (42, 44, 49, 67, 113). In fact, severe malaria is characterized by multi-organ failure (64). Here, we have discussed the compounds that interrupt the redox system of the parasite and lead to cell death. In general, compounds that disturb the redox system of the parasite could be categorized into three different groups: (i) molecules which inhibit the activities of enzymes that are responsible for the maintenance of the redox balance of the parasite; (ii) molecules that prevent the inherent scavenging of pro-oxidant metabolic products (*i.e.*, hemozoin [Hz] formation in malaria

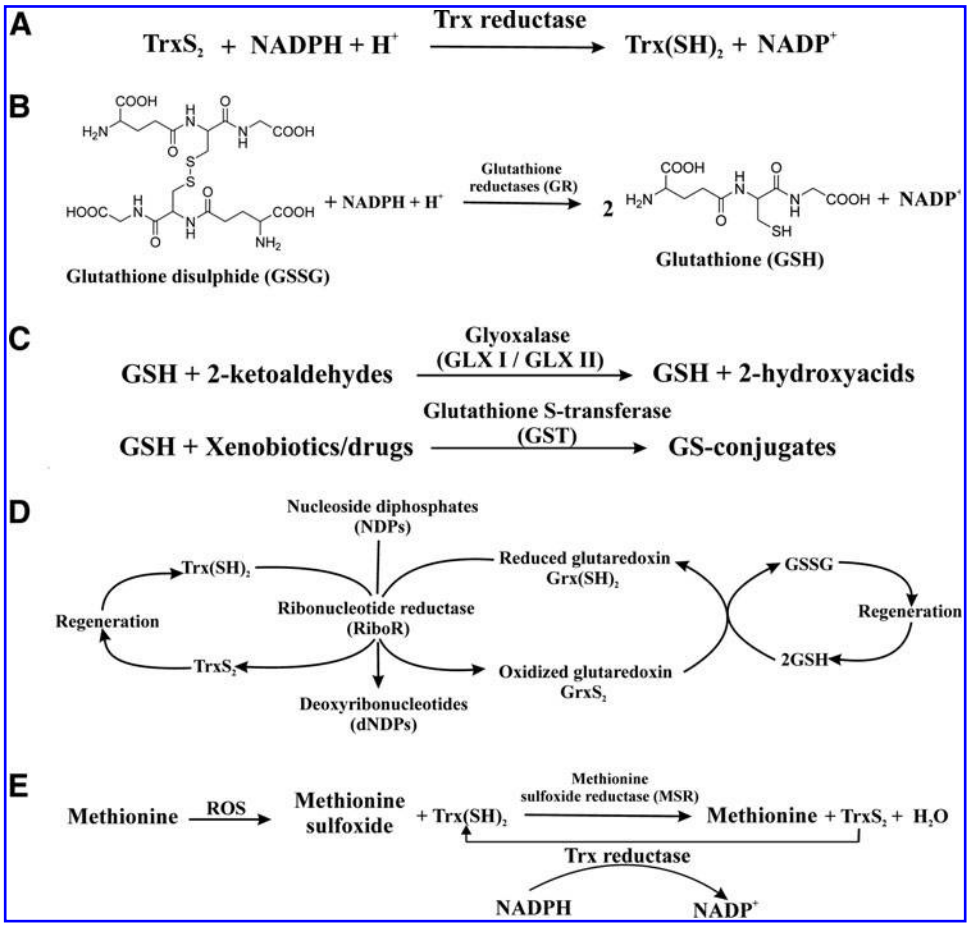


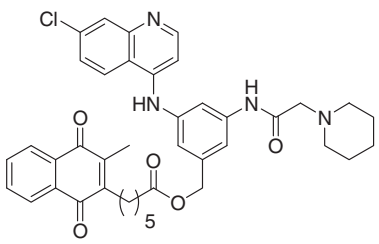
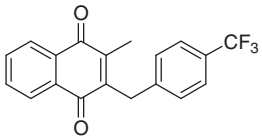
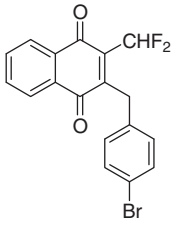
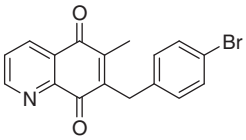
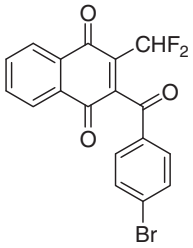
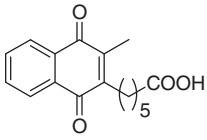
FIG. 2. GSH, TrxR and MSR-dependent reactions identified in *Plasmodium* and other protozoa. (A) The NADPH-dependent reduction of $TrxS_2$ by Trx reductase in *Plasmodium falciparum*. **(B)** The reduction of glutathione disulfide (GSSG) by GR in presence of NADPH in *P. falciparum*. **(C)** GSH-specific reactions to remove 2-ketoaldehydes by the glyoxalase (GLX) system (GLX I and II) and the detoxification of drugs/xenobiotics by GST in *P. falciparum*. **(D)** A well-known function of both Trx and GSH is the reduction of NDPs to dNDPs catalyzed by RiboR. $Trx(SH)_2$ directly interacts with RiboR to bring about this reduction. GSH does not directly interact with RiboR. It converts oxidized glutaredoxin to its reduced form, which subsequently interacts with RiboR. **(E)** Detoxification of MetSO in *Schistosoma mansoni*. Methionine residues are oxidized by ROS to form MetSOs. The product is then converted back to its previous form by the action of the MSR system in the presence of $Trx(SH)_2$. GSH, Glutathione; TrxR, thioredoxin reductase; Trx, thioredoxin; $Trx(SH)_2$, reduced thioredoxin; $TrxS_2$, oxidized thioredoxin; ROS, reactive oxygen species; GR, glutathione reductase; NADPH, nicotinamide adeninine dinucleotide phosphate; GST, glutathione S-transferase.

parasites) and lead to oxidative stress to induce parasite death; and (iii) molecules that produce ROS by themselves and, thus, lead to parasite death.

Drugs inhibiting enzymes in the redox system

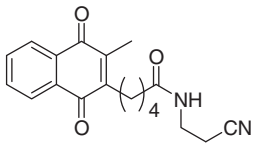
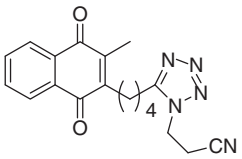
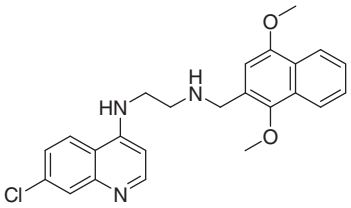
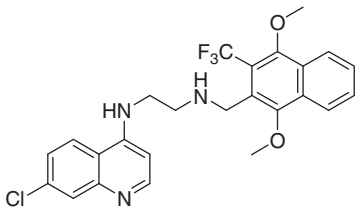
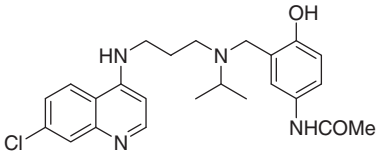
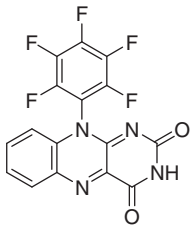
GSH plays important roles in the maintenance of the redox and antioxidant status of protein-SH moieties in *P. falciparum* (84). An elevation of GSH content in parasites leads to an increased resistance toward chloroquine (CQ), while GSH depletion in resistant *P. falciparum* strains is expected to restore the sensitivity to CQ. GSH is involved in the reductive detoxification of free heme (11, 110) and also directly participates in the termination of radical-based chain reactions in which a single electron is transferred from thiyl radicals or disulfide radicals (53). GR is a key enzyme in the cell's defense mechanisms against oxidative stress, and based on this notion, several compounds were synthesized. They show antimalarial activity and inhibit *P. falciparum* GR (PfGR) (57, 65). A quinol-quinoline hybrid (**2a**) shows an antimalarial effect against CQ-sensitive (CQ-S) D6 strain as well as CQ-resistant (CQ-R) *P. falciparum* FcB1R strain and inhibits PfGR in a reversible manner (Table 2) (39). Compound **2a** also shows an antimalarial effect *in vivo* and does not show cytotoxicity as evaluated using human diploid embryonic lung cell line (hMRC-5, Bio-Whittaker 72211D). Compound **2a** suppresses >99.9% parasitemia in mice (*Plasmodium berghei* strain) at a dose of 40 mg/kg (39). Some synthesized compounds (**2b–e**) having quinine scaffold irreversibly inhibit PfGR and show excellent antimalarial activity against multidrug-resistant (MDR) *P. falciparum* clone Dd2 strain (112). **2e** shows antimalarial activity *in vivo* and does not show any cytotoxicity as evaluated in human cell lines such as the buccal carcinoma cell line (KB) and the lung MRC-5 fibroblasts. **2e** suppresses >34.9% parasitemia in mice (*P. berghei* strain) at a dose of 30 mg/kg (112). **2b**, **2c**, and **2e** inhibit human GR (hGR) with

Table 2. Compounds Inhibiting *Plasmodium falciparum* Glutathione Reductase and *Plasmodium falciparum* Growth

Compound	*Structure*	*PfGR inhibition [IC_{50} (μM)] M ± SEM*	*Mode of inhibition*	*Antimalarial activity [EC_{50} (μM)] M ± SEM*	*(Ref)*
Quinol-quinoline hybrid compound **2a**		-	Reversible inhibition	0.027 ± 0.003 (CQ-S) 0.0231 ± 0.006 (CQ-R)	(39)
BenzylNQ **2b**		> 50	Irreversible inhibition	0.029 ± 0.002 (MDR)	(112)
F-BenzylNQ **2c**		13	Irreversible inhibition ($k_i = 0.025\ min^{-1}$)	> 16 (MDR)	(112)
AzaNQ **2d**		-	-	0.608 (MDR)	(112)
F-benzoylNQ **2e**		12	Irreversible inhibition ($k_i = 9.0\ min^{-1}$)	> 1.6	(112)
Quinone derivatives (M5) **2f**		4.5	Reversible uncompetitive inhibition	4.0 (CQ-R)	(21)

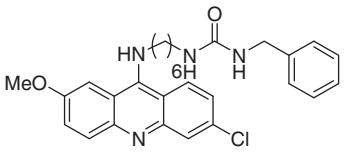
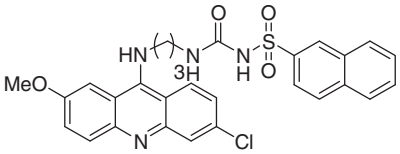
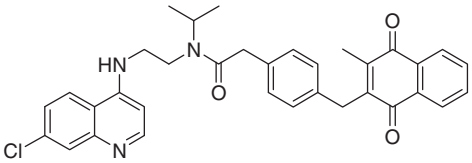
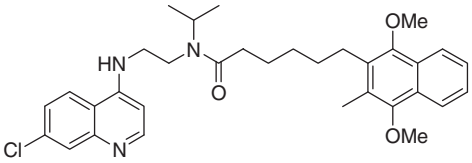
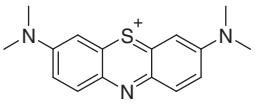
(*continued*)

Table 2. (Continued)

Compound	*Structure*	*PfGR inhibition [IC_{50} (μM)] M ± SEM*	*Mode of inhibition*	*Antimalarial activity [EC_{50} (μM)] M ± SEM*	*(Ref)*
2g		57.0	Reversible uncompetitive inhibition	1.5 ± 0.4 (CQ-R)	(21)
2h		10.8	Reversible uncompetitive inhibition	1.4 ± 0.2 (CQ-R)	(21)
Phenolic Mannich bases **2i**		-	-	0.023 (CQ-S) 0.028 (CQ-R)	(54)
2j		-	-	0.127 (CQ-S) 0.016 (CQ-R)	(54)
2k		-	-	0.022 (CQ-S) 0.317 (CQ-R)	(54)
Isoalloxazines **2l**		-	Noncompetitive inhibition $k_i = 1.9\ \mu M$	-	(131)

(*continued*)

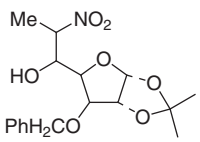
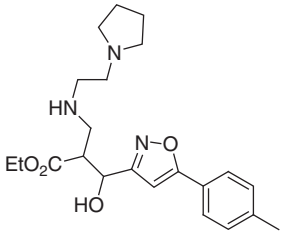
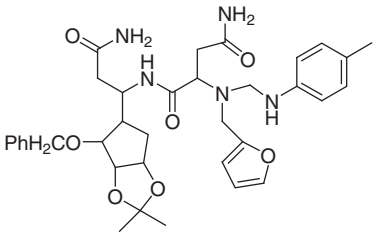
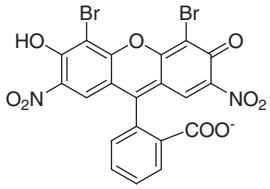
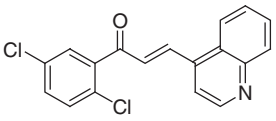
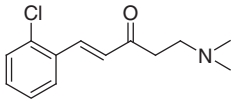
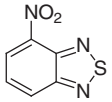
Table 2. (Continued)

Compound	Structure	PfGR inhibition [IC_{50} (μM)] M ± SEM	Mode of inhibition	Antimalarial activity [EC_{50} (μM)] M ± SEM	(Ref)
Quinacrines **2m**		-	-	0.14 (CQ-S)	(29)
2n		-	-	0.0005 (CQ-S) 0.015 (CQ-R)	(29)
Tertiary amides **2o**		-	-	0.012 (CQ-S) 0.005 (CQ-R)	(54)
2p		-	-	0.052 (CQ-S) 0.3 (CQ-R)	(54)
Peroxynitrite **2q**	$ONOO^-$	15	Inactivates PfGR through the nitration of Tyr86 and Tyr94.	-	(90, 132)
Methylene blue **2r**		5.4	Irreversible noncompetitive inhibition	0.003 (CQ-S) 0.004 (CQ-R)	(21, 50, 90, 134)

-, not evaluated; CQ-S, chloroquine-sensitive; CQ-R, chloroquine-resistant; MDR, multidrug-resistant; PfGR, *Plasmodium falciparum* glutathione reductase.

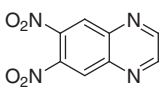
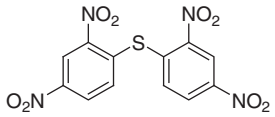
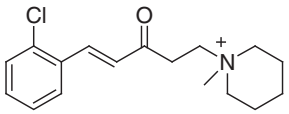
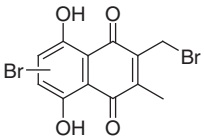
IC_{50} values of 16, 17, and 12 μM, respectively (112). 6-[2′-(3′-methyl)-1′,4′-naphthoquinolyl] hexanoic acid [M5] (**2f**) and **2f** derivatives (**2g, h**) also show antimalarial activity against the CQ-R strain (FcB1R) and inhibit PfGR in a reversible manner (21). **2f–h** do not exhibit any cytotoxicity against the hMRC-5 cell line. **2f–h** inhibit hGR with IC_{50} 3.2, 22.7, and 27.0 μM, respectively (21). These data indicate that **2f–h** by inhibiting PfGR offer antimalarial activity. Some synthesized phenolic Mannich bases (**2i–k**) possess antimalarial activity against CQ-S (3D7 strain) as well as CQ-R (K1 strain) parasites and do not show any cytotoxicity against human KB cells (54). Isoalloxazines (**2l**), quinacrines (**2m, n**), and tertiary amides (**2o, p**) are potential PfGR inhibitors and offer antimalarial activity at low doses against the CQ-S *P. falciparum* strain 3D7 and the CQ-R strain K1 (29, 54, 131). **2l** also inhibits hGR, and the inhibition constant (k_i) of **2l** for hGR is 2.5 μM. Peroxynitrite (**2q**) has antimalarial activity and inactivates PfGR through the nitration of Tyr86 and Tyr94. By oxidizing the catalytic dithiol to a disulfide, peroxynitrite itself can act as a substrate of unmodified and bisnitrated PfGR (90, 132). These indicate that **2q** shows antimalarial activity by inhibiting PfGR. Methylene blue (MB) (**2r**), a noncompetitive inhibitor as well as subversive substrate of PfGR, shows antiplasmodial activity. **2r** does not show toxicity against mammalian cells and

Table 3. Compounds Inhibiting Enzymes Involved in the Redox System of *Plasmodium falciparum* As Well As Growth of *Plasmodium falciparum*

Compound	*Structure*	*Target enzyme*	*Enzyme inhibition [IC_{50} (μM)] M ± SEM*	*Mode of inhibition*	*Antimalarial activity [EC_{50} (μM)] M ± SEM*	*(Ref)*
Glycoconjugate **3a**		PfGST	-	-	74.4 (CQ-R)	(3)
Isoxazole derivative **3b**		PfGST	100	-	100 (CQ-R)	(3)
Glycosyl urea **3c**		PfGST	50	-	50 (CQ-R)	(3)
Eosin B **3d**		PfTrxR	4.2	Reversible uncompetitive inhibition	21.9 (CQ-S) 0.11 (CQ-R)	(105)
Chalcone derivative **3e**		PfTrxR	-	-	0.20 (CQ-R)	(100)
Mannich bases **3f**		PfTrxR	1.9	Irreversible competitive inhibition $k_i = 147 \pm 2.8\ \mu M$		(41)
Nitro compound **3g**		PfTrxR	2	Reversible uncompetitive inhibition $k_i = 1\ \mu M$	11	(8)

(*continued*)

Table 3. (Continued)

Compound	Structure	Target enzyme	Enzyme inhibition [IC_{50} (μM)] M ± SEM	Mode of inhibition	Antimalarial activity [EC_{50} (μM)] M ± SEM	(Ref)
3h		PfTrxR	2	Reversible uncompetitive inhibition	15 (CQ-R)	(8)
3i		PfTrxR	0.5	Reversible uncompetitive inhibition $k_i = 0.2\ \mu M$	18 (CQ-R)	(8)
Mannich base **3j**		PfTrxR	-	Irreversible inhibition	16.2 (CQ-S)	(97)
Naphthazarine derivative **3k**		PfTrxR	-	Competitive inhibition $k_i = 0.5\ \mu M$	-	(90)

PfTrxR, *Plasmodium falciparum* thioredoxin reductase; PfGST, *P. falciparum* glutathione S-transferase.

inhibits hGR ($IC_{50} = 16\ \mu M$) (21, 25, 50, 90, 134). **2r** is active against all blood stages of both CQ-S and CQ-R *P. falciparum* strains (4, 12, 15, 58). The comparative effects of compounds inhibiting PfGR and their antiplasmodial activity are presented (Table 2). It is evident that **2n** ($EC_{50} = 0.0005\ \mu M$) is the most effective antimalarial agent compared with the other PfGR inhibitors (**2a–r**) depicted in Table 2.

GST is one of the vital components of the GSH system. It uses GSH as a substrate and catalyzes the conjugation of GSH to a variety of electrophilic substrates (130). GST is a good target for designing antiparasitic drugs. Glycoconjugate (**3a**), isoxazole (**3b**), and glycosyl urea (**3c**) inhibit *P. falciparum* GST (PfGST) and show antimalarial activity (Table 3) (3, 128). *P. falciparum* thioredoxin reductase (PfTrxR) may be a promising antimalarial drug target as well (16). Inhibition of PfTrxR may affect the parasite at several vulnerable points, resulting in enhanced oxidative stress, ineffective DNA synthesis, hindrance in cell division, and disturbed redox regulatory processes (16). Eosin B (**3d**) and chalcone derivatives (**3e**) exhibit antiplasmodial activity and also inhibit PfTrxR (100, 105). Mannich bases (**3f**) and nitro compounds (**3g–i**), which are PfTrxR inhibitors, show antimalarial activity against CQ-R (K1) strain and no toxicity against mammalian cells (8, 41, 97). These data suggested that **3g–i** show antimalarial activity by inhibiting PfTrxR. **3g–i** also inhibit human TrxR (hTrxR) with IC_{50} values of 50, 140, and 4 μM, respectively (8). Mannich base (**3j**) and naphthazarine derivatives (**3k**) competitively inhibit PfTrxR and demonstrate antimalarial activity against the CQ-S *P. falciparum* 3D7 strain (90). **3k** also inhibits hTrxR ($k_i = 0.005\ \mu M$) (90). The comparative effects of compounds inhibiting different redox enzymes and their antiplasmodial activities are presented (Table 3). The PfTrxR inhibitor Eosin B (**3d**) [$EC_{50} = 0.11\ \mu M$] is the most effective antimalarial agent as compared with other agents within this series (**3a–c**, **3e–k**) (Table 3).

Drugs inhibiting Hz formation and inducing oxidative stress

Hemoglobin (Hb) is the major protein inside the erythrocyte, and the parasite has evolved a unique metabolic pathway to digest Hb. Hb degradation occurs inside the food vacuole (FV), which involves many enzymes (112). Heme is the degradation product of Hb, which is extremely toxic to the parasite. The released free heme (Fe^{+III}) can offer a major toxic insult to the parasite through the generation of ROS (106, 112). A number of antimalarial drugs are known to act as inhibitors of Hz formation by binding to heme. Inhibition of Hz formation may develop oxidative stress in *P. falciparum* due to the accumulation of free heme and, thus, cause parasite death (106).

Quinolines, azoles, isonitriles, xanthones, and their derivatives adopt the aforementioned strategy to kill the parasites. Quinoline-containing derivatives such as CQ, amodiaquine, amopyroquine, tebuquine, mepacrine, pyronaridine, halofantrine, quinine, epiquinine, quinidine, and bisquinoline show antimalarial effects and inhibit Hz formation (Fig. 3) (48, 89, 114, 152). It is evident that amopyroquine ($EC_{50} = 5.3$ nM) and halofantrine ($EC_{50} = 2.8$ nM) are the most active antimalarial agents against CQ-S (3D7) and CQ-R (K1) strains, respectively (Fig. 3). Amodiaquine shows *in vivo* antimalarial activity against the *Plasmodium yoelii* NS strain in mice at a low dose ($ED_{50} = 7.65$ mg/kg) (114). Azole derivatives such as clotrimazole (CLT), ketoconazole, and miconazole (15, 125)

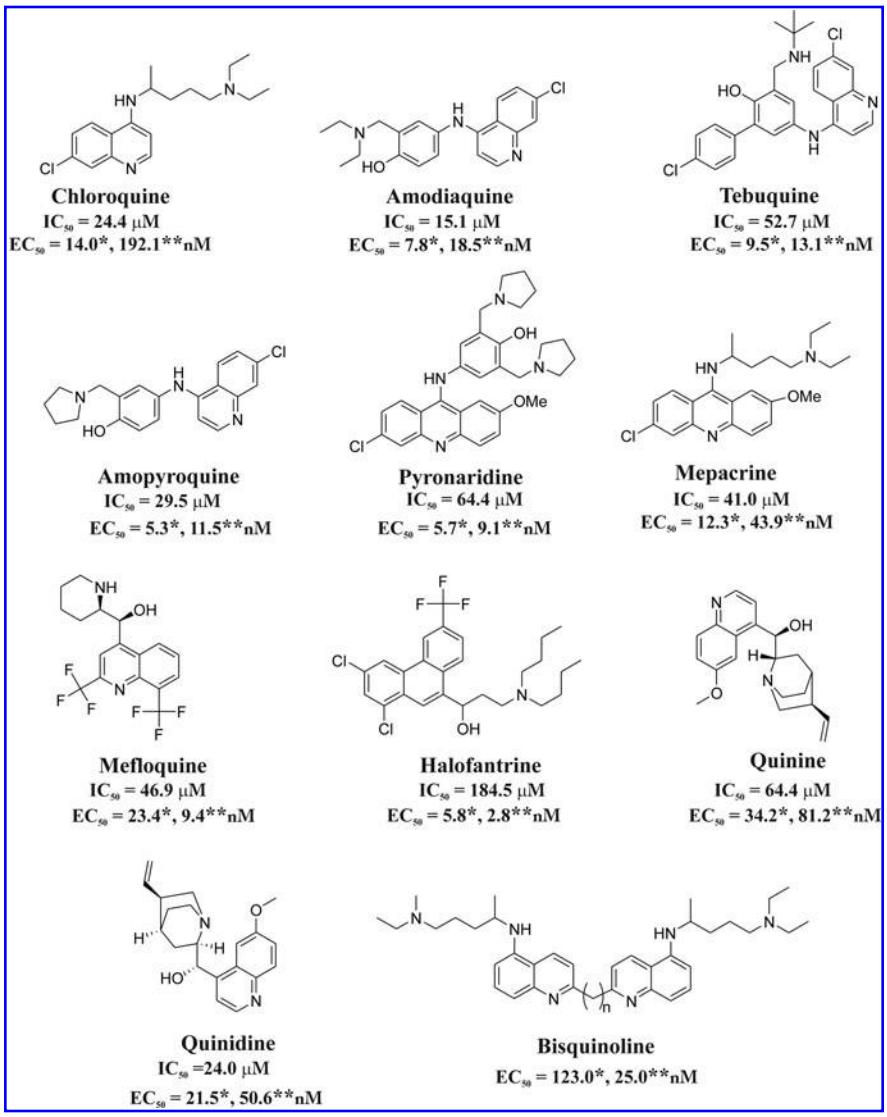


FIG. 3. Antimalarial quinoline compounds inhibiting hemozoin formation. IC_{50}, inhibitory concentration required to inhibit 50% hemozoin formation; EC_{50}, effective concentration required to inhibit 50% *P. falciparum* growth; *Chloroquine-sensitive (CQ-S); **Chloroquine-resistant (CQ-R) *P. falciparum* strain.

inhibit Hz formation and show antimalarial effect against both CQ-S and CQ-R *P. falciparum* strains (62) (Fig. 4A). The azoles, including CLT (IC_{50} = 12.9 μM), ketoconazole (IC_{50} = 6.5 μM), and miconazole (IC_{50} = 21.4 μM), reversibly block the growth of ferriprotoporphyrin crystals and induce oxidative stress in malaria parasites (30). CLT shows low *in vitro* toxicity in human and murine cell lines (61–62).

Xanthones have been identified as a novel class of antimalarial compounds (45). The antimalarial activity of xanthone and its derivatives (Fig. 4B) is based on the ability to interact with heme and, hence, prevent Hz formation (76, 162). Antimalarial activity is positively correlated with the number of hydroxyl substituents (51). 2,3,4,5,6-Pentahydroxyxanthone was found to be active against both the sensitive (3D7) and resistant strains (K1) of *P. falciparum* (76) (Fig. 4B) 1,3,6,8-Tetrahydroxyxanthone is very potent against *P. berghei in vivo* and was found to be better than the other oxygenated xanthones in this series (51). Xanthones bearing a hydroxyl group at any peri-position (1 or 8) show decreased antimalarial activity. These derivatives lose their affinity for heme due to intramolecular H-bond formation between the OH group and the carbonyls (77).

Several isonitrile derivatives (Fig. 4C) exhibit antimalarial activity and inhibit Hz formation (95, 161). Synthetic isonitriles were screened for their antimalarial activity against *P. falciparum* and MDR *P. yoelii* in the Swiss mice model (142). These isonitriles show antimalarial activity at a very low concentration (EC_{50} = 9.02 – 196.65 nM) (Fig. 4C). A series of synthesized [(aryl)arylsufanylmethyl]pyridines (AASMP) derivatives (15, 94) (Fig. 4D) show antimalarial activity. These compounds inhibit Hz formation, form complexes (K_D = 12 to 20 μM) with free heme (ferriprotoporphyrin IX) at a pH close to the pH of the parasite FV, and exhibit antimalarial activity *in vitro* against *P. falciparum* (MDR strain). AASMP developed oxidative stress in the parasite by reducing the level of GSH and increasing the formation of lipid peroxide H_2O_2 and OH in *P. falciparum*. AASMP also exhibits profound antimalarial activity *in vivo* against CQ resistant *P. yoelii*. These AASMP derivatives suppressed the day 4 mean parasitemia by 30%, 50%, and 80% at doses of 25, 50, and 100 mg/kg, respectively, against MDR strain *P. yoelii* in BALB/c mice models (94). AASMP derivatives do not show any cytotoxicity against mammalian MCF-7 cells. Benzylmenadione (benzylNQ) derivatives have antimalarial

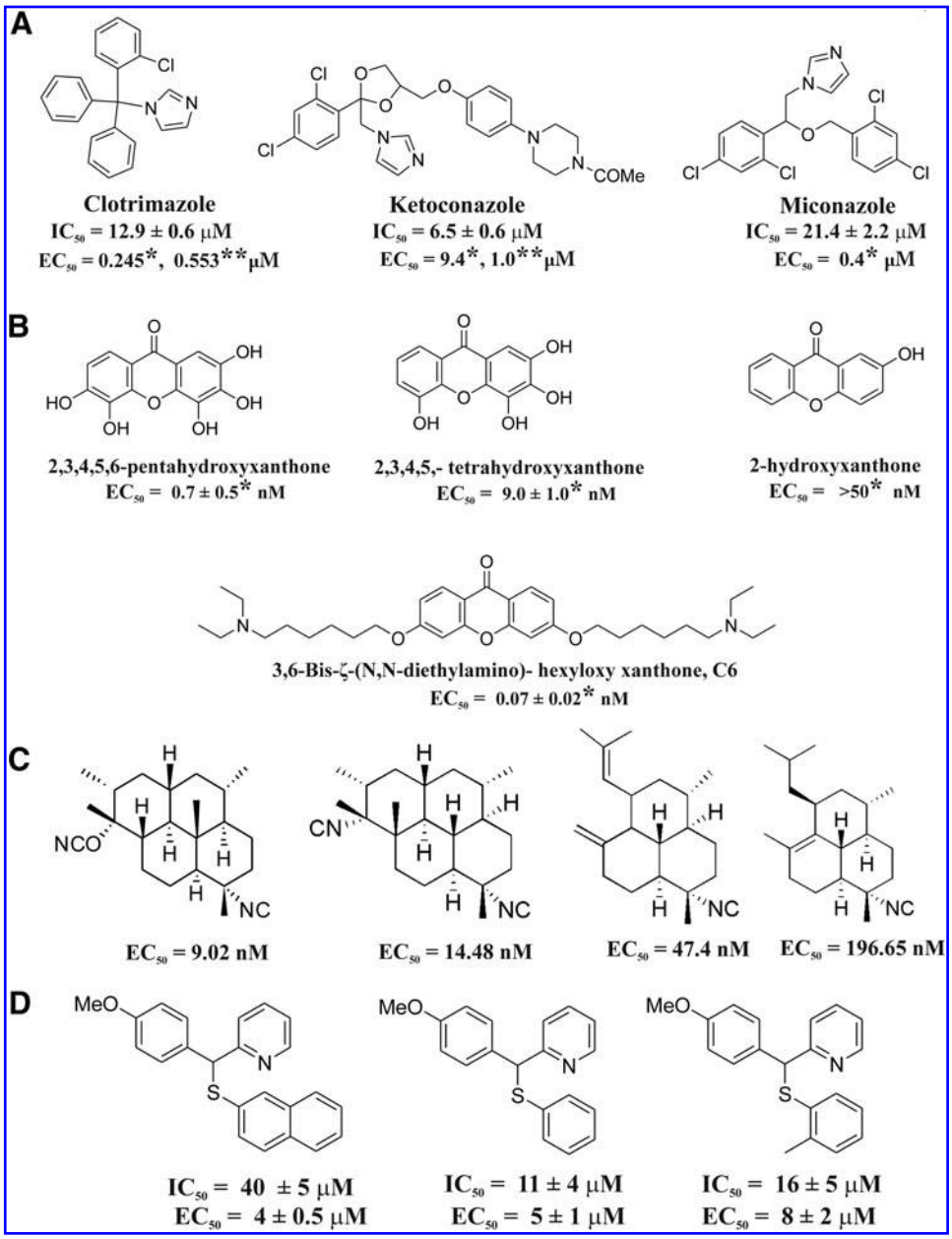


FIG. 4. Selected nonquinoline antimalarial compounds inhibiting hemozoin formation. (A) Azoles **(B)** Xanthones **(C)** Isonitriles **(D)** (Aryl)-arylsufanylmethyl]pyridines. *Chloroquine-sensitive (CQ-S); **Chloroquine-resistant (CQ-R) *P. falciparum* strain; IC_{50} and EC_{50} are presented as M ± SEM.

activity against the CQ-R *P. falciparum* strain (Dd2) by inhibiting Hz formation and do not show any cytotoxicity against two human cell lines, the buccal carcinoma cell line cell (KB) and the human lung MRC-5 fibroblasts (Fig. 5A) (112). It is suggested that the benzylNQ are initially oxidized at the benzylic chain to benzoyl naphthoquinones in a heme-catalyzed reaction within the FV of the parasite. The major putative benzoyl metabolites are found to function as redox cyclers. The benzoyl metabolites (benzoylNQ, metabolite 1) are reduced by NADPH in GR-catalyzed reactions within the cytosol of infected red blood cells, and those benzoyl metabolites (reduced benzoylNQ, metabolite 2) can convert methemoglobin to oxyhemoglobin. This ultimately leads to the inhibition of Hz formation (Fig. 5A) (40). Since such inhibition is a validated antimalarial strategy (106), synthesis of a new inhibitor of Hz formation or identification of a new inhibitor against it from natural products will be helpful in developing novel antimalarials (106, 148). Liu *et al.* (103) suggested that 8-AQ causes heme toxicity by oxidizing Hb to methemoglobin. Both iron and 8-AQ donate an electron to the π^* orbital of O_2, which facilitates the formation of H_2O_2 in the parasite and leads to cell death (103). Since the pH in the FV is ~5.2, in order to enter the compartment and exhibit antimalarial activity, compounds should be alkaline and stable in an acidic environment. Thus, synthesis of compounds (high pK_a) specific for FV is necessary for antimalarial drug development, capitalizing Hz formation as a target.

Drugs self-generating ROS and causing oxidative stress in the parasite

Many compounds kill *Plasmodium* spp. by self-generating ROS in the parasite. Fluoromenadione shows an antimalarial

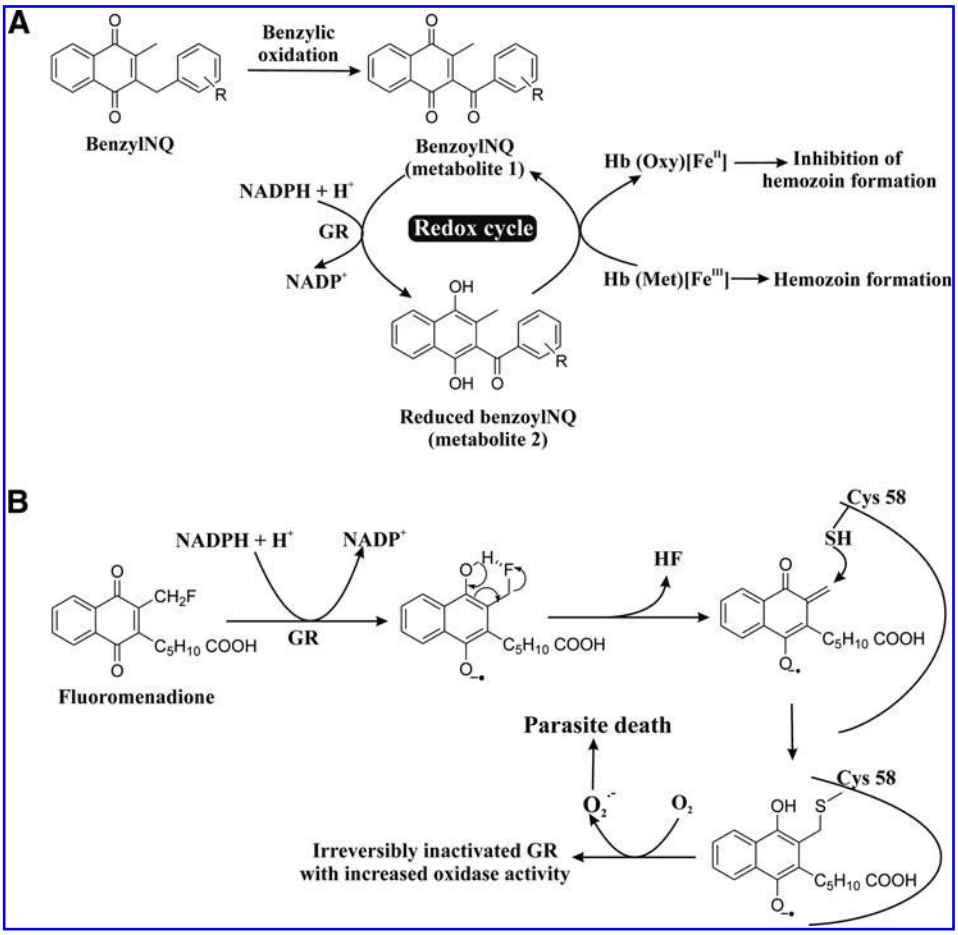


FIG. 5. Proposed mechanism of antimalarial activity of 3-benzylmenadione (BenzylNQ) and fluoromenadione derivatives. (A) Putative redox cascade through which the BenzylNQ derivatives inhibit *P. falciparum* trophozoite development and hemzoin formation. **(B)** Mechanism of ROS production by fluoromenadione in *P. falciparum.* Fluoromenadione is activated *via* GR-catalyzed one-electron reduction (two-electron transfer is also possible). Two molecules of fluoromenadione are reduced by one NADPH to two semihydroquinones. Elimination of HF due to the formation of an intramolecular hydrogen bond results in the formation of a quinone methide radical. Nucleophilic attack of the catalytic Cys58 of GR on the quinone methide causes covalent modification of the enzyme. The radical can further react with oxygen, leading to the formation of superoxide, which can kill the parasite. Hb (oxy)Fe^{II}; oxyhemoglobin, Hb (met)Fe^{II}; methemoglobin; HF, hydrogen fluoride.

effect against the CQ-S strain 3D7 as well as the CQ-R strain K1 through the generation of ROS ($O_2^{\bullet -}$) but much lower cytotoxicity against human cells (18). Fluoromenadione is activated *via* GR-catalyzed one-electron reduction (two-electron transfer is also possible). Hydrogen fluoride is released from the activated fluoromenadione to form a quinone methide radical. Nucleophilic attack of the catalytic Cys58 of GR leads to covalent modification of the GR. The radical can react with oxygen, leading to the formation of $O_2^{\bullet -}$, which causes oxidative stress and parasite death (Fig. 5B) (40). MB, a well-known PfGR inhibitor, shows antimalarial activity by inducing oxidative stress in malaria parasite. MB not only inhibits the physiological reaction but also serves as a subversive substrate of PfGR. PfGR catalyzes the reduction of MB to leuco MB by NADPH. The product leuco MB is auto-oxidized back to MB with the concomitant production of H_2O_2 (ROS) resulting in oxidative stress in malaria parasite (Fig. 6A) (25, 40, 71). Curcumin (Fig. 6B) shows activity against both CQ-susceptible (3D7) and resistant (Dd2) *P. falciparum* strains by increasing ROS in the parasite (34). Curcumin's cytotoxic effect could be antagonized by co-incubation with antioxidants and ROS scavengers (34). Pyrimethamine (Fig. 6B) shows an antimalarial effect by inducing oxidative stress in parasites both *in vivo* and in *P. yoelii* 17XL-infected mice at a dose of 10 mg/kg (98). The minimum inhibiting concentration of pyrimethamine for activity against the *P. falciparum* NF-54 strain is 10 μg/ml (2).

Redox-Active Drugs Against Trypanosomiasis

Trypanosomes are parasitic protozoa within the order Kinetoplastida that comprise the causative agents of African sleeping sickness (*Trypanosoma brucei gambiense* and *T. b. rhodesiense*), South American Chagas disease (*T. cruzi*), and Nagana cattle disease (*Trypanosoma congolense*) (60, 88). Human African trypanosomiasis (HAT) or sleeping sickness is caused by two subspecies of *T. brucei*. In West and Central Africa, *T. b. gambiense* causes the chronic form of sleeping sickness, while in East Africa, *T. b. rhodesiense* causes the more fulminant form (79). *T. cruzi* is the causative agent of Chagas

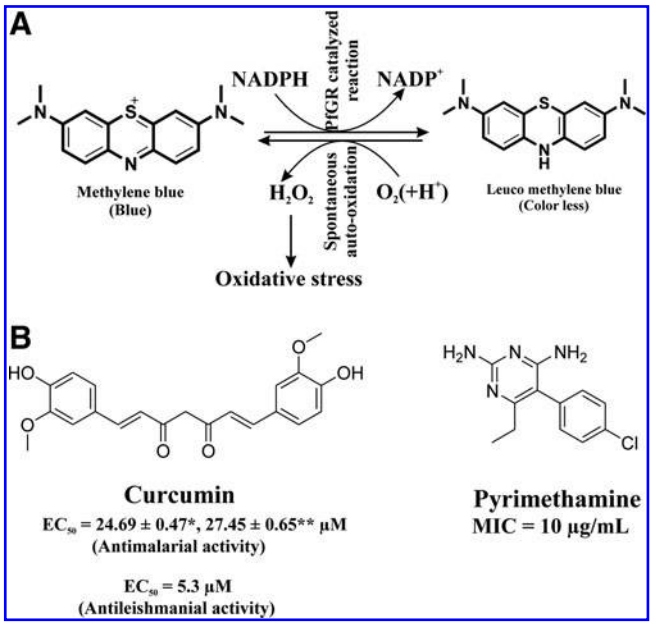


FIG. 6. Compounds self-producing ROS. (A) Mechanism of MB mediated production of ROS in *P. falciparum*. *P. falciparum* GR catalyzes the reduction of MB to leuco MB in the presence of NADPH. Leuco MB is auto-oxidized back to MB with the concomitant production of H_2O_2 (ROS), which causes oxidative stress in malaria parasite. **(B)** Compounds self-producing ROS in parasites. MIC, minimum inhibitory concentration for inhibiting the development of schizont stage from ring stage parasites; *Chloroquine-sensitive (CQ-S); **Chloroquine-resistant (CQ-R) *P. falciparum* strain. MB, methylene blue.

disease, the most important parasitic infection in Latin America. Approximately 8 million people are thought to be infected (124). *T. cruzi* infects many mammalian species (163) and is transmitted to humans primarily by the infected feces of hematophagous triatomine bugs coming in contact with mucosal membranes or damaged skin. Transmission also occurs *via* blood transfusion, congenitally, or, rarely, by ingestion of food contaminated by infected triatomine feces (5, 164). Chemotherapy against all forms of trypanosomiasis is very limited and unsatisfactory (85). Sleeping sickness is transmitted by the tsetse fly in sub-Saharan Africa with an estimated incidence of 70,000–80,000 cases and ~30,000 deaths per annum (145). Once the infection has spread to the central nervous system, the disease is invariably fatal without treatment (145). Trypanothione reductase (TR) is an NADPH-dependent flavoprotein disulfide oxidoreductase unique to and essential for growth of trypanosomes, whose function is to convert TS_2 into the physiologically relevant reduced form $T[SH]_2$ (72, 146). In trypanosomes, $T[SH]_2$ serves as a substitute for many of the metabolic and antioxidant functions ascribed to GSH in mammalian cells (32). Mammalian GR is the nearest homolog to TR. However, both enzymes of the host and parasite have significant differences in their active site architecture, resulting in a pronounced ability to discriminate between their respective disulfide substrates. These features make TR an attractive target for selective drug design (144). *T. brucei* TR and *T. cruzi* TR are the key enzymes for controlling the redox system of their respective parasites (Table 1) (36). Thus, inhibitors of *T. brucei* TR and *T. cruzi* TR are suitable candidates for drug development against HAT and American trypanosomiasis, respectively (28, 90).

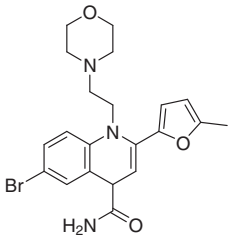
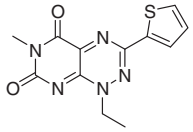
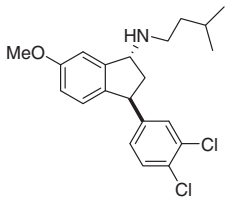
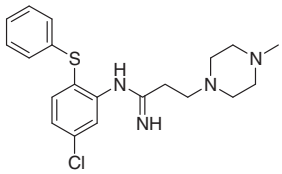
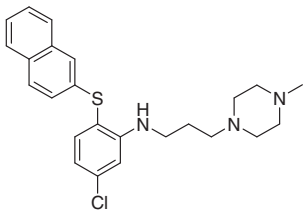
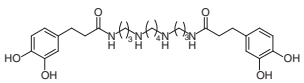
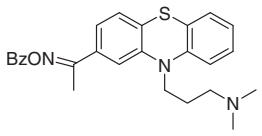
Drugs inhibiting enzyme activity in the redox system

In this section, we discuss the drugs that inhibit the activity of *T. brucei* TR. Quinolines (**4a**), synthesized from substituted isatine derivatives show antitrypanosomal activity, and inhibit *T. brucei* TR ($IC_{50} = 4.2\ \mu M$) (144). Pyrimidopyridazines (**4b**) and indatraline derivatives (**4c**) are used for the treatment of HAT and inhibit *T. brucei* TR (Table 4). **4a–c** show antitrypanosomal activity by inhibiting *T. brucei* TR. IC_{50} of **4b** and **4c** for the inhibition of *T. brucei* TR are 12.8 and $2.23 \pm 0.66\ \mu M$, respectively (144, 156). Dimethylamine-bearing diaryl sulfides (**4d**) and piperazine-bearing diaryl sulfides (**4e**) show antitrypanosomal activity against the *T. b. rhodesiense* STIB900 strain and inhibit *T. b. rhodesiense* TR (146). Polyamines such as kukoamine (**4f**) and reversible mixed type *T. brucei* TR inhibitor ($k_i = 1.8\ \mu M$, $k_i' = 13\ \mu M$) show antitrypanosomal activity (120, 143). 2-Substituted promazines (**4g**) are also effective against HAT and inhibit *T. brucei* TR (27). Naphthoquinone derivatives (**4h**), Mannich bases (**4i**), and acridines (**4j–k**) show activity against American trypanosomiasis and inhibit *T. cruzi* TR. These molecules do not show toxicity against human cell lines (23, 97). Benzylammonium compounds (**4l**) and N-(3-phenylpropyl) polyamine (**4m**), competitive inhibitors of *T. cruzi* TR, show activity against American trypanosomiasis (101). 1-Phenethyl-4-aminopiperidine derivatives (**4n**) show antitrypanosomal activity against *T. b. rhodesiense* STIB900 strain and inhibit *T. b. rhodesiense* TR (37). Some metal complexes such as Pt complex-acridone conjugate (**4o**) inhibit *T. cruzi* TR at low concentrations ($IC_{50} = 1\ \mu M$) (78). Triflupromazine (**4p**), *T. cruzi* TR inhibitor ($IC_{50} = 110 \pm 18\ \mu M$) has been found effective against American trypanosomiasis (27). Tricyclic derivative (**4q**) inhibits both *T. brucei* TR ($IC_{50} = 11.5 \pm 0.4\ \mu M$) and *T. cruzi* TR ($IC_{50} = 14.6 \pm 0.8\ \mu M$) (118). Quaternary alkylammonium phenothiazine derivatives (**4r**) inhibit *T. cruzi* TR at low concentrations ($IC_{50} = 1.2 \pm 1.20\ \mu M$) (86). Thus, it appears that **4p–r** show antitrypanosomal activity by inhibiting *T. cruzi* TR. The comparative effects of compounds on the antitrypanosomal activity are presented (Table 4). From the comparative studies, it is evident that **4r** is the most effective ($EC_{50} = 0.062\ \mu M$) antitrypanosomal agent compared with other antitrypanosomal agents (**4a–q**) (Table 4).

Drugs self-inducing oxidative stress in the parasite

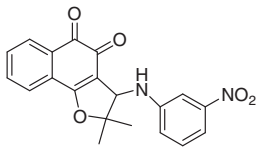
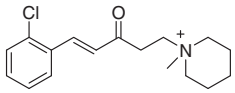
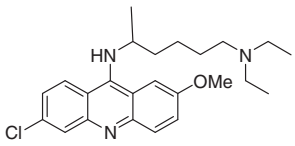
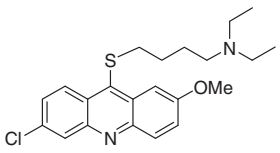
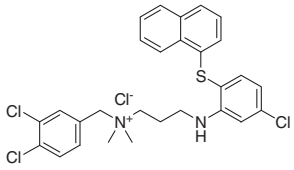
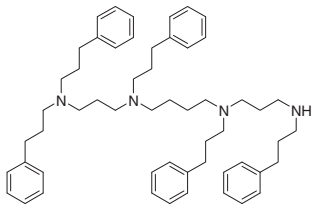
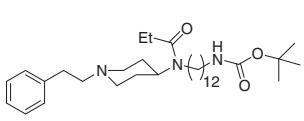
Chinifur [**1**] (Fig. 7), a noncompetitive inhibitor and subversive substrate of *T. congolense* TR, shows antitrypanosomal activity by generating $O_2^{\bullet -}$ anion radicals in the parasite (26). Aromatic nitro compounds ($ArNO_2$) show antitrypanosomal activity by producing ROS in the trypanosomatid protozoa (68, 159). The key step in this process involves reactions catalyzed by a group of nitroreductases (NTRs). Based on oxygen sensitivity, the enzymes can be divided into Type I NTRs and Type II NTRs (126). Type I NTRs mediate the sequential reduction of the nitro group *via* a series of 2-electron transfers from NAD(P)H through a nitroso (ArNO) intermediate to produce hydroxylamine derivatives. This nitroso compound scavenges thiols of the parasite. Type II NTRs are ubiquitous

Table 4. Redox-Active Antitrypanosomal Compounds

Compound	*Structure*	*Target enzyme*	*Mode of inhibition*	*Inhibition constant (μM) M ± SEM*	*Antitrypanosomal activity [EC_{50} (μM)] M ± SEM*	*(Ref)*
Quinoline derivative **4a**		*T. brucei* TR	Linear mixed type inhibition	$k_i = 3.0 \pm 0.2$ $k_i' = 4.3 \pm 0.3$	25.6	(144)
Pyrimidopyridazine derivative **4b**		*T. brucei* TR	Linear uncompetitive inhibition	$k_i = 3.0 \pm 0.2$	1.43	(144)
Indatraline derivative **4c**		*T. brucei* TR	Linear mixed type inhibition	$k_i = 3.5 \pm 0.4$ $k_i' = 16.8 \pm 3.3$	1.26 ± 0.13	(156)
Dimethylamine-bearing diaryl sulfide **4d**		*T. b. rhodesiense* TR	-	-	1.56	(146)
Piperazine-bearing diaryl sulfide **4e**		*T. b. rhodesiense* TR	-	-	0.70	(146)
Polyamines Kukoamine **(4f)**		*T. brucei* TR	Reversible mixed type inhibition	$k_i = 1.8$ $k_i' = 13$	-	(120, 143)
2-Substituted Promazine **4g**		*T. cruzi* TR	Reversible inhibition	$k_i = 204 \pm 16$	> 30	(27)

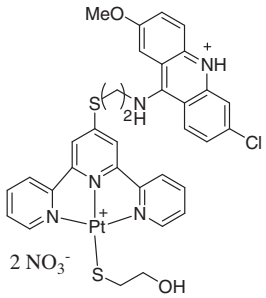
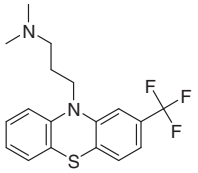
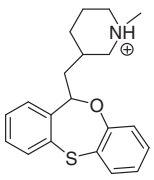
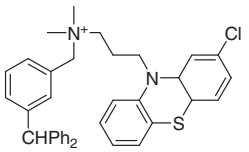
(*continued*)

Table 4. (Continued)

Compound	*Structure*	*Target enzyme*	*Mode of inhibition*	*Inhibition constant (μM) M ± SEM*	*Antitrypanosomal activity [EC_{50} (μM)] M ± SEM*	*(Ref)*
Naphthoquinone derivative **4h**		*T. cruzi* TR	-	-	86.3 ± 4.6	(36)
Mannich base **4i**		*T. cruzi* TR	Irreversible inhibition	$k_i = 8$	11.8	(97)
Acridines **4j**		*T. cruzi* TR	Competitive inhibition	$k_i = 5.5 \pm 1$	-	(23)
4k		*T. cruzi* TR	Mixed type inhibition	$k_i = 21 \pm 3$ $k_i' = 67 \pm 3$	-	(23)
Benzylammonium compounds **4l**		*T. cruzi* TR	Competitive inhibition	$k_i = 9 \pm 5$		(146)
N-(3-phenylpropyl) substituted polyamines **4m**		*T. cruzi* TR	Competitive reversible inhibition	$k_i = 0.151$	3.05	(101)
1-Phenethyl-4-aminopiperidine derivative **4n**		*T. b. rhodesiense TR*	-	-	3.16	(37)

(*continued*)

Table 4. (Continued)

Compound	*Structure*	*Target enzyme*	*Mode of inhibition*	*Inhibition constant (μM) M ± SEM*	*Antitrypanosomal activity [EC_{50} (μM)] M ± SEM*	*(Ref)*
Pt complex-acridone conjugate **4o**		*T. cruzi* TR	Mixed type inhibition	$k_i = 2$ $k_i' = 2.8$	-	(78)
Triflupromazine **4p**		*T. cruzi* TR	Reversible inhibition	$k_i = 30.2 \pm 2.6$	3.2	(27)
Tricyclic derivative **4q**		*T. brucei* TR *T. cruzi* TR	Reversible inhibition	$k_i = 0.1$–50	-	(118)
Quaternary Alkylammonium Phenothiazine **4r**		*T. cruzi* TR	Linear competitive inhibition	$k_i = 0.71 \pm 0.1$	0.062	(86)

oxygen-sensitive enzymes that contain flavin adenine dinucleotide as a cofactor. They function by mediating the one electron reduction of the nitro group to form an unstable nitro-radical. In the presence of oxygen, this radical undergoes futile cycling to produce $O_2^{\bullet -}/H_2O_2$ (ROS), with the subsequent regeneration of the parent nitro-compound. ROS oxidizes $T(SH)_2$ into TS_2 in the parasite (22, 68, 159) (Fig. 8). In trypanosomes, Type II NTR activity has been proposed to be the main source of ROS (45, 154). Type I NTR has the capacity to metabolize a wide range of nitroheterocyclic drugs and that a reduction in this activity in both *T. cruzi* and *T. brucei* confers resistance to these trypanocidal agents (159).

Molecules having nitro group as well as metal complexes have the potential to become drugs against trypanosomiasis. N-oxide-containing heterocycles such as benzofuroxans [**2**] ($EC_{50} = 7.0\ \mu M$), benzimidazoles [**3**] ($EC_{50} = 12.5\ \mu M$), and indazole derivatives [**4**] ($EC_{50} = 25.0\ \mu M$) show antitrypanosomal activity against *T. cruzi* epimastigotes (Tulahuen 2 strain) by inducing oxidative stress in and depleting free thiol in *T. cruzi* (Fig. 7) (22). Some metal-based compounds such as pyridine-2-thiol N-oxide Pd complex [**5**], pyridine-2-thiol N-oxide Pt-complex [**6**], and Pt-complex [**7**] show activity against *T. cruzi* by producing intraparasitic nitro anion radicals (Fig. 7) (153). Nifurtimox [**8**] ($EC_{50} = 7.0\ \mu M$) and benznidazole [**9**] ($EC_{50} = 1.97\ \mu M$) show anti trypanosomal activity by inducing oxidative stress *via* production of nitro anion radicals as well as depletion of free thiol in the parasite (Fig. 7) (35, 37, 153). Nifurtimox [**8**] leads not only to cellular damage to *T. cruzi*, but also to mammalian tissues by the formation of free radicals and redox cycling. The electron transfer from drugs generates $O_2^{\bullet -}$ and other ROS (H_2O_2 and OH) (121). ROS interacts with macromolecules and causes cellular damage (lipid peroxidation, membrane destruction, DNA damage, and enzyme inactivation) (121). Benznidazole [**9**] and its metabolites are thought to disturb the $T(SH)_2$ metabolism of *T. cruzi*, and an involvement of

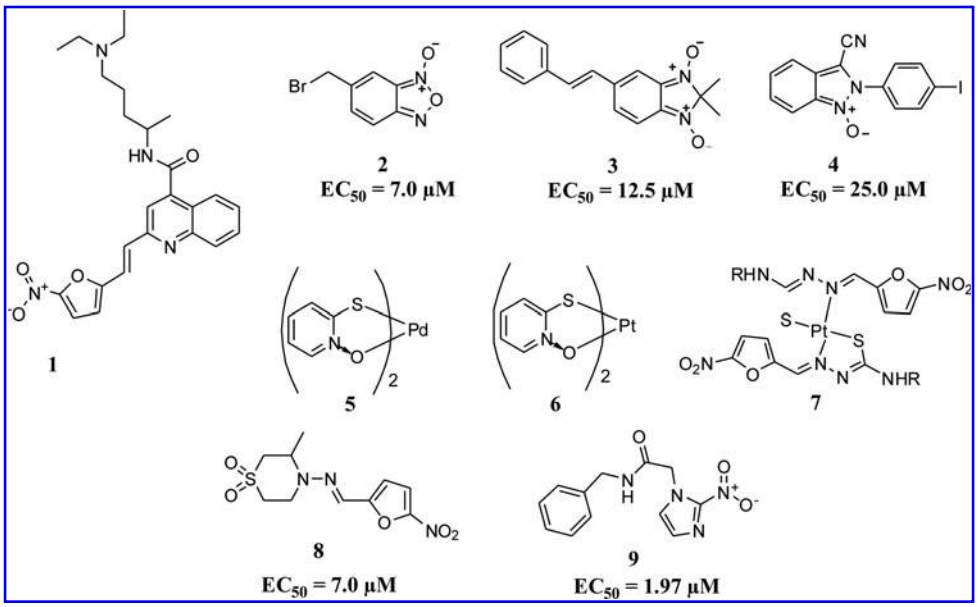


FIG. 7. Redox-active compounds induce oxidative stress in *Trypanosoma cruzi*. EC_{50}, effective concentration required to inhibit 50% *T. cruzi* growth *in vitro*.

several free radical species similar to nifurtimox may lead to various types of cellular damage (121). Type I NTRs activate nifurtimox and benznidazole to offer antitrypanosomal activity (159).

Redox-Active Antileishmanial Drugs

The leishmaniases are a group of diseases caused by infection with the protozoan parasite *Leishmania*, transmitted by the sand fly. The most fatal form of the disease is visceral leishmaniasis, which results from infection with *L. donovani* and *L. infantum*. The amastigote form replicates in macrophages of the liver, spleen, and bone marrow, causing persistent fever, hepatosplenomegaly, weight loss, and pancytopenia. If untreated, it eventually becomes fatal and is thought to account for 41,000 deaths per year (66, 119). According to current WHO statistics, about 12 million people living in 88 countries, mainly of 5 continents, that is, Asia, Europe, Africa, South America, and North America are suffering from leishmaniasis with 1.5–2 million new cases annually (139). This disease is endemic in the low-income population of Central and South American countries. Thus, there is an urgent need for new and less toxic treatments for leishmaniasis (139). Targeting the redox system of the parasite *via* small molecules would be a good strategy for drug development against *Leishmania*. In this section, we refer to a series of such small molecules that inhibit the enzyme activity, which maintains the redox system of *L. donovani*.

L. donovani TR is a validated drug target against leishmaniasis (Table 1). Doxorubicin (**5a**) and mitomycin C (**5b**) show antileishmanial effect and inhibit *L. donovani* TR (Table 5) (139). These compounds act as subversive substrates and subvert the physiological function of *L. donovani* TR by converting it from an antioxidant to a pro-oxidant. **5a** and **5b** also show significant effect on redox homeostasis of the parasite (139). It has been documented that **5a** and **5b** generate ROS in *L. donovani* by inhibiting *L. donovani* TR (139). The N-substituted phenothiazine **5c** shows an antileishmanial effect and reversibly inhibits *L. donovani* TR (27). Dinitrodiphenylthioethers (**5d–e**) generate ROS in *L. donovani* promastigotes and reduce the level of parasitemia in peritoneal macrophages (43, 149). Alterations to the 1,3-dinitro-5-(trifluoromethyl) benzene ring of **5d** have more influence on antiparasitic activity with two aromatic nitro groups and a third electron-withdrawing group. These structural orientations increase the ability to generate ROS in the parasites (43). **5e** shows *in vivo* antileishmanial activity and causes a 28%

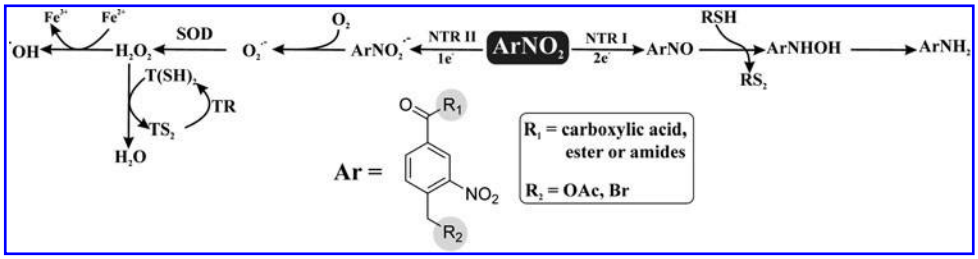
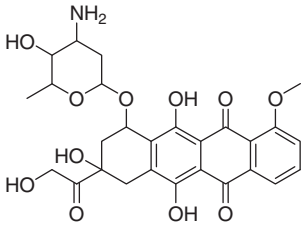
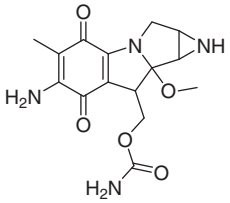
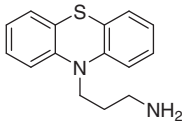
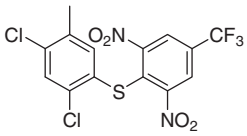
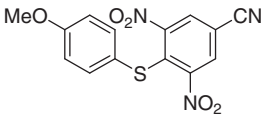
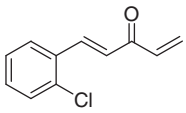
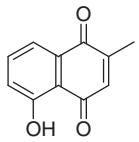
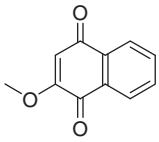


FIG. 8. Mechanism of the production of ROS by aromatic nitro compounds ($ArNO_2$) in trypanosomatids. One-electron reduction of $ArNO_2$ by Type II NTR promotes formation of ROS ($O_2^{\bullet -}$ and H_2O_2), which oxidize parasite thiol. Two-electron reduction of $ArNO_2$ by Type I NTR yields the nitrosocompound (ArNO), a good scavenger of thiols. SOD, superoxide dismutase; NTR, nitroreductases.

Table 5. Redox-Active Antileishmanial Compounds

Compound	Structure	Mode of action	Antileishmanial activity [EC_{50} (μM)] M ± SEM	(Ref)
Doxorubicin **5a**		Inhibits *L. donovani* TR and generates intraparasitic O_2^-	11.76 ± 0.11	(139)
Mitomycin C **5b**		Inhibits *L. donovani* TR and generates intraparasitic O_2^-	11.52 ± 0.15	(139)
N-Substituted Phenothiazines **5c**		Reversible inhibition of *L. donovani* TR	3.30	(27)
Dinitrodiphenylthioethers **5d**		Generates intraparasitic ROS	0.56 ± 0.12	(43)
5e		Generates intraparasitic ROS	0.67 ± 0.24	(43)
Antimonial drugs **5f** Sb(III)		Inhibits *L. infantum* TR	1.5 ± 0.4	(14)
Divinyl Ketone **5g**		Inhibits *L. donovani* TR	68.0	(97)
Plumbagin **5h**		Noncompetitive inhibition of *L. donovani* TR (k_i = 2.55 ± 0.35 μM) and generates intraparasitic ROS	0.34 ± 0.11	(138)
2-methoxy 1, 4-naphthoquinone **5i**		Noncompetitive inhibition of *L. donovani* TR (k_i = 1.092 ± 0.14 μM) and generates intraparasitic ROS	0.614 ± 0.20	(138)

ROS, reactive oxygen species.

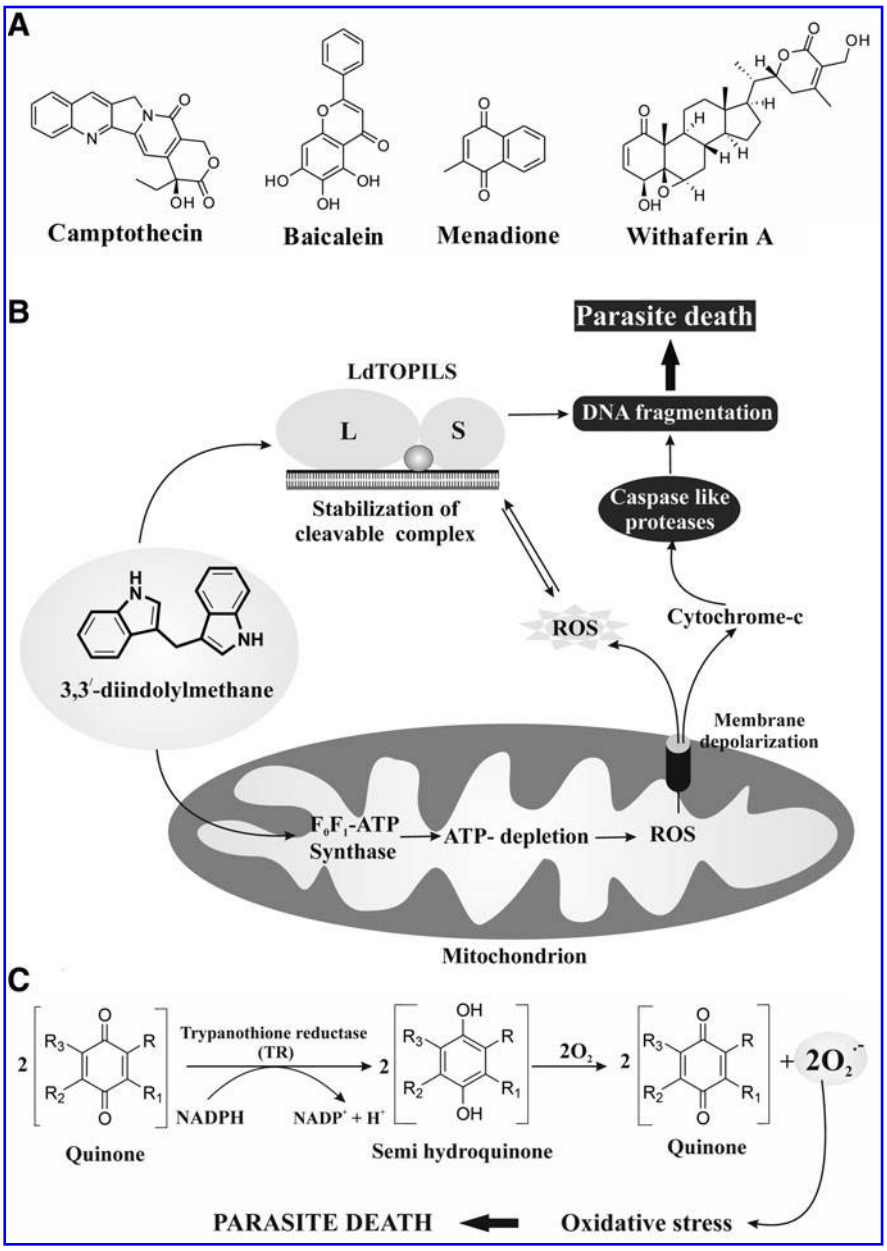


FIG. 9. Redox-active antileishmanial compounds and their mode of action. (A) Compounds self-producing ROS in *Leishmania donovani*. **(B)** Mode of action of antileishmanial DIM. DIM mediated inhibition of F_0F_1-ATP synthase causes depletion of mitochondrial ATP and significant stimulation of mitochondrial ROS production, followed by depolarization of the mitochondrial membrane ($\Delta\Psi$). Loss of the membrane potential results in depletion of cellular ATP level that promotes cellular ROS generation, which, in turn, leads to parasite death. **(C)** Mechanism of quinone compounds producing ROS in *L. donovani*. TR reduces quinone derivative to hydroquinone, which is re-oxidized by molecular oxygen and generates $O_2^{\bullet -}$. TR, trypanothione reductase; LdTOP1LS, *Leishmania donovani* topoisomerase I; DIM, 3,3′-diindolylmethane.

decrease in parasitemia on average, at a dose 25 mg/kg/day for 5 days (Table 5) (43).

Antimonial drugs (**5f**) show antileishmanial activity by inhibiting *L. infantum* TR (Table 5) (14). The crystal structures of the complex of reduced TR with Sb(III) suggested that Sb(III) is coordinated by the two redox-active catalytic cysteine residues (Cys52 and Cys57), one threonine residue (Thr335), and one histidine residue (His461) of the 2-fold symmetry related subunit of the dimer and strongly inhibits TR activity (14). Divinyl ketone (**5g**) inhibits *L. donovani* TR and shows an antileishmanial effect against *L. donovani* (strains MHOM/ET/67/HU3) (97). Plumbagin (**5h**), a plant-derived naphthoquinone, and its derivative 2-methoxy 1, 4-naphthoquinone (**5i**) are reported to possess antileishmanial properties by inhibiting TR. The k_i values for **5h** and **5i** are found to be 2.55 ± 0.35 and $1.092 \pm 0.14\ \mu M$, respectively. These compounds also act as subversive substrates and subvert the physiological function of TR by converting it from an antioxidant to a prooxidant. Both compounds show a significant effect on redox homeostasis, resulting in morphological changes and parasite death. The EC_{50} values of **5h** against promastigotes and axenic amastigotes are found to be 0.34 ± 0.11 and $0.214 \pm 0.15\ \mu M$, respectively. **5i** shows EC_{50} values of 0.614 ± 0.20 and $0.47 \pm 0.15\ \mu M$ for promastigotes and axenic amastigotes, respectively (138). **5h** and **5i** also produce ROS in *L. donovani* by inhibiting *L. donovani* TR (138). The comparative studies suggest that **5h** is the most effective ($EC_{50} = 0.34 \pm 0.11\ \mu M$) antileishmanial agent compared with other redox-active agents (**5a–g, 5i**) (Table 5). Curcumin (Fig. 6B), a polyphenol, shows antileishmanial activity by generating ROS and elevating cytosolic calcium through the release of calcium ions from intracellular stores as well as by the influx of extracellular calcium (38). Elevation of cytosolic calcium is responsible for depolarization of mitochondrial

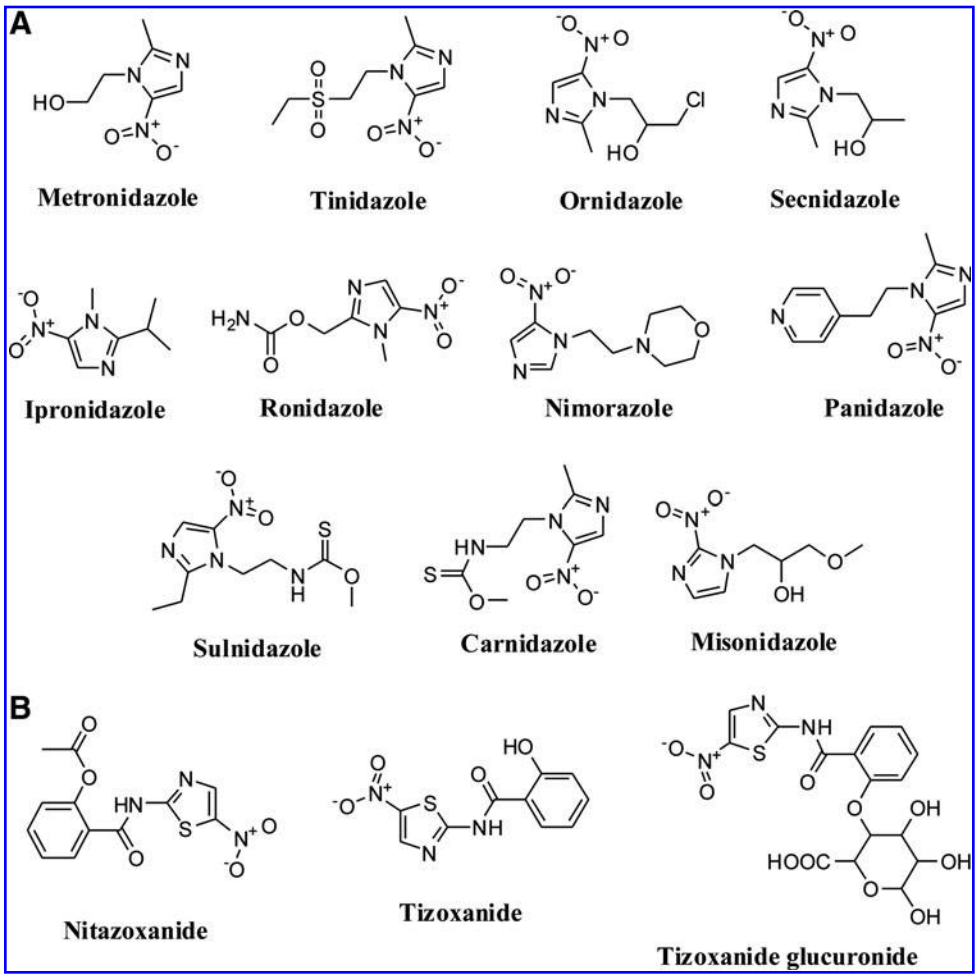


FIG. 10. Redox-active compounds against amoebiasis and trichomoniasis. (A) Compounds induce oxidative stress in *Entamoeba histolytica.* **(B)** Compounds inhibit *Trichomonas vaginalis* pyruvate: ferredoxin/flavodoxin oxidoreductases.

membrane potential ($\Delta\Psi$) and release of cytochrome c into the cytosol, which promotes apoptosis of the parasite (38). Curcumin shows an average EC_{50} of 5.3 μM against promastigotes of various leishmanial strains (129). Antimony sodium gluconate induces the generation of ROS and nitric oxide by activating phosphoinositide 3-kinase as well as mitogen-activated protein kinase in *L. donovani*-infected macrophages (109). These activated components of the intracellular signaling pathway are responsible for an early wave of ROS-dependent parasite killing and a stronger late wave of NO-dependent parasite killing (109).

Camptothecin (CPT) (Fig. 9A), an inhibitor of DNA topoisomerase I, shows an antileishmanial effect against the *L. donovani* AG83 strain by inducing ROS both in the amastigotes and promastigotes of *L. donovani* (137). CPT inhibits growth of *L. donovani* AG83 to 65% at a concentration of 5 μM. CPT-induced cellular dysfunction in *L. donovani* promastigotes is characterized by several cytoplasmic and nuclear features of apoptosis. It has been proposed that CPT-induced apoptosis-like death is due to mitochondrial dysfunction and ROS generation. (137). Baicalein (BLN) (Fig. 9A) shows its antileishmanial potency against *L. donovani* AG83 strain through ROS generation in parasites without involving caspases (24). BLN inhibits 73% growth of *L. donovani* AG83 promastigotes at a concentration of 10 μM (24). Menadione and withaferin A, potent inhibitors of protein kinase C (PKC), show an antileishmanial effect by inducing ROS in *Leishmania chagasi* and *L. donovani*, respectively, (Fig. 9A) (107, 136). In *L. donovani* AG83 promastigotes, the inhibition of PKC by withaferin A causes depolarization of $\Delta\Psi$ and generates ROS inside cells. Loss of $\Delta\Psi$ leads to the release of cytochrome c into the cytosol and subsequently activates caspase-like proteases and oligonucleosomal DNA cleavage and causes parasite death (136). At 6 h, withaferin A inhibits 85% growth of *L. donovani* AG83 promastigotes at a concentration of 15 μM (136). Mitochondria are the principal site for the generation of cellular ATP by oxidative phosphorylation (127). 3,3′-diindolylmethane, a DNA topoisomerase I inhibitor, inhibits mitochondrial F_0F_1-ATP synthase of *L. donovani*. This, in turn, causes depletion of mitochondrial ATP levels and significant stimulation of mitochondrial ROS production, leading to oxidation and fragmentation of DNA and hence, death of the parasite (Fig. 9B) (127). Quinone compounds act as subversive substrates and are reduced by *L. donovani* TR to give reduced semihydroquinones (139). These semihydroquinones are further oxidized by oxygen to quinone and $O_2^{\bullet -}$, which kills the parasite (Fig. 9C) (80).

Redox-Active Drugs Against Amoebiasis and Trichomoniasis

E. histolytica, which is responsible for amoebiasis infects, >10% of the world's population, primarily in the tropics and regions with poor sanitation. Approximately 10% of those

infected will become clinically symptomatic, resulting in an annual toll of 50 million to 100 million cases of invasive colitis and liver abscess and up to 100,000 deaths (99, 104, 151). Targeting the redox system of *E. histolytica* is a strategy for the development of drugs against amoebiasis. Different nitroimidazoles such as metronidazole, tinidazole, ornidazole, secnidazole, sulnidazole, carnidazole, misonidazole, ipronidazole, ronidazole, nimorazole, and panidazole show activity against amoebiasis (13, 47, 56, 121) (Fig. 10A). EC_{50} value of metronidazole and tinidazole for their antiamoebic activity against *E. histolytica* HM1: IMSS strain are 1.84 and 10.2 μM, respectively (17, 70).

Nitroimidazoles, through the formation of highly reactive nitro radical anions, damage susceptible pathogens by radical-mediated mechanisms (59). Unlike aerobic organisms, these pathogens possess the electron transport protein ferredoxin (small Fe-S protein). Once the drug has entered the parasitic trophozoite and is within the cell, ferredoxin donates electrons to the nitro group of the drug. The drug becomes activated by the reduction of the nitro group. The active drug binds covalently to DNA, resulting in DNA damage and, subsequently, to the death of the trophozoite. The reductive activation of metronidazole, an important nitroimidazole drug, may also lead to toxic radicals reacting with essential cellular components. In addition to these effects, the drug also inhibits trophozoite respiration. O_2 is a competitor of 5-nitroimidazoles. O_2 is able to generate both a decrease in the reductive activation of a 5-nitroimidazole drug and an increase in the catalytic recycling of the activated drug (1, 121).

Trichomoniasis is a common sexually transmitted disease caused by *Trichomonas vaginalis*, a protozoan parasite. *T. vaginalis* infection has been associated with problems in pregnancy, premature birth, and low birth weight (157). *T. vaginalis* pyruvate: ferredoxin/flavodoxin oxidoreductases (PFORs) are key enzymes that maintain the redox system of *T. vaginalis* (Table 1). *T. vaginalis* derives energy from the oxidative fermentation of pyruvate. PFOR, an important enzyme of the intermediary metabolisms of these organisms, catalyzes pyruvate decarboxylation. This process releases electrons that reduce ferredoxin, and the latter, in turn, catalytically donates its electrons to biological electron acceptors (121). Nitazoxanide shows activity against trichomoniasis and inhibits *T. vaginalis* PFORs in a noncompetitive manner (74). Tizoxanilide and tizoxanilide glucuronide are also redox-active antiparasitic drugs against trichomoniasis (Fig. 10B) (1). Recent studies in anaerobic protozoa (*T. vaginalis*) have shown that nitazoxanide inhibits *T. vaginalis* PFOR. Unlike nitroimidazoles, nitazoxanide is independent of reduced ferredoxin, that is, it appears to interact directly with PFOR. The different mechanisms of action and resistance may explain the therapeutic efficacy of nitazoxanide against organisms showing resistance to 5-nitroimidazoles (*e.g.*, *T. vaginalis*), especially metronidazole. In helminths, the mechanism of nitazoxanide activity is not yet fully understood, but the enzymes involved in anaerobic electron transport appear to be potential targets. The products of nitazoxanide activation do not induce mutations in DNA (63).

Redox-Active Drugs Against Multicellular Parasites

The muticellular parasite *S. mansoni* is responsible for schistosomiasis, affecting more than 200 million people in >70 countries (115–116). The parasites can survive for up to decades in the human host, as it has a unique set of antioxidant enzymes that continuously degrade the ROS produced by the host's innate immune response. Since schistosomes do not have catalase (108), other mechanisms should exist within the parasite to degrade H_2O_2. The principal component of this defense system, thioredoxin-GR (TGR), has been recently identified and validated as a target for antischistosomiasis drug development (Table 1) (96). TGR is a multifunctional selenocysteine-containing enzyme that catalyzes the inter conversion between reduced and oxidized forms of both GSH and Trx, which are major contributors to the maintenance of redox balance (6). Auranofin [**10**], a gold-containing molecule, shows activity against schistosomiasis (Fig. 11). Auranofin inhibits TGR in an irreversible manner and substantially

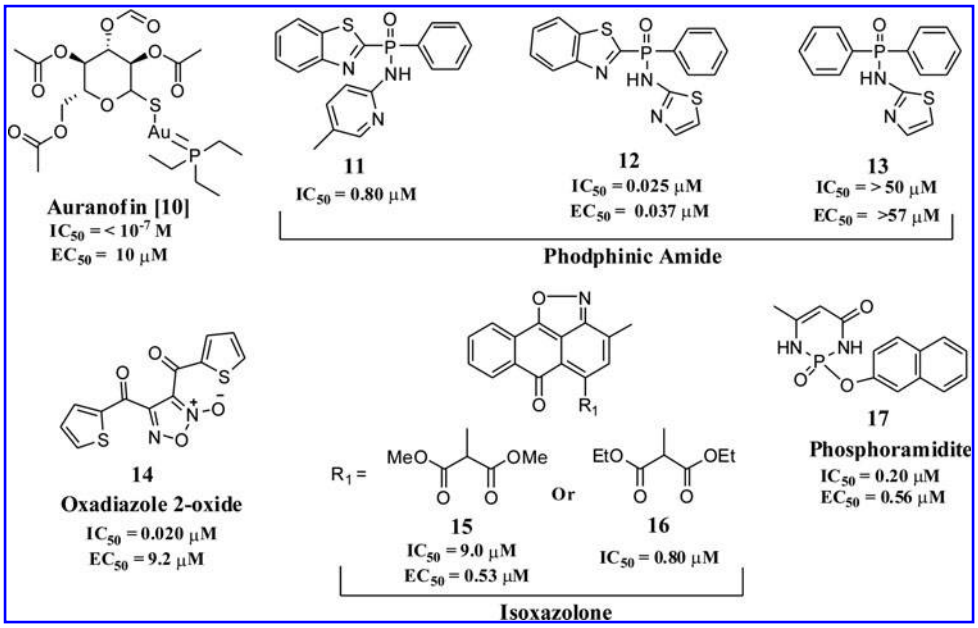


FIG. 11. Redox-active compounds against schistosomiasis. IC_{50} = inhibitory concentration required to inhibit 50% *S. mansoni* thioredoxin-glutathione reductase activity; EC_{50}, effective concentration required to inhibit 50% *S. mansoni* growth.

reduces worm burden in mice. From the X-ray crystallographic analysis, it is reported that the gold of auranofin plays a vital role in the inhibition of TGR. Selenium of TGR also plays an important role in the inhibition of TGR by auranofin. Gold from auranofin transfers to the redox-active Cys couples of TGR during inhibition (9). Some synthesized compounds such as phodphinic amide [**11–13**], oxadiazole 2-oxide [**14**], isoxazolone [**15–16**], and phosphoramidite [**17**] (Fig. 11) also show activity against schistosomiasis by inhibiting *S. mansoni* TGR (9, 140).

Conclusions and Future Perspectives

The major problem of current chemotherapy for parasitic diseases is the emergence of drug resistance against available drugs. This situation warrants the identification of functionally validated targets in order to discover novel, chemotherapeutically viable molecules that treat resistant parasites. The available parasite genome data provide a scope to search for new targets. Different approaches, such as evolutionary patterning, gene networks, system biology, and synthetic biology, will be extremely helpful for the identification of suitable targets. There are so many reported molecules having antiparasitic activity; however, not all of these are therapeutically viable drug-like molecules due to various limitations such as toxicity, low bioavailability, rapid inactivation under *in vivo* conditions, and development of resistance. Thus, synthesis of new molecules by cutting down the toxophore part of active antiparasitic molecules and simultaneously adding valuable moieties/scaffold to them with a view to overcome the above-mentioned limitations might be fruitful for developing novel antiparasitic drugs for a future generation. Furthermore, studies on drug synergism should receive special attention, which can open new avenues to improve the efficacy of antiparasitic drugs in combination with others. Since parasites such as *P. falciparum*, *L. chagasi*, *T. brucei*, *T. cruzi*, and *S. mansoni* are very susceptible to oxidative stress (33, 55, 107, 115, 133, 150), the identification of new scaffolds that affect the redox systems of these parasites and induce oxidative stress will be a valid rationale to develop new drugs. However, extreme care should be taken so that the designed scaffolds/molecules cannot affect the redox system of the host and the products formed from these molecules after metabolic turn-over by the parasitic machinery should not be toxic. Ethnopharmacology is one of the important areas for new antiparasitic drug development. Ethnopharmacology is the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man. Medicinal plants are important source of indigenous medical systems in many parts of the world, and these resources will be useful for the development of antiparasitic drugs. We have tried to incorporate the maximum number of known redox-active antiparasitic molecules, but the number of reported lead molecules may be much higher than indicated in this article. It can be anticipated that effective research in the near future capitalizing redox-active enzymes and molecules in parasites will open new avenues for the development of novel antiparasitic drugs that combat resistant parasites.

Acknowledgments

The authors thank Council of Scientific and Industrial Research (CSIR), New Delhi, for providing funds to carry out this work. They thank Sumanta Dey, Samik Bindu, Souvik Sarkar, Rahul Kumar, Manish Goyal, Mohd. Shameel Iqbal, and Athar Alam for critically reading and editing the article.

References

1. Adagu IS, Nolder D, Warhurst DC, and Rossignol JF. *In vitro* activity of nitazoxanide and related compounds against isolates of Giardia intestinalis, *Entamoeba histolytica* and *Trichomonas vaginalis. J Antimicrob Chemother* 49: 103–111, 2002.
2. Agarwal A, Srivastava K, Puri SK, and Chauhan PM. Synthesis of 4-pyrido-6-aryl-2-substituted amino pyrimidines as a new class of antimalarial agents. *Bioorg Med Chem* 13: 6226–6232, 2005.
3. Ahmad R, Srivastava AK, Tripathi RP, Batra S, and Walter RD. Synthesis and biological evaluation of potential modulators of malarial glutathione-S-transferase(s). *J Enzym Inhib Med Chem* 22: 327–342, 2007.
4. Akoachere M, Buchholz K, Fischer E, Burhenne J, Haefeli WE, Schirmer RH, and Becker K. *In vitro* assessment of methylene blue on chloroquine-sensitive and -resistant *Plasmodium falciparum* strains reveals synergistic action with artemisinins. *Antimicrob Agents Chemother* 49: 4592–4597, 2005.
5. Alarcon de Noya B, Diaz-Bello Z, Colmenares C, Ruiz-Guevara R, Mauriello L, Zavala-Jaspe R, Suarez JA, Abate T, Naranjo L, Paiva M, Rivas L, Castro J, Marques J, Mendoza I, Acquatella H, Torres J, and Noya O. Large urban outbreak of orally acquired acute Chagas disease at a school in Caracas, Venezuela. *J Infect Dis* 201: 1308–1315, 2010.
6. Alger HM and Williams DL. The disulfide redox system of *Schistosoma mansoni* and the importance of a multifunctional enzyme, thioredoxin glutathione reductase. *Mol Biochem Parasitol* 121: 129–139, 2002.
7. Ali V and Nozaki T. Current therapeutics, their problems, and sulfur-containing-amino-acid metabolism as a novel target against infections by "amitochondriate" protozoan parasites. *Clin Microbiol Rev* 20: 164–187, 2007.
8. Andricopulo AD, Akoachere MB, Krogh R, Nickel C, McLeish MJ, Kenyon GL, Arscott LD, Williams CH, Jr., Davioud-Charvet E, and Becker K. Specific inhibitors of *Plasmodium falciparum* thioredoxin reductase as potential antimalarial agents. *Bioorg Med Chem Lett* 16: 2283–2292, 2006.
9. Angelucci F, Sayed AA, Williams DL, Boumis G, Brunori M, Dimastrogiovanni D, Miele AE, Pauly F, and Bellelli A. Inhibition of *Schistosoma mansoni* thioredoxin-glutathione reductase by auranofin: structural and kinetic aspects. *J Biol Chem* 284: 28977–28985, 2009.
10. Arias DG, Cabeza MS, Erben ED, Carranza PG, Lujan HD, Tellez Inon MT, Iglesias AA, and Guerrero SA. Functional characterization of methionine sulfoxide reductase A from Trypanosoma spp. *Free Radic Biol Med* 50: 37–46, 2011.
11. Atamna H and Ginsburg H. Heme degradation in the presence of glutathione. A proposed mechanism to account for the high levels of non-heme iron found in the membranes of hemoglobinopathic red blood cells. *J Biol Chem* 270: 24876–24883, 1995.
12. Atamna H, Krugliak M, Shalmiev G, Deharo E, Pescarmona G, and Ginsburg H. Mode of antimalarial effect of methylene blue and some of its analogues on *Plasmodium falciparum* in culture and their inhibition of *P. vinckei petteri* and *P. yoelii* nigeriensis *in vivo. Biochem Pharmacol* 51: 693–700, 1996.
13. Mata-Cárdenas BD, Vargas-Villarreal JM. González-Garza MT, and Said-Fernández S. In-vitro high anti-amoebic

potency of secnidazole and dimetridazole. *Pharm Pharmacol Commun* 2: 513–514, 1996.
14. Baiocco P, Colotti G, Franceschini S, and Ilari A. Molecular basis of antimony treatment in leishmaniasis. *J Med Chem* 52: 2603–2612, 2009.
15. Bandyopadhyay U and Dey S. Antimalarial drugs and molecules inhibiting hemozoin formation. In: *Apicomplexan Parasites: Molecular Approaches Toward Targeted Drug Development*, edited by Becker K. Weinheim, Germany: Wiley-VCH Verlag & Co. KGaA, 2011, pp. 205–234.
16. Banerjee AK, Arora N, and Murty US. Structural model of the *Plasmodium falciparum* thioredoxin reductase: a novel target for antimalarial drugs. *J Vector Borne Dis* 46: 171–183, 2009.
17. Bansal D, Sehgal R, Chawla Y, Mahajan RC, and Malla N. *In vitro* activity of antiamoebic drugs against clinical isolates of *Entamoeba histolytica* and Entamoeba dispar. *Ann Clin Microbiol Antimicrob* 3: 27, 2004.
18. Bauer H, Fritz-Wolf K, Winzer A, Kuhner S, Little S, Yardley V, Vezin H, Palfey B, Schirmer RH, and Davioud-Charvet E. A fluoro analogue of the menadione derivative 6-[2′-(3′-methyl)-1′,4′-naphthoquinolyl]hexanoic acid is a suicide substrate of glutathione reductase. Crystal structure of the alkylated human enzyme. *J Am Chem Soc* 128: 10784–10794, 2006.
19. Becker K, Kanzok SM, Iozef R, Fischer M, Schirmer RH, and Rahlfs S. Plasmoredoxin, a novel redox-active protein unique for malarial parasites. *Eur J Biochem* 270: 1057–1064, 2003.
20. Becker K, Rahlfs S, Nickel C, and Schirmer RH. Glutathione—functions and metabolism in the malarial parasite *Plasmodium falciparum. Biol Chem* 384: 551–566, 2003.
21. Biot C, Bauer H, Schirmer RH, and Davioud-Charvet E. 5-substituted tetrazoles as bioisosteres of carboxylic acids. Bioisosterism and mechanistic studies on glutathione reductase inhibitors as antimalarials. *J Med Chem* 47: 5972–5983, 2004.
22. Boiani M, Piacenza L, Hernandez P, Boiani L, Cerecetto H, Gonzalez M, and Denicola A. Mode of action of nifurtimox and N-oxide-containing heterocycles against *Trypanosoma cruzi*: is oxidative stress involved? *Biochem Pharmacol* 79: 1736–1745, 2010.
23. Bonse S, Santelli-Rouvier C, Barbe J, and Krauth-Siegel RL. Inhibition of *Trypanosoma cruzi* trypanothione reductase by acridines: kinetic studies and structure-activity relationships. *J Med Chem* 42: 5448–5454, 1999.
24. BoseDasgupta S, Das BB, Sengupta S, Ganguly A, Roy A, Dey S, Tripathi G, Dinda B, and Majumder HK. The caspase-independent algorithm of programmed cell death in Leishmania induced by baicalein: the role of LdEndoG, LdFEN-1 and LdTatD as a DNA "degradesome". *Cell Death Differ* 15: 1629–1640, 2008.
25. Buchholz K, Schirmer RH, Eubel JK, Akoachere MB, Dandekar T, Becker K, and Gromer S. Interactions of methylene blue with human disulfide reductases and their orthologues from *Plasmodium falciparum. Antimicrob Agents Chemother* 52: 183–191, 2008.
26. Cenas N, Bironaite D, Dickancaite E, Anusevicius Z, Sarlauskas J, and Blanchard JS. Chinifur, a selective inhibitor and "subversive substrate" for *Trypanosoma congolense* trypanothione reductase. *Biochem Biophys Res Commun* 204: 224–229, 1994.
27. Chan C, Yin H, Garforth J, McKie JH, Jaouhari R, Speers P, Douglas KT, Rock PJ, Yardley V, Croft SL, and Fairlamb AH. Phenothiazine inhibitors of trypanothione reductase as potential antitrypanosomal and antileishmanial drugs. *J Med Chem* 41: 148–156, 1998.
28. Chan C, Yin H, McKie JH, Fairlamb AH, and Douglas KT. Peptoid inhibition of trypanothione reductase as a potential antitrypanosomal and antileishmanial drug lead. *Amino Acids* 22: 297–308, 2002.
29. Chibale K, Haupt H, Kendrick H, Yardley V, Saravanamuthu A, Fairlamb AH, and Croft SL. Antiprotozoal and cytotoxicity evaluation of sulfonamide and urea analogues of quinacrine. *Bioorg Med Chem Lett* 11: 2655–2657, 2001.
30. Chong CR and Sullivan DJ, Jr. Inhibition of heme crystal growth by antimalarials and other compounds: implications for drug discovery. *Biochem Pharmacol* 66: 2201–2212, 2003.
31. Chowdhury S, Mukherjee T, Sengupta S, Chowdhury SR, Mukhopadhyay S, and Majumder HK. Novel betulin derivatives as antileishmanial agents with mode of action targeting type IB DNA topoisomerase. *Mol Pharmacol* 80: 694–703, 2011.
32. Ciccarelli A, Araujo L, Batlle A, and Lombardo E. Effect of haemin on growth, protein content and the antioxidant defence system in *Trypanosoma cruzi. Parasitology* 134: 959–965, 2007.
33. Clark IA and Hunt NH. Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria. *Infect Immun* 39: 1–6, 1983.
34. Cui L and Miao J. Cytotoxic effect of curcumin on malaria parasite *Plasmodium falciparum*: inhibition of histone acetylation and generation of reactive oxygen species. *Antimicrob Agents Chemother* 51: 488–494, 2007.
35. Cunha EFFD, Ramalho TC, Mancini DT, Fonsecs EMB, and Oliveira AA. New approaches to the development of antiprotozoan drug candidates: a review of patents. *J Braz Chem Soc* 21: 1787–1806, 2010.
36. da Silva Junior EN, de Souza MC, Fernandes MC, Menna-Barreto RF, Pinto Mdo C, de Assis Lopes F, de Simone CA, Andrade CK, Pinto AV, Ferreira VF, and de Castro SL. Synthesis and anti-*Trypanosoma cruzi* activity of derivatives from nor-lapachones and lapachones. *Bioorg Med Chem* 16: 5030–5038, 2008.
37. Dardonville C, Fernandez-Fernandez C, Gibbons SL, Jagerovic N, Nieto L, Ryan G, Kaiser M, and Brun R. Antiprotozoal activity of 1-phenethyl-4-aminopiperidine derivatives. *Antimicrob Agents Chemother* 53: 3815–3821, 2009.
38. Das R, Roy A, Dutta N, and Majumder HK. Reactive oxygen species and imbalance of calcium homeostasis contributes to curcumin induced programmed cell death in *Leishmania donovani. Apoptosis* 13: 867–882, 2008.
39. Davioud-Charvet E, Delarue S, Biot C, Schwobel B, Boehme CC, Mussigbrodt A, Maes L, Sergheraert C, Grellier P, Schirmer RH, and Becker K. A prodrug form of a *Plasmodium falciparum* glutathione reductase inhibitor conjugated with a 4-anilinoquinoline. *J Med Chem* 44: 4268–4276, 2001.
40. Davioud-Charvet E and Lanfranchi D. Subversive substrates of glutathione reductases from plasmodium falciparum-infected red blood cells as antimalarial agents. In: *Apicomplexan Parasites: Molecular Approaches Toward Targeted Drug Development*, edited by Becker K. Weinheim, Germany: Wiley-VCH Verlag & Co. KGaA, 2011, pp. 375–396.
41. Davioud-Charvet E, McLeish MJ, Veine DM, Giegel D, Arscott LD, Andricopulo AD, Becker K, Muller S, Schirmer RH, Williams CH, Jr., and Kenyon GL. Mechanism-based inactivation of thioredoxin reductase from *Plasmodium falciparum* by Mannich bases. Implication for cytotoxicity. *Biochemistry* 42: 13319–13330, 2003.

42. De Franceschi L, Sada S, Andreoli A, Angheben A, Marocco S, and Bisoffi Z. Sickle cell disease and hyperreactive malarial splenomegaly (HMS) in young immigrants from Africa. *Blood* 106: 4415–4417, 2005.
43. Delfin DA, Morgan RE, Zhu X, and Werbovetz KA. Redox-active dinitrodiphenylthioethers against Leishmania: synthesis, structure-activity relationships and mechanism of action studies. *Bioorg Med Chem* 17: 820–829, 2009.
44. Dey S, Guha M, Alam A, Goyal M, Bindu S, Pal C, Maity P, Mitra K, and Bandyopadhyay U. Malarial infection develops mitochondrial pathology and mitochondrial oxidative stress to promote hepatocyte apoptosis. *Free Radic Biol Med* 46: 271–281, 2009.
45. Docampo R. Sensitivity of parasites to free radical damage by antiparasitic drugs. *Chem Biol Interact* 73: 1–27, 1990.
46. Dodean RA, Kelly JX, Peyton D, Gard GL, Riscoe MK, and Winter RW. Synthesis and heme-binding correlation with antimalarial activity of 3,6-bis-(omega-N,N-diethylaminoamyloxy)-4,5-difluoroxanthone. *Bioorg Med Chem* 16: 1174–1183, 2008.
47. Edwards DI. Mechanisms of selective toxicity of metronidazole and other nitroimidazole drugs. *Br J Vener Dis* 56: 285–290, 1980.
48. Egan TJ, Hunter R, Kaschula CH, Marques HM, Misplon A, and Walden J. Structure-function relationships in aminoquinolines: effect of amino and chloro groups on quinoline-hematin complex formation, inhibition of beta-hematin formation, and antiplasmodial activity. *J Med Chem* 43: 283–291, 2000.
49. Elsheikha HM and Sheashaa HA. Epidemiology, pathophysiology, management and outcome of renal dysfunction associated with plasmodia infection. *Parasitol Res* 101: 1183–1190, 2007.
50. Farber PM, Arscott LD, Williams CH, Jr., Becker K, and Schirmer RH. Recombinant *Plasmodium falciparum* glutathione reductase is inhibited by the antimalarial dye methylene blue. *FEBS Lett* 422: 311–314, 1998.
51. Fotie J, Nkengfack AE, Rukunga G, Tolo F, Peter MG, Heydenreich M, and Fomum ZT. *In-vivo* antimalarial activity of some oxygenated xanthones. *Ann Trop Med Parasitol* 97: 683–688, 2003.
52. Frearson JA, Wyatt PG, Gilbert IH, and Fairlamb AH. Target assessment for antiparasitic drug discovery. *Trends Parasitol* 23: 589–595, 2007.
53. Frey PA. Radicals in enzymatic reactions. *Curr Opin Chem Biol* 1: 347–356, 1997.
54. Friebolin W, Jannack B, Wenzel N, Furrer J, Oeser T, Sanchez CP, Lanzer M, Yardley V, Becker K, and Davioud-Charvet E. Antimalarial dual drugs based on potent inhibitors of glutathione reductase from *Plasmodium falciparum. J Med Chem* 51: 1260–1277, 2008.
55. Friedman MJ. Oxidant damage mediates variant red cell resistance to malaria. *Nature* 280: 245–247, 1979.
56. Fung HB and Doan TL. Tinidazole: a nitroimidazole antiprotozoal agent. *Clin Ther* 27: 1859–1884, 2005.
57. Gallo V, Schwarzer E, Rahlfs S, Schirmer RH, van Zwieten R, Roos D, Arese P, and Becker K. Inherited glutathione reductase deficiency and *Plasmodium falciparum* malaria—a case study. *PLoS One* 4: e7303, 2009.
58. Garavito G, Bertani S, Rincon J, Maurel S, Monje MC, Landau I, Valentin A, and Deharo E. Blood schizontocidal activity of methylene blue in combination with antimalarials against *Plasmodium falciparum. Parasite* 14: 135–140, 2007.
59. Gardner TB and Hill DR. Treatment of giardiasis. *Clin Microbiol Rev* 14: 114–128, 2001.
60. Gelb MH and Hol WG. Parasitology. Drugs to combat tropical protozoan parasites. *Science* 297: 343–344, 2002.
61. Gemma S, Campiani G, Butini S, Kukreja G, Coccone SS, Joshi BP, Persico M, Nacci V, Fiorini I, Novellino E, Fattorusso E, Taglialatela-Scafati O, Savini L, Taramelli D, Basilico N, Parapini S, Morace G, Yardley V, Croft S, Coletta M, Marini S, and Fattorusso C. Clotrimazole scaffold as an innovative pharmacophore towards potent antimalarial agents: design, synthesis, and biological and structure-activity relationship studies. *J Med Chem* 51: 1278–1294, 2008.
62. Gemma S, Campiani G, Butini S, Kukreja G, Joshi BP, Persico M, Catalanotti B, Novellino E, Fattorusso E, Nacci V, Savini L, Taramelli D, Basilico N, Morace G, Yardley V, and Fattorusso C. Design and synthesis of potent antimalarial agents based on clotrimazole scaffold: exploring an innovative pharmacophore. *J Med Chem* 50: 595–598, 2007.
63. Gilles HM and Hoffman PS. Treatment of intestinal parasitic infections: a review of nitazoxanide. *Trends Parasitol* 18: 95–97, 2002.
64. Greenwood BM, Fidock DA, Kyle DE, Kappe SH, Alonso PL, Collins FH, and Duffy PE. Malaria: progress, perils, and prospects for eradication. *J Clin Invest* 118: 1266–1276, 2008.
65. Grellier P, Maroziene A, Nivinskas H, Sarlauskas J, Aliverti A, and Cenas N. Antiplasmodial activity of quinones: roles of aziridinyl substituents and the inhibition of *Plasmodium falciparum* glutathione reductase. *Arch Biochem Biophys* 494: 32–39, 2010.
66. Guerin PJ, Olliaro P, Sundar S, Boelaert M, Croft SL, Desjeux P, Wasunna MK, and Bryceson AD. Visceral leishmaniasis: current status of control, diagnosis, and treatment, and a proposed research and development agenda. *Lancet Infect Dis* 2: 494–501, 2002.
67. Guha M, Kumar S, Choubey V, Maity P, and Bandyopadhyay U. Apoptosis in liver during malaria: role of oxidative stress and implication of mitochondrial pathway. *FASEB J* 20: 1224–1226, 2006.
68. Hall BS, Wu X, Hu L, and Wilkinson SR. Exploiting the drug-activating properties of a novel trypanosomal nitroreductase. *Antimicrob Agents Chemother* 54: 1193–1199, 2010.
69. Harwaldt P, Rahlfs S, and Becker K. Glutathione S-transferase of the malarial parasite *Plasmodium falciparum*: characterization of a potential drug target. *Biol Chem* 383: 821–830, 2002.
70. Hayat F, Salahuddin A, Umar S, and Azam A. Synthesis, characterization, antiamoebic activity and cytotoxicity of novel series of pyrazoline derivatives bearing quinoline tail. *Eur J Med Chem* 45: 4669–4675, 2010.
71. Haynes RK, Chan WC, Wong HN, Li KY, Wu WK, Fan KM, Sung HH, Williams ID, Prosperi D, Melato S, Coghi P, and Monti D. Facile oxidation of leucomethylene blue and dihydroflavins by artemisinins: relationship with flavoenzyme function and antimalarial mechanism of action. *ChemMedChem* 5: 1282–1299, 2010.
72. Henderson GB, Ulrich P, Fairlamb AH, Rosenberg I, Pereira M, Sela M, and Cerami A. "Subversive" substrates for the enzyme trypanothione disulfide reductase: alternative approach to chemotherapy of Chagas disease. *Proc Natl Acad Sci U S A* 85: 5374–5378, 1988.
73. Hirt RP, Muller S, Embley TM, and Coombs GH. The diversity and evolution of thioredoxin reductase: new perspectives. *Trends Parasitol* 18: 302–308, 2002.

74. Hoffman PS, Sisson G, Croxen MA, Welch K, Harman WD, Cremades N, and Morash MG. Antiparasitic drug nitazoxanide inhibits the pyruvate oxidoreductases of *Helicobacter pylori*, selected anaerobic bacteria and parasites, and *Campylobacter jejuni. Antimicrob Agents Chemother* 51: 868–876, 2007.
75. Huynh TT, Huynh VT, Harmon MA, and Phillips MA. Gene knockdown of gamma-glutamylcysteine synthetase by RNAi in the parasitic protozoa *Trypanosoma brucei* demonstrates that it is an essential enzyme. *J Biol Chem* 278: 39794–39800, 2003.
76. Ignatushchenko MV, Winter RW, Bachinger HP, Hinrichs DJ, and Riscoe MK. Xanthones as antimalarial agents; studies of a possible mode of action. *FEBS Lett* 409: 67–73, 1997.
77. Ignatushchenko MV, Winter RW, and Riscoe M. Xanthones as antimalarial agents: stage specificity. *Am J Trop Med Hyg* 62: 77–81, 2000.
78. Inhoff O, Richards JM, Briet JW, Lowe G, and Krauth-Siegel RL. Coupling of a competitive and an irreversible ligand generates mixed type inhibitors of *Trypanosoma cruzi* trypanothione reductase. *J Med Chem* 45: 4524–4530, 2002.
79. Jamonneau V, Bucheton B, Kabore J, Ilboudo H, Camara O, Courtin F, Solano P, Kaba D, Kambire R, Lingue K, Camara M, Baelmans R, Lejon V, and Buscher P. Revisiting the immune trypanolysis test to optimise epidemiological surveillance and control of sleeping sickness in West Africa. *PLoS Negl Trop Dis* 4: e917, 2010.
80. Jockers-Scherubl MC, Schirmer RH, and Krauth-Siegel RL. Trypanothione reductase from *Trypanosoma cruzi*. Catalytic properties of the enzyme and inhibition studies with trypanocidal compounds. *Eur J Biochem* 180: 267–272, 1989.
81. Kanzok SM, Rahlfs S, Becker K, and Schirmer RH. Thioredoxin, thioredoxin reductase, and thioredoxin peroxidase of malaria parasite *Plasmodium falciparum. Methods Enzymol* 347: 370–381, 2002.
82. Kanzok SM, Schirmer RH, Turbachova I, Iozef R, and Becker K. The thioredoxin system of the malaria parasite *Plasmodium falciparum*. Glutathione reduction revisited. *J Biol Chem* 275: 40180–40186, 2000.
83. Kawazu S, Komaki K, Tsuji N, Kawai S, Ikenoue N, Hatabu T, Ishikawa H, Matsumoto Y, Himeno K, and Kano S. Molecular characterization of a 2-Cys peroxiredoxin from the human malaria parasite *Plasmodium falciparum. Mol Biochem Parasitol* 116: 73–79, 2001.
84. Kehr S, Jortzik E, Delahunty C, Yates JR, Rahlfs S, and Becker K. Protein s-glutathionylation in malaria parasites. *Antioxid Redox Signal* 15: 2855–2865, 2011.
85. Keiser J, Stich A, and Burri C. New drugs for the treatment of human African trypanosomiasis: research and development. *Trends Parasitol* 17: 42–49, 2001.
86. Khan MO, Austin SE, Chan C, Yin H, Marks D, Vaghjiani SN, Kendrick H, Yardley V, Croft SL, and Douglas KT. Use of an additional hydrophobic binding site, the Z site, in the rational drug design of a new class of stronger trypanothione reductase inhibitor, quaternary alkylammonium phenothiazines. *J Med Chem* 43: 3148–3156, 2000.
87. Khaw M and Panosian CB. Human antiprotozoal therapy: past, present, and future. *Clin Microbiol Rev* 8: 427–439, 1995.
88. Koliwer-Brandl H, Gbem TT, Waespy M, Reichert O, Mandel P, Drebitz E, Dietz F, and Kelm S. Biochemical characterization of trans-sialidase TS1 variants from *Trypanosoma congolense. BMC Biochem* 12: 39, 2011.
89. Kotecka BM, Barlin GB, Edstein MD, and Rieckmann KH. New quinoline di-Mannich base compounds with greater antimalarial activity than chloroquine, amodiaquine, or pyronaridine. *Antimicrob Agents Chemother* 41: 1369–1374, 1997.
90. Krauth-Siegel RL, Bauer H, and Schirmer RH. Dithiol proteins as guardians of the intracellular redox milieu in parasites: old and new drug targets in trypanosomes and malaria-causing plasmodia. *Angew Chem Int Ed Engl* 44: 690–715, 2005.
91. Krauth-Siegel RL, Meiering SK, and Schmidt H. The parasite-specific trypanothione metabolism of trypanosoma and leishmania. *Biol Chem* 384: 539–549, 2003.
92. Krnajski Z, Gilberger TW, Walter RD, Cowman AF, and Muller S. Thioredoxin reductase is essential for the survival of *Plasmodium falciparum* erythrocytic stages. *J Biol Chem* 277: 25970–25975, 2002.
93. Krnajski Z, Walter RD, and Muller S. Isolation and functional analysis of two thioredoxin peroxidases (peroxiredoxins) from *Plasmodium falciparum. Mol Biochem Parasitol* 113: 303–308, 2001.
94. Kumar S, Das SK, Dey S, Maity P, Guha M, Choubey V, Panda G, and Bandyopadhyay U. Antiplasmodial activity of [(aryl)arylsulfanylmethyl]Pyridine. *Antimicrob Agents Chemother* 52: 705–715, 2008.
95. Kumar S, Guha M, Choubey V, Maity P, and Bandyopadhyay U. Antimalarial drugs inhibiting hemozoin (beta-hematin) formation: a mechanistic update. *Life Sci* 80: 813–828, 2007.
96. Kuntz AN, Davioud-Charvet E, Sayed AA, Califf LL, Dessolin J, Arner ES, and Williams DL. Thioredoxin glutathione reductase from *Schistosoma mansoni*: an essential parasite enzyme and a key drug target. *PLoS Med* 4: e206, 2007.
97. Lee B, Bauer H, Melchers J, Ruppert T, Rattray L, Yardley V, Davioud-Charvet E, and Krauth-Siegel RL. Irreversible inactivation of trypanothione reductase by unsaturated Mannich bases: a divinyl ketone as key intermediate. *J Med Chem* 48: 7400–7410, 2005.
98. Legorreta-Herrera M, Retana-Ugalde R, Ventura-Gallegos JL, and Narvaez V. Pyrimethamine induces oxidative stress in *Plasmodium yoelii* 17XL-infected mice: a novel immunomodulatory mechanism of action for an old antimalarial drug? *Exp Parasitol* 126: 381–388, 2010.
99. Leitsch D, Kolarich D, Wilson IB, Altmann F, and Duchene M. Nitroimidazole action in *Entamoeba histolytica*: a central role for thioredoxin reductase. *PLoS Biol* 5: e211, 2007.
100. Li R, Kenyon GL, Cohen FE, Chen X, Gong B, Dominguez JN, Davidson E, Kurzban G, Miller RE, Nuzum EO, *et al. In vitro* antimalarial activity of chalcones and their derivatives. *J Med Chem* 38: 5031–5037, 1995.
101. Li Z, Fennie MW, Ganem B, Hancock MT, Kobaslija M, Rattendi D, Bacchi CJ, and O'Sullivan MC. Polyamines with N-(3-phenylpropyl) substituents are effective competitive inhibitors of trypanothione reductase and trypanocidal agents. *Bioorg Med Chem Lett* 11: 251–254, 2001.
102. Liebau E, Bergmann B, Campbell AM, Teesdale-Spittle P, Brophy PM, Luersen K, and Walter RD. The glutathione S-transferase from *Plasmodium falciparum. Mol Biochem Parasitol* 124: 85–90, 2002.
103. Liu H, Walker LA, Nanayakkara NP, and Doerksen RJ. Methemoglobinemia caused by 8-aminoquinoline drugs: DFT calculations suggest an analogy to H4B's role in nitric oxide synthase. *J Am Chem Soc* 133: 1172–1175, 2011.

104. Loftus B, Anderson I, Davies R, Alsmark UC, Samuelson J, Amedeo P, Roncaglia P, Berriman M, Hirt RP, Mann BJ, Nozaki T, Suh B, Pop M, Duchene M, Ackers J, Tannich E, Leippe M, Hofer M, Bruchhaus I, Willhoeft U, Bhattacharya A, Chillingworth T, Churcher C, Hance Z, Harris B, Harris D, Jagels K, Moule S, Mungall K, Ormond D, Squares R, Whitehead S, Quail MA, Rabbinowitsch E, Norbertczak H, Price C, Wang Z, Guillen N, Gilchrist C, Stroup SE, Bhattacharya S, Lohia A, Foster PG, Sicheritz-Ponten T, Weber C, Singh U, Mukherjee C, El-Sayed NM, Petri WA, Jr., Clark CG, Embley TM, Barrell B, Fraser CM, and Hall N. The genome of the protist parasite *Entamoeba histolytica. Nature* 433: 865–868, 2005.
105. Massimine KM, McIntosh MT, Doan LT, Atreya CE, Gromer S, Sirawaraporn W, Elliott DA, Joiner KA, Schirmer RH, and Anderson KS. Eosin B as a novel antimalarial agent for drug-resistant *Plasmodium falciparum. Antimicrob Agents Chemother* 50: 3132–3141, 2006.
106. Meunier B and Robert A. Heme as trigger and target for trioxane-containing antimalarial drugs. *Acc Chem Res* 43: 1444–1451, 2010.
107. Miller MA, McGowan SE, Gantt KR, Champion M, Novick SL, Andersen KA, Bacchi CJ, Yarlett N, Britigan BE, and Wilson ME. Inducible resistance to oxidant stress in the protozoan *Leishmania chagasi. J Biol Chem* 275: 33883–33889, 2000.
108. Mkoji GM, Smith JM, and Prichard RK. Antioxidant systems in *Schistosoma mansoni*: correlation between susceptibility to oxidant killing and the levels of scavengers of hydrogen peroxide and oxygen free radicals. *Int J Parasitol* 18: 661–666, 1988.
109. Mookerjee Basu J, Mookerjee A, Sen P, Bhaumik S, Banerjee S, Naskar K, Choudhuri SK, Saha B, Raha S, and Roy S. Sodium antimony gluconate induces generation of reactive oxygen species and nitric oxide via phosphoinositide 3-kinase and mitogen-activated protein kinase activation in *Leishmania donovani*-infected macrophages. *Antimicrob Agents Chemother* 50: 1788–1797, 2006.
110. Muller S. Thioredoxin reductase and glutathione synthesis in *Plasmodium falciparum. Redox Rep* 8: 251–255, 2003.
111. Muller S, Liebau E, Walter RD, and Krauth-Siegel RL. Thiol-based redox metabolism of protozoan parasites. *Trends Parasitol* 19: 320–328, 2003.
112. Muller T, Johann L, Jannack B, Bruckner M, Lanfranchi DA, Bauer H, Sanchez C, Yardley V, Deregnaucourt C, Schrevel J, Lanzer M, Schirmer RH, and Davioud-Charvet E. A glutathione reductase-catalyzed cascade of redox reactions to bioactivate potent antimalarial 1,4-naphthoquinones—a new strategy to combat malarial parasites. *J Am Chem Soc* 133: 11557–11571, 2011.
113. Newton CR and Krishna S. Severe falciparum malaria in children: current understanding of pathophysiology and supportive treatment. *Pharmacol Ther* 79: 1–53, 1998.
114. O'Neill PM, Mukhtar A, Stocks PA, Randle LE, Hindley S, Ward SA, Storr RC, Bickley JF, O'Neil IA, Maggs JL, Hughes RH, Winstanley PA, Bray PG, and Park BK. Isoquine and related amodiaquine analogues: a new generation of improved 4-aminoquinoline antimalarials. *J Med Chem* 46: 4933–4945, 2003.
115. Oke TT, Moskovitz J, and Williams DL. Characterization of the methionine sulfoxide reductases of *Schistosoma mansoni. J Parasitol* 95: 1421–1428, 2009.
116. Olds GR and Dasarathy S. Recent advances in schistosomiasis. *Curr Infect Dis Rep* 3: 59–67, 2001.
117. Oza SL, Ariyanayagam MR, and Fairlamb AH. Characterization of recombinant glutathionylspermidine synthetase/amidase from *Crithidia fasciculata. Biochem J* 364: 679–686, 2002.
118. Perez-Pineiro R, Burgos A, Jones DC, Andrew LC, Rodriguez H, Suarez M, Fairlamb AH, and Wishart DS. Development of a novel virtual screening cascade protocol to identify potential trypanothione reductase inhibitors. *J Med Chem* 52: 1670–1680, 2009.
119. Perry MR, Wyllie S, Prajapati VK, Feldmann J, Sundar S, Boelaert M, and Fairlamb AH. Visceral leishmaniasis and arsenic: an ancient poison contributing to antimonial treatment failure in the Indian subcontinent? *PLoS Negl Trop Dis* 5: e1227, 2011.
120. Ponasik JA, Strickland C, Faerman C, Savvides S, Karplus PA, and Ganem B. Kukoamine A and other hydrophobic acylpolyamines: potent and selective inhibitors of Crithidia fasciculata trypanothione reductase. *Biochem J* 311 (Pt 2): 371–375, 1995.
121. Raether W and Hanel H. Nitroheterocyclic drugs with broad spectrum activity. *Parasitol Res* 90 Supp 1: S19–S39, 2003.
122. Rahlfs S, Fischer M, and Becker K. *Plasmodium falciparum* possesses a classical glutaredoxin and a second, glutaredoxin-like protein with a PICOT homology domain. *J Biol Chem* 276: 37133–37140, 2001.
123. Rahlfs S, Schirmer RH, and Becker K. The thioredoxin system of *Plasmodium falciparum* and other parasites. *Cell Mol Life Sci* 59: 1024–1041, 2002.
124. Rassi A, Jr., Rassi A, and Marin-Neto JA. Chagas disease. *Lancet* 375: 1388–1402, 2010.
125. Rodrigues JR, Lourenco D, and Gamboa N. Disturbance in hemoglobin metabolism and *in vivo* antimalarial activity of azole antimycotics. *Rev Inst Med Trop Sao Paulo* 53: 25–29, 2011.
126. Roldan MD, Perez-Reinado E, Castillo F, and Moreno-Vivian C. Reduction of polynitroaromatic compounds: the bacterial nitroreductases. *FEMS Microbiol Rev* 32: 474–500, 2008.
127. Roy A, Ganguly A, BoseDasgupta S, Das BB, Pal C, Jaisankar P, and Majumder HK. Mitochondria-dependent reactive oxygen species-mediated programmed cell death induced by 3,3′-diindolylmethane through inhibition of F0F1-ATP synthase in unicellular protozoan parasite *Leishmania donovani. Mol Pharmacol* 74: 1292–1307, 2008.
128. Royer RE, Deck LM, Campos NM, Hunsaker LA, and Vander Jagt DL. Biologically active derivatives of gossypol: synthesis and antimalarial activities of peri-acylated gossylic nitriles. *J Med Chem* 29: 1799–1801, 1986.
129. Saleheen D, Ali SA, Ashfaq K, Siddiqui AA, Agha A, and Yasinzai MM. Latent activity of curcumin against leishmaniasis *in vitro. Biol Pharm Bull* 25: 386–389, 2002.
130. Salinas AE and Wong MG. Glutathione S-transferases—a review. *Curr Med Chem* 6: 279–309, 1999.
131. Sarma GN, Savvides SN, Becker K, Schirmer M, Schirmer RH, and Karplus PA. Glutathione reductase of the malarial parasite *Plasmodium falciparum*: crystal structure and inhibitor development. *J Mol Biol* 328: 893–907, 2003.
132. Savvides SN, Scheiwein M, Bohme CC, Arteel GE, Karplus PA, Becker K, and Schirmer RH. Crystal structure of the antioxidant enzyme glutathione reductase inactivated by peroxynitrite. *J Biol Chem* 277: 2779–2784, 2002.
133. Sayed AA, Cook SK, and Williams DL. Redox balance mechanisms in *Schistosoma mansoni* rely on peroxiredoxins and albumin and implicate peroxiredoxins as novel drug targets. *J Biol Chem* 281: 17001–17010, 2006.

134. Schirmer RH, Coulibaly B, Stich A, Scheiwein M, Merkle H, Eubel J, Becker K, Becher H, Muller O, Zich T, Schiek W, and Kouyate B. Methylene blue as an antimalarial agent. *Redox Rep* 8: 272–275, 2003.
135. Seeber F, Aliverti A, and Zanetti G. The plant-type ferredoxin-NADP+reductase/ferredoxin redox system as a possible drug target against apicomplexan human parasites. *Curr Pharm Des* 11: 3159–3172, 2005.
136. Sen N, Banerjee B, Das BB, Ganguly A, Sen T, Pramanik S, Mukhopadhyay S, and Majumder HK. Apoptosis is induced in leishmanial cells by a novel protein kinase inhibitor withaferin A and is facilitated by apoptotic topoisomerase I-DNA complex. *Cell Death Differ* 14: 358–367, 2007.
137. Sen N, Das BB, Ganguly A, Mukherjee T, Tripathi G, Bandyopadhyay S, Rakshit S, Sen T, and Majumder HK. Camptothecin induced mitochondrial dysfunction leading to programmed cell death in unicellular hemoflagellate *Leishmania donovani*. *Cell Death Differ* 11: 924–936, 2004.
138. Sharma N, Shukla AK, Das M, and Dubey VK. Evaluation of plumbagin and its derivative as potential modulators of redox thiol metabolism of Leishmania parasite. *Parasitol Res* 2011 [Epub ahead of print]: DOI: 10.1007/s00436-011-2498-x.
139. Shukla AK, Patra S, and Dubey VK. Evaluation of selected antitumor agents as subversive substrate and potential inhibitor of trypanothione reductase: an alternative approach for chemotherapy of Leishmaniasis. *Mol Cell Biochem* 352: 261–270, 2011.
140. Simeonov A, Jadhav A, Sayed AA, Wang Y, Nelson ME, Thomas CJ, Inglese J, Williams DL, and Austin CP. Quantitative high-throughput screen identifies inhibitors of the *Schistosoma mansoni* redox cascade. *PLoS Negl Trop Dis* 2: e127, 2008.
141. Singh C, Hassam M, Naikade NK, Verma VP, Singh AS, and Puri SK. Synthesis and antimalarial assessment of a new series of orally active amino-functionalized spiro 1,2,4-trioxanes. *J Med Chem* 53: 7587–7598, 2010.
142. Singh C, Srivastav NC, and Puri SK. *In vivo* active antimalarial isonitriles. *Bioorg Med Chem Lett* 12: 2277–2279, 2002.
143. Smith HK and Bradley M. Comparison of resin and solution screening methodologies in combinatorial chemistry and the identification of a 100 nM inhibitor of trypanothione reductase. *J Comb Chem* 1: 326–332, 1999.
144. Spinks D, Shanks EJ, Cleghorn LA, McElroy S, Jones D, James D, Fairlamb AH, Frearson JA, Wyatt PG, and Gilbert IH. Investigation of trypanothione reductase as a drug target in *Trypanosoma brucei*. *ChemMedChem* 4: 2060–2069, 2009.
145. Stuart K, Brun R, Croft S, Fairlamb A, Gurtler RE, McKerrow J, Reed S, and Tarleton R. Kinetoplastids: related protozoan pathogens, different diseases. *J Clin Invest* 118: 1301–1310, 2008.
146. Stump B, Eberle C, Kaiser M, Brun R, Krauth-Siegel RL, and Diederich F. Diaryl sulfide-based inhibitors of trypanothione reductase: inhibition potency, revised binding mode and antiprotozoal activities. *Org Biomol Chem* 6: 3935–3947, 2008.
147. Thornalley PJ, Strath M, and Wilson RJ. Antimalarial activity *in vitro* of the glyoxalase I inhibitor diester, S-p-bromobenzylglutathione diethyl ester. *Biochem Pharmacol* 47: 418–420, 1994.
148. Trivedi V, Chand P, Srivastava K, Puri SK, Maulik PR, and Bandyopadhyay U. Clotrimazole inhibits hemoperoxidase of *Plasmodium falciparum* and induces oxidative stress. Proposed antimalarial mechanism of clotrimazole. *J Biol Chem* 280: 41129–41136, 2005.
149. Tsumori Y, Ndounga M, Sunahara T, Hayashida N, Inoue M, Nakazawa S, Casimiro P, Isozumi R, Uemura H, Tanabe K, Kaneko O, and Culleton R. *Plasmodium falciparum*: differential selection of drug resistance alleles in contiguous urban and peri-urban areas of Brazzaville, Republic of Congo. *PLoS One* 6: e23430, 2011.
150. Turrens JF. Oxidative stress and antioxidant defenses: a target for the treatment of diseases caused by parasitic protozoa. *Mol Aspects Med* 25: 211–220, 2004.
151. van Hal SJ, Stark DJ, Fotedar R, Marriott D, Ellis JT, and Harkness JL. Amoebiasis: current status in Australia. *Med J Aust* 186: 412–416, 2007.
152. Vennerstrom JL, Ellis WY, Ager AL, Jr., Andersen SL, Gerena L, and Milhous WK. Bisquinolines. 1. N,N-bis(7-chloroquinolin-4-yl)alkanediamines with potential against chloroquine-resistant malaria. *J Med Chem* 35: 2129–2134, 1992.
153. Vieites M, Smircich P, Parajon-Costa B, Rodriguez J, Galaz V, Olea-Azar C, Otero L, Aguirre G, Cerecetto H, Gonzalez M, Gomez-Barrio A, Garat B, and Gambino D. Potent *in vitro* anti-*Trypanosoma cruzi* activity of pyridine-2-thiol N-oxide metal complexes having an inhibitory effect on parasite-specific fumarate reductase. *J Biol Inorg Chem* 13: 723–735, 2008.
154. Viode C, Bettache N, Cenas N, Krauth-Siegel RL, Chauviere G, Bakalara N, and Perie J. Enzymatic reduction studies of nitroheterocycles. *Biochem Pharmacol* 57: 549–557, 1999.
155. Wagner JT, Ludemann H, Farber PM, Lottspeich F, and Krauth-Siegel RL. Glutamate dehydrogenase, the marker protein of *Plasmodium falciparum*—cloning, expression and characterization of the malarial enzyme. *Eur J Biochem* 258: 813–819, 1998.
156. Walton JG, Jones DC, Kiuru P, Durie AJ, Westwood NJ, and Fairlamb AH. Synthesis and evaluation of indatraline-based inhibitors for trypanothione reductase. *ChemMedChem* 6: 321–328, 2011.
157. Westrop GD, Georg I, and Coombs GH. The mercaptopyruvate sulfurtransferase of *Trichomonas vaginalis* links cysteine catabolism to the production of thioredoxin persulfide. *J Biol Chem* 284: 33485–33494, 2009.
158. White NJ. Antimalarial drug resistance. *J Clin Invest* 113: 1084–1092, 2004.
159. Wilkinson SR, Taylor MC, Horn D, Kelly JM, and Cheeseman I. A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes. *Proc Natl Acad Sci U S A* 105: 5022–5027, 2008.
160. Williams CH, Arscott LD, Muller S, Lennon BW, Ludwig ML, Wang PF, Veine DM, Becker K, and Schirmer RH. Thioredoxin reductase two modes of catalysis have evolved. *Eur J Biochem* 267: 6110–6117, 2000.
161. Wright AD, Wang H, Gurrath M, Konig GM, Kocak G, Neumann G, Loria P, Foley M, and Tilley L. Inhibition of heme detoxification processes underlies the antimalarial activity of terpene isonitrile compounds from marine sponges. *J Med Chem* 44: 873–885, 2001.
162. Xu Kelly J, Winter R, Riscoe M, and Peyton DH. A spectroscopic investigation of the binding interactions between 4,5-dihydroxyxanthone and heme. *J Inorg Biochem* 86: 617–625, 2001.
163. Yeo M, Acosta N, Llewellyn M, Sanchez H, Adamson S, Miles GA, Lopez E, Gonzalez N, Patterson JS, Gaunt MW, de Arias AR, and Miles MA. Origins of Chagas disease: Didelphis species are natural hosts of *Trypanosoma cruzi* I and armadillos hosts of *Trypanosoma cruzi* II, including hybrids. *Int J Parasitol* 35: 225–233, 2005.
164. Yeo M, Mauricio IL, Messenger LA, Lewis MD, Llewellyn MS, Acosta N, Bhattacharyya T, Diosque P, Carrasco HJ, and Miles MA. Multilocus sequence typing (MLST) for

lineage assignment and high resolution diversity studies in *Trypanosoma cruzi. PLoS Negl Trop Dis* 5: e1049, 2011.

Address correspondence to:
Dr. Uday Bandyopadhyay
Department of Infectious Diseases and Immunology
Indian Institute of Chemical Biology
4 Raja S. C. Mullick Road
Jadavpur
Kolkata 700032
India

E-mail: ubandyo_1964@yahoo.com

Date of first submission to ARS Central, November 25, 2011; date of acceptance, November 28, 2011.

Abbreviations Used

AASMP = [(aryl)arylsufanylmethyl]pyridines
benzylNQ = benzylmenadione
BLN = baicalein
CLT = clotrimazole
CPT = camptothecin
CQ = chloroquine
CQ-R = chloroquine-resistant
CQ-S = chloroquine-sensitive
DIM = 3,3′-diindolylmethane
dNDP = deoxynucleoside diphosphates
FV = food vacuole
GLX = glyoxalase
GR = glutathione reductase
GSH = glutathione
GspS = glutathionylspermidine synthetase
GSSG = glutathione disulfide
GST = glutathione S-transferase
HAT = human African trypanosomiasis
Hb = hemoglobin
HF = hydrogen fluoride
hGR = human glutathione reductase
hTrxR = human TrxR
Hz = hemozoin
LdTOP1LS = *Leishmania donovani* topoisomerase I
MB = methylene blue
MDR = multidrug-resistant
Met = methionine
MetSO = methionine sulfoxide
MIC = minimum inhibitory concentration
MSR = methionine sulfoxide reductase
NADPH = nicotinamide adeninine dinucleotide phosphate
NDP = nucleoside diphosphate
NTR = nitroreductases
PfGR = *Plasmodium falciparum* glutathione reductase
PfGST = *P. falciparum* glutathione S-transferase
PFOR = pyruvate: ferredoxin/flavodoxin oxidoreductases
PfTrxR = *Plasmodium falciparum* thioredoxin reductase
PKC = protein kinase C
RiboR = ribonucleotide reductase
ROS = reactive oxygen species
SOD = superoxide dismutase
TGR = thioredoxin-glutathione reductase
TR = trypanothione reductase
$Trx(SH)_2$ = reduced thioredoxin
TrxR = thioredoxin reductase
$TrxS_2$ = oxidized thioredoxin
$T(SH)_2$ = trypanothione
TS_2 = trypanothione disulfide
TXN = tryparedoxin