Review

Mode of action of natural and synthetic drugs against *Trypanosoma cruzi* and their interaction with the mammalian host[☆]

Juan Diego Maya [a], Bruce K. Cassels [b], Patricio Iturriaga-Vásquez [b], Jorge Ferreira [a],
Mario Faúndez [a], Norbel Galanti [a], Arturo Ferreira [a], Antonio Morello [a,*]

[a] *Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, P.O. Box 70000, Santiago 7, Santiago, Chile*
[b] *Departamento de Química, Facultad de Ciencias, Universidad de Chile, Chile*

Abstract

Current knowledge of the biochemistry of *Trypanosoma cruzi* has led to the development of new drugs and the understanding of their mode of action. Some trypanocidal drugs such as nifurtimox and benznidazole act through free radical generation during their metabolism. *T. cruzi* is very susceptible to the cell damage induced by these metabolites because enzymes scavenging free radicals are absent or have very low activities in the parasite. Another potential target is the biosynthetic pathway of glutathione and trypanothione, the low molecular weight thiol found exclusively in trypanosomatids. These thiols scavenge free radicals and participate in the conjugation and detoxication of numerous drugs. Inhibition of this key pathway could render the parasite much more susceptible to the toxic action of drugs such as nifurtimox and benznidazole without affecting the host significantly. Other drugs such as allopurinol and purine analogs inhibit purine transport in *T. cruzi*, which cannot synthesize purines de novo. Nitroimidazole derivatives such as itraconazole inhibit sterol metabolism. The parasite's respiratory chain is another potential therapeutic target because of its many differences with the host enzyme complexes. The pharmacological modulation of the host's immune response against *T. cruzi* infection as a possible chemotherapeutic target is discussed. A large set of chemicals of plant origin and a few animal metabolites active against *T. cruzi* are enumerated and their likely modes of action are briefly discussed.
© 2006 Elsevier Inc. All rights reserved.

Keywords: Review; *Trypanosoma cruzi*; Nifurtimox; Benznidazole; Glutathione; Trypanothione; Immune system; Natural antichagasic compounds; Macrophages; Nitric oxide; Prostaglandins

Contents

In 1909 Carlos Chagas, a Brazilian scientist, discovered a parasitic flagellate that he named *Trypanosoma cruzi* and which is the causative agent of American trypanosomiasis, now known as Chagas'disease. Chagas' disease affects 24 million people from Southern California to Argentina and Chile (Chagas, 1909; WHO Expert Committee on the Control of Chagas Disease, 2002). The most important mode of transmission of the disease is associated with the feces of several species of triatomine bugs that are strictly hematophagous. Blood transfusion also plays a role in Chagas' disease transmission, since serological tests in blood banks of areas where the disease is endemic give 10–50% positivity, and of that percentage around 10% of the blood contains infective parasites. This disease has been present in the American continent for more than 9000 years (Aufderheide et al., 2004).

1. The life cycle of *T. cruzi*

The parasite's biological cycle includes three fundamental forms characterized by the relative positions of the flagellum, kinetoplast, and nucleus (Prata, 2001): (1) Trypomastigotes: 20 μm long, fusiform, subterminal kinetoplast, constitute the infecting form, and are found in mammalian blood and the hindgut of triatomine bugs; they do not multiply. In mammals they are the disseminators of blood-borne infection (Prata, 2001). (2) Epimastigotes: Also 20 μm long, kinetoplast anterior to the nucleus, fusiform. They represent the parasite's multiplicative form in the triatomid's intestine, and are the predominant form in culture. For this reason it is the form most commonly used in biochemical studies (Prata, 2001). (3) Amastigotes: Approximately 2 μm in diameter, round, without an emergent flagellum. They multiply by means of binary fission inside mammalian host cells, producing their rupture, and liberating trypomastigotes into the bloodstream that can once again invade any nucleated cell (Prata, 2001). They can be grown in culture in muscle cells, fibroblasts, and macrophages among others (Faúndez et al., 2005; Morello, 1988).

2. Epidemiology

American trypanosomiasis or Chagas' disease is a major public health concern in Latin America. It takes second place after malaria in prevalence and mortality due to vector-associated diseases (WHO Expert Committee on the Control of Chagas Disease, 2002). At least 25 million people are considered to be at risk of exposure to infection, with a total estimate of 8 million infected cases, with Chile contributing to this number with 150,000 presumably infected cases (WHO Expert Committee on the Control of Chagas Disease, 2002). Furthermore, according to World Health Organization reports, mortality rates vary from 8% to 12% depending on the country studied, age, patients' health conditions, and treatment received (WHO Expert Committee on the Control of Chagas Disease, 2002). This report also states that recent studies have shown approximately 200,000 new cases per year and 21,000 deaths per year associated with Chagas' disease (WHO Expert Committee on the Control of Chagas Disease, 2002).

Chagas' disease is controlled at present through the elimination of the vectors with insecticides; better housing and educational campaigns are also fruitful approaches. Chagas' disease, as well as other parasitic diseases, is associated with poverty and low educational levels. The development of vaccines has thus far been unsuccessful. The chemotherapy of Chagas' disease is inadequate since the treatment of patients with the drugs nifurtimox and benznidazole presents serious toxic side effects; there are also doubts as to whether these drugs are capable of achieving parasitological cure. Gentian violet (Hiratake et al., 2002) is used to treat transfusion blood, its main disadvantage being the purple colouring of the blood and the staining of the patients' tissues. Hundreds of *T. cruzi* "strains" have been isolated from different countries and geographical zones. Important differences in resistance or susceptibility to drugs in use, in laboratory experimentation, or in clinical studies, have been described among different strains of the parasite. This situation makes the development of new antichagasic drugs even more difficult (Morello et al., 1994).

Currently, most antiparasitic drugs are considered orphan drugs with the main exception of antimalarials. Economic considerations of the pharmaceutical industry outweigh all others, because of the very low return of the developmental costs. Therefore, it is necessary to find alternative and cheaper ways to approach the treatment of Chagas' disease. This could be achieved by increasing the activity of the antichagasic drugs presently used or by modifying the host's immune response, which would render current therapies more effective.

3. Treatment of Chagas' disease

3.1. Clinical approach

The drugs currently used to treat Chagas' disease are nifurtimox (4[(5-nitrofurfurylidene)amino]-3-methylthiomorpholine-

1,1-dioxide), derived from nitrofuran, and benznidazole (*N*-benzyl-2-nitroimidazole-1-acetamide), a nitroimidazole derivative. The recommended dose in the acute phase is 8–10 mg/kg/day for nifurtimox and 5 mg/kg/day for 90 days for benznidazole. The average duration of treatment is about 60 days, but when chronic disease is reactivated, such as in immunocompromised patients, treatment can last 5 months or longer. Only in cases of accidental and presumed infections, by means of a vector, blood transfusion, or contamination in a laboratory, is the duration of prophylactic treatment limited to 10 days.

Nifurtimox and benznidazole are trypanocidal to all forms of the parasite (Rodriques Coura and de Castro, 2002). However, they can cause systemic toxicity and adverse effects that include anorexia, nausea, vomiting, headache, central nervous system depression or maniacal symptoms, seizures, vertigo, paresthesias, peripheral polyneuropathies, and dermatitis (Kirchhoff, 2000). In addition, there are reports of mutagenesis and DNA damage (Zahoor et al., 1987). In large series of patients treated with these drugs, no major problems have been found related to the latter point (Apt, 1999), and therefore the risk seems to be more theoretical than clinical. An additional element that complicates the pharmacological management of this disease, and a cause of treatment failure, is the different susceptibility of different parasite strains to these drugs (Filardi and Brener, 1987).

4. Mechanism of action of nifurtimox and benznidazole

Nifurtimox and benznidazole act through the formation of free radicals and/or electrophilic metabolites (Fig. 1). The nitro group of both drugs is reduced to an amino group by the action of nitroreductases, with the formation of various free radical intermediates and electrophilic metabolites. This process begins

with a reaction catalyzed by NADPH-cytochrome P-450 reductase, that acts on the nitro group of $R\text{-}NO_2$-type molecules, producing an intermediary nitro anion radical ($R\text{-}NO_2^-$) (Moreno et al., 1982). This radical undergoes redox recycling with molecular oxygen, which partially reduces it and regenerates the drug (Mason and Holtzman, 1975). As seen in Fig. 1, the superoxide anion (O_2^{U-}) undergoes superoxide dismutase-catalyzed dismutation to afford O_2 and H_2O_2 (Temperton et al., 1998). The superoxide anion (O_2^{U-}) and hydrogen peroxide (H_2O_2), in the presence of Fe^{3+}, form the hydroxyl free radical (Haber-Weiss reaction). These free radicals, mainly OH^U, bind to lipids, proteins, and DNA, damaging them (Díaz de Toranzo et al., 1988).

When nifurtimox is added to *T. cruzi* infected cells, a characteristic ESR spectrum corresponding to the nitro anion appears (Docampo et al., 1981; Docampo and Moreno, 1984). Furthermore, the nifurtimox concentration (10–20 μM) at which epimastigote culture is inhibited is similar to that required for maximum production of superoxide anion, and for the exit of hydrogen peroxide from the cell to begin (Docampo and Moreno, 1984; Docampo and Stoppani, 1979). These and other experiments (Docampo and Stoppani, 1980) suggest that the intracellular reduction of nifurtimox, generating the nitro radical, followed by redox cycling, and production of O_2^- and H_2O_2, is the main mechanism of action of nifurtimox against *T. cruzi*.

The trypanocidal effect of benznidazole does not depend on oxygen radicals, as does that of nifurtimox. The generation of O_2^- and H_2O_2 at concentrations inhibiting epimastigote growth has not been observed. In addition, *T. cruzi* homogenates generate a weak ESR signal corresponding to the nitro anion (Docampo and Moreno, 1984; Moreno et al., 1982). It is likely that the reduced metabolites of benznidazole are involved in its
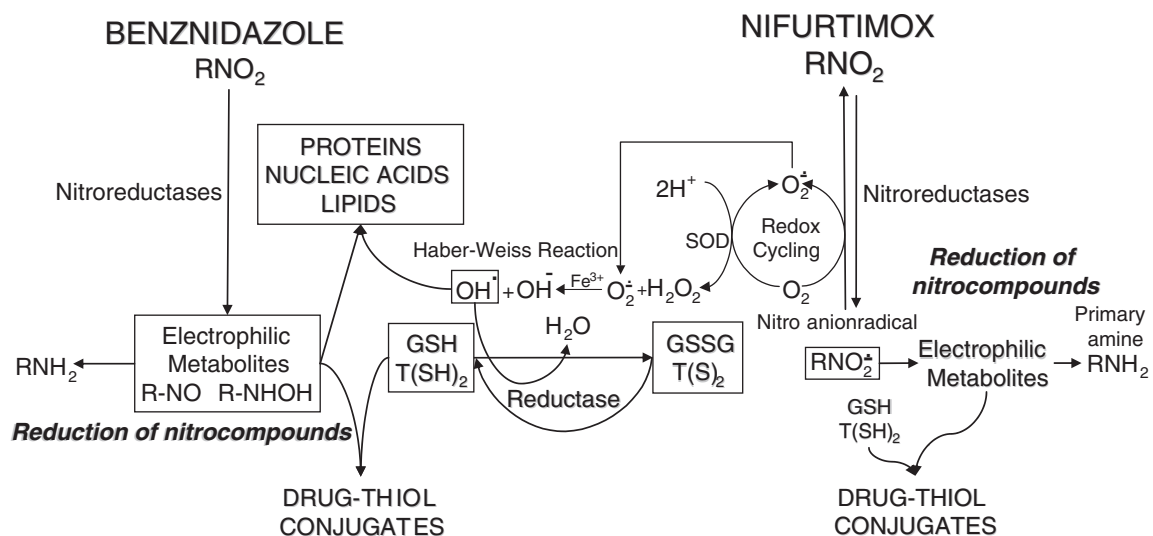
Fig. 1. Role of glutathione and trypanothione in the action and metabolism of the antichagasic drugs nifurtimox and benznidazole. The nitro group of both antichagasic drugs is reduced to free radicals or electrophilic metabolites by *T. cruzi* cytochrome P450-related nitroreductases. The nifurtimox-derived free radicals may undergo redox cycling with oxygen and it is produced H_2O_2 by the further action of superoxide dismutase (SOD). The produced oxygen derived free radicals and electrophilic metabolites bind to intracellular macromolecules damaging them. In the parasite, trypanothione (T(SH)$_2$) and glutathione (GSH) neutralize the nifurtimox and benznidazole derived metabolites by conjugation producing drug-thiol conjugates that will be further metabolized to mercapturates in the mammal host. Free radicals are neutralized by oxidation of reduced GSH or T(SH)$_2$. Trypanothione reductase (EC 1.8.1.12) reduces oxidized trypanothione (T(S)$_2$).

trypanocidal effects by covalent bonding to macromolecules, (Fig. 1) (Díaz de Toranzo et al., 1988; Maya et al., 2004). It has also been shown that benznidazole improves phagocytosis, increases trypanosomal death through IFN-γ (Romanha et al., 2002), and inhibits T. cruzi NADH-fumarate reductase (Turrens et al., 1996).

Several imidazole and nitrofuran analogues (Pozas et al., 2005) have been tested against T. cruzi, showing similar activity against the epimastigote and the trypomastigote forms. Megazol, a 5-nitroimidazole derivative, has been used for the therapy of T. brucei infection (Darsaud et al., 2004), and at least one study points to its utility in T. cruzi (Maya et al., 2003), although its usefulness is doubtful because of reports on chromosomal alterations and DNA damage with its use (Enanga et al., 2003; Nesslany et al., 2004).

5. Thiol metabolism and defense against free radicals

Mammalian cells defend themselves efficiently against free radicals in diverse ways (Gutteridge and Halliwell, 2000). Enzymatic defense mechanisms include the use of superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferases. Non-enzymatic mechanisms include the use of reductive compounds such as α-tocopherol, ascorbate, β-carotene, and reduced glutathione (GSH). Metallothioneins can also participate in the metabolism of free radicals or electrophilic agents, due to their elevated content of –SH groups (Viarengo et al., 2000). In contrast, the parasite's defense mechanisms against oxidative stress are defective (Krauth-Siegel et al., 2003; Turrens, 2004). No catalase or glutathione peroxidase activity has been detected in T. cruzi (Turrens, 2004; Wilkinson and Kelly, 2003), and superoxide dismutase activity is very much diminished (Turrens, 2004). In addition, there is no published evidence of the existence of β-carotene or α-tocopherol in the parasite. However, activity of ascorbate reductase and dehydroascorbate reductase has been described in T. cruzi, suggesting the presence of an ascorbic acid redox cycle in these parasites (Wilkinson et al., 2002). Therefore, T. cruzi's principal mechanisms of defense against free radicals are reduced glutathione and a glutathione–spermidine conjugate called trypanothione, characteristic of all trypanosomatids, and indispensable for glutathione reduction (Ariyanayagam and Fairlamb, 2001; Turrens, 2004). GSH is the most abundant low molecular weight thiol in mammalian cells participating, among other things, in protection from the toxic effects produced by highly electrophilic compounds such as certain xenobiotics and their metabolites, and is an effective non-enzymatic trapper of hydrogen peroxide and reactive free radicals (Jones et al., 2003a,b). It is also a substrate for reactions catalyzed by glutathione-S-transferase that conjugate GSH with electrophilic xenobiotics such as heavy metals, herbicides, and insecticides (Pastore et al., 2003; Valko et al., 2005). In addition, it plays an important role in DNA protection (Mazur, 2000), and in protection of the lipid membrane against damage produced by free radicals (Hayes and McLellan, 1999; Kuhn and Borchert, 2002).

In mammals, oxidized GSH (GSSG) is reduced by glutathione reductase, the enzyme responsible for maintaining a high GSH:GSSG ratio. In fact, this enzyme can catalyze the reduction of mixed disulfides composed of GSH and other compounds, such as GSH and γ-glutamyl cysteine or GSH and coenzyme A (Fernandes and Holmgren, 2004). The enzyme GSSG reductase has not been found in T. cruzi, but reduction of GSSG by trypanothione has been demonstrated (Krauth-Siegel et al., 2003).

GSH is synthesized by the successive action of the enzymes γ-glutamyl-cysteinyl synthetase (GGCS) and GSH synthetase. Both enzymes require ATP. Reduced GSH inhibits GGCS in a non-allosteric fashion through negative feedback. GSH degradation is carried out by the action of the enzymes γ-glutamyl transpeptidase, γ-glutamyl cyclotransferase, and 5-oxo-prolinase. All these enzymes make up the so-called γ-glutamyl cycle (Griffith and Mulcahy, 1999) (Fig. 2). GGCS, the limiting enzyme in GSH synthesis, can be inhibited by L-buthionine[S,R] sulfoximine (BSO; Anderson and Reynolds, 2002), a glutamate analog, with high selectivity for the enzyme without affecting other metabolic functions (Anderson, 1998). BSO or its ATP phosphorylated derivative strongly bonds, although not covalently, to the active site of GGCS, thus inhibiting it. In various strains of T. cruzi it has been seen that when they are treated with BSO, the GSH concentration falls by 50%, and both nifurtimox and benznidazole toxicity is elevated (Faúndez et al., 2005) (Fig. 2).

Trypanothione (N1,N8-bis(glutathionyl)spermidine, (T(SH)$_2$) is a low molecular weight thiol synthesized by the conjugation of two reduced molecules of GSH and spermidine, in a reaction characteristic of trypanosomatids. The reaction is ATP-dependent and catalyzed by trypanothione synthetase (Fairlamb and Cerami, 1992; Krauth-Siegel et al., 2003; Oza et al., 2002) (Fig. 2). Trypanothione requires two electrons in order to be reduced. At physiologic pH it has a +1 charge and its redox potential is slightly more electronegative than that of GSH, which gives it important reducing power (Fairlamb and Cerami, 1992).

T(SH)$_2$ is the molecule in trypanosomatids that reduces GSSG to GSH, and it also has a role equivalent to that of GSH in mammals, that is to say it acts as an intracellular protector against endogenous and exogenous oxidative agents (Steenkamp, 2002). Other functions of T(SH)$_2$ include ascorbate homeostasis (Krauth-Siegel and Ludemann, 1996), reduction of hydroperoxides (Thomson et al., 2003; Wilkinson et al., 2000), synthesis of deoxyribonucleotides (Dormeyer et al., 2001), and conjugation with metals and drugs (Maya et al., 1997).

In summary, defense mechanisms against oxidative stress in T. cruzi are deficient compared to those of mammals. T. cruzi only presents low superoxide dismutase activity, lack of glutathione peroxidase and catalase, and absence of α-tocopherol and β-carotene (Aldunate and Morello, 1993). Defense against oxidative stress is carried out by GSH and T(SH)$_2$, the latter compound being exclusive to trypanosomatids.
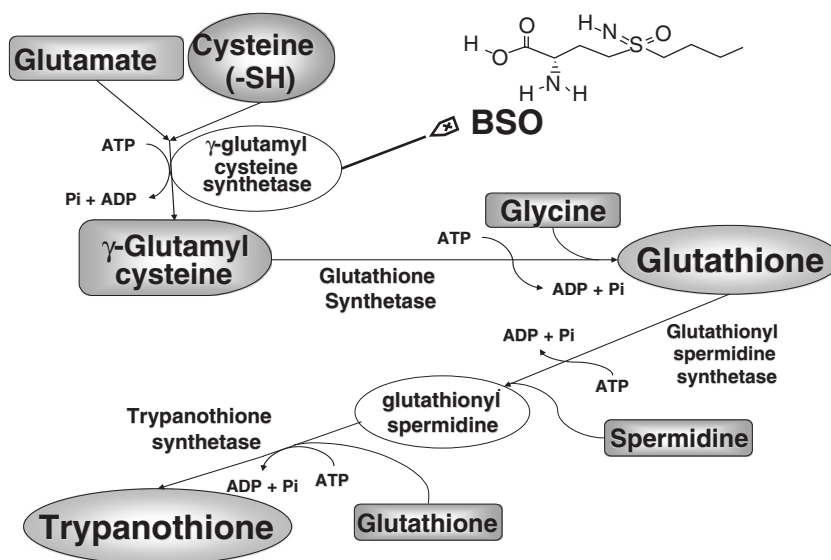
Fig. 2. Biosynthesis of glutathione and trypanothione in the *Trypanosoma cruzi* parasite. Glutathione is synthesized by the consecutive action of γ-glutamylcysteine synthetase and glutathione synthetase in an ATP dependent reaction. In *T. cruzi*, two molecules of glutathione are conjugated with spermidine to synthetize trypanothione (N1,N8-bisglutahionyl spermidine, T(SH)$_2$). The host is unable to synthesyze T(SH)$_2$. γ-Glutamylcysteine synthetase is the step limiting enzyme in this process and can be inhibited by buthionine sulfoximine (BSO).

The different thiol content in *T. cruzi* and the host is an important biochemical difference. For this reason, conjugation of electrophilic metabolites of nifurtimox and benznidazole with thiols reduces the thiol content in the parasite even further, worsening the parasite's already precarious defense against oxidative stress and electrophilic metabolites (Ariyanayagam et al., 2003; Maya et al., 1997). In mammals, GSH synthesis can be inhibited by 80–90% without apparent toxicity, due to the existence of α-tocopherol, β-carotene, and ascorbate among others as defense mechanisms against oxidative stress and electrophilic agents. In fact, when 20 mmol BSO/kg p.o. is administered to mice, it inhibits glutathione synthesis in all tissues examined (Watanabe et al., 2003).

γ-Glutamylcysteine synthetase has been isolated from different species, from bacteria to humans, and the K_i of BSO for this enzyme can vary greatly from one species to another (Hiratake et al., 2002). Although it has been reported that BSO is a potent inhibitor compared to other sulfoximine analogs in *Escherichia coli*, this enzyme can also be inhibited by other compounds such as alkyl derivatives (Griffith and Mulcahy, 1999; Kelly et al., 2002). This suggests that the selective inhibition of the synthesis of GSH is possible and has therapeutic potential, since mice infected with *T. brucei brucei* have been cured by the sole administration of 2–4 mmol/kg BSO (Arrick et al., 1981). A similar approach has been attempted in trypanosomatid protozoans where dependence on GSH and trypanothione is essential to survival (Huynh et al., 2003). Apparently, the same strategy has also worked partially with *Leishmania*, at least in vitro (Kapoor et al., 2000).

All the above indicates structural differences in GGCS that could be exploited in Chagas' disease chemotherapy. Even more so, phase I and phase II clinical studies have shown that BSO can be used to revert resistance to or stimulate the effect of antineoplastic agents such as doxorubicin (Vanhoefer et al., 1996), melphalan (Anderson et al., 2001; Anderson and Reynolds, 2002; Bailey et al., 1994, 1997; Calvert et al., 1998; O'Dwyer et al., 1996), and cyclophosphamide and its derivatives (Sipos et al., 2001), which is in agreement with observations made in our laboratory, where BSO accentuated the effects of nifurtimox and benznidazole in in vitro models against *T. cruzi*. Nevertheless, BSO can also elevate the production of proinflammatory cytokines, due to the fact that the mechanism of the redox signal depends on glutathione content (Haddad, 2002). This last point is important because in acute *T. cruzi* infection an anti-inflammatory state exists that facilitates parasite evasion.

Apart from thiol metabolism and its association with the action of nifurtimox and benznidazole, *T. cruzi* has a number of different metabolic pathways that differ from those of the hosts and could therefore be potentially exploited as therapeutic targets. Indeed, purine and ergosterol biosynthesis have been explored clinically as trypanocidal targets (Apt et al., 2003). Other targets are under active research.

6. Purine metabolism

Trypanosomal nucleic acid metabolism is unusual in several ways. First, trypanosomatids incorporate a large proportion of the DNA produced into a unique organelle known as the kinetoplast. This disc-like structure located within the matrix of a single trypanosomal mitochondrion contains a mass of circular DNA which represents up to 25% of the cellular DNA (Opperdoes, 1985). Second, they lack the ability to synthesize purines de novo. However, they have efficient salvage pathways for preformed purine bases and nucleosides.

In contrast to purines, pyrimidine nucleotides can be synthesized by trypanosomes de novo. Several of the enzymes

involved in this synthesis are located in glycosomes, whereas the analogous mammalian counterparts are in the cytosol (Hammond et al., 1981).

Differences in the metabolism of purines between *T. cruzi* and the host have been utilized for the development of a rational approach to the chemotherapy of Chagas' disease (Berens et al., 1981; Marr et al., 1978). Although *T. cruzi* does not synthesize purines de novo as humans do, the parasite is able to concentrate pyrazolopyrimidines within the cell and metabolize them as purines through the salvage pathway ultimately incorporating them into nucleic acids (Gutteridge and Davies, 1981). This does not occur in mammals. The pyrazolopyrimidine base allopurinol (4-hydroxypyrazolo[3,4-a]pyrimidine, HPP, a structural analog of hypoxanthine, is activated by phosphoribosyl transferase to the ribonucleotide-5′ monophosphate (HPPR-MP). HPPR-MP is aminated to 4-aminopyrazolopyrimidine ribonucleotide (APPR-MP) and subsequently phosphorylated to the triphosphate form and probably incorporated into RNA. The conversion of IMP to AMP is mediated by the enzymes succino-AMP synthetase and succino-AMP lyase. In 1982 the kinetic properties of both enzymes in *T. cruzi* were described (Spector et al., 1982). The substrate specificity and K_m of succino-AMP synthetase are clearly distinguishable from those of the mammalian enzyme. HPPR-MP, an analog of IMP, is converted by the *T. cruzi* enzyme to succino-APPR-MP; this reaction does not occur with the mammalian enzyme. The succino-AMP lyase from *T. cruzi* has the same broad substrate specificity (Spector et al., 1982) of the enzymes characterized in other organisms.

The growth of *T. cruzi* epimastigotes is inhibited by allopurinol (Avila and Avila, 1981; Marr et al., 1978) and *T. cruzi*-infected mice treated with this drug showed significant increases in survival times when compared with controls (Avila and Avila, 1981). However, some *T. cruzi* strains are not responsive to this type of compound, which suggests metabolic differences present in these strains (Avila et al., 1984; Avila and Avila, 1981; Marr et al., 1978). This raises doubts as to the efficacy of these drugs to treat Chagas' disease in man. Allopurinol ribonucleoside and another inosine analogue, formycin B have been tested against *Leishmania* with some success (Nelson et al., 1982). Allopurinol is a relatively nontoxic drug which has been used in humans for many years as a treatment for gout. In mammals, it acts by inhibiting the enzyme xanthine oxidase, thereby inhibiting the production of uric acid. In man, about 60% is converted to oxipurinol and 30% of it is excreted in the urine (Elion et al., 1968; Nelson et al., 1973). No effects of allopurinol on purine synthesis in man have been noted (Elion and Nelson, 1974).

Allopurinol and purine analogs 3′-desoxyinosine and 3′-desoxyadenosine inhibited amastigote proliferation in HeLa cells (Nakajima-Shimada et al., 1996). Other purine analogs were shown to interact with the enzyme hypoxanthine-guanine phosphoribosyl transferase, and some of them were effective against the intracellular forms of *T. cruzi* (Eakin et al., 1997; Freymann et al., 2000). In studies with chronic patients (Apt et al., 1998, 2003), allopurinol was administered during 60 days

and parasitological cure was achieved in 44% of treated patients.

7. Inhibition of ergosterol synthesis

T. cruzi shares with fungi the requirement of ergosterol for cell viability and proliferation. Inhibition of ergosterol biosynthesis has been proved to be effective against this parasite. Triazoles, clinically used as antifungal agents, act through cytochrome P-450-dependent C14α sterol demethylase inhibition. This leads to 14-α-methylsterol accumulation which is toxic for many membrane-bound enzyme systems including the electron transport system (Bennett, 2001). This may explain why these groups of drugs are trypanocidal. The first triazoles available, ketoconazole and itraconazole, were partially effective against *T. cruzi* infection: they reduced parasitemia but serology remained positive (Apt et al., 2003). Newer and probably more effective trypanocidal triazoles are under study, such as posaconazole and ravuconazole (Molina et al., 2000; Urbina et al., 2003), and different strategies that also affect ergosterol biosynthesis such as oxidosqualene cyclase (lanosterol synthase) or squalene synthase inhibition (Urbina et al., 2004) could be useful.

8. The respiratory chain

The respiratory chain of *T. cruzi* was intensively investigated (Stoppani and de Boiso, 1973), and important differences were identified between the respiratory chains of the parasite and its mammalian hosts that could be exploited as chemotherapeutic targets.

BHA (*t*-butyl-4-hydroxyanisole) inhibited the growth of *T. cruzi* epimastigotes (Aldunate et al., 1986; Ferreira et al., 1988). BHA is a known and safe antioxidant food additive (Branen, 1975). This compound inhibited oxygen consumption of epimastigotes by 70%. The redox level of NAD(P) was shifted to a more reduced state and conversely the redox level of cytochrome b changed to a more oxidized state (Aldunate et al., 1986). This hydroxyanisole is thus a new electron transport chain inhibitor. Accordingly, BHT is capable of sterilizing human blood contaminated with trypomastigotes of *T. cruzi* (Letelier et al., 1990).

Other chemicals, such as pyrimidine derivatives, have been found to inhibit *T. cruzi* growth and parasite respiration (Maya et al., 2000, 2001). These derivatives are in clinical use for other diseases such as hypertension; however, the therapeutic doses needed to treat these conditions are lower than those required to achieve the trypanocidal effect.

9. Miltefosine and phospholipid analogues

Phospholipid analogues are promising. This class of compounds was originally developed as anticancer drugs, as such substances can suppress tumor growth. Later, their inhibitory activity against *Leishmania* was tested. They probably act through the inhibition of phosphatidylcholine biosynthesis and sphingomyelin biosynthesis, which in turn trigger apoptosis. Other potential targets are signal transduction

cascades and/or plasma membranes of the parasites. Currently miltefosine is approved for *Leishmania* in India. However, it is necessary to assess its clinical efficacy in Chagas' disease, especially because congenital transmission is a real problem in this disease and miltefosine is teratogenic.

10. Other drug targets

Trypanothione reductase is present only in trypanosomatids and is responsible for the reduction of oxidized trypanothione and glutathione in the parasite. Diverse nitrofuran, naphthoquinone and phenothiazine derivatives are able to inhibit this enzyme (Augustyns et al., 2001; Fairlamb and Cerami, 1992; Paulino et al., 2005).

Cruzipain belongs to the cysteine protease family and shares homology with cathepsins S and C (Cazzulo et al., 2001). These proteases are important in the host/parasite relationship; inhibitors of cruzipain kill the parasite and cure infected mice. There are also other proteinases involved in Ca^{2+} signalling during mammalian cell invasion. Enzymes of this type are very promising targets for the development of new drugs against Chagas' disease (Cazzulo, 2002).

The enzymes involved in nucleotide synthesis, dihydrofolate reductase (Machado and Ayala, 2002) and thymidylate synthetase are well known therapeutic targets for cancer, malaria and bacterial infections. These two widespread enzymes have also been characterized in trypanosomatids (Machado and Ayala, 2002). Several synthetic inhibitors have been developed (Chowdhury et al., 2002; Khabnadideh et al., 2005; Pez et al., 2003), including some methotrexate derivatives (Zuccotto et al., 1999). However, the mammalian host might also be susceptible to these drugs. Pteridine reductase also reduces folate to dihydrofolate as well as unconjugated pterins. There is a second class of pteridine reductases that only reduces dihydropterin, but not folate, and is only known to be expressed in *T. cruzi* (Senkovich et al., 2003), and hence this could be a more suitable target. Intracellular amastigotes obtain their energy from glycolysis. One enzyme, glyceraldehyde-3-phosphate dehydrogenase, from the *T. cruzi* glycolytic pathway, has structural dissimilarities when compared with its host counterpart (Ladame et al., 2003). There are potential antitrypanosomal inhibitors for this enzyme (de Marchi et al., 2004; Ladame et al., 2005).

Pamidronate, a nitrogen-containing bisphosphonate, inhibits the farnesylpyrophosphate synthase involved in the synthesis of a variety of sterols and polyisoprenoids in pathogenic protozoa, including *T. cruzi* (Szajnman et al., 2003). One of its most remarkable characteristics is its accumulation in the acidocalciosomes of *T. cruzi* (Montalvetti et al., 2004). This compound has been tested in an animal model (Montalvetti et al., 2001).

None of the above-mentioned strategies has been proved more efficacious than nifurtimox and benznidazole, especially against the intracellular amastigotes.

11. Natural compounds with potential antichagasic activity

Plant metabolites active against *T. cruzi* were extensively reviewed almost a decade ago (Sepúlveda-Boza and Cassels, 1996). A more recent update, without chemical structures or details regarding potencies, is that of Rodriques Coura and de Castro (2002), while Paulino et al. (2005) only mention a small number of natural products with antitrypanosomal activity. We will concentrate here on progress in this field since 2001, when the Coura and de Castro review was written. Unfortunately, little has been published regarding the mechanisms of action of these compounds and clinical–or even in vivo–studies are almost completely lacking. In addition, most of the natural products mentioned in the more recent literature have rather low potency. Large variations in the susceptibility of *T. cruzi* strains are well documented, and the different life stages differ in their sensitivity to drugs. Furthermore, reported potencies depend on the duration of exposure of the parasite. However, considering that the standard therapeutic entities benznidazole and nifurtimox exhibit IC_{50} values of about 10 μM (less than 3 μg/mL) in the usual preliminary epimastigote assays (Cuéllar et al., 2003), for practical reasons we will limit our discussion to natural products active at concentrations of 100 μg/mL or less.

The potent antioxidative flavanols catechin, epicatechin, gallocatechin, epigallocatechin, and some of their gallates were tested against *T. cruzi* trypomastigote and amastigote forms. Gallocatechin 3,3′-digallate (**1**) and epigallocatechin 3-gallate caused trypomastigote lysis with a minimal lytic concentration (MBC_{50}) below 1 pM, while the corresponding values for gallocatechin and epigallocatechin were 10.5 and 13 pM, respectively, and the values for catechin gallate, epicatechin gallate, catechin and epicatechin rose from 48 to 85 pM. However, to achieve 50% lysis of amastigotes, 100 nM concentrations of gallocatechin digallate or epigallocatechin gallate were necessary. These compounds were also tested as inhibitors of the parasite's arginine kinase, a key enzyme in its energy metabolism, but only catechin gallate and gallocatechin gallate inhibited the enzyme by about 50% at 1 nM concentrations (Paveto et al., 2004). The β-oxygenated chalcones praecansone B and demethylpraecansone A (**2**) inhibited trypomastigotes with IC_{50} = 7.6 and 6.0 μg/mL (20.7 and 17.0 μM), respectively (Tarus et al., 2002). A chalcone-flavone dimer named cissampeloflavone (**3**) inhibited *T. cruzi* amastigotes in peritoneal exudate macrophages with ED_{50} = 2.09 μg/mL (3.42 μM) (Ramírez et al., 2003). Although no direct evidence is available, the activity of these phenolic compounds may well be related to their free radical-scavenging behavior, as found for other synthetic and natural antioxidants (Letelier et al., 1990; Morello et al., 1994).

In this regard, it is intriguing that other fairly potent natural antioxidants such as the ellagitannin punicalagin only inhibited amastigotes growing in peritoneal exudate macrophages by 50% at 30 μg/mL (27.6 μM) (Asres et al., 2001), and the flavone isosakuranetin showed only weak inhibition of *T. cruzi* with IC_{50} (trypomastigote) = 248 μM (da Silva Filho et al., 2004). Heptadecyl-5-methoxyphenol and embelin (3,6-dihydroxy-2-undecylbenzophenone) also seemed to have rather low potency, causing 100% trypomastigote lysis at 50 and 100 μg/mL (138 and 340 μM), respectively. These two compounds are constituents of *Oxalis erythrorhiza*, a plant used traditionally for the treatment of "heart complaints" that are a common

Table 1
Xanthones tested against epimastigotes (epi) and trypomastigotes (tryp) of *T. cruzi* (Minimal Inhibitory Concentration (MIC) values are shown in µM)

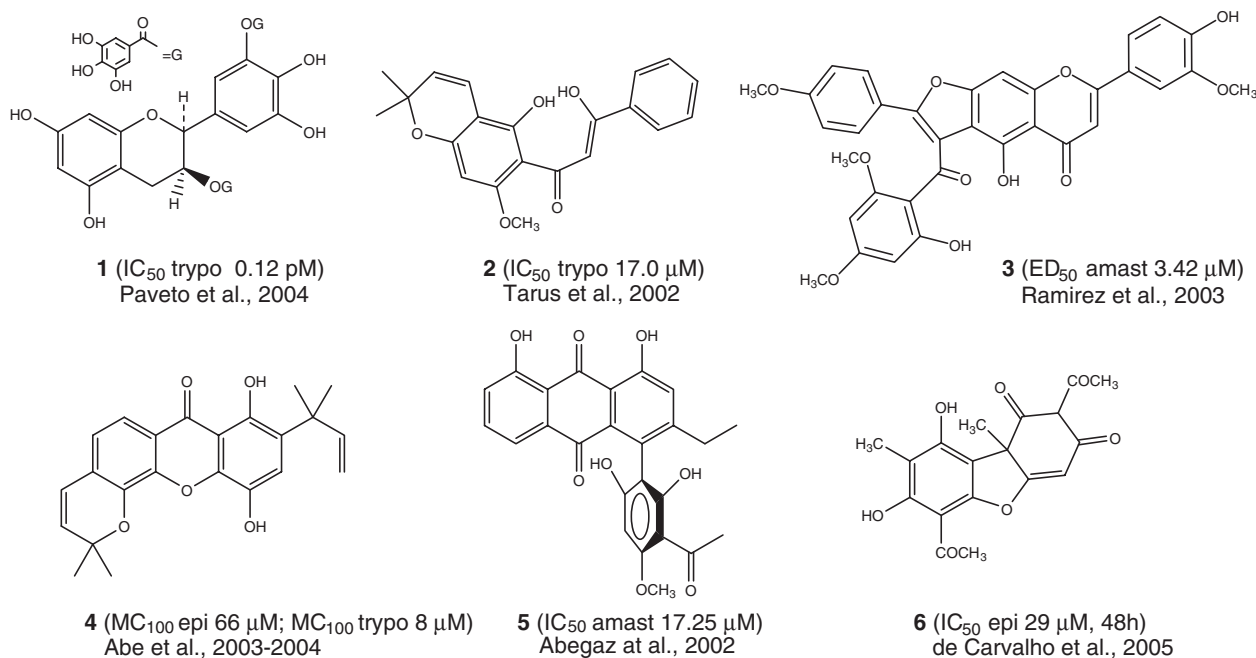| Xanthones	MIC (µM)
garciniaxanthone B (**4**) [a]	66 (epi); 8 (tryp)
garciniaxanthone A [a]	158 (epi); 16 (tryp)
subelliptenone H [a]	190 (epi); 114 (tryp)
subelliptenone B [a]	51 (epi); 25 (tryp)
subelliptenone A [a]	162 (epi); 54 (tryp)
4-hydroxybrasilixanthone B [a]	196 (epi); 147 (tryp)
isogarciniaxanthone E [a]	172 (epi); 54 (tryp)
1,4,5-trihydroxy-2-(1,1-dimethyl-2-propenyl)xanthone [a]	128 (epi); 48 (tryp)
8-desoxygartanin [a]	118 (epi); 131 (tryp)
jacareubin [b]	153 (epi); 46 (tryp)
6-deoxyjacareubin [b]	161 (epi); 645 (tryp)
1,3,5,6-tetrahydroxy-2-(3-methyl-2-butenyl)xanthone [b]	213 (epi); 122 (tryp)

[a] Abe et al. (2003).
[b] Abe et al. (2004).

symptomatology related to Chagas' disease in the area where the plant is used (Feresin et al., 2003).

Xanthones, dibenzofuranones and anthraquinones have practically flat molecules and can be presumed to act as DNA intercalators, although little direct evidence is available for most representatives of these classes. Minimal immobilizing concentrations (MIC_{100}) were determined for *T. cruzi* treated with a fairly extensive series of xanthones. The recorded values for epimastigotes and trypomastigotes are shown in Table 1 (Abe et al., 2003, 2004). In the latter paper the activities (MIC_{100}) of guttiferone A, a related benzophenone derivative, were also determined as 100 and 83 µM (60 and 50 µg/mL) for epimastigotes and trypomastigotes, respectively. The phenylanthraquinones knipholone (**5**), 4′-*O*-demethylknipholone-4′-*O*-β-D-glucopyranoside, and gaboroquinones A and B, were

active against amastigotes in L-6 rat myoblasts with $IC_{50} = 7.6$, 6.8, 33.1, and >90 µg/mL (17.25, 11.8, 72.5, >200 µM), respectively (Abegaz et al., 2002). The widespread lichen metabolite usnic acid, a dibenzofurandione, inhibited the growth of *T. cruzi* epimastigotes by more than 50% after 48h or longer incubations at 10 µg/mL (29 µM), and 100% trypomastigote death was observed at 24 h with 40 µg/mL (120 µM), at 48 h with 20 µg/mL (60 µM), and at 72 h with 10 µg/mL (29 µM). Incubation of infected peritoneal macrophages for 24 h with 40 or 80 µg/mL usnic acid (**6**) also caused marked changes in the ultrastructure of the parasites (De Carvalho et al., 2005). Another possible mechanism of action of these electrophilic and redox-active molecules is the inhibition of trypanothione reductase (Paulino et al., 2005), but none of them seem to have been tested directly against the enzyme. Some representative structures are shown in Scheme 1.

In a similar vein, the icetexane diterpenes cyclocoulterone and komaroviquinone (**7**), and the 20-norabietane diterpene dracocephalone A, were tested against epimastigotes of *T. cruzi*, showing minimum lethal concentrations (MLC) in the broad range of 20, 0.2, and 200 µM, respectively (Uchiyama et al., 2003). The high potency of komaroviquinone (**7**) is unusual and warrants more extensive study. The core skeleton of this compound has been synthesized quite recently by Padwa et al. (2005). Three aporphine alkaloids, actinodaphnine, cassythine, and dicentrine, exhibited moderate in vitro activity at lower concentrations than bulbocapnine, glaucine, isocorydine or boldine (Hoet et al., 2004). The authors suggest that the antitrypanosomal activity of the more potent alkaloids may be related to their ability to inhibit topoisomerases. This seems to be more clearly the case of the benzo-δ-carboline alkaloids quindoline and cryptolepine (**8**), which were tested against epimastigotes, amastigotes (growing in L-6 rat myoblasts) and

1 (IC_{50} trypo 0.12 pM)
Paveto et al., 2004

2 (IC_{50} trypo 17.0 µM)
Tarus et al., 2002

3 (ED_{50} amast 3.42 µM)
Ramirez et al., 2003

4 (MC_{100} epi 66 µM; MC_{100} trypo 8 µM)
Abe et al., 2003-2004

5 (IC_{50} amast 17.25 µM)
Abegaz at al., 2002

6 (IC_{50} epi 29 µM, 48h)
de Carvalho et al., 2005

Scheme 1. The most potent inhibitor of *T. cruzi* in each series is shown as an example for catechins, chalcones, flavones, xanthones, anthraquinones and arylphloroglucinols.
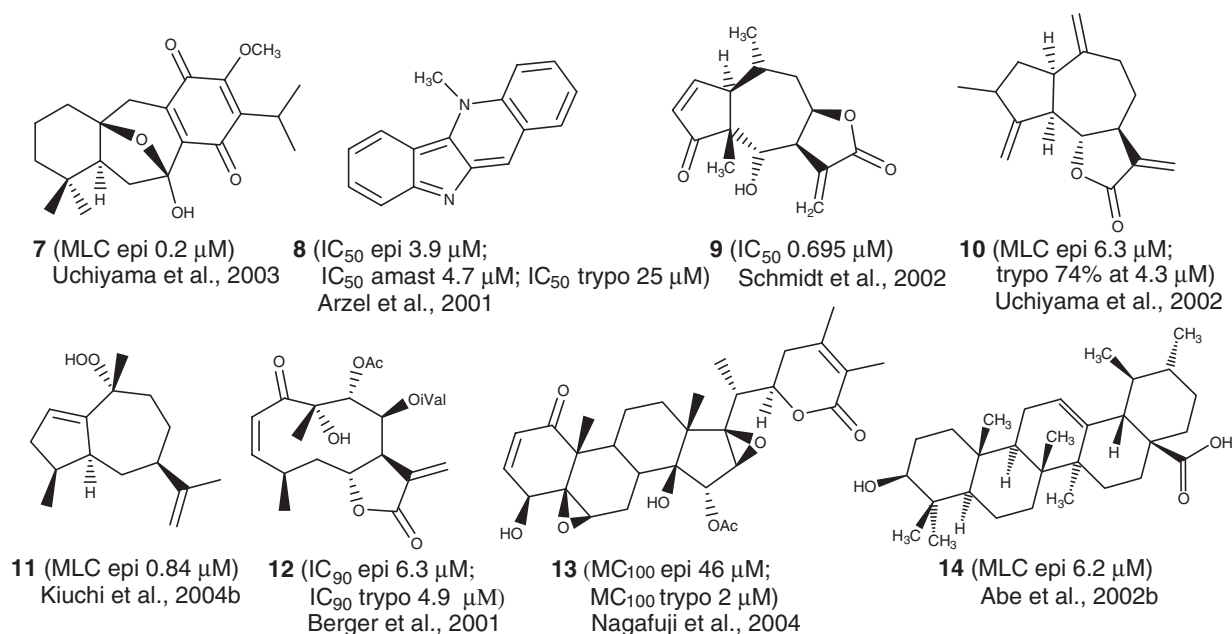
trypomastigotes: the IC_{50} values reported for quindoline are 8.7 and 14.2 μM for epimastigotes and amastigotes, respectively, while 50 μM had no effect on trypomastigote motility. For cryptolepine, the respective values are 3.9, 4.7 and 25 μM (Arzel et al., 2001). The isomeric benzo-α-carboline neocryptolepine exhibited $IC_{50} = 4.0$ μM against intracellular amastigotes (Jonckers et al., 2002).

Conjugated exomethylene sesquiterpene lactones have a long history of bioactivities related to conjugate addition of a variety of cellular nucleophiles. Helenalin (9) exhibited an IC_{50} value of 0.695 μM against *T. cruzi* (Schmidt et al., 2002). The minimal lethal concentrations against epimastigotes of dehydrocostus lactone (10) and zaluzanin D, and of 10α-hydroperoxy-guaia-1,11-diene (11), 1α-hydroperoxy-guaia-10 (15),11-diene, and 15α-hydroperoxy-guaia-1(10),11-diene, were shown to be 6.3, 2.5, 0.84, 1.7, and 1.7 μM, respectively (Uchiyama et al., 2002; Kiuchi et al., 2004b). Dehydrocostus lactone also inhibited the infection of HeLa cells by trypomastigotes by 74 % at 1 μg/mL (4.3 μM) (Uchiyama et al., 2002). The sesquiterpene lactone cynaropicrin was weakly active ($IC_{50} = 93.4$ μg/mL or 680 μM) in a trypomastigote lysis assay (Schinor et al., 2004). The germacranolides neurolenin B (12), C and D (the two latter as a mixture) had potent trypanocidal activity against epimastigotes ($IC_{90} = 6.3$ and 11.7 μM, respectively) and trypomastigotes ($IC_{90} = 4.9$ and 6.1 μM, respectively) (Berger et al., 2001). Another group of sesquiterpenoids for which recent data are available are the furoheliangolides, but of several compounds of this type tested, only goyazensolide showed almost complete lysis of trypomastigotes at 100 μg/mL ($IC_{50} = 56.9$ μM) (Grael et al., 2005).

Withanolides are steroid derivatives that are commonly cytotoxic, presumably because of their α,β-unsaturated carbonyl groups, as in the above sesquiterpenoids. Some withanolides show activity against *T. cruzi*. 18-Acetoxywithanolide D decreased epimastigote concentration in culture by 50% at 50 μg/mL (94.5 μM), while 18-acetoxy-5,6-deoxy-5-withanolide D achieved total lysis of the parasites at 25 μg/mL (48.7 μM) (Bravo et al., 2001). Minimal immobilizing concentrations (MC_{100}) were determined for several other withanolides, with both epimastigotes and trypomastigotes. Six of these compounds: physagulin A, physagulin B, physagulin C (13), physagulin H (4-deoxyphysagulin C), the chlorinated physagulin I, and withangulatin A, showed minimal immobilizing concentration (MC_{100}) values against epimastigotes in the 38–91 μM range, which are very close to their cytotoxicities as determined toward HeLa cells. Their MC_{100} values for trypomastigotes, however, are considerably lower, between 2 and 5 μM, suggesting that in vivo work may be warranted (Nagafuji et al., 2004).

The tetracyclic triterpene baccharis oxide inhibited the Y strain of *T. cruzi* trypomastigotes with $IC_{50} = 250$ μM (da Silva Filho et al., 2004). The oleanane glycosides arjunglucoside (4-*epi*-sericoside) and sericoside, with a pentacyclic triterpene skeleton, inhibited amastigote growth in peritoneal exudate macrophages by only 31.8 and 25.4% at 30 μg/mL (45 and 61 μM) (Asres et al., 2001), and the related aglycone oleanolic acid immobilized epimastigotes with the rather high MC_{100} of about 250 μg/mL (>500 μM) (Abe et al., 2002b). However, the isomeric ursolic acid (14) exhibited an MC_{100} of 40 μg/mL (88 μM). Betulinic acid, another closely related compound, was practically inactive The latter report is in disturbing contrast with a more recent one, according to which ursolic acid, oleanolic acid, and colosolic acid all immobilized epimastigotes with minimal lethal concentrations (MLC) of 6.2 μM, in a very similar assay (Saeidnia et al., 2004). The pentacyclic triterpenes are well-known inhibitors of protein kinase C, and this property

7 (MLC epi 0.2 μM)
Uchiyama et al., 2003

8 (IC_{50} epi 3.9 μM;
IC_{50} amast 4.7 μM; IC_{50} trypo 25 μM)
Arzel et al., 2001

9 (IC_{50} 0.695 μM)
Schmidt et al., 2002

10 (MLC epi 6.3 μM;
trypo 74% at 4.3 μM)
Uchiyama et al., 2002

11 (MLC epi 0.84 μM)
Kiuchi et al., 2004b

12 (IC_{90} epi 6.3 μM;
IC_{90} trypo 4.9 μM)
Berger et al., 2001

13 (MC_{100} epi 46 μM;
MC_{100} trypo 2 μM)
Nagafuji et al., 2004

14 (MLC epi 6.2 μM)
Abe et al., 2002b

Scheme 2. The most potent inhibitor of *T. cruzi* in each series is shown as an example for icetexane diterpenes, benzo-δ-carboline alkaloids, sesquiterpene lactones and hydroperoxides, withanolides, and pentacyclic triterpenes.

may be related to their antiparasitic activity. A number of structures representative of the compounds discussed in these paragraphs are shown in Scheme 2.

A fairly large number of novel naphthylisoquinoline alkaloids have been isolated for the first time and tested against intracellular amastigotes in L-6 rat myoblasts. Some of them approach benzimidazole and nifurtimox in potency (Bringmann et al., 2002a,b, 2003a,b,c, 2004a,b, 2005). The results obtained with these compounds are collated in Table 2 and compared with earlier data from the same group (Bringmann et al., 2000).

The tetrahydrofuran lignans austrobailignan-7 and fragransin E completely immobilized epimastigotes at 75 and 50 μg/mL (219 and 149 μM), respectively, after incubating for 48 h (Abe et al., 2002a,b). The previously assayed grandisin was reexamined against trypomastigotes, together with the new rel-($7R,8R,7'R,8'R$)-3′,4′-methylenedioxy-3,4,5,5′-tetra-methoxy-7,7′-epoxylignan and rel-($7R,8R,7'R,8'R$)-3,4,3′,4′-dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan. The reported IC_{50} values for these and related compounds (Martins et al., 2003; da Silva Filho et al., 2004; de Souza et al., 2005; Gertsch et al., 2003) are shown in Table 3. The neolignans eupomatenoid-7 and licarin A immobilized epimastigotes completely at 25 and 40 μg/mL (77 and 123 μM), respectively, after incubating for 48 h, while the related eupomatenoid-1 and

Table 2
Novel naphthylisoquinoline alkaloids tested against *T. cruzi* intracellular amastigotes in L-6 rat myoblasts (IC_{50} values are shown in μM)

Naphthylisoquinoline alkaloids	IC_{50} amastigotes (μM)
ancistrocongoline A [a]	101
ancistrocongoline B [a]	41.3
ancistrocongoline C [a]	>200
ancistrocongoline D [a]	71.4
korupensamine [a]	36.8
dioncophylline E [b]	40.7
habropetaline A [c]	inactive
ancistrolikokine D [d]	32.4
ancistrotanzanine A (**15**) [e]	4.2.
ancistrotanzanine B (**16**) [e]	3.6
ancistrotectoriline A [e]	42.2
ancistrocladinine [f]	57.7
ancistrotectorine (**17**) [f]	10.2
ancistrotanzanine C [f]	34.3
O-methylancistrocladinine [f]	144
O,N-dimethylancistrocladine [f]	150
ancistrobenomine A [g]	11.5
6-O-demethylancistrobenomine A [g]	57.5
5′-O-demethylancistrocline [g]	89.3
ent-dioncophylleine A [h]	144
5′-O-demethyl-ent-dioncophylleine A [h]	60.6
dioncophylleine D [h]	65
ancistroealaine A (**18**) [i]	5.6
ancistroealaine B [i]	43.2

[a] Bringmann et al. (2002a).
[b] Bringmann et al. (2002b).
[c] Bringmann et al. (2003a).
[d] Bringmann et al. (2003b).
[e] Bringmann et al. (2003c).
[f] Bringmann et al. (2004a).
[g] Bringmann et al. (2004b).
[h] Bringmann et al. (2005).
[i] Bringmann et al. (2000).

Table 3
Lignans tested against epimastigotes (epi), trypomastigotes (tryp) and amastigotes (amast) of *T. cruzi* (IC_{50} values are shown in μM)

Lignans	IC_{50} (μM)
austrobailignan-7 [a]	219 (epi)
fragransin E [a]	149 (epi)
grandisin [b]	20.2 (tryp)
rel-($7R,8R,7'R,8'R$)-3′,4′-methylenedioxy-3,4,5,5′-tetramethoxy-7,7′-epoxylignan [b]	42.3 (tryp)
rel-($7R,8R,7'R,8'R$)-3,4,3,3′,4′-dimethylenedioxy-5,5′-dimethoxy-7,7′-epoxylignan [b]	8.7 (tryp)
machilin G (**19**) [c]	2.2 (tryp)
galgravin [c]	4.4 (tryp)
nectandrin B [c]	47.3 (tryp)
calopiptin [c]	12.6 (tryp)
aristolignin [c]	34.8 (tryp)
ganschisandrine [c]	12.2 (tryp)
nectandrin A [c]	Inactive (tryp)
(−)-hinokinin (**20**) [d]	0.7 (amast)
justicidin B [e]	7.1 (epi)
piscatorin [e]	Inactive (epi)

[a] Abe et al. (2002a,b).
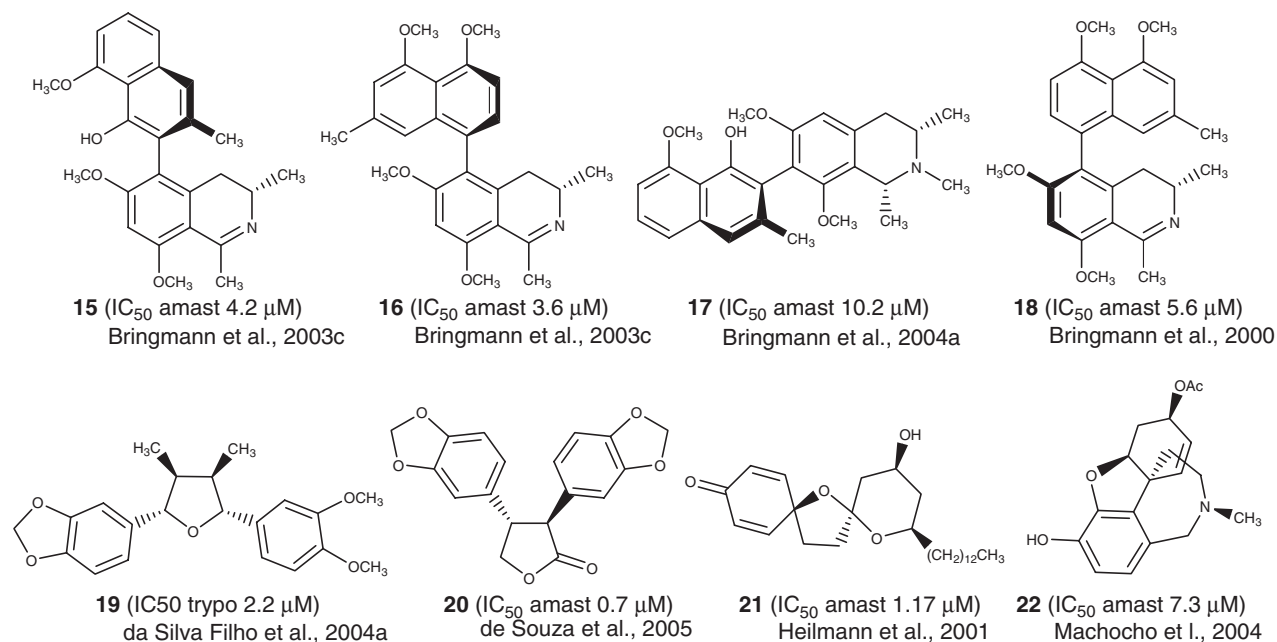[b] Martins et al. (2003).
[c] da Silva Filho et al. (2004).
[d] de Souza et al. (2005).
[e] Gertsch et al. (2003).

licarin B were inactive (Abe et al., 2002a). An unusual study showed that the structurally different neolignan burchellin, when fed to *T. cruzi*-infected *Triatoma infestans* larvae, significantly reduced the population density of the parasite in the insects' rectum, decreasing the number of both epimastigotes and trypomastigotes at 147 μM (Cabral et al., 2001). It is difficult to suggest a mechanism of action for lignoids, but in some cases it seems possible that they can interfere with mitosis in a similar way to *epi*-podophyllotoxin and its semisynthetic derivatives.

A number of low molecular weight natural products with widely different structures exhibited varying degrees of antitrypanosomal activity that cannot be ascribed to any particular mode of action at this time. Thus, the stilbenoid isonotholaenic acid inhibited epimastigotes with $IC_{50} = 50$ μg/mL or 166 μM, but was practically inactive against trypomastigotes (del Olmo et al., 2001). The dioxa-dispiroketal aculeatin D (**21**) showed potent activity against *T. cruzi* amastigotes in L-6 rat skeletal myoblasts: $IC_{50} = 0.49$ μg/mL (1.17 μM) (Heilmann et al., 2001). In contrast with an early report of the potent antitrypanosomal activity of quinine, cited in Sepúlveda-Boza and Cassels (1996), a very recent publication indicates that the ED_{50} of this alkaloid inhibiting epimastigote growth is about 50 μg/mL (154 μM), while the related cupreine, cinchonine and acetylcupreine are practically inactive (Ruiz-Mesía et al., 2005). The 5,11-methanomorphanthridine Amaryllidaceous alkaloids pancracine and (new) nangustine have IC_{50} values of 7.1 and 54.6 μg/mL (24.5 and 189 μM), respectively, against amastigotes in L-6 rat skeletal myoblasts (Labraña et al., 2002). 3-O-acetylsanguinine (**22**) was active in the same test with $IC_{50} = 2.3$ μg/mL (7.3 μM), but the related sanguinine, 1,2-di-O-acetyllycorine, hippadine, kirkine, amabiline and noraugustamine were inactive (Machocho et al., 2004). Representative

15 (IC$_{50}$ amast 4.2 μM)
Bringmann et al., 2003c

16 (IC$_{50}$ amast 3.6 μM)
Bringmann et al., 2003c

17 (IC$_{50}$ amast 10.2 μM)
Bringmann et al., 2004a

18 (IC$_{50}$ amast 5.6 μM)
Bringmann et al., 2000

19 (IC50 trypo 2.2 μM)
da Silva Filho et al., 2004a

20 (IC$_{50}$ amast 0.7 μM)
de Souza et al., 2005

21 (IC$_{50}$ amast 1.17 μM)
Heilmann et al., 2001

22 (IC$_{50}$ amast 7.3 μM)
Machocho et l., 2004

Scheme 3. The most potent inhibitor of *T. cruzi* in each series is shown as an example for naphthylisoquinolines (four isomeric series), lignans (two different types), a dispiroketal, and amaryllidaceous alkaloids.

structures of naphthylisoquinolines, lignans, and aculeatin (**21**) and 3-*O*-acetylsanguinine (**22**) are shown in Scheme 3. The black pepper alkaloid piperine (**23**) inhibited epimastigotes and amastigotes (in Y-strain macrophages) with IC$_{50}$=7.36 and 4.91 μM, respectively (Ribeiro et al., 2004).

The simple monoterpenoid aldehydes limonen-10-al, geranial and neral immobilized epimastigotes with minimal lethal concentrations (MLC) of 3.1 μM (Saeidnia et al., 2004). The monoterpene endoperoxide ascaridole and the related (−)-(2S,4S)- and (−)-(2R,4S)-*p*-mentha-1(7),8-dien-2-hydroperoxide and (−)-(1R,4S)- and (−)-(1S,4S)-*p*-mentha-2,8-dien-1-hydroperoxide (**24**) and (−)-(1R,4S)-hydroperoxy-*p*-menth-2-en-8-ol acetate were tested against epimastigotes, immobilizing the parasites with MLC of 23, 1.2, 1.6, 3.1, 0.8, and 1.4 μM, respectively. At 1 μg/mL, (−)-(1S,4S)-*p*-mentha-2,8-dien-1-hydroperoxide and (−)-(1R,4S)-hydroperoxy-*p*-menth-2-en-8-ol acetate almost completely inhibited the infection of HeLa cells by trypomastigotes, while the inhibition by (−)-(2R,4S)-*p*-mentha-1(7),8-dien-2-hydroperoxide and (−)-(1R,4S)-*p*-mentha-2,8-dien-1-hydroperoxide at this concentration was 63% and 88%, respectively, and the effect of ascaridole was not significant. None of the compounds inhibited the proliferation of amastigotes in infected cells (Kiuchi et al., 2002; Uchiyama et al., 2002). The *seco*-iridoids 7-methoxydiderroside, 6′-*O*-acetyldiderroside, secoxyloganin and diderroside were weakly active against trypomastigotes with IC$_{50}$ values of 59.0, 90.2, 74.7, and 84.9 μg/mL (123, 178, 184 and 177 μM), respectively (Cardona Zuleta et al., 2003).

A series of new cassane diterpenes: 18-hydroxycassan-13,15-diene (**25**), 6β-18-dihydroxycassan-13,15-diene, 6β-hydroxy-18-acetoxycassan-13,15-diene, 18-acetoxy-13,15-diene-19-cassanoic acid, and 6β,13β-dihydroxy-18-acetoxycassan-14(17),15-diene, were tested against epimastigotes and amasti-

gotes (in human foreskin fibroblasts). The test results for these compounds (Mendoza et al., 2003) and others (Kiuchi et al., 2004a; Espindola et al., 2004) are shown in Table 4. Five diterpenoids with unusual skeletons characteristic of the genera *Azorella* and *Mulinum* (Apiaceae): were tested against three different strains of *T. cruzi* epimastigotes, trypomastigotes and amastigotes (in VeRo cells). Only azorellanol (**27**) and mulin-11,13-dien-20-oic acid displayed strong activity at 10 μM against all stages and strains tested (Araya et al., 2003). Some representative structures are shown in Scheme 4.
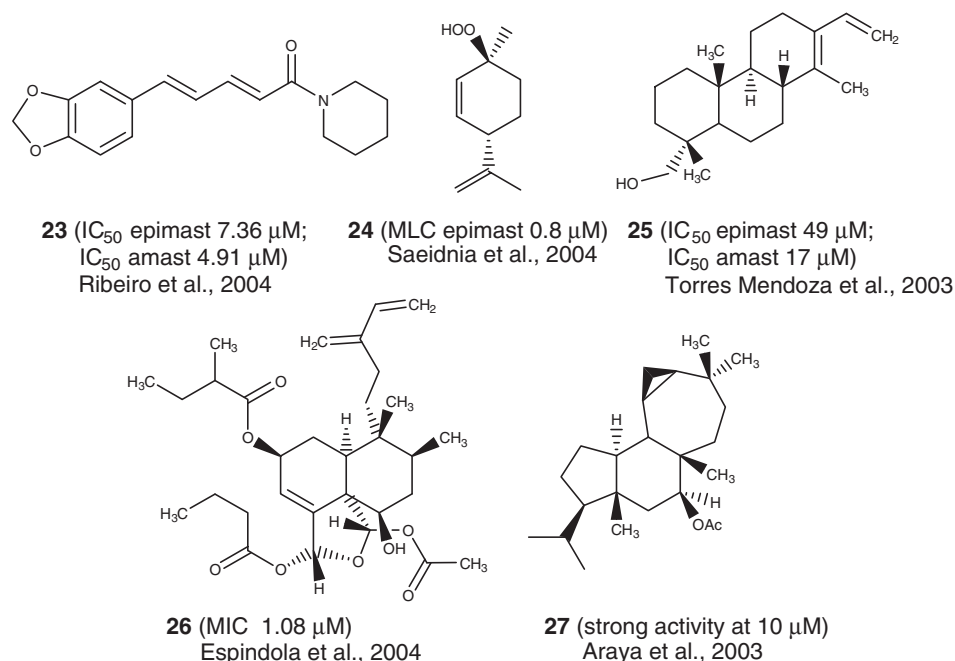
Table 4
Cassane, labdane and clerodane diterpenes tested against *T. cruzi* epimastigotes (epi) and amastigotes (amast) cultured in human foreskin fibroblasts (IC$_{50}$, MLC and MIC values are shown in μM)

Diterpenes	IC$_{50}$, MLC or MIC (μM)
18-hydroxycassan-13,15-diene (**25**) [a]	IC$_{50}$ 49 (epi); 17 (amast)
6β–18-dihydroxycassan-13,15-diene [a]	IC$_{50}$ 56 (epi); 17 (amast)
6β−hydroxy-18-acetoxycassan-13,15-diene [a]	IC$_{50}$ 12 (epi); 26 (amast)
18-acetoxy-13,15-diene-19-cassanoic acid [a]	IC$_{50}$ 104 (epi)
6β,13β−dihydroxy-18-acetoxycassan-14(17),15-diene [a]	IC$_{50}$ 17 (epi); 36 (amast)
9,13-epoxy-16-norlabd-13(E)-en-15-al [b]	MLC 11 (epi)
6-acetoxy-9,13-epoxy-16-norlabd-13(E)-en-15-al [b]	MLC 36 (epi)
vitexifolin E [b]	MLC 34 (epi)
vitexifolin F [b]	MLC 34 (epi)
vitexilactone [b]	MLC 66 (epi)
6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide [b]	MLC 66 (epi)
rel-(2S,5R,6R,8S,9S,10R,18S,19R)-19-acetoxy-18,19-epoxy-6-hydroxy-18-butanoyloxy-2-(2-methylbutanoyloxy)cleroda-3,13(16),14-triene (**26**)Espindola et al. (2004).	MIC 1.08 (epi)

[a] Mendoza et al. (2003).
[b] Kiuchi et al. (2004a).

23 (IC$_{50}$ epimast 7.36 μM;
IC$_{50}$ amast 4.91 μM)
Ribeiro et al., 2004

24 (MLC epimast 0.8 μM)
Saeidnia et al., 2004

25 (IC$_{50}$ epimast 49 μM;
IC$_{50}$ amast 17 μM)
Torres Mendoza et al., 2003

26 (MIC 1.08 μM)
Espindola et al., 2004

27 (strong activity at 10 μM)
Araya et al., 2003

Scheme 4. The most potent inhibitor of *T. cruzi* in each series is shown as an example for pepper alkaloids, cyclic monoterpene hydroperoxides, cassane, labdane and azorellane diterpenes.

Several amphiphilic, basic peptides with molecular masses ranging from 1.7 to 2.9 kDa, isolated from the skin of frogs belonging to the *Phyllomedusa* genus, were shown to clear trypomastigotes from blood at low micromolar concentrations that do not produce hemolysis (Brand et al., 2002; Leite et al., 2005). Cyclosporin A, which exacerbates parasitic infections due to its immunosuppressive action, was used as a template for the synthesis of several nonimmunosuppressive analogs. In spite of its deleterious effect on antichagasic chemotherapy, cyclosporin A inhibited epimastigote growth with IC$_{50}$=5.39 μM and lysed trypomastigotes with IC$_{50}$=7.19 μM (Bua et al., 2004). A protein with 164 amino acid residues, isolated from *Bauhinia bauhinioides* seeds, was found to inhibit the *T. cruzi* cysteine proteinase cruzipain with a low dissociation constant (K_i=1.2 nM). This protein also inhibits the highly homologous cathepsin L and cruzain, but not cathepsin B, papain, bromelain or ficin (de Oliveira et al., 2001).

An unusual positive development in this period was the experimental treatment of chronic *T. cruzi* infection in mice with the previously identified 2-*n*-propylquinoline. Parasitological cure was achieved in a smaller fraction of the animals than with benznidazole (at the same daily oral dose of 25 mg/kg for 30 days, beginning 60 days after infection). Nevertheless, 35 days after beginning the treatment, a serological ELISA test indicated significantly less circulating parasite antigen in the 2-*n*-propylquinoline-treated animals than in the positive (benznidazole) controls, a difference which intriguingly disappeared by the 85th day (Nakayama et al., 2001). However, as with most of the natural products discussed above, there is no information allowing a mechanism of action to be identified.

12. Pharmacological modulation of the host's immune response against *T. cruzi* infection

T. cruzi infection can induce apoptosis in T lymphocytes and neutrophils (De Souza et al., 2003). Phagocytosis of these cells induces the production of TGF-β, which is an anti-inflammatory response that promotes permissiveness to *T. cruzi* infection (Waghabi et al., 2005) (Fig. 3). In the same fashion, TGF-β blocks the production of IFN-γ induced NO, responsible for the trypanocidal effect of macrophages (Ramos-Ligonio et al., 2004; Waghabi et al., 2005). Production of prostaglandin E$_2$ is also elevated, and consequently there is an elevation of arginase and ornithine decarboxylase (ODC), which together with the reduction in NO production leads to elevation in the production of polyamines (Freire-de-Lima et al., 2000) that can be captured by *T. cruzi* in order to elevate, among others, the production of nucleic acids and T(SH)$_2$ (Fig. 3). Inhibition of cyclooxygenase (Fong et al., 2000) in macrophages reduces the production of ornithine decarboxylase and, in *T. cruzi*-infected mice, lowers parasitemia in a dose-dependent fashion (Freire-de-Lima et al., 2000).

13. Mechanism of acute phase response to *T. cruzi* infection in the host

Once trypomastigotes enter the dermis or conjunctival membrane, they invade a great number of host cells. Initially, they invade macrophages, where they transform into amastigotes. In *T. cruzi*, the host–parasite interaction rests mainly on the presence of glycosylphosphatidylinositol molecules (GPIs) that belong to the group of molecular patterns associated to the pathogen (PAMPs). The exact role of these molecules in *T. cruzi* pathogenesis and molecular biology is still unknown, but what
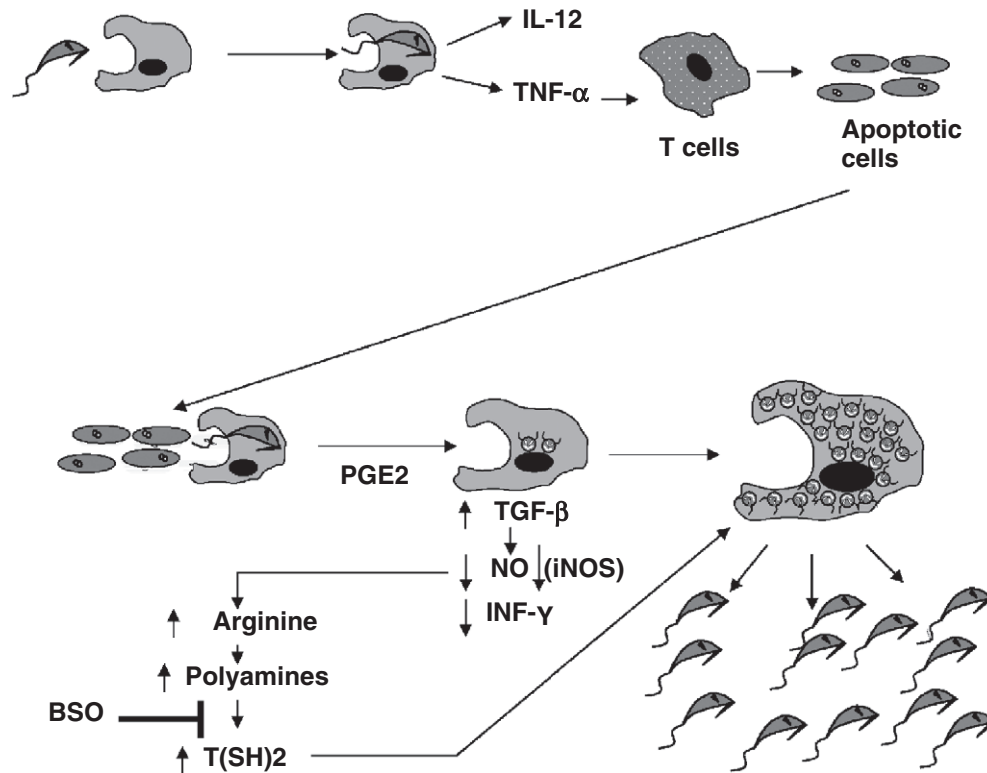
Fig. 3. Effect of *T. cruzi* induced-apoptotic cells upon *T. cruzi* growth in macrophages. During the acute infection with *T. cruzi*, the invasion of macrophages with trypomastigotes trigger the release of TNF-α that induce apoptosis of T lymphocytes. Further phagocytosis of the apoptotic bodies by *T. cruzi* infected macrophages induces production of prostaglandins (PGE2). Simultaneously, the parasite activates the transformig growth factor beta (TGF-β). These two substances inhibit interferon-γ (IFN-γ) production and inducible nitric oxide synthase (iNOS) activity. The diminished nitric oxide (NO) production renders the arginine pool susceptible to be used by the polyamine synthesis pathway. The macrophage produced spermidine is used by *T. cruzi* to synthetize trypanthione (TSH)$_2$. The consequence of all these events is evasion of the antiparasitc activity of macrophages and proliferation of the parasite (Freire-de-Lima et al., 2000). Buthionine sulfoximine (BSO) inhibits trypanothione synthesis even in the presence of increased polyamine synthesis in non-stimulated macrophages.

is known is that this glycolipid produces different responses in lymphocytes and macrophages, facilitating a permissive cellular response for the parasite (Previato et al., 2004).

In the first place, parasite adhesion to the macrophage's surface induces activation of, among others, the signals necessary to proceed with parasite invasion of cells. The mechanism of invasion used by trypomastigotes involves two strategies. The first involves induction of the cascade of calcium signals induced by phosphatidylinositol 3 (IP3), recruitment and fusion of lysosomes in the area of the plasma membrane where invasion is occurring, and the formation of a vacuole with lysosomal properties (Andrade and Andrews, 2004). A second mechanism of entry has been suggested that also involves recruitment and fusion of lysosomes, but mediated by the activation of phosphatidylinositol-3 kinase (Woolsey et al., 2003). Fusion of lysosomes is crucial for the retention and subsequent replication of the parasite (Andrade and Andrews, 2004). After the trypomastigote enters the host cell, the parasitic vacuole ruptures due to the action of a lytic protein dependent on acid pH, and the parasite escapes into the cytosol where it transforms into an amastigote (Andrade and Andrews, 2004; Vieira et al., 2002). This process requires actin (Rosestolato et al., 2002), especially in the case of macrophages (Caler et al., 2000), but also in non-phagocytic cells.

In addition to signals related with phosphorylation of tyrosine, IP3, and MAPK, parasite adhesion to host cells can also trigger other signals such as (a) the phospholipase C pathway (PLC-C), (b) elevation of intracellular calcium, essential for the process of phagocytosis, (c) elevation of cyclic 3′,5′-AMP, closely related to the elevation of calcium levels induced by parasite adhesion to the cell membrane, (d) signaling mediated by nuclear factor kappa beta (Nf-κβ) (Burleigh and Woolsey, 2002; Campos et al., 2001; DosReis et al., 2002; Ropert et al., 2001), and by transforming growth factor beta (TGF-β) (Waghabi et al., 2005) that is involved in the mechanisms of evasion of the immune response. In general, interaction between *T. cruzi* and the host results in a variety of consequences that range from control of infection by the host cells to immune response evasion, depending on the route activated in the macrophage (Peluffo et al., 2004).

In the same fashion, the interaction of *T. cruzi* GPIs with specific receptors on macrophage membranes triggers diverse intracellular signals via Toll-2 (TLR2)-type receptors (Ropert and Gazzinelli, 2004). These receptors belong to the family of receptors that recognize PAMPs. Activation of TLR2s induces macrophage activation and elevates macrophage anti-parasitic activity. During this process various signaling systems are activated that finally lead to activation of the synthesis of nitric oxide (NO) and proinflammatory cytokines (Magez et al.,

1998), through the activation of MAP kinase-dependent pathways (Ropert et al., 2001). Among the most important biochemical changes resulting from macrophage activation is the capacity to secrete hydrogen peroxide (H_2O_2), NO, and production of pro-inflammatory cytokines such as interleukins (ILs) 1, 6, 8, 12, and TNF-α. These last two interleukins induce synthesis of interferon-gamma (INF-γ) in NK cells (Une et al., 2003). Circulating monocytes and splenic macrophages in *T. cruzi*-infected animals show an important rise in oxygen uptake, and consequently, in H_2O_2 production (Melo et al., 2003). This respiratory "burst" is an important mechanism of macrophage trypanocidal activity. However, this is not the only mechanism and NO plays a central role in this activity.

14. Role of NO in *T. cruzi* infection

When trypomastigotes escape into the cytosol from macrophage phagosomes, they transform into amastigotes and proliferate. Under these circumstances, the infection triggers a modest production of inducible nitric oxide synthetase (iNOS), independent of interferon, and capable of favoring parasite proliferation. Moreover, it has been described that NO-donating drugs facilitate the proliferation of amastigotes (Rottenberg et al., 1996), reason for which low intracellular levels of NO in the host or parasite are beneficial for *T. cruzi*. Once the parasitic infection has been established, and T helper-derived cytokine activation has occurred through IL-12 and IFN-γ, iNOS is induced along with the consequent elevation of NO production, with the effect of controlling parasite invasion (Rodrigues et al., 2000; Fabrino et al., 2004; Michailowsky et al., 2001). Solid evidence supports the idea that NO is the mechanism through which IFN-γ controls *T. cruzi* infection. In fact, it has been proven that NO blocks the *T. cruzi* life cycle both in vivo and in vitro (Ramos-Ligonio et al., 2004), due to the fact that *T. cruzi* directly, or through parasite-derived glucoconjugates, induces IFN-γ production in first place, and subsequently the induction of iNOS that generates millimolar concentrations of NO. The proposed antiparasitic mechanism for NO implies the formation of peroxynitrite ion ($ONOO^-$) through the oxidative "burst", which supplies the superoxide anion necessary for the formation of $ONOO^-$ (Alvarez et al., 2002). This point is still controversial because of recent reports where iNOS-deficient mice were shown to be resistant to *T. cruzi* infection (Cummings and Tarleton, 2004; Fabrino et al., 2004), demonstrating the active participation of other components of the immune system.

Nevertheless, it has been observed that NO produced during *T. cruzi* infection also plays an important role in at least two of the processes that facilitate parasite evasion from the cellular immune response during the acute phase of disease: (i) Proapoptotic activity has been demonstrated with trypomastigote-derived ceramide-glycolipids; these glycolipids act synergistically with IFN-γ, via Toll-type receptors. In consequence, NO production increases which on the one hand promotes macrophage apoptosis (Brodskyn et al., 2002), and on the other, intracellular parasite proliferation (De Souza et al., 2003); during this process some parasites also undergo apoptosis. (ii)

During acute *T. cruzi* infection, marked immunosuppression of the host is observed that is induced by different mechanisms. One of these mechanisms implies the inhibition of IL-2 synthesis and reduction in IL-2R expression in blood cells mediated by prostaglandins (Kierszenbaum et al., 2002). In addition, through modulation of iNOS activity (Ramos-Ligonio et al., 2004), either through immature myeloid cells that suppress the high levels of IFN-γ stimulated NO production (Goñi et al., 2002), or by the modulation of activity of anti-inflammatory cytokines such as transforming growth factor beta (TGF-β). TGF-β is found as a latent complex that is activated by specific proteases, integrins, or thrombospondins. *T. cruzi* is capable of elevating TGF-β activation through proteases secreted by the parasite (Waghabi et al., 2005). In addition to the previous process, macrophage infection with *T. cruzi* induces TNF-α production, which in turn induces apoptosis of cells such as T lymphocytes. At the same time, phagocytosis of these apoptotic bodies induces TGF-β production that consequently lowers NO macrophage content (Freire-de-Lima et al., 2000; Lopes and DosReis, 2000) (Fig. 3). Other components of this complex process include cytokines such as IL-10, IL-12, and prostaglandins. In fact, prostaglandins synthesized by activated macrophages inhibit cellular proliferation and regulate cytokine synthesis (Brandonisio et al., 2001; Pinge-Filho et al., 1999), which in turn can reduce iNOS function (Plum et al., 2002). In vitro observations have shown that cyclooxygenase inhibition induces cellular proliferation (de Barros-Mazon et al., 2004), reduces IFN-γ without affecting IL-4 and IL-5 production (Une et al., 2003), and can in addition modify synthesis of IL-1, TNF-α, and IL-12 in macrophages while elevating the production of IL-10 (Shinomiya et al., 2001). On the other hand, prostaglandin E_2 (PGE$_2$) is capable of inducing TGF-β production in macrophages exposed to apoptotic cells (Fadok et al., 1998). In the same way, TGF-β is capable of inducing PGE$_2$ production in non-phagocytic inflammatory cells (Fong et al., 2000). In any case, exposure of *T. cruzi*-infected macrophages to apoptotic cells elevates the production of both cytokines, and the inhibition of COX with aspirin is capable not only of blocking PGE$_2$, but also TGF-β (Freire-de-Lima et al., 2000), and of elevating TNF-α production (Kim and Hahn, 2000), thus facilitating the antiparasitic activity of macrophages.

In synthesis, contact of specific *T. cruzi* surface glycophospholipids with host cells via Toll-type receptors triggers IP3 and IP3-kinase-type intracellular signals producing aggregation of lysosomal vesicles on the plasma membrane, thus favoring entrance of the parasite into the cell. Simultaneously, proinflammatory signals are produced through IFN-γ, IL-12, and TNF-α secretion that consequently elevate intracellular NO, essential for macrophage anti-parasitic activity. On the other hand, the parasite produces an inflammatory response leading to evasion of the immune response of the host. Among the mechanisms used to evade the immune response of the host is the induction of apoptosis in diverse cells and host immunosuppression. In both cases, NO production is compromised, because levels of proinflammatory cytokines like TGF-β and PGE$_2$ are elevated.

In conclusion, at present curative treatments for Chagas' disease do not exist. Nifurtimox and benznidazole are the only antichagasic drugs with high clinical efficacy, but they are far from optimal, because of adverse events. The knowledge of the parasite's biology has led to the identification of potential useful drug targets that could be exploited for the treatment of Chagas' disease. However, increasing the trypanocidal activity of nifurtimox and benznidazole through glutathione biosynthesis inhibition is a rational approach. It also seems logical to modify the host's response to *T. cruzi* infection with the goal of further increasing the activities of nifurtimox and benznidazole.

Acknowledgements

References

Abe, F., Nagafuji, S., Yamauchi, T., Okabe, H., Maki, J., Higo, H., Akahane, H., Aguilar, A., Jimenez-Estrada, M., Reyes-Chilpa, R., 2002a. Trypanocidal constituents in plants: 1. Evaluation of some Mexican plants for their trypanocidal activity and active constituents in Guaco, roots of *Aristolochia taliscana*. Biol. Pharm. Bull. 25, 1188–1191.

Abe, F., Yamauchi, T., Nagao, T., Kinjo, J., Okabe, H., Higo, H., Akahane, H., 2002b. Ursolic acid as a trypanocidal constituent in rosemary. Biol. Pharm. Bull. 25, 1485–1487.

Abe, F., Nagafuji, S., Okabe, H., Higo, H., Akahane, H., 2003. Trypanocidal constituents in plants: 2. Xanthones from the stem bark of *Garcinia subelliptica*. Biol. Pharm. Bull. 26, 1730–1733.

Abe, F., Nagafuji, S., Okabe, H., Akahane, H., Estrada-Muniz, E., Huerta-Reyes, M., Reyes-Chilpa, R., 2004. Trypanocidal constituents in plants: 3. Leaves of *Garcinia intermedia* and heartwood of *Calophyllum brasiliense*. Biol. Pharm. Bull. 27, 141–143.

Abegaz, B.M., Bezabih, M., Msuta, T., Brun, R., Menche, D., Muhlbacher, J., Bringmann, G., 2002. Gaboroquinones A and B and 4′-O-demethylknipholone-4′-O-beta-D-glucopyranoside, phenylanthraquinones from the roots of *Bulbine frutescens*. J. Nat. Prod. 65, 1117–1121.

Aldunate, J., Morello, A., 1993. Free radicals in the mode of action of parasitic drugs. In: Aruoma, O.I. (Ed.), Free Radicals in Tropical Diseases. Harwood Academic Publishers, Switzerland, pp. 137–165.

Aldunate, J., Ferreira, J., Letelier, M.E., Repetto, Y., Morello, A., 1986. t-Butyl-4-hydroxyanisole, a novel respiratory chain inhibitor. Effects on *Trypanosoma cruzi* epimastigotes. FEBS Lett. 195, 295–297.

Alvarez, M.N., Trujillo, M., Radi, R., 2002. Peroxynitrite formation from biochemical and cellular fluxes of nitric oxide and superoxide. Methods Enzymol. 359, 353–366.

Anderson, M.E., 1998. Glutathione: an overview of biosynthesis and modulation. Chem.-Biol. Interact. 111–112, 1–14.

Anderson, C.P., Reynolds, C.P., 2002. Synergistic cytotoxicity of buthionine sulfoximine (BSO) and intensive melphalan (L-PAM) for neuroblastoma cell lines established at relapse after myeloablative therapy. Bone Marrow Transplant. 30, 135–140.

Anderson, C.P., Seeger, R.C., Satake, N., Monforte-Muñoz, H.L., Keshelava, N., Bailey, H.H., Reynolds, C.P., 2001. Buthionine sulfoximine and myeloablative concentrations of melphalan overcome resistance in a melphalan-resistant neuroblastoma cell line. J. Pediatr. Hematol./Oncol. 23, 500–505.

Andrade, L.O., Andrews, N.W., 2004. Lysosomal fusion is essential for the retention of *Trypanosoma cruzi* inside host cells. J. Exp. Med. 200, 1135–1143.

Apt, W., 1999. Tratamiento de la Enfermedad de Chagas. Parasitol. Día 23, 100–112.

Apt, W., Aguilera, X., Arribada, A., Perez, C., Miranda, C., Sanchez, G., Zulantay, I., Cortes, P., Rodriguez, J., Juri, D., 1998. Treatment of chronic Chagas' disease with itraconazole and allopurinol. Am. J. Trop. Med. Hyg. 59, 133–138.

Apt, W., Arribada, A., Zulantay, I., Sanchez, G., Vargas, S.L., Rodriguez, J., 2003. Itraconazole or allopurinol in the treatment of chronic American trypanosomiasis: the regression and prevention of electrocardiographic abnormalities during 9 years of follow-up. Ann. Trop. Med. Parasitol. 97, 23–29.

Araya, J.E., Neira, I., da Silva, S., Mortara, R.A., Manque, P., Cordero, E., Sagua, H., Loyola, A., Bórquez, J., Morales, G., González, J., 2003. Diterpenoids from *Azorella compacta* (Umbelliferae) active on *Trypanosoma cruzi*. Mem. Inst. Oswaldo Cruz 98, 413–418.

Ariyanayagam, M.R., Fairlamb, A.H., 2001. Ovothiol and trypanothione as antioxidants in trypanosomatids. Mol. Biochem. Parasitol. 115, 189–198.

Ariyanayagam, M.R., Oza, S.L., Mehlert, A., Fairlamb, A.H., 2003. Bis (glutathionyl)spermine and other novel trypanothione analogues in *Trypanosoma cruzi*. J. Biol. Chem. 278, 27612–27619.

Arrick, B.A., Griffith, O.W., Cerami, A., 1981. Inhibition of glutathione synthesis as a chemotherapeutic strategy for trypanosomiasis. J. Exp. Med. 153, 720–725.

Arzel, E., Rocca, P., Grellier, P., Labaeid, M., Frappier, F., Gueritte, F., Gaspard, C., Marsais, F., Godard, A., Queguiner, G., 2001. New synthesis of benzo-delta-carbolines, cryptolepines, and their salts: in vitro cytotoxic, antiplasmodial, and antitrypanosomal activities of delta-carbolines, benzo-delta-carbolines, and cryptolepines. J. Med. Chem. 44, 949–960.

Asres, K., Bucar, F., Knauder, E., Yardley, V., Kendrick, H., Croft, S.L., 2001. In vitro antiprotozoal activity of extract and compounds from the stem bark of *Combretum molle*. Phytother. Res. 15, 613–617.

Aufderheide, A.C., Salo, W., Madden, M., Streitz, J., Buikstra, J., Guhl, F., Arriaza, B., Renier, C., Wittmers Jr., L.E., Fornaciari, G., Allison, M., 2004. A 9000-year record of Chagas' disease. Proc. Natl. Acad. Sci. U. S. A. 101, 2034–2039.

Augustyns, K., Amssoms, K., Yamani, A., Rajan, P.K., Haemers, A., 2001. Trypanothione as a target in the design of antitrypanosomal and antileishmanial agents. Curr. Pharm. Des. 7, 1117–1141.

Avila, J.L., Avila, A., 1981. *Trypanosoma cruzi*: allopurinol in the treatment of mice with experimental acute Chagas disease. Exp. Parasitol. 51, 204–208.

Avila, J.L., Avila, A., Monzón, H., 1984. Differences in allopurinol and 4-aminopyrazolo(3,4-d) pyrimidine metabolism in drug-sensitive and insensitive strains of *Trypanosoma cruzi*. Mol. Biochem. Parasitol. 11, 51–60.

Bailey, H.H., Mulcahy, R.T., Tutsch, K.D., Arzoomanian, R.Z., Alberti, D., Tombes, M.B., Wilding, G., Pomplun, M., Spriggs, D.R., 1994. Phase I clinical trial of intravenous L-buthionine sulfoximine and melphalan: an attempt at modulation of glutathione. J. Clin. Oncol. 12, 194–205.

Bailey, H.H., Ripple, G., Tutsch, K.D., Arzoomanian, R.Z., Alberti, D., Feierabend, C., Mahvi, D., Schink, J., Pomplun, M., Mulcahy, R.T., Wilding, G., 1997. Phase I study of continuous-infusion L-S,R-buthionine sulfoximine with intravenous melphalan. J. Natl. Cancer Inst. 89, 1789–1796.

Bennett, J.E., 2001. Antimicrobial agents: antifungal agents. In: Goodman, L.S., Gilman, A.G., Hardman, J.G., Limbird, L.E. (Eds.), Goodman & Gilman's The Pharmacological Basis of Therapeutics. McGraw-Hill, Medical Publishing Division, New York, pp. 1295–1312.

Berens, R.L., Marr, J.J., LaFon, S.W., Nelson, D.J., 1981. Purine metabolism in *Trypanosoma cruzi*. Mol. Biochem. Parasitol. 3, 187–196.

Berger, I., Passreiter, C.M., Cáceres, A., Kubelka, W., 2001. Antiprotozoal activity of *Neurolaena lobata*. Phytother. Res. 15, 327–330.

Brand, G.D., Leite, J.R., Silva, L.P., Albuquerque, S., Prates, M.V., Azevedo, R.B., Carregaro, V., Silva, J.S., Sa, V.C., Brandão, R.A., Bloch Jr., C., 2002. Dermaseptins from *Phyllomedusa oreades* and *Phyllomedusa distincta*. Anti-*Trypanosoma cruzi* activity without cytotoxicity to mammalian cells. J. Biol. Chem. 277, 49332–49340.

Brandonisio, O., Panaro, M.A., Sisto, M., Acquafredda, A., Fumarola, L., Leogrande, D., Mitolo, V., 2001. Nitric oxide production by Leishmania-infected macrophages and modulation by cytokines and prostaglandins. Parassitologia 43 (Suppl 1), 1–6.

Branen, A.L., 1975. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. J. Am. Oil Chem. Soc. 52, 59–63.

Bravo, B.J., Sauvain, M., Giménez, T.A., Balanza, E., Serani, L., Laprevôte, O., Massiot, G., Lavaud, C., 2001. Trypanocidal withanolides and withanolide glycosides from *Dunalia brachyacantha*. J. Nat. Prod. 64, 720–725.

Bringmann, G., Hamm, A., Günther, C., Michel, M., Brun, R., Mudogo, V., 2000. Ancistroealaines A and B, two new bioactive naphthylisoquinolines, and related naphthoic acids from *Ancistrocladus ealaensis*. J. Nat. Prod. 63, 1465–1470.

Bringmann, G., Messer, K., Brun, R., Mudogo, V., 2002a. Ancistrocongolines A-D, new naphthylisoquinoline alkaloids from *Ancistrocladus congolensis*. J. Nat. Prod. 65, 1096–1101.

Bringmann, G., Messer, K., Wolf, K., Muhlbacher, J., Grune, M., Brun, R., Louis, A.M., 2002b. Dioncophylline E from *Dioncophyllum thollonii*, the first 7,3′-coupled *Dioncophyllaceous naphthylisoquinoline* alkaloid. Phytochemistry 60, 389–397.

Bringmann, G., Dreyer, M., Faber, J.H., Dalsgaard, P.W., Staerk, D., Jaroszewski, J.W., Ndangalasi, H., Mbago, F., Brun, R., Reichert, M., Maksimenka, K., Christensen, S.B., 2003a. Ancistrotanzanine A, the first 5,3′-coupled naphthylisoquinoline alkaloid, and two further, 5,8′-linked related compounds from the newly described species *Ancistrocladus tanzaniensis*. J. Nat. Prod. 66, 1159–1165.

Bringmann, G., Messer, K., Schwobel, B., Brun, R., Ake, A.L., 2003b. Habropetaline A, an antimalarial naphthylisoquinoline alkaloid from *Triphyophyllum peltatum*. Phytochemistry 62, 345–349.

Bringmann, G., Saeb, W., Ruckert, M., Mies, J., Michel, M., Mudogo, V., Brun, R., 2003c. Ancistrolikokine D, a 5,8′-coupled naphthylisoquinoline alkaloid, and related natural products from *Ancistrocladus likoko*. Phytochemistry 62, 631–636.

Bringmann, G., Dreyer, M., Faber, J.H., Dalsgaard, P.W., Staerk, D., Jaroszewski, J.W., Ndangalasi, H., Mbago, F., Brun, R., Christensen, S.B., 2004a. Ancistrotanzanine C and related 5,1′- and 7,3′-coupled naphthylisoquinoline alkaloids from *Ancistrocladus tanzaniensis*. J. Nat. Prod. 67, 743–748.

Bringmann, G., Dreyer, M., Rischer, H., Wolf, K., Hadi, H.A., Brun, R., Meimberg, H., Heubl, G., 2004b. Ancistrobenomine a, the first naphthylisoquinoline oxygenated at Me-3, and related 5,1′-coupled alkaloids, from the "new" plant species *Ancistrocladus benomensis*. J. Nat. Prod. 67, 2058–2062.

Bringmann, G., Dreyer, M., Kopff, H., Rischer, H., Wohlfarth, M., Hadi, H.A., Brun, R., Meimberg, H., Heubl, G., 2005. ent-Dioncophylleine A and related dehydrogenated naphthylisoquinoline alkaloids, the first Asian Dioncophyllaceae-type alkaloids, from the "new" plant species *Ancistrocladus benomensis*. J. Nat. Prod. 68, 686–690.

Brodskyn, C., Patricio, J., Oliveira, R., Lobo, L., Arnholdt, A., Mendonça-Previato, L., Barral, A., Barral-Netto, M., 2002. Glycoinositolphospholipids from *Trypanosoma cruzi* interfere with macrophages and dendritic cell responses. Infect. Immun. 70, 3736–3743.

Bua, J., Ruiz, A.M., Potenza, M., Fichera, L.E., 2004. In vitro anti-parasitic activity of Cyclosporin A analogs on *Trypanosoma cruzi*. Bioorg. Med. Chem. Lett. 14, 4633–4637.

Burleigh, B.A., Woolsey, A.M., 2002. Cell signalling and *Trypanosoma cruzi* invasion. Cell. Microbiol. 4, 701–711.

Cabral, M.M., Azambuja, P., Gottlieb, O.R., Kleffmann, T., García, E.S., Schaub, G.A., 2001. Burchellin: effects on *Triatoma infestans* and on *Trypanosoma cruzi* within this vector. Parasitol. Res. 87, 730–735.

Caler, E.V., Morty, R.E., Burleigh, B.A., Andrews, N.W., 2000. Dual role of signaling pathways leading to Ca(2+) and cyclic AMP elevation in host cell invasion by *Trypanosoma cruzi*. Infect. Immun. 68, 6602–6610.

Calvert, P., Yao, K.S., Hamilton, T.C., O'Dwyer, P.J., 1998. Clinical studies of reversal of drug resistance based on glutathione. Chem. Biol. Interact. 111–112, 213–224.

Campos, M.A., Almeida, I.C., Takeuchi, O., Akira, S., Valente, E.P., Procopio, D.O., Travassos, L.R., Smith, J.A., Golenbock, D.T., Gazzinelli, R.T., 2001. Activation of Toll-like receptor-2 by glycosylphosphatidylinositol anchors from a protozoan parasite. J. Immunol. 167, 416–423.

Cardona Zuleta, L.M., Cavalheiro, A.J., Siqueira Silva, D.H., Furlan, M., Marx Young, M.C., Albuquerque, S., Castro-Gamboa, I., da Silva Bolzani, V.,

2003. Seco-iridoids from *Calycophyllum spruceanum* (Rubiaceae). Phytochemistry 64, 549–553.

Cazzulo, J.J., 2002. Proteinases of *Trypanosoma cruzi*: potential targets for the chemotherapy of Chagas desease. Curr. Top. Med. Chem. 2, 1261–1271.

Cazzulo, J.J., Stoka, V., Turk, V., 2001. The major cysteine proteinase of *Trypanosoma cruzi*: a valid target for chemotherapy of Chagas disease. Curr. Pharm. Des. 7, 1143–1156.

Chagas, C., 1909. Nova tripanosomiase humana. Estudios sobre a morfología e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen, n. sp., agente etiológico do nova entidae mórbida do homen. Mem. Inst. Oswaldo Cruz. Mem. Inst. Oswaldo Cruz 1, 159–218.

Chowdhury, S.F., Guerrero, R.H., Brun, R., Ruiz-Pérez, L.M., Pacanowska, D.G., Gilbert, I.H., 2002. Synthesis and testing of 5-benzyl-2,4-diaminopyrimidines as potential inhibitors of leishmanial and trypanosomal dihydrofolate reductase. J. Enzyme Inhib. Med. Chem. 17, 293–302.

Cuéllar, M.A., Salas, C., Cortés, M.J., Morello, A., Maya, J.D., Preite, M.D., 2003. Synthesis and in vitro trypanocide activity of several polycyclic drimane-quinone derivatives. Bioorg. Med. Chem. 11, 2489–2497.

Cummings, K.L., Tarleton, R.L., 2004. Inducible nitric oxide synthase is not essential for control of *Trypanosoma cruzi* infection in mice. Infect. Immun. 72, 4081–4089.

Darsaud, A., Chevrier, C., Bourdon, L., Dumas, M., Buguet, A., Bouteille, B., 2004. Megazol combined with suramin improves a new diagnosis index of the early meningo-encephalitic phase of experimental African trypanosomiasis. Trop. Med. Int. Health 9, 83–91.

da Silva Filho, A.A., Pires Bueno, P.C., Gregorio, L.E., Andrade e Silva, M.L., Albuquerque, S., Bastos, J.K., 2004. In-vitro trypanocidal activity evaluation of crude extract and isolated compounds from *Baccharis dracunculifolia* D.C. (Asteraceae). J. Pharm. Pharmacol. 56, 1195–1199.

de Barros-Mazon, S., Guariento, M.E., da Silva, C.A., Coffman, R.L., Abrahamsohn, I.A., 2004. Differential regulation of lymphoproliferative responses to *Trypanosoma cruzi* antigen in patients with the cardiac or indeterminate form of Chagas disease. Clin. Immunol. 111, 137–145.

De Carvalho, E.A., Andrade, P.P., Silva, N.H., Pereira, E.C., Figueiredo, R.C., 2005. Effect of usnic acid from the lichen *Cladonia substellata* on *Trypanosoma cruzi* in vitro: an ultrastructural study. Micron 36, 155–161.

de Marchi, A.A., Castilho, M.S., Nascimento, P.G., Archanjo, F.C., del Ponte, G., Oliva, G., Pupo, M.T., 2004. New 3-piperonylcoumarins as inhibitors of glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) from *Trypanosoma cruzi*. Bioorg. Med. Chem. 12, 4823–4833.

de Oliveira, C., Santana, L.A., Carmona, A.K., Cezari, M.H., Sampaio, M.U., Sampaio, C.A., Oliva, M.L., 2001. Structure of cruzipain/cruzain inhibitors isolated from *Bauhinia bauhinioides* seeds. Biol. Chem. 382, 847–852.

De Souza, E.M., Araujo-Jorge, T.C., Bailly, C., Lansiaux, A., Batista, M.M., Oliveira, G.M., Soeiro, M.N., 2003. Host and parasite apoptosis following *Trypanosoma cruzi* infection in in vitro and in vivo models. Cell Tissue Res. 314, 223–235.

de Souza, V., da Silva, R., Pereira, A.C., Royo, V.A., Saraiva, J., Montanheiro, M., de Souza, G.H., da Silva Filho, A.A., Grando, M.D., Donate, P.M., Bastos, J.K., Albuquerque, S., Silva, M.L., 2005. Trypanocidal activity of (−)-cubebin derivatives against free amastigote forms of *Trypanosoma cruzi*. Bioorg. Med. Chem. Lett. 15, 303–307.

del Olmo, E., Armas, M.G., López-Pérez, J.L., Ruiz, G., Vargas, F., Giménez, A., Deharo, E., San Feliciano, A., 2001. Anti-Trypanosoma activity of some natural stilbenoids and synthetic related heterocyclic compounds. Bioorg. Med. Chem. Lett. 11, 2755–2757.

Díaz de Toranzo, E.G., Castro, J.A., Franke de Cazzulo, B.M., Cazzulo, J.J., 1988. Interaction of benznidazole reactive metabolites with nuclear and kinetoplastic DNA, proteins and lipids from *Trypanosoma cruzi*. Experientia 44, 880–881.

Docampo, R., Stoppani, A.O., 1979. Generation of superoxide anion and hydrogen peroxide induced by nifurtimox in *Trypanosoma cruzi*. Arch. Biochem. Biophys. 197, 317–321.

Docampo, R., Stoppani, A.O., 1980. Mechanism of the trypanocidal action of nifurtimox and other nitro-derivatives on *Trypanosoma cruzi*. Medicina (B Aires) 40 (Suppl. 1), 10–16.

Docampo, R., Moreno, S.N., 1984. Free radical metabolites in the mode of action of chemotherapeutic agents and phagocytic cells on *Trypanosoma cruzi*. Rev. Infect. Dis. 6, 223–238.

Docampo, R., Moreno, S.N., Stoppani, A.O., León, W., Cruz, F.S., Villalta, F., Muñiz, R.F., 1981. Mechanism of nifurtimox toxicity in different forms of *Trypanosoma cruzi*. Biochem. Pharmacol. 30, 1947–1951.

Dormeyer, M., Reckenfelderbaumer, N., Ludemann, H., Krauth-Siegel, R.L., 2001. Trypanothione-dependent synthesis of deoxyribonucleotides by *Trypanosoma brucei* ribonucleotide reductase. J. Biol. Chem. 276, 10602–10606.

DosReis, G.A., Pecanha, L.M., Bellio, M., Previato, J.O., Mendonça-Previato, L., 2002. Glycoinositol phospholipids from *Trypanosoma cruzi* transmit signals to the cells of the host immune system through both ceramide and glycan chains. Microbes Infect. 4, 1007–1013.

Eakin, A.E., Guerra, A., Focia, P.J., Torres-Martínez, J., Craig III, S.P., 1997. Hypoxanthine phosphoribosyltransferase from *Trypanosoma cruzi* as a target for structure-based inhibitor design: crystallization and inhibition studies with purine analogs. Antimicrob. Agents Chemother. 41, 1686–1692.

Elion, G.B., Nelson, D.J., 1974. Ribonucleotides of allopurinol and oxipurinol in rat tissues and their significance in purine metabolism. Adv. Exp. Med. Biol. 41, 639–652.

Elion, G.B., Yu, T.F., Gutman, A.B., Hitchings, G.H., 1968. Renal clearance of oxipurinol, the chief metabolite of allopurinol. Am. J. Med. 45, 69–77.

Enanga, B., Ariyanayagam, M.R., Stewart, M.L., Barrett, M.P., 2003. Activity of megazol, a trypanocidal nitroimidazole, is associated with DNA damage. Antimicrob. Agents Chemother. 47, 3368–3370.

Espindola, L.S., Vasconcelos Jr., J., de Mesquita, M.L., Marquie, P., de Paula, J. E., Mambu, L., Santana, J.M., 2004. Trypanocidal activity of a new diterpene from *Casearia sylvestris* var. *lingua*. Planta Med. 70, 1093–1095.

Fabrino, D.L., Leon, L.L., Parreira, G.G., Genestra, M., Almeida, P.E., Melo, R. C., 2004. Peripheral blood monocytes show morphological pattern of activation and decreased nitric oxide production during acute Chagas' disease in rats. Nitric Oxide 11, 166–174.

Fadok, V.A., Bratton, D.L., Konowal, A., Freed, P.W., Westcott, J.Y., Henson, P. M., 1998. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J. Clin. Invest. 101, 890–898.

Fairlamb, A.H., Cerami, A., 1992. Metabolism and functions of trypanothione in the Kinetoplastida. Annu. Rev. Microbiol. 46, 695–729.

Faúndez, M., Pino, L., Letelier, P., Ortiz, C., López, R., Seguel, C., Ferreira, J., Pavani, M., Morello, A., Maya, J.D., 2005. Buthionine sulfoximine increases the toxicity of nifurtimox and benznidazole to *Trypanosoma cruzi*. Antimicrob. Agents Chemother. 49, 126–130.

Feresin, G.E., Tapia, A., Sortino, M., Zacchino, S., de Arias, A.R., Inchausti, A., Yaluff, G., Rodríguez, J., Theoduloz, C., Schmeda-Hirschmann, G., 2003. Bioactive alkyl phenols and embelin from *Oxalis erythrorhiza*. J. Ethnopharmacol. 88, 241–247.

Fernandes, A.P., Holmgren, A., 2004. Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. Antioxid. Redox Signal. 6, 63–74.

Ferreira, J., Coloma, L., Fones, E., Letelier, M.E., Repetto, Y., Morello, A., Aldunate, J., 1988. Effects of t-butyl-4-hydroxyanisole and other phenolic antioxidants on tumoral cells and Trypanosoma parasites. FEBS Lett. 234, 485–488.

Filardi, L.S., Brener, Z., 1987. Susceptibility and natural resistance of *Trypanosoma cruzi* strains to drugs used clinically in Chagas disease. Trans. R. Soc. Trop. Med. Hyg. 81, 755–759.

Fong, C.Y., Pang, L., Holland, E., Knox, A.J., 2000. TGF-beta1 stimulates IL-8 release, COX-2 expression, and PGE(2) release in human airway smooth muscle cells. Am. J. Physiol., Lung Cell. Mol. Physiol. 279, L201–L207.

Freire-de-Lima, C.G., Nascimento, D.O., Soares, M.B., Bozza, P.T., Castro-Faria-Neto, H.C., de Mello, F.G., DosReis, G.A., Lopes, M.F., 2000. Uptake of apoptotic cells drives the growth of a pathogenic trypanosome in macrophages. Nature 403, 199–203.

Freymann, D.M., Wenck, M.A., Engel, J.C., Feng, J., Focia, P.J., Eakin, A.E., Craig, S.P., 2000. Efficient identification of inhibitors targeting the closed active site conformation of the HPRT from *Trypanosoma cruzi*. Chem. Biol. 7, 957–968.

Gertsch, J., Tobler, R.T., Brun, R., Sticher, O., Heilmann, J., 2003. Antifungal, antiprotozoal, cytotoxic and piscicidal properties of Justicidin B and a new

arylnaphthalide lignan from *Phyllanthus piscatorum*. Planta Med. 69, 420–424.

Goñi, O., Alcaide, P., Fresno, M., 2002. Immunosuppression during acute *Trypanosoma cruzi* infection: involvement of Ly6G (Gr1(+))CD11b(+) immature myeloid suppressor cells. Int. Immunol. 14, 1125–1134.

Grael, C.F., Albuquerque, S., Lopes, J.L., 2005. Chemical constituents of *Lychnophora pohlii* and trypanocidal activity of crude plant extracts and of isolated compounds. Fitoterapia 76, 73–82.

Griffith, O.W., Mulcahy, R.T., 1999. The enzymes of glutathione synthesis: gamma-glutamylcysteine synthetase. Advan. Enzymol. Relat. Areas Mol. Biol. 73, 209–267 (xii).

Gutteridge, W.E., Davies, M.J., 1981. Enzymes of purine salvage in *Trypanosoma cruzi*. FEBS Lett. 127, 211–214.

Gutteridge, J.M., Halliwell, B., 2000. Free radicals and antioxidants in the year 2000. A historical look to the future. Ann. N.Y. Acad. Sci. 899, 136–147.

Haddad, J.J., 2002. The involvement of L-gamma-glutamyl-L-cysteinyl-glycine (glutathione/GSH) in the mechanism of redox signaling mediating MAPK (p38)-dependent regulation of pro-inflammatory cytokine production. Biochem. Pharmacol. 63, 305–320.

Hammond, D.J., Gutteridge, W.E., Opperdoes, F.R., 1981. A novel location for two enzymes of de novo pyrimidine biosynthesis in trypanosomes and Leishmania. FEBS Lett. 128, 27–29.

Hayes, J.D., McLellan, L.I., 1999. Glutathione and glutathione-dependent enzymes represent a coordinately regulated defence against oxidative stress. Free Radic. Res. 31, 273–300.

Heilmann, J., Brun, R., Mayr, S., Rali, T., Sticher, O., 2001. Minor cytotoxic and antibacterial compounds from the rhizomes of *Amomum aculeatum*. Phytochemistry 57, 1281–1285.

Hiratake, J., Irie, T., Tokutake, N., Oda, J., 2002. Recognition of a cysteine substrate by *E. coli* gamma-glutamylcysteine synthetase probed by sulfoximine-based transition-state analogue inhibitors. Biosci. Biotechnol. Biochem. 66, 1500–1514.

Hoet, S., Stevigny, C., Block, S., Opperdoes, F., Colson, P., Baldeyrou, B., Lansiaux, A., Bailly, C., Quetin-Leclercq, J., 2004. Alkaloids from *Cassytha filiformis* and related aporphines: antitrypanosomal activity, cytotoxicity, and interaction with DNA and topoisomerases. Planta Med. 70, 407–413.

Huynh, T.T., Huynh, V.T., Harmon, M.A., Phillips, M.A., 2003. 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.

Jonckers, T.H., van Miert, S., Cimanga, K., Bailly, C., Colson, P., De Pauw-Gillet, M.C., van den Heuvel, H., Claeys, M., Lemiere, F., Esmans, E.L., Rozenski, J., Quirijnen, L., Maes, L., Dommisse, R., Lemiere, G.L., Vlietinck, A., Pieters, L., 2002. Synthesis, cytotoxicity, and antiplasmodial and antitrypanosomal activity of new neocryptolepine derivatives. J. Med. Chem. 45, 3497–3508.

Jones, C.M., Lawrence, A., Wardman, P., Burkitt, M.J., 2003a. Kinetics of superoxide scavenging by glutathione: an evaluation of its role in the removal of mitochondrial superoxide. Biochem. Soc. Trans. 31, 1337–1339.

Jones, S.P., Hoffmeyer, M.R., Sharp, B.R., Ho, Y.S., Lefer, D.J., 2003b. Role of intracellular antioxidant enzymes after in vivo myocardial ischemia and reperfusion. Am. J. Physiol, Heart Circ. Physiol. 284, H277–H282.

Kapoor, P., Sachdev, M., Madhubala, R., 2000. Inhibition of glutathione synthesis as a chemotherapeutic strategy for leishmaniasis. Trop. Med. Int. Health 5, 438–442.

Kelly, B.S., Antholine, W.E., Griffith, O.W., 2002. Escherichia coli gamma-glutamylcysteine synthetase. Two active site metal ions affect substrate and inhibitor binding. J. Biol. Chem. 277, 50–58.

Khabnadideh, S., Pez, D., Musso, A., Brun, R., Pérez, L.M., González-Pacanowska, D., Gilbert, I.H., 2005. Design, synthesis and evaluation of 2,4-diaminoquinazolines as inhibitors of trypanosomal and leishmanial dihydrofolate reductase. Bioorg. Med. Chem. 13, 2637–2649.

Kierszenbaum, F., Fresno, M., Sztein, M.B., 2002. The *Trypanosoma cruzi* membrane glycoprotein AGC10 inhibits human lymphocyte activation by a mechanism preceding translation of both, interleukin-2 and its high-affinity receptor subunits. Mol. Biochem. Parasitol. 125, 91–101.

Kim, J.G., Hahn, Y.S., 2000. IFN-gamma inhibits the suppressive effects of PGE2 on the production of tumor necrosis factor-alpha by mouse macrophages. Immunol. Invest. 29, 257–269.

Kirchhoff, L.V., 2000. American trypanosomiasis (Chagas' disease). In: Rakel, R.E. (Ed.), Conn's Current Therapy. W. B. Saunders, New York, pp. 101–102.

Kiuchi, F., Itano, Y., Uchiyama, N., Honda, G., Tsubouchi, A., Nakajima-Shimada, J., Aoki, T., 2002. Monoterpene hydroperoxides with trypanocidal activity from Chenopodium ambrosioides. J. Nat. Prod. 65, 509–512.

Kiuchi, F., Matsuo, K., Ito, M., Qui, T.K., Honda, G., 2004a. New norditerpenoids with trypanocidal activity from Vitex trifolia. Chem. Pharm. Bull. (Tokyo) 52, 1492–1494.

Kiuchi, F., Matsuo, K., Ito, M., Qui, T.K., Honda, G., 2004b. New sesquiterpene hydroperoxides with trypanocidal activity from Pogostemon cablin. Chem. Pharm. Bull. (Tokyo) 52, 1495–1496.

Krauth-Siegel, R.L., Ludemann, H., 1996. Reduction of dehydroascorbate by trypanothione. Mol. Biochem. Parasitol. 80, 203–208.

Krauth-Siegel, R.L., Meiering, S.K., Schmidt, H., 2003. The parasite-specific trypanothione metabolism of Trypanosoma and Leishmania. Biol. Chem. 384, 539–549.

Kuhn, H., Borchert, A., 2002. Regulation of enzymatic lipid peroxidation: the interplay of peroxidizing and peroxide reducing enzymes. Free Radic. Biol. Med. 33, 154–172.

Labraña, J., Machocho, A.K., Kricsfalusy, V., Brun, R., Codina, C., Viladomat, F., Bastida, J., 2002. Alkaloids from Narcissus angustifolius subsp. transcarpathicus (Amaryllidaceae). Phytochemistry 60, 847–852.

Ladame, S., Castilho, M.S., Silva, C.H., Denier, C., Hannaert, V., Perie, J., Oliva, G., Willson, M., 2003. Crystal structure of Trypanosoma cruzi glyceraldehyde-3-phosphate dehydrogenase complexed with an analogue of 1,3-bisphospho-D-glyceric acid. Eur. J. Biochem. 270, 4574–4586.

Ladame, S., Faure, R., Denier, C., Lakhdar-Ghazal, F., Willson, M., 2005. Selective inhibition of Trypanosoma cruzi GAPDH by "bi-substrate" analogues. Org. Biomol. Chem. 3, 2070–2072.

Leite, J.R., Silva, L.P., Rodrigues, M.I., Prates, M.V., Brand, G.D., Lacava, B.M., Azevedo, R.B., Bocca, A.L., Albuquerque, S., Bloch Jr., C., 2005. Phylloseptins: a novel class of anti-bacterial and anti-protozoan peptides from the Phyllomedusa genus. Peptides 26, 565–573.

Letelier, M.E., Rodríguez, E., Wallace, A., Lorca, M., Repetto, Y., Morello, A., Aldunate, J., 1990. Trypanosoma cruzi: a possible control of transfusion-induced Chagas' disease by phenolic antioxidants. Exp. Parasitol. 71, 357–363.

Lopes, M.F., DosReis, G.A., 2000. Experimental Chagas disease: phagocytosis of apoptotic lymphocytes deactivates macrophages and fuels parasite growth. Apoptosis 5, 221–224.

Machado, C.A., Ayala, F.J., 2002. Sequence variation in the dihydrofolate reductase-thymidylate synthase (DHFR-TS) and trypanothione reductase (TR) genes of Trypanosoma cruzi. Mol. Biochem. Parasitol. 121, 33–47.

Machocho, A.K., Bastida, J., Codina, C., Viladomat, F., Brun, R., Chhabra, S.C., 2004. Augustamine type alkaloids from Crinum kirkii. Phytochemistry 65, 3143–3149.

Magez, S., Stijlemans, B., Radwanska, M., Pays, E., Ferguson, M.A., De Baetselier, P., 1998. The glycosyl-inositol-phosphate and dimyristoylgly-cerol moieties of the glycosylphosphatidylinositol anchor of the trypanosome variant-specific surface glycoprotein are distinct macrophage-activating factors. J. Immunol. 160, 1949–1956.

Marr, J.J., Berens, R.L., Nelson, D.J., 1978. Antitrypanosomal effect of allopurinol: conversion in vivo to aminopyrazolopyrimidine nucleotides by Trypanosoma cruzi. Science 201, 1018–1020.

Martins, R.C., Lago, J.H., Albuquerque, S., Kato, M.J., 2003. Trypanocidal tetrahydrofuran lignans from inflorescences of Piper solmsianum. Phytochemistry 64, 667–670.

Mason, R.P., Holtzman, J.L., 1975. The role of catalytic superoxide formation in the O_2 inhibition of nitroreductase. Biochem. Biophys. Res. Commun. 67, 1267–1274.

Maya, J.D., Repetto, Y., Agosin, M., Ojeda, J.M., Téllez, R., Gaule, C., Morello, A., 1997. Effects of nifurtimox and benznidazole upon glutathione and trypanothione content in epimastigote, trypomastigote and amastigote forms of Trypanosoma cruzi. Mol. Biochem. Parasitol. 86, 101–106.

Maya, J.D., Morello, A., Repetto, Y., Téllez, R., Rodríguez, A., Zelada, U., Puebla, P., Caballero, E., Medarde, M., Núñez-Vergara, L.J., Squella, J.A., Bonta, M., Bollo, S., San Feliciano, A., 2000. Effects of 3-chloro-phenyl-1,4-dihydropyridine derivatives on Trypanosoma cruzi epimastigotes. Comp. Biochem. Physiol., C. Toxicol. Pharmacol. 125, 103–109.

Maya, J.D., Morello, A., Repetto, Y., Rodríguez, A., Puebla, P., Caballero, E., Medarde, M., Núñez-Vergara, L.J., Squella, J.A., Ortiz, M.E., Fuentealba, J., San Feliciano, A., 2001. Trypanosoma cruzi: inhibition of parasite growth and respiration by oxazolo(thiazolo)pyridine derivatives and its relationship to redox potential and lipophilicity. Exp. Parasitol. 99, 1–6.

Maya, J.D., Bollo, S., Núñez-Vergara, L.J., Squella, J.A., Repetto, Y., Morello, A., Perie, J., Chauvière, G., 2003. Trypanosoma cruzi: effect and mode of action of nitroimidazole and nitrofuran derivatives. Biochem. Pharmacol. 65, 999–1006.

Maya, J.D., Rodríguez, A., Pino, L., Pabon, A., Ferreira, J., Pavani, M., Repetto, Y., Morello, A., 2004. Effects of buthionine sulfoximine nifurtimox and benznidazole upon trypanothione and metallothionein proteins in Trypanosoma cruzi. Biol. Res. 37, 61–69.

Mazur, L., 2000. Radioprotective effects of the thiols GSH and WR-2721 against X-ray-induction of micronuclei in erythroblasts. Mutat. Res. 468, 27–33.

Melo, R.C., Fabrino, D.L., D'Avila, H., Teixeira, H.C., Ferreira, A.P., 2003. Production of hydrogen peroxide by peripheral blood monocytes and specific macrophages during experimental infection with Trypanosoma cruzi in vivo. Cell Biol. Int. 27, 853–861.

Mendoza, D.T., Ureña Gonzalez, L.D., Ortega-Barría, E., Capson, T.L., Ríos, L.C., 2003. Five new cassane diterpenes from Myrospermum frutescens with activity against Trypanosoma cruzi. J. Nat. Prod. 66, 928–932.

Michailowsky, V., Silva, N.M., Rocha, C.D., Vieira, L.Q., Lannes-Vieira, J., Gazzinelli, R.T., 2001. Pivotal role of interleukin-12 and interferon-gamma axis in controlling tissue parasitism and inflammation in the heart and central nervous system during Trypanosoma cruzi infection. Am. J. Pathol. 159, 1723–1733.

Molina, J., Martins-Filho, O., Brener, Z., Romanha, A.J., Loebenberg, D., Urbina, J.A., 2000. Activities of the triazole derivative SCH 56592 (posaconazole) against drug-resistant strains of the protozoan parasite Trypanosoma (Schizotrypanum) cruzi in immunocompetent and immunosuppressed murine hosts. Antimicrob. Agents Chemother. 44, 150–155.

Montalvetti, A., Bailey, B.N., Martin, M.B., Severin, G.W., Oldfield, E., Docampo, R., 2001. Bisphosphonates are potent inhibitors of Trypanosoma cruzi farnesyl pyrophosphate synthase. J. Biol. Chem. 276, 33930–33937.

Montalvetti, A., Rohloff, P., Docampo, R., 2004. A functional aquaporin co-localizes with the vacuolar proton pyrophosphatase to acidocalcisomes and the contractile vacuole complex of Trypanosoma cruzi. J. Biol. Chem. 279, 38673–38682.

Morello, A., 1988. The biochemistry of the mode of action of drugs and the detoxication mechanisms in Trypanosoma cruzi. Comp. Biochem. Physiol. C 90, 1–12.

Morello, A., Lipchenca, I., Cassels, B.K., Speisky, H., Aldunate, J., Repetto, Y., 1994. Trypanocidal effect of boldine and related alkaloids upon several strains of Trypanosoma cruzi. Comp. Biochem. Physiol., Pharmacol. Toxicol. Endocrinol. 107, 367–371.

Moreno, S.N., Docampo, R., Mason, R.P., León, W., Stoppani, A.O., 1982. Different behaviors of benznidazole as free radical generator with mammalian and Trypanosoma cruzi microsomal preparations. Arch. Biochem. Biophys. 218, 585–591.

Nagafuji, S., Okabe, H., Akahane, H., Abe, F., 2004. Trypanocidal constituents in plants: 4. Withanolides from the aerial parts of Physalis angulata. Biol. Pharm. Bull. 27, 193–197.

Nakajima-Shimada, J., Hirota, Y., Aoki, T., 1996. Inhibition of Trypanosoma cruzi growth in mammalian cells by purine and pyrimidine analogs. Antimicrob. Agents Chemother. 40, 2455–2458.

Nakayama, H., Ferreira, M.E., Rojas de Arias, A., Vera de Bilbao, N., Torres, S., Schinini, A., Fournet, A., 2001. Experimental treatment of chronic Trypanosoma cruzi infection in mice with 2-n-propylquinoline. Phytother. Res. 15, 630–632.

Nelson, D.J., Bugge, C.J., Krasny, H.C., Elion, G.B., 1973. Formation of nucleotides of (6–14C)allopurinol and (6–14C)oxipurinol in rat tissues and effects on uridine nucleotide pools. Biochem. Pharmacol. 22, 2003–2022.

Nelson, D.J., LaFon, S.W., Jones, T.E., Spector, T., Berens, R.L., Marr, J.J., 1982. The metabolism of formycin B in *Leishmania donovani*. Biochem. Biophys. Res. Commun. 108, 349–354.

Nesslany, F., Brugier, S., Mouries, M.A., Le, C.F., Marzin, D., 2004. In vitro and in vivo chromosomal aberrations induced by megazol. Mutat. Res. 560, 147–158.

O'Dwyer, P.J., Hamilton, T.C., LaCreta, F.P., Gallo, J.M., Kilpatrick, D., Halbherr, T., Brennan, J., Bookman, M.A., Hoffman, J., Young, R.C., Comis, R.L., Ozols, R.F., 1996. Phase I trial of buthionine sulfoximine in combination with melphalan in patients with cancer. J. Clin. Oncol. 14, 249–256.

Opperdoes, F.R., 1985. Biochemical peculiarities of trypanosomes, African and South American. Br. Med. Bull. 41, 130–136.

Oza, S.L., Tetaud, E., Ariyanayagam, M.R., Warnon, S.S., Fairlamb, A.H., 2002. A single enzyme catalyses formation of Trypanothione from glutathione and spermidine in *Trypanosoma cruzi*. J. Biol. Chem. 277, 35853–35861.

Padwa, A., Boonsombat, J., Rashatasakhon, P., Willis, J., 2005. Efficient construction of the oxatricyclo[6.3.1.0(0,0)]dodecane core of komaroviquinone using a cyclization/cycloaddition cascade of a rhodium carbenoid intermediate. Org. Lett. 7, 3725–3727.

Pastore, A., Federici, G., Bertini, E., Piemonte, F., 2003. Analysis of glutathione: implication in redox and detoxification. Clin. Chim. Acta 333, 19–39.

Paulino, M., Iribarne, F., Dubin, M., Aguilera-Morales, S., Tapia, O., Stoppani, A.O., 2005. The chemotherapy of Chagas' disease: an overview. Mini Rev. Med. Chem. 5, 499–519.

Paveto, C., Guida, M.C., Esteva, M.I., Martino, V., Coussio, J., Flawia, M. M., Torres, H.N., 2004. Anti-*Trypanosoma cruzi* activity of green tea (*Camellia sinensis*) catechins. Antimicrob. Agents Chemother. 48, 69–74.

Peluffo, G., Piacenza, L., Irigoin, F., Alvarez, M.N., Radi, R., 2004. L-arginine metabolism during interaction of *Trypanosoma cruzi* with host cells. Trends Parasitol. 20, 363–369.

Pez, D., Leal, I., Zuccotto, F., Boussard, C., Brun, R., Croft, S.L., Yardley, V., Ruiz Perez, L.M., González, P.D., Gilbert, I.H., 2003. 2,4-Diaminopyrimidines as inhibitors of Leishmanial and Trypanosomal dihydrofolate reductase. Bioorg. Med. Chem. 11, 4693–4711.

Pinge-Filho, P., Tadokoro, C.E., Abrahamsohn, I.A., 1999. Prostaglandins mediate suppression of lymphocyte proliferation and cytokine synthesis in acute *Trypanosoma cruzi* infection. Cell. Immunol. 193, 90–98.

Plum, J., Huang, C., Grabensee, B., Schror, K., Meyer-Kirchrath, J., 2002. Prostacyclin enhances the expression of LPS/INF-gamma-induced nitric oxide synthase in human monocytes. Nephron 91, 391–398.

Pozas, R., Carballo, J., Castro, C., Rubio, J., 2005. Synthesis and in vitro antitrypanosomal activity of novel Nifurtimox analogues. Bioorg. Med. Chem. Lett. 15, 1417–1421.

Prata, A., 2001. Clinical and epidemiological aspects of Chagas disease. Lancet, Infect. Dis. 1, 92–100.

Previato, J.O., Wait, R., Jones, C., DosReis, G.A., Todeschini, A.R., Heise, N., Previato, L.M., 2004. Glycoinositolphospholipid from *Trypanosoma cruzi*: structure, biosynthesis and immunobiology. Adv. Parasitol. 56, 1–41.

Ramírez, I., Carabot, A., Meléndez, P., Carmona, J., Jiménez, M., Patel, A.V., Crabb, T.A., Blunden, G., Cary, P.D., Croft, S.L., Costa, M., 2003. Cissampeloflavone, a chalcone-flavone dimer from *Cissampelos pareira*. Phytochemistry 64, 645–647.

Ramos-Ligonio, A., López-Monteon, A., Talamas-Rohana, P., Rosales-Encina, J.L., 2004. Recombinant SSP4 protein from *Trypanosoma cruzi* amastigotes regulates nitric oxide production by macrophages. Parasite Immunol. 26, 409–418.

Ribeiro, T.S., Freire-de-Lima, L., Previato, J.O., Mendonça-Previato, L., Heise, N., de Lima, M.E., 2004. Toxic effects of natural piperine and its derivatives on epimastigotes and amastigotes of *Trypanosoma cruzi*. Bioorg. Med. Chem. Lett. 14, 3555–3558.

Rodrigues, M.M., Ribeirao, M., Boscardin, S.B., 2000. CD4 Th1 but not Th2 clones efficiently activate macrophages to eliminate *Trypanosoma cruzi* through a nitric oxide dependent mechanism. Immunol. Lett. 73, 43–50.

Rodrigues Coura, J., de Castro, S.L., 2002. A critical review on Chagas disease chemotherapy. Mem. Inst. Oswaldo Cruz 97, 3–24.

Romanha, A.J., Alves, R.O., Murta, S.M., Silva, J.S., Ropert, C., Gazzinelli, R.T., 2002. Experimental chemotherapy against *Trypanosoma cruzi* infection: essential role of endogenous interferon-gamma in mediating parasitologic cure. J. Infect. Dis. 186, 823–828.

Ropert, C., Gazzinelli, R.T., 2004. Regulatory role of Toll-like receptor 2 during infection with *Trypanosoma cruzi*. J. Endotoxin Res. 10, 425–430.

Ropert, C., Almeida, I.C., Closel, M., Travassos, L.R., Ferguson, M.A., Cohen, P., Gazzinelli, R.T., 2001. Requirement of mitogen-activated protein kinases and I kappa B phosphorylation for induction of proinflammatory cytokines synthesis by macrophages indicates functional similarity of receptors triggered by glycosylphosphatidylinositol anchors from parasitic protozoa and bacterial lipopolysaccharide. J. Immunol. 166, 3423–3431.

Rosestolato, C.T., Dutra, J.M., De Souza, W., de Carvalho, T.M., 2002. Participation of host cell actin filaments during interaction of trypomastigote forms of *Trypanosoma cruzi* with host cells. Cell Struct. Funct. 27, 91–98.

Rottenberg, M.E., Castaños-Vélez, E., de Mesquita, R., Laguardia, O.G., Biberfeld, P., Orn, A., 1996. Intracellular co-localization of *Trypanosoma cruzi* and inducible nitric oxide synthase (iNOS): evidence for dual pathway of iNOS induction. Eur. J. Immunol. 26, 3203–3213.

Ruiz-Mesía, L., Ruiz-Mesia, W., Reina, M., Martínez-Díaz, R., de, I.C., Guadaño, A., González-Coloma, A., 2005. Bioactive Cinchona alkaloids from *Remijia peruviana*. J. Agric. Food Chem. 53, 1921–1926.

Saeidnia, S., Gohari, A.R., Uchiyama, N., Ito, M., Honda, G., Kiuchi, F., 2004. Two new monoterpene glycosides and trypanocidal terpenoids from *Dracocephalum kotschyi*. Chem. Pharm. Bull. (Tokyo) 52, 1249–1250.

Schinor, E.C., Salvador, M.J., Ito, I.Y., de Albuquerque, S., Dias, D.A., 2004. Trypanocidal and antimicrobial activities of *Moquinia kingii*. Phytomedicine 11, 224–229.

Schmidt, T.J., Brun, R., Willuhn, G., Khalid, S.A., 2002. Anti-trypanosomal activity of helenalin and some structurally related sesquiterpene lactones. Planta Med. 68, 750–751.

Senkovich, O., Pal, B., Schormann, N., Chattopadhyay, D., 2003. *Trypanosoma cruzi* genome encodes a pteridine reductase 2 protein. Mol. Biochem. Parasitol. 127, 89–92.

Sepúlveda-Boza, S., Cassels, B.K., 1996. Plant metabolites active against *Trypanosoma cruzi*. Planta Med. 62, 98–105.

Shinomiya, S., Naraba, H., Ueno, A., Utsunomiya, I., Maruyama, T., Ohuchida, S., Ushikubi, F., Yuki, K., Narumiya, S., Sugimoto, Y., Ichikawa, A., Oh-ishi, S., 2001. Regulation of TNFalpha and interleukin-10 production by prostaglandins I(2) and E(2): studies with prostaglandin receptor-deficient mice and prostaglandin E-receptor subtype-selective synthetic agonists. Biochem. Pharmacol. 61, 1153–1160.

Sipos, E.P., Witham, T.F., Ratan, R., Burger, P.C., Baraban, J., Li, K.W., Piantadosi, S., Brem, H., 2001. L-buthionine sulfoximine potentiates the antitumor effect of 4-hydroperoxycyclophosphamide when administered locally in a rat glioma model. Neurosurgery 48, 392–400.

Spector, T., Berens, R.L., Marr, J.J., 1982. Adenylosuccinate synthetase and adenylosuccinate lyase from *Trypanosoma cruzi*, Specificity studies with potential chemotherapeutic agents. Biochem. Pharmacol. 31, 225–229.

Steenkamp, D.J., 2002. Trypanosomal antioxidants and emerging aspects of redox regulation in the trypanosomatids. Antioxid. Redox Signal. 4, 105–121.

Stoppani, A.O., de Boiso, J.F., 1973. Oxidative phosphorylation in *Trypanosoma cruzi*. Experientia 29, 1494–1496.

Szajnman, S.H., Montalvetti, A., Wang, Y., Docampo, R., Rodríguez, J.B., 2003. Bisphosphonates derived from fatty acids are potent inhibitors of *Trypanosoma cruzi* farnesyl pyrophosphate synthase. Bioorg. Med. Chem. Lett. 13, 3231–3235.

Tarus, P.K., Machocho, A.K., Lang'at-Thoruwa, C.C., Chhabra, S.C., 2002. Flavonoids from *Tephrosia aequilata*. Phytochemistry 60, 375–379.

Temperton, N.J., Wilkinson, S.R., Meyer, D.J., Kelly, J.M., 1998. Overexpression of superoxide dismutase in *Trypanosoma cruzi* results in

increased sensitivity to the trypanocidal agents gentian violet and benznidazole. Mol. Biochem. Parasitol. 96, 167–176.

Thomson, L., Denicola, A., Radi, R., 2003. The trypanothione-thiol system in *Trypanosoma cruzi* as a key antioxidant mechanism against peroxynitrite-mediated cytotoxicity. Arch. Biochem. Biophys. 412, 55–64.

Turrens, J.F., 2004. Oxidative stress and antioxidant defenses: a target for the treatment of diseases caused by parasitic protozoa. Mol. Aspects Med. 25, 211–220.

Turrens, J.F., Watts Jr., B.P., Zhong, L., Docampo, R., 1996. Inhibition of *Trypanosoma cruzi* and *T. brucei* NADH fumarate reductase by benznidazole and anthelmintic imidazole derivatives. Mol. Biochem. Parasitol. 82, 125–129.

Uchiyama, N., Matsunaga, K., Kiuchi, F., Honda, G., Tsubouchi, A., Nakajima-Shimada, J., Aoki, T., 2002. Trypanocidal terpenoids from *Laurus nobilis* L. Chem. Pharm. Bull. (Tokyo) 50, 1514–1516.

Uchiyama, N., Kiuchi, F., Ito, M., Honda, G., Takeda, Y., Khodzhimatov, O.K., Ashurmetov, O.A., 2003. New icetexane and 20 norabietane diterpenes with trypanocidal activity from *Dracocephalum komarovi*. J. Nat. Prod. 66, 128–131.

Une, C., Andersson, J., Orn, A., 2003. Role of IFN-alpha/beta and IL-12 in the activation of natural killer cells and interferon-gamma production during experimental infection with *Trypanosoma cruzi*. Clin. Exp. Immunol. 134, 195–201.

Urbina, J.A., Payares, G., Sanoja, C., Lira, R., Romanha, A.J., 2003. In vitro and in vivo activities of ravuconazole on *Trypanosoma cruzi*, the causative agent of Chagas disease. Int. J. Antimicrob. Agents 21, 27–38.

Urbina, J.A., Concepción, J.L., Caldera, A., Payares, G., Sanoja, C., Otomo, T., Hiyoshi, H., 2004. In vitro and in vivo activities of E5700 and ER-119884, two novel orally active squalene synthase inhibitors, against *Trypanosoma cruzi*. Antimicrob. Agents Chemother. 48, 2379–2387.

Valko, M., Morris, H., Cronin, M.T., 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem. 12, 1161–1208.

Vanhoefer, U., Cao, S., Minderman, H., Toth, K., Skenderis, B.S., Slovak, M.L., Rustum, Y.M., 1996. D,L-Buthionine-(*S,R*)-sulfoximine potentiates in vivo the therapeutic efficacy of doxorubicin against multidrug resistance protein-expressing tumors. Clin. Cancer Res. 2, 1961–1968.

Viarengo, A., Burlando, B., Ceratto, N., Panfoli, I., 2000. Antioxidant role of metallothioneins: a comparative overview. Cell. Mol. Biol. (Noisy-le-grand) 46, 407–417.

Vieira, M., Dutra, J.M., Carvalho, T.M., Cunha-e-Silva, N.L., Souto-Padron, T., Souza, W., 2002. Cellular signaling during the macrophage invasion by *Trypanosoma cruzi*. Histochem. Cell Biol. 118, 491–500.

Waghabi, M.C., Keramidas, M., Feige, J.J., Araujo-Jorge, T.C., Bailly, S., 2005. Activation of transforming growth factor beta by *Trypanosoma cruzi*. Cell. Microbiol. 7, 511–517.

Watanabe, T., Sagisaka, H., Arakawa, S., Shibaya, Y., Watanabe, M., Igarashi, I., Tanaka, K., Totsuka, S., Takasaki, W., Manabe, S., 2003. A novel model of continuous depletion of glutathione in mice treated with L-buthionine (*S,R*)-sulfoximine. J. Toxicol. Sci. 28, 455–469.

Wilkinson, S.R., Kelly, J.M., 2003. The role of glutathione peroxidases in trypanosomatids. Biol. Chem. 384, 517–525.

Wilkinson, S.R., Temperton, N.J., Mondragon, A., Kelly, J.M., 2000. Distinct mitochondrial and cytosolic enzymes mediate trypanothione-dependent peroxide metabolism in *Trypanosoma cruzi*. J. Biol. Chem. 275, 8220–8225.

Wilkinson, S.R., Obado, S.O., Mauricio, I.L., Kelly, J.M., 2002. *Trypanosoma cruzi* expresses a plant-like ascorbate-dependent hemoperoxidase localized to the endoplasmic reticulum. Proc. Natl. Acad. Sci. U. S. A. 99, 13453–13458.

Woolsey, A.M., Sunwoo, L., Petersen, C.A., Brachmann, S.M., Cantley, L.C., Burleigh, B.A., 2003. Novel PI 3-kinase-dependent mechanisms of trypanosome invasion and vacuole maturation. J. Cell. Sci. 116, 3611–3622.

World Health Organization, 2002. Control of Chagas disease: second report of the WHO expert committee. Technical Report Series, vol. 905. WHO, Geneva.

Zahoor, A., Lafleur, M.V., Knight, R.C., Loman, H., Edwards, D.I., 1987. DNA damage induced by reduced nitroimidazole drugs. Biochem. Pharmacol. 36, 3299–3304.

Zuccotto, F., Brun, R., González, P.D., Ruiz Pérez, L.M., Gilbert, I.H., 1999. The structure-based design and synthesis of selective inhibitors of *Trypanosoma cruzi* dihydrofolate reductase. Bioorg. Med. Chem. Lett. 9, 1463–1468.