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REVIEW ARTICLE

Polyamines and Related Nitrogen Compounds in the Chemotherapy of Neglected Diseases Caused by Kinetoplastids

Juan A. Bisceglia*, María C. Mollo, Nadia Gruber and Liliana R. Orelli*

Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. CONICET, Buenos Aires, Argentina

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Abstract: Neglected diseases due to the parasitic protozoa Leishmania and Trypanosoma (kinetoplastids) affect millions of people worldwide, and the lack of suitable treatments has promoted an ongoing drug discovery effort to identify novel nontoxic and cost-effective chemotherapies. Polyamines are ubiquitous small organic molecules that play key roles in kinetoplastid parasites metabolism, redox homeostasis and in the normal progression of cell cycles, which differ from those found in the mammalian host. These features make polyamines attractive in terms of antiparasitic drug development. The present work provides a comprehensive insight on the use of polyamine derivatives and related nitrogen compounds in the chemotherapy of kinetoplastid diseases. The amount of literature on this subject is considerable, and a classification considering drug targets and chemical structures were made. Polyamines, aminoalcohols and basic heterocycles designed to target the relevant parasitic enzyme trypanothione reductase are discussed in the first section, followed by compounds directed to less common targets, like parasite SOD and the aminopurine P2 transporter. Finally, the third section comprises nitrogen compounds structurally derived from antimalaric agents. References on the chemical synthesis of the selected compounds are reported together with their *in vivo* and/or *in vitro* IC_{50} values, and structure-activity relationships within each group are analyzed. Some favourable structural features were identified from the SAR analyses comprising protonable sites, hydrophobic groups and optimum distances between them. The importance of certain pharmacophoric groups or amino acid residues in the bioactivity of polyamine derived compounds is also discussed.

Keywords: Neglected diseases, Leishmania, Trypanosoma, Polyamines, Nitrogen compounds, Drug research.

1. INTRODUCTION

African trypanosomiasis, Chagas disease and the leishmaniases are neglected tropical diseases [1] according to the WHO. These vector-borne diseases of public health concern are caused by closely related protozoan parasites called trypanosomatids or kinetoplastids: *Trypanosoma brucei* (TB), *Trypanosona cruzi* (TC) and *Leishmania spp* (L), respectively. Trypanosomatids share certain structural features, *i. e.* they possess a single mitochondrion with a structured DNA body, the kinetoplast, located near the flagellum, and specific organelles for glycolysis called glycosomes. Kinetoplastids have metabolic pathways and cellular functions that diverge from those found in mammals, among which their specific thiol metabolism is the most relevant. These differential biochemical features are attractive for drug discovery, and considerable efforts have been devoted to the development of drugs selectively targeting the parasites unique metabolism. On the other hand, the similarities between the three lineages have led to a unified approach in drug discovery, in which new potential therapeutic agents are tested against two or more kinetoplastids.

A brief account of each individual kinetoplastid parasitic disease is given below, including their description, prevalence and parasitic cell cycles [2].

Human leishmaniasis is caused by more than 20 species of obligate intracellular protozoa of the genus *Leishmania* that infect mammals, including *L. donovani*, *L. infantum* (also known as *L. chagasi* in the New World), *L. amazonensis*, *L. tropica* and *L. major*, among others. These species are morphologically indistinguishable, but they can be differentiated by molecular markers, and also show different drug sensitivities [3]. Leishmaniasis is the second-largest parasitic killer in the world (after malaria) [4], responsible for near one million infections each year worldwide [5].

Leishmaniasis is transmitted by the bite of infected sandflies, which inject the infective stage of the parasite (promastigotes) during blood meals. Promastigotes are then phagocytized by macrophages, within which they transform into amastigotes, the tissue stage of the parasite. Amastigotes multiply within phagolysosomes by binary fission and infect other mononuclear phagocytic cells. Sandflies become in-

*Address correspondence to this author at the Departamento de Química Orgánica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. CONICET, Buenos Aires, Argentina;
E-mails: lorell@ffyb.uba.ar (L.R.O); jab@ffyb.uba.ar (J.A.B.)

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fected by ingesting infected cells during blood meals. In the vector, amastigotes transform into promastigotes, develop in the gut, and migrate to the proboscis [6].

There are three types of leishmaniasis. The most common is cutaneous leishmaniasis [7], which exists in many different forms. In most cases, patients self-cure after 6-18 months, leaving scarred tissue. As most disfiguring lesions are located in the face, arms and legs, it is a stigmatizing disease. Visceral leishmaniasis, also known as kala-azar [8], is potentially fatal and affects several internal organs (usually spleen, liver, and bone marrow). The disease develops within months or even years after the sandfly bite, causing fever, weight loss, spleno and hepatomegaly, anemia, leukopenia and thrombocytopenia [9]. Some patients develop, after treatment, a diffuse cutaneous form termed post kala-azar leishmaniasis. Finally, mucosal leishmaniasis is less common and causes deformative ulcerations in the mucous membranes (nose, mouth, and throat). Different factors (parasite and host, among others) determine if the infection will become symptomatic and whether cutaneous or visceral leishmaniasis results.

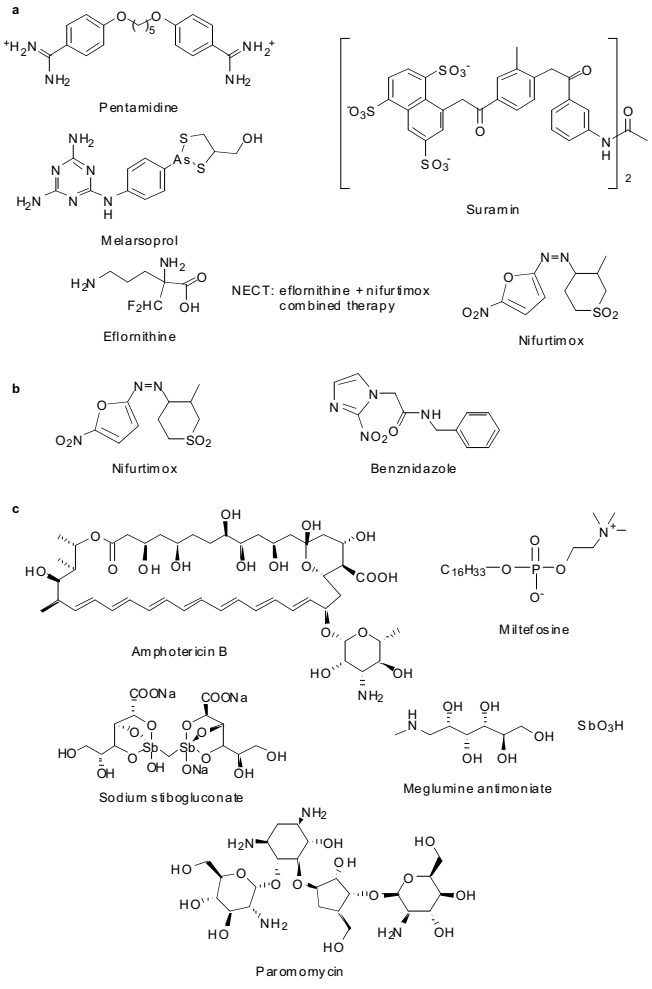


Fig. (1). Medication currently in use for HAT (a), Chagas disease (b) and visceral leishmaniasis (c).

Human African Trypanosomiasis (HAT) [5], also known as sleeping sickness, is restricted to the natural habitat of the vector (tsetse fly, genus *Glossina*), in sub-Saharan Africa. The HAT can be caused by two parasites. *Trypanosoma brucei gambiense* (*TB gambiense*), which causes about 95% of reported infections and is anthroponotic and chronic. *Trypanosoma brucei rhodesiense* (TB *rhodesiense*) is a zoonotic infection affecting wild ungulates and cattle. A third species, *T.b. brucei* (*TB brucei*), does not infect humans.

The TB *gambiense* HAT disease progresses through an early haemo-lymphatic stage (stage 1) to a late CNS infection (stage 2). Most patients undergo a relatively asymptomatic first stage and come for treatment with second stage disease. The stage 2 CNS infection is characterized by an immunopathological response in the brain and a breakdown of neurological function [5].

The life cycle of TB [10] initiates when an infected tsetse fly injects metacyclic trypomastigotes into skin tissue of the host. The parasites enter the lymphatic system and into the bloodstream, where they transform into bloodstream trypomastigotes, are carried to other localizations, reaching other fluids (lymph, spinal fluid), where they continue their replication by binary fission. The vector then becomes infected with bloodstream trypomastigotes during a blood meal on an infected mammalian host. In the fly's midgut, the parasites differentiate into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission. The life cycle of TB does not involve intracellular stages.

Chagas disease [11, 12] or South American Trypanosomiasis is caused by the parasite *Trypanosoma cruzi* (TC) and transmitted by *Triatoma infestans* (kissing bugs). It is restricted mainly to Central and South America, with an estimated 8-10 million infected people and about 50,000 new cases each year. Recently, the disease has extended to populations both in Europe and the US due to migrations. *Trypanosoma cruzi* is divided into six lineages with a different distribution that can be characterized by genetic markers. The disease has three different stages, the acute, indeterminate and chronic phases. In the acute phase, after infection, the parasite spreads throughout the mammalian host (parasitemia), causing inflammatory lesions at the site of infection, followed by a self-limited febrile state and/or allergic reaction, although this stage may be asymptomatic. The indeterminate stage may last 5-25 years, being characterized by a very low parasite burden, and is generally asymptomatic. During this stage, the parasites establish in the target organs, forming amastigote nests. In the last stage (chronic phase), various pathologies emerge due to damage to muscle or nerve tissues. Among them, Chagasic cardiomyopathy is the most frequent, followed by digestive tract pathology (megacolon, megaesophagus, hepatomegaly). An important element in Chagas disease is a chronic and diffuse inflammation in the infected tissues, as a result of immune response maintained by a minimum parasite load [13].

The life cycle of TC [14] initiates when an infected vector takes a blood meal and releases trypomastigotes in its faeces near the bite wound. Trypomastigotes enter the host through the wound or through intact mucosal membranes. Inside the host, they invade cells near the site of inoculation, where they differentiate into intracellular amastigotes, which multiply by binary fission, differentiate into trypomastigotes,

and are then released into the circulation as bloodstream trypomastigotes. Trypomastigotes infect cells from a variety of tissues and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle. The bloodstream trypomastigotes do not replicate. Replication resumes only when the parasites enter another cell or are ingested by another vector, which becomes infected by feeding on human or animal blood containing circulating parasites. The ingested trypomastigotes transform into epimastigotes in the vector's midgut. The parasites multiply in the midgut and differentiate into infective metacyclic trypomastigotes in the hindgut. *Trypanosoma cruzi* can also be transmitted through blood transfusions, organ transplantation, transplacentally, by ingestion of contaminated food and in laboratory accidents.

In spite of the sanitary burden that kinetoplastid diseases inflict on the affected countries and of the research efforts devoted to the problem, very little significant progress has been attained regarding the discovery of new therapeutic agents for their treatment. Medication currently in use for the HAT, Chagas disease and leishmaniasis [15] is listed in Fig. **1**. The existing treatments, however, suffer from serious disadvantages. Most of the available drugs show toxicity either in their acute or chronic administration, and very few among them are available as oral formulations. Furthermore, their efficacy is variable for different parasitic strains and also in the different stages of the diseases, and many cases of drug resistance have been reported [3]. Therefore, there is an urgent need to identify and develop new therapeutic alternatives.

Drug discovery against kinetoplastid [2] has followed three main approaches [15]. Target-based approaches involve screening for inhibitors against a specific enzyme, which are subsequently optimized to be active in a cellular model. This strategy has in general not been successful for kinetoplastid diseases. Very often, compounds with high affinity for the target enzyme display poor bioactivity against the parasite. Alternatively, phenotypic approaches involve screening for compounds with growth inhibition or biocidal properties against the intact parasite, usually *in vitro*. In the third approach, drug repurposing, molecules previously developed (and often approved) for an alternative use are tested as new anti-trypanosomatid agents [15].

As previously mentioned, for neglected diseases in general target-based approaches have led to poor results, although for some compounds the molecular targets are known [5]. On the other hand, phenotypic approaches have produced interesting structures to be used in kinetoplastid diseases, like nitroheterocycles (Fexinidazole, DNDi-0690, Delamanid), oxaboroles (SCYX-7158 and DNDi6148), diamidines (Parafuramidine) and the 8-aminoquinoline derivative Sitamaquine [5].

Finally, many drugs currently in use for the treatment of neglected tropical diseases were 'repositioned' from anticancer, antipsychotic, antibacterial, antifungal and antihelminthic agents, including amphotericin B (an antifungal), miltefosine (an antineoplastic agent) and the antibiotic paromomycin, among others [16, 17].

In spite of the significant efforts over the last decades towards the discovery of new suitable antikinetoplastid agents, and the support from both public and private initiatives to address the problem, currently no new candidates are in clinical development for leishmaniasis or Chagas disease, and there is still a great need for new (ideally oral) drugs to treat each trypanosomatid disease.

Polyamines are small, water-soluble organic molecules containing two or more amino groups that are found in virtually all living cells. These molecules play key roles in the normal progression of cell cycles, including cellular proliferation, growth, differentiation, survival and signalling [18]. Naturally occurring polyamines include the diamine putrescine, the triamine spermidine and the tetraamine spermine. Less common polyamines have also been reported: 1,3-diaminopropane, cadaverine (1,5-diaminopentane), symnorspermidine, symhomospermidine, aminopropylcadaverine, aminobutylcadaverine, norspermine, thermospermine, among others. Chemical structures are displayed in Fig. (**2**).

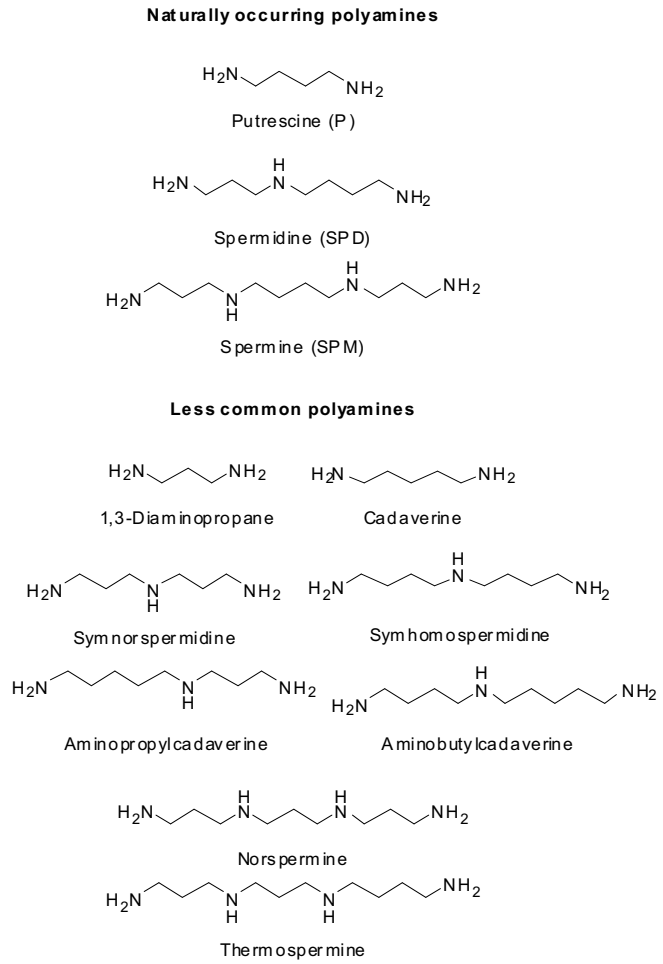


Fig. (2). Natural polyamines.

Due to their chemical nature, polyamines bind both specifically and non-specifically to different kinds of macromolecules such as nucleic acids, soluble and membrane proteins, enzymes, lipids, and also to small, negatively charged molecules present in the nucleus and cytoplasm. After many years from their initial isolation and characterization, and in spite of the considerable amount of research efforts devoted

to the study of polyamines [19-22], their ubiquity and multifunctionality has hampered the unequivocal identification of their specific biochemical functions, and a unifying concept to interpret their molecular roles is still missing [23].

It is however well established that polyamines are involved in many pathological states, the most important and probably best studied of which is cancer. In fact, abnormal levels of polyamines are nearly always associated with tumour initiation and growth [24], and synthetic polyamine analogues or specific inhibitors disrupting their biosynthetic pathways possess antineoplastic activity [25]. The involvement of polyamines in cancer derives, although not exclusively, from their ability to interact with nucleic acids altering gene expression regulation at the transcriptional and post-transcriptional level, chromatin structure and stability, RNA stabilization and apoptosis [26], among other relevant biochemical processes [27]. Due to their multiple biochemical functions, polyamines have been related to many conditions, including neurodegenerative and infectious diseases, metabolic disorders and aging [28]. The most relevant among them are Alzheimer´s and Parkinson´s diseases, rheumatoid arthritis, systemic lupus, psoriasis, parasitic infections, chronic renal failure, liver cirrhosis and cystic fibrosis [29].

Another essential role of polyamines in eukaryotic cells is the biosynthesis of hypusine, which requires spermidine as a substrate [30]. Hypusination of protein eIF5A is necessary for its activity, related to protein synthesis and other biochemical processes involved in cell growth and viability. Hypusination also plays an important role in viral replication, in which polyamines regulate DNA and RNA polymerization, nucleic acid packaging, and protein synthesis [31]. Consequently, blockage of polyamine synthesis is emerging as a promising broad-spectrum antiviral approach [32]. Polyamines are also capable of interacting with ion channels [33], acting as modulators of NMDA, AMPA and cholinergic receptors and regulating K^+ and Ca^{++} channels [34].

Biosynthetically, polyamines derive from aminoacids, and the levels of putrescine, spermidine and spermine are highly controlled by complex interactions between their *de novo* synthesis, retro-conversion, degradation, efflux and uptake [35].

Biosynthesis of polyamines [23] starts from arginine, which is hydrolyzed to ornithine by arginase. Decarboxylation of ornithine to putrescine is catalyzed by Ornithine Decarboxylase (ODC), the rate-limiting enzyme of the pathway. *S*-Adenosylmethionine (SAM) is decarboxylated by *S*-Adenosylmethionine Decarboxylase (AdoMetDC) to Decarboxylated *S*-Adenosylmethionine (dcSAM), the aminopropyl group donor for spermidine and spermine synthesis. Then, the aminopropyl transferase spermidine synthase (SpdSyn) catalyzes the reaction between putrescine and dcSAM leading to spermidine. A second diaminopropyl unit is then added to spermidine by Spermine Synthase (SpmS) to produce SPM. The catabolic enzymes SSAT (spermidine/spermine-N^1-acetyltransferase) and PAO (Polyamine Oxidase) provide a pathway for back conversion of spermine to spermidine and of the latter to putrescine. SSAT converts spermidine and spermine to their *N*-acetylation products, which are then transformed respectively to spermidine and putrescine by PAO, releasing H_2O_2 and acetoamidopropanol. Polyamine uptake occurs by a caveolar endocytotic pathway [36] and their efflux involves the amino acid transporter SLC3A2, which is colocalized with the polyamine catabolic enzyme SSAT, facilitating excretion of acetylated polyamines [37].

In mammals, the enzyme ODC is the limiting factor for the biosynthetic path leading to putrescine and the higher polyamines. Its level is tightly regulated at different stages (transcription and translation), and it changes in response to different stimuli among which polyamines concentration is important. ODC has a short half-life (< 1h) [38] and its turnover is ubiquitine independent and takes place *via* the 26S proteasome [39]. Degradation of ODC is catalyzed by the protein antizyme (AZ), which inhibits ODC monomers dimerization and catalyzes its degradation by the proteasome [40]. Antizyme also acts on polyamine levels by suppressing their intake and increasing their efflux [45] Its activity is in turn regulated by polyamine levels and by the expression of Antizyme Inhibitor (AZi) [41]. Another important enzyme regulating polyamines homeostasis is AdoMetDC, which is upregulated by putrescine and downregulated by spermidine and spermine [42]. The key catabolic enzyme SSAT can be induced by different stimuli including high polyamine levels, hormones, cytokines and stress, among other factors [43]. A detailed description of polyamine biosynthesis and its regulation is provided in two recent reviews [23, 44].

Polyamine biosynthesis and homeostasis have attracted much attention in terms of drug development [45-47]. In contrast to the detailed description now available of polyamine regulation in mammals, the mechanisms by which kinetoplastids maintain polyamine homeostasis are still under investigation. In spite of this, some key differences between parasite and host are well understood [48]. For example, TC lacks ODC and SpmS and depends on efficient putrescine uptake and AdoMetDC for spermidine synthesis. TB and L also lack SpmS, and ODC and AdoMetDC have long half-lives (>6 h) if compared to the same enzymes in mammals [46]. In fact, the selectivity of the ODC inhibitor eflornithine has been attributed to the rapid turnover of mammalian ODC. In mammals, the drug covalently binds to the enzyme, and the eflornithine-ODC complex is rapidly replaced by newly synthesized ODC. In contrast, the inactivated enzyme within the parasite is slowly replaced, resulting in critically low putrescine levels which can eventually stop parasite growth and multiplication. Moreover, homologues of ODC Antizyme and its inhibitor have not been found in kinetoplastids. It has been demonstrated that ODC, AdoMetDC or spermidine synthase are essential for L and TB, while TC depends on exogenous putrescine or spermidine for its survival [46].

Another distinctive feature of trypanosomatids, which has been targeted for drug design, is their thiol metabolism, which is linked to polyamines [49]. Trypanothione (N^1,N^8-bis(glutathionyl)spermidine, $T(SH)_2$, or TS_2, in its oxidized form, Fig. **3**) is a low molecular weight glutathione-spermidine conjugate unique to kinetoplastids [50] that plays a key role in the parasites redox homeostasis [51].

Similar to the function of glutathione in mammals, in trypanosomatids, $T(SH)_2$ participates in several processes

that require reduction equivalents [52]. Together with Tryparedoxin (TXN) and Tryparedoxin Peroxidase (TXNPx), $T(SH)_2$ participates in a complex peroxidase system that mediates the reduction of several proteins, ribonucleotides, and different peroxides and hydroperoxides [53]. It also reacts with electrophiles, being involved in detoxification of several species like heavy metals and xenobiotics, including electrophilic metabolites from nifurtimox and benznidazole [54]. The biosynthesis of $T(SH)_2$ starts from spermidine and involves two reactions in which two molecules of glutathione are consecutively added to the terminal nitrogens of the polyamine. Both steps are catalyzed by the enzyme Trypanothione Synthetase (TryS) and require ATP for glutathione activation [55]. The reduction reactions in which $T(SH)_2$ is involved originate Trypanothione Disulfide (TS_2), which is recycled in trypanosomatids to $T(SH)_2$ by trypanothione reductase (TR). TR is an NADPH-dependent flavoenzyme located mainly in the cytosol, but that can also be found in small proportions in the mitochondria and in glycosomes. TR, together with TryS and tryparedoxin-dependent peroxidase are absent in humans and essential for kinetoplastids in order to maintain the parasites redox environment and address oxidative stress [56]. The crystal structure of *L. infantum* TR has been solved in its oxidized state and in the reduced state in complex with NADPH and Sb(III) [57]. The enzyme consists of two identical monomers, each of which contains three different domains, namely the FAD-binding domain, the NADPH-binding and the interface domain [58]. The trypanothione-binding site is located at the dimeric interface, which is mostly nonpolar and is contains the NADP site (N-site) and the active site (G-site), connected by the flavin ring of FAD and a redox active disulfide bridge. Besides, a putative binding site (allosteric site) exists in the dimer interface region [59].

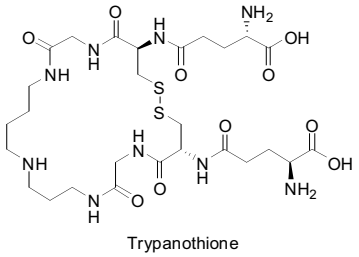


Fig. (3). Trypanothione structure (TS_2).

The strategic importance of parasites TR together with the differences between TR and the homologous enzyme in the host (GR) has encouraged extensive research on potential selective inhibitors of this enzyme [60]. Other targets related to the thiol metabolism of kinetoplastids have also been explored. These include the enzyme Glutamate-Cysteine Ligase (GCL), which is the limiting step in glutathione synthesis and crucial for the parasite's survival, and polyamine uptake in TC [57, 61].

The literature both on polyamines in trypanosomatids and on kinetoplastid diseases is very abundant. However, no specific material on the use of polyamines and related compounds in the therapy of neglected diseases caused by kinetoplastids is available up to date. This prompted us to compile and systematize the existing information, providing a comprehensive insight into this subject. The present review covers the literature from 2000 to 2016 and is organized in different sections taking into account the main research lines explored by different groups in this field.

2. POLYAMINES

2.1. Polyamines Designed to Target Trypanothione Reductase

As mentioned before, Trypanothione Reductase (TR) is an interesting drug target for the treatment of *T. cruzi* (TC) infections, since the substrate scope is different from the equivalent (Human) Glutathione Reductase (HGR) although both enzymes share 40% of their primary structures [62]. In this section, some examples will be presented of compounds pondered as selective inhibitors of TCTR.

Several studies have shown that structurally diverse compounds can bind reversibly to the active site of TR, including some devoid of a cyclic structure, a disulfide or γ-glutamyl residues. Compounds that bind to the active site, however, generally contain amino or other groups bearing a positive charge at physiological pH, and/or hydrophobic residues.

Considering these general structural requisites for competitive inhibitors of TCTR, in 1995 Sullivan designed a series of structures based on *N*-arylmethyl or *N*-acyl derivatives of spermine and spermidine. All the compounds but N^1, N^8-dibenzoylspermidine **8** proved to be competitive inhibitors of a recombinant TR preparation (SG5 *E. coli* strain). The compounds were neither TR substrates nor HGR inhibitors, thus complying with the selectivity requirement. Best results were obtained with N^1, N^8-bis(2-naphtylmethyl)spermidine **12**, N^4, N^8-bis(2-naphtylmethyl/3-phenylpropyl)spermines **14** and **15** (K_i = 9.5, 3.5 and 5.5 μM, respectively), while free or acylated terminal amino groups in a spermidine scaffold led to compounds with the lowest activities [63].

In subsequent work of this group, the inhibitory effects of several spermidine derivatives were determined. As expected, those containing hydrophobic aromatic substituents were competitive inhibitors of TCTR, and N^4-acyl spermidines were less effective than the corresponding N^4-alkyl derivatives. None of the new compounds was more potent than N^1, N^8-bis(2-naphthylmethyl)spermidine **12**. The differential inhibitory effects observed between alkylated and acylated spermidines were attributed to differences in their protonation states and to loss of conformational flexibility caused by hindered rotation about the (O)C-N bond. The second assumption would explain why N^4-(benzyloxycarbonyl)spermidine **2** shows some inhibitory activity (K_i = 280 μM) while the benzoyl analogue **6** is inactive [64].

A new screening involving a wider number of compounds was carried out later by the same group, and several spermine and spermidine derivatives were synthesized and tested both in TCTR and *in vitro* growth inhibition assays. Although some compounds were potent trypanocides *in vitro* (Table **1**), they displayed no trypanocidal properties *in vivo*. Additionally, *Ki* and IC_{50} did not correlate satisfactorily [65].

The low *Ki* and IC_{50} values displayed by spermine **14** in the preceding work prompted Li to study several *N*-(3-

phenylpropyl) spermidine and spermine derivatives. Thus, compounds with different degrees of *N*-3-phenylpropyl (PhPr) substitution were prepared (Table **2**) and found to be potent and selective competitive inhibitors of TCTR and also to possess trypanocidal effect.

The authors found that inhibition of TCTR increased with increasing number of 3-phenylpropyl substituents, as shown by comparison of the K_i values for compounds **25** and **28**, but the hexa(phenylpropyl) derivative **29** was less effective, indicating that at this point the beneficial effect of an increased lipophilicity is limited by excessive steric hindrance. A comparison between the K_i of positional isomers suggests that, for this class of compounds, the hydrophobic interactions have a greater influence on the binding to TR than the net charge of the inhibitor. The studied compounds displayed *in vitro* trypanocidal activities which correlated loosely with TCTR inhibition ability. The observed discrepancies were attributed to the presence of polyamine oxidase in the culture medium. However, the consideration of differential trypanosomal cell membrane permeability cannot be overlooked [66].

Following the known selective TCTR polyamine-hydrophobic group basic structure, Salmon *et al*. selected a weak PKC inhibitor, the monoindolylmaleimide modified polyamine **33**, as the lead compound (IC_{50} = 38 μM). Three different alternative coupling of the building blocks was intended: one or two polyamine arms linked to the maleimide scaffold or a single polyamine bearing two maleimide moieties. Three different polyamines were used, two of them (norspermidine and 1,4-bis(3-aminopropyl)piperazine) possessing two terminal primary amino groups and one (4-methyl-1-(3-aminopropyl)piperazine) having a single primary amino group. Table **3** shows the structures of the assayed compounds together with their IC_{50} values. Although some of the compounds showed promising inhibition values, one of them being a stronger inhibitor than clomipramine, none was active against TC and TB trypomastigotes or LI promastigotes in the 1.5-12.5 μM range [67].

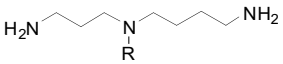
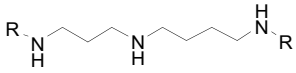
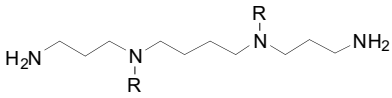
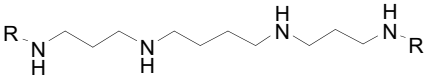
Chibale designed a series of novel tricyclic polyamines based on the xanthene nucleus as a hydrophobic motif. Starting from polyamine **41** (Table **4**) as a core bifunctional molecule [68], sulfonyl and carbamoyl derivatives (**42-46**) were prepared. These compounds resulted in lower inhibition than the parent compound, while tetraamino and bis(carbamoylvinyl) derivatives **47-49** showed IC_{50} values comparable to promazine and clomipramine [69].

For some time, the most potent inhibitor described was a tricyclic amine, clomipramine (with an approximate *Ki* of 6.5 μM against TCTR) [70]. This and other tricyclic drugs are known to inhibit TCTR although their CNS activity and numerous drug interactions render them inappropriate as parasiticides [71]. Bearing this in mind, and using 5-aminodibenzocycloheptane as a surrogate for the dibenzoazepine ring [72]. O'Sullivan studied a series of *N*-(5-dibenzosuberyl) di and polyamines. All of the compounds were selective and competitive TR inhibitors (Table **5**). Since N^4, N^8-bis(dibenzosuberyl)spermine **55**, the analogous spermidine **54** and norspermidine **53** were more potent than clomipramine, the importance of additional protonable nitrogens plus a hydrophobic N^4-substituent was emphasized. These results are in line with the previously discussed ones and suggest the importance of the interaction of a suitable flat substituent with a hydrophobic cleft in the vicinity of the binding site for competitive TCTR inhibitors. In a close analogy with 3-phenylpropyl and 2-naphtylmethyl derivatives (**12**, **14** and **15**) discussed above, the lower K_i values corresponded to spermidine **54** and spermine **55**, being the latter the most potent inhibitor in the series. Interestingly, N^4-(5-dibenzosuberyl)norspermidine **53** showed a K_i slightly lower than clomipramine itself, but almost twofold higher than the spermidine analogue **54**, highlighting the importance of an additional protonation site and also the existence of distance requirements between cationic groups. The compounds were also tested against the bloodstream form of *T. brucei spp*. In all cases, IC_{50} values were lower than 10 μM. No correlation was found between K_i and IC_{50}, reflecting that other factors besides inhibitor potency (probably related to cell membrane permeability and compound solubility) may play an important role in the bioactivity of these compounds. The spermine and spermidine derivatives were also tested *in vivo* but did not increase the infected animals survival. Moreover, at higher doses and although the animals lived longer than the control, symptoms of cytotoxicity appeared. A docking study of these compounds showed that the chlorine atom in clomipramine offers additional stabilization to enzyme-inhibitor complexes compared to DBS substituted polyamines. The increased binding ability of concave-shaped DBS compared to planar naphthyl groups was also recognized [73].

An alternative approach to the investigation of trypanocidal compounds related to tricyclic antidepressants but devoid of central effects is a molecular simplification of phenothiazines by heterocyclic ring opening leading to diarylsulfides and related compounds.

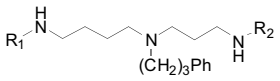
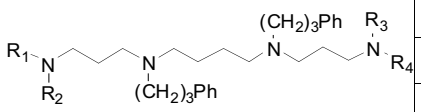
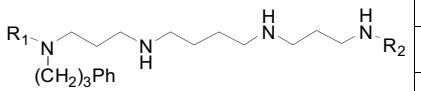
Various groups devoted their efforts to the rational design and computational study of the interaction of this family of compounds with TR. In this line, Baillet *et al*. chose compound **56** (Table **6**) as hit for TR inhibition assays by means of high-throughput screening. Since the active site of TR is richer in hydrophobic and cationic binding sites than HGR, a model of interaction of **56** with TR in which the piperazine moiety interacts with Glu465' and Glu466' was originally proposed by Baillet and then improved by Stump [74,75]. The aromatic phenylthio moiety undergoes favourable interactions with Trp21 and Met113, as already proposed for tricyclic TR inhibitors [72b]. It is worth noting that interactions of the arylamino group with Glu18 are related to TR specificity and might be of importance for the selective binding of diaryl sulfide-based inhibitors. This hypothesis was supported by the co-crystal structure of TR with mepacrine, where the inhibitor takes part in a water-mediated H-bonding to Glu18 [76]. Synthetic derivatives of **56** with modifications in the side chain of the diphenyl sulphide were proposed by Baillet with the aim of unveiling the optimal distance for the interaction with both acidic regions, Glu 18, Glu465'-Glu466'. An additional amino group of **56** did not modify inhibition, but its acylation suppressed anti-TR activity. Although some derivatives acted as inhibitors, they did not display *in vitro* activity for *T. cruzi* or *T. brucei* except for compound **57**, which showed good anti-*T. brucei* activity.

Table 1. TCTR inhibitory properties of miscellaneous acyl and aralkyl polyamines [65].

Polyamine	Nº	R	*Ki* (μM)	IC_{50} (μM)	
				TBb[a]	TBr[b]
	**1**	H	>2000	>100	>100
	2	Cbz	280		
	3	2-Naphtylmethyl	108		
	4	Boc	>2000		
	5	Ac	>2000		
	6	Bz	>2000		
	7	Bn	185		
	**8**	Bz	>2000		
	9	F_3CCO	>2000	0.63	0.61
	10	Ac	>2000		
	11	CBz	311		
	12	2-Naphtylmethyl	9.5		
	**13**	Bn	19		
	14	3-Phenylpropyl	3.5	0.66	0.66
	15	2-Naphtylmethyl	5.5	0.82	0.82
	16	Boc	>2000		
	17	Cbz	81		
	**18**	H	>2000	-	-
	19	Phenylacetyl	114		
	20	Bn	153		

a: Lab 110 is a drug sensitive *T. b. brucei* strain; b*:* K.243-As-10-3 is a pentamidine and melarsoprol resistant clinical TB *rhodesiense* isolate.

Table 2. TCTR inhibitory properties phenylpropyl substituted polyamines [66].

-	-					K_i (μM)	IC_{50} (μM)	
	-	R_1	R_2	-	-		TBb[a]	TBr[b]
	**22**	H	H			14.1	ND	ND
	23	PhPr[c] H	H PhPr	*Mixture of isomers*		4.67	0.31	0.57
	24	PhPr	PhPr			3.50	0.22	0.46
		R_1	R_2	R_3	R_4			
	**25**	H	H	H	H	3.48	0.66	0.66
	26	PhPr	H	H	H	1.38	0.27	0.16
	27	PhPr	PhPr	H	H	0.614	0.12	0.15
	28	PhPr	PhPr	PhPr	H	0.151	3.05	5.5
	29	PhPr	PhPr	PhPr	PhPr	10.3	22	24
		R_1	R_2	-	-	-	-	-
	**30**	H	H	-	-	37.6	-	-
	31	H	PhPr	-	-	12.3	0.81	2.2
	32	PhPr	PhPr	-	-	3.62	0.40	0.53

a*:* Lab 110 is a drug sensitive *T. b. brucei* strain.
b:K.243-As-10-3 is a pentamidine and melarsoprol resistant clinical *T. b. rhodesiense* isolate.
c: 3-phenyl-1-propyl

Table 3. TCTR inhibitory properties of indolylmaleimides [67].

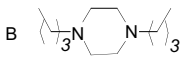
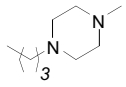
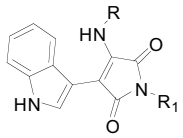
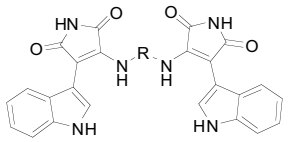
-	-	Nº	R	R_1	IC_{50} (μM)
Amines A -$(CH_2)_3$-NH-$(CH_2)_3$- B  C 		**33**	A-NH_2	H	38
		34	B-NH_2	H	29
		35	C	H	>57
		36	A-NH_2	A-NH_2	26
		37	B-NH_2	B-NH_2	5.4
		38	C	C	>57
		**39**	A	-	>57
		40	B	-	16
Clomipramine		-	-	-	12

Table 4. TCTR inhibitory properties of xanthene derivatives [68].

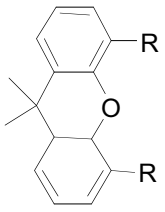
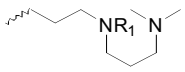
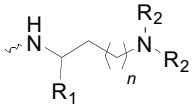
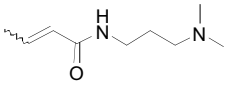
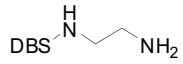
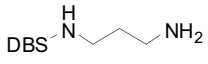
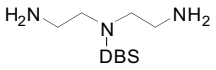
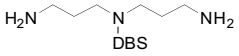
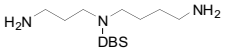
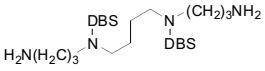
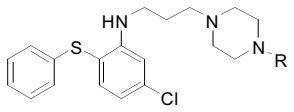
-	R	Nº	R_1	R_2	n	% inh. @ 100 μM	IC_{50} μM
		**41**	H	-	-	63.0	-
		42	4-$MePhSO_2$-	-	-	13.9	-
		43	2-$CH_{10}H_7SO_2$-	-	-	26.8	-
		44	BnNHCO-	-	-	21.5	-
		45	4-ClPhNHCO-	-	-	11.8	-
		46	4-FPhNHCO-	-	-	11.1	-
		**47**	H	CH_3	1	-	108
	-	**48**	CH_3	C_2H_5	2	-	105
		**49**	-	-	-	-	35.7
Promazine	-	-	-	-	-	-	108
Clomipramine	-	-	-	-	-	-	32.4

Table 5. TCTR inhibitory properties of dibenzosuberyl substituted di and polyamines [73].

-	N°	K_i (μM)	IC_{50} (μM)	
			TBb [a]	TBr[b]
Clomipramine		8.40	5.05	ND[c]
	**50**	136	1.68	ND
	**51**	81.6	1.38	ND
	**52**	27.9	2.8	4.0
	**53**	7.62	ND	ND
	**54**	4.00	0.62	8.3
	**55**	0.25	2.55	22

a: Lab 110 is a drug-sensitive TB *brucei* strain. b: K.243-As-10-3 is a pentamidine and melarsoprol resistant clinical TB *rhodesiense* isolate. c*:* Not determined

Table 6. TCTR inhibitory properties of simplified clomipramine analogues [73].



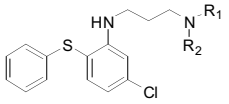
-	R	% ITCTR (10 μM)
56	CH_3	22
57	$(CH_2)_2NH_2$	22
58	$(CH_2)_2NH\text{-}CO\text{-}CH_3$	5
59	$(CH_2)_2NH\text{-}CO\text{-}C_6H_5$	5
60	H	18
Clomipramine		45

Replacement of the piperazinyl group by flexible alkyl chains, the addition of an amino group or branched side chains modified the inhibition profile. Compounds **63**, **65** and **67** displayed the highest TR inhibitory activities (Table **7**) although they were inactive against *T. cruzi*. Only **62**, **64** and **65** inhibited *T. brucei* trypomastigotes growth. Compounds **63**, **65** and **67** showed toxicity during *in vivo* assays [74].

Taking into account the differences between TCTR and HGR, Girault designed a series of putative inhibitors derived from bis(2-aminophenylsulfides) and selected **56** and related structures **69** and **70** for further studies [77]. In order to increase the number of proton-accepting amino groups in **70**, the authors prepared series **71** (Fig. **4**), where the most specific and potent TR inhibitor (*n* = 3, X = Br, R = *N*-methylpiperazine) showed IC_{50} of 0.55 μM at $[TS_2]$ = 57 μM [78].

The effect of each aryl moiety was then evaluated by the synthesis of dissymmetrically substituted analogues **72-80**, in which one aromatic sulphide is replaced by different amines (Table **8**). TR inhibition studies revealed the importance of the aromatic rings and of the amino groups in the side chains for potent inhibition, since its replacement by aliphatic, alicyclic or heterocyclic amines strongly decreased TR inhibition. The presence of an amino group in the side chain of each aromatic moiety is essential for recognition as revealed by the inhibitory activities of **78** and **79**. Although quinonic moieties were introduced with the aim of conferring redox-cycling properties to the TR inhibitors, the compounds did not act as subversive substrates [79].

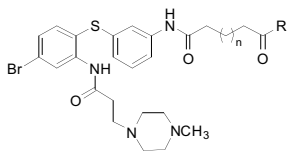
Table 7. TCTR inhibitory properties of non-piperazinic clomipramine analogues [74].

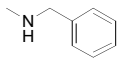
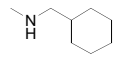
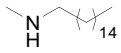
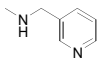
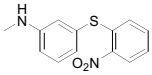
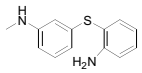
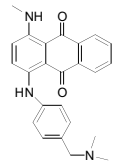
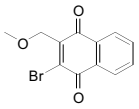


Nº	R_1	R_2	% TCTRI[a]	% TBI[b]
61	H	$(CH_2)_2NH_2$	0	0
62	H	$(CH_2)_3NH_2$	33	100
63	H	$(CH_2)_4NH_2$	67	0
64	H	$(CH_2)_2NH\text{-}(CH_2)_2\text{-}NH_2$	12	100
65	H	$(CH_2)_3NH\text{-}(CH_2)_3\text{-}NH_2$	53	100
66	H	$(CH_2)_2N\text{-}(CH_2\text{-}CH_2\text{-}NH_2)_2$	4	0
67	$(CH_2)_2NH_2$	$(CH_2)_2NH_2$	75	0
68	H	$(CH_2)_2NH\text{-}C(=NH)\text{-}NH_2$	30	0
Clomipramine			45	0

a: TCTR inhibition % at 10 µM; b: *T brucei* inhibition % at 1.56 µM.

Table 8. TCTR inhibitory properties of dissymmetrically substituted diarylsulfides [79].



Nº	*n*	R	IC_{50} (µM)
72	1		35
73	1		47
74	1		>60
75	1		>60
76	3		14
77	3		>60
78	3		16
79	3		3.3
80	3		41

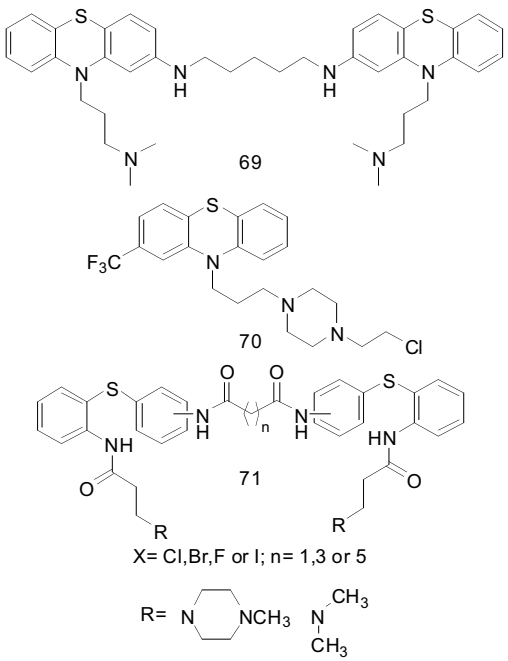


Fig. (4). Bis(2-aminophenylsulfides) and clomipramine analogues

Stump *et al*. continued the study on phenyl disulphides, highlighting that a simultaneous H-bonding to Glu18 and Glu465'/Glu466' plays a critical role in TR binding. To prove this hypothesis, a series of compounds varying the proximal amino group of reference compound **56** were synthesized to evaluate the relevance of the interaction with Glu 18 (Table **9**).

The replacement of A = CH_2 in **56** by a more basic group (**82**) almost doubled the activity. The authors suggested, based on docking studies, that the substrate interacts simultaneously through H-bonding with Glu 18 and Glu465'/Glu466' and that this conformation is stabilized in the active site by an intramolecular H-bond between the amidine C = NH and the unprotonated proximal piperazine nitrogen. Introduction of an amide moiety as in **81** and **86** led to inactive compounds. Molecular modelling studies suggested that the amine H-bond is favoured, but any interaction of the ligand with Glu465'/Glu466' is unlikely. Replacement of the NH functionality (B, Table **9**) in **56** by a NCH_3 (**83**), O (**84**) or CH_2 (**85**), reduced TCTR inhibition. Regarding the *in vitro* antiprotozoan activity, the authors noticed that the compounds did not inhibit growth of intracellular forms of *T. cruzi* and *L. donovani* (data not shown). On the other hand, piperazine derivatives showed a good correlation between TR inhibition and their activity against *T. brucei* trypomastigotes, displaying also good SIs.

Additionally, eight analogues of **56** with different arylthio moieties were sinthetized and tested (Table **10**). Compounds displayed IC_{50} between 2.03-0.70 μM. Replacement of the phenylthio substituent by a pyridinethio moiety annihilates anti *T. b. rhodesiense* activity. A second set of analogues in which the *N*-methyl piperazine was replaced by a dimethylamino group (**96-104**, Table **10**) displayed IC_{50} values in the low micromolar range but were cytotoxic against human L-6 myoblast cells.

Sulphides containing quaternary ammonium groups (Fig. **5**) with improved TR affinity due to a better interaction with the anionic aminoacid residues, also showing better solubility were reported [80]. Replacement of the piperazine ring in **56** by an *N,N*-dimethyl-3,4-dichlorobenzylammonium motif (**105**) doubled TCTR inhibition. Varying the substitution pattern on the phenyl moiety or replacing it with other aromatic groups did not affect the inhibition or the affinity constant (around 5-10 μM). Despite this, they were inactive against *T. brucei* trypomastigotes *in vitro* (data not shown). Interestingly, 5-nitrofurfuryl derivatives were subversive TR substrates with very good anti *T. brucei* activity (0.59-1.02 μM) [81].

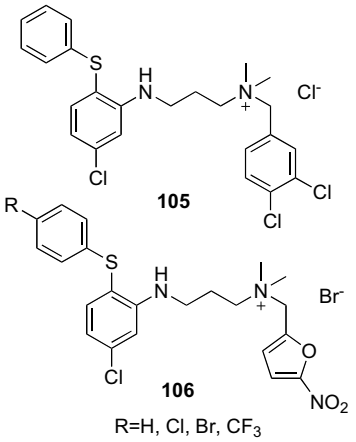
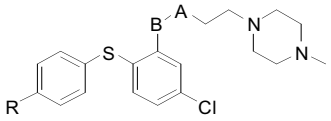


Fig. (5). Sulphides containing quaternary ammonium groups.

Although simple acyl derivatives (Ac, Bz, Boc) of natural polyamines offered only marginal results, other amide derivatives like the natural product kukoamine A (N^1,N^{12}-bis(dihydrocaffeoyl)spermine) [82], are active inhibitors of TCTR. Taking it as the lead structure, Chitkul developed a directed solid phase synthesis of acylated spermine, spermidine and norspermidine derivatives related to kukoamine A using various acids as building blocks. Preliminar testing of a first set of compounds including (het)aralkanoyl, (het)aralkenoyl, (het)arylcarboxy primary amine capped derivatives of natural polyamines led to the identification of two quinolinecarboxyl and one indolylacetyl derivatives as submicromolar inhibitors of TCTR. N^1-N^{12}-bis(3-indolilacetyl)spermine **110**, a 3-fold more potent TR inhibitor than kukoamine A, was selected for further structure refining. Thus, a second group of 3-indolylalkanoic acid capped spermine derivatives was then prepared using the same methodology. The 5-bromo-3-indoleacetyl derivative **115** showed a 5-fold increase in potency with respect to the parent compound (K_i = 76 nM). None of the compounds of this second set showed inhibition of YGR over 20% at 50 μM level. Additionally, nine of the most potent compounds, six of them based on the spermine scaffold, showed inhibitory activities exceeding 90% at 10 μM. Table **11** shows the most potent TCTR inhibitors. In comparison, lead compound kukoamine A shows 82% inhibition at the same concentration. All the tested compounds behaved as TCTR noncompetitive inhibitors [83].

Another approach to compounds targeting the TR function is by means of peptide modification of a polyamine scaffold. This alternative seemed promising since

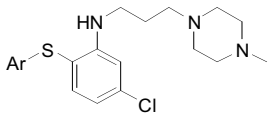
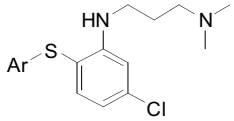
Table 9. Influence of basicity on aminophenylsulfide activity.



Nº	A	B	R	TRI%[a]	TB IC_{50} (SI) [c]
56	CH_2	NH	H	33	0.98 (10.7)
81	C=O	NH	H	0	NA
82	C=NH	NH	H	64	1.56 (50.3)
83	CH_2	NCH_3	H	26	ND
84	CH_2	O	H	15	ND
85	CH_2	CH_2	H	-[b]	ND
86	C=O	NH	Br	5	NA
87	CH_2	NH	Br	39	1.32 (6.9)

a: % TR inhibition; 43 µM TS_2, 100 µM inhibitor, *ca.* 4 $mU.cm^{-3}$ TR, 25 °C; b*:* Not soluble; c: IC_{50} in µM, STIB900 TB *rhodesiense* trypomastigotes; NA: Not active; ND: Not determined.

Table 10. Influence of the arylsulfide moiety on piperazine and dimethylamine clomipramine analogues.

-						
Ar	**Nº**	**TB[a] IC_{50} (µM)**	**SI**	**Nº**	**TB[a] IC_{50} (µM)**	**SI**
C_6H_5	**56**	0.98	10.7	**96**	NA	-
4-BrC_6H_4	**88**	1.32	6.9	**97**	2.33	4.1
4-ClC_6H_4	**89**	0.97	9.4	**98**	1.44	5.3
1-$C_{10}H_7$	**90**	0.70	12.1	**99**	1.89	4.6
2-$C_{10}H_7$	**91**	0.70	12.4	**100**	1.62	5.7
2-C_5H_4N	**92**	NA	-	**101**	-	-
2-$CF_3C_6H_4$	**93**	0.90	10.4	**102**	2.06	5.9
3-$CF_3C_6H_4$	**94**	1.06	9.2	**103**	NA	-
4-$CF_3C_6H_4$	**95**	2.03	4.8	**104**	1.6	8.5

a*: T. brucei* STIB900; NA: Not active

trypanothione itself is a polyamine-aminoacid conjugate. Besides, high throughput combinatorial methods are available for peptide synthesis. Polyamine-aminoacid conjugates libraries can be prepared by the split and mix method using a specific resin-bound polyamine scaffold [84]. However, when the resulting complex mixtures are screened for biological activity, the actual active component must be determined after a deconvolution procedure [85]. In some cases, the observed effects cannot be reproduced by any single compound, a situation that is regarded as a false positive. Marsh was faced with such situation when testing a 576 compound library [86]. In spite of the considerable amount of work, none of the tested bis(acylaminocyl)spermidine/norspermidine derivatives had inhibitory properties on TCTR.

A slight modification of this approach, employing dipeptide and protected dipeptide capping groups, yielded some potent TCTR inhibitors both in solution and in resin-bound phase. In this study, the importance of a hydrophobic aminoacid residue was brought to light. Interestingly, the most potent inhibitors assayed incorporated the Pmc (2,2,5,7,8-pentamethylchroman-6-sulfonyl) protecting group in their structure. Kinetic analysis of the active compounds

showed that they behaved as noncompetitive TCTR inhibitors. Table **12** shows the structure of the best hits in this series.

All active compounds contained diterminal Trp residues, followed by Arg in the most potent derivatives. Interestingly, the presence of two Pmc capped Arg residues increased the activity by two orders of magnitude (compare **120** and **117**), whilst N^4-Cbz protection had less impact (compare **120** and **118**), highlighting the relevance of hydrophobic interactions in these compounds. Removal of one Pmc group in **116**, however, increased activity, suggesting that the binding modes of these compounds may not be comparable. Unfortunately, no computational study is available to account for the observed results [87].

Further refinement was intended in subsequent work, in which a focused library of putative TCTR inhibitors was designed on the basis of the previously identified, non-competitive lead **117**. Selected structures comprised two *N*-Pmc capped Arg residues linked by a spermidine bridge. The arginine residue was conserved throughout the series and its alpha amino group was modified with substituted indoleacetyl groups or tryptophan. Compounds with 5-methoxyindole-3-acetic acid capping displayed good activity. The most potent inhibitor contained 4-methoxy-2,3,6-trimethylbenzenesulphonyl as Arg protecting group and a Trp capping group (K_i = 530 nM). This compound behaved as a competitive inhibitor, but it did not exceed the activity of the lead [88].

In 2005, the same group reported a SPS of new related compounds containing norspermidine as the polyamine scaffold and Arg, Trp, Trp-Arg, acetyl and hexanoyl *N*-capping groups (Table **13**). Additionaly, Pmc-Arg was also explored as a building block. Nine compounds displayed K_i values under 10 μM, among which the most active had submicromolar values, comparable to compound **116**. Kinetic analysis of library members established that minimal structural changes switched the mechanism of action from competitive to non-competitive inhibition. Evidence was found for an allosteric mechanism, indicating that binding sites and mode of action in TR inhibitors should not be inferred from their chemical structure [89].

In order to avoid TCTR inhibition reversion due to the build-up of intracellular TS_2 to millimolar levels, a strictly competitive inhibitor should possess a low nanomolar K_i value. This prompted investigations of irreversible inhibitors. *Lunaria biennis* alkaloid lunarine **144** is a conjugate of a dibenzofurandiacrylic acid and spermidine and it is a competitive, time-dependent TCTR inhibitor which ultimately

Table 11. Selected indolylacyl polyamine compounds with ITCTR≥95% at 10 μM.

RCO(NH-PA-NH)COR			
-	**PA**	**R**	**ITCTR %**
107	NSP	3-indolylmethyl	97
108		5-methoxy-3-indolylmethyl	96
109	SPD	3-indolylmethyl	95
110	SPM	3-indolylmethyl	97
111		5-hydroxy-3-indolylmethyl	95
112		2-(3-indolyl)ethyl	96
113		5-methoxy-3-indolylmethyl	96
114		3-(3-indolyl)butyl	97
115		5-bromo-3-indolylmethyl	98
Kukoamine A		-	82

Table 12. TCTR inhibition by tryptophanylaminoacyl spermidine derivatives [87].

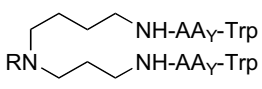
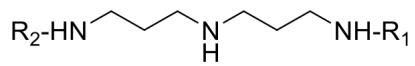
-	**Nº**	**AA_Y**	**R**	**K_i (μM)**
	**116**	Arg/Arg(Pmc)	H	0.10
	117	Arg(Pmc)	H	0.19
	118	Arg	Cbz	1.40
	119	Trp	H	3.10
	120	Arg	H	16
	121	Phg	H	83

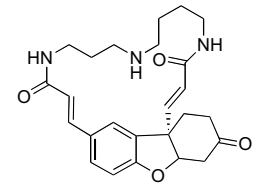
Table 13. TCTR inhibition by acyl, aminoacyl and peptidyl norspermidine derivatives [89].



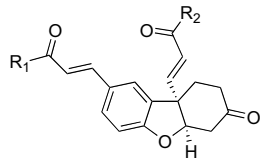
Nº	R_1	R_2	Inhibition type[a]	*Ki* (μM)
122	Trp-Arg(Pmc)	Trp-Arg(Pmc)	NC	0.16
123	Trp-Arg(Pmc)	Trp-Arg	NC	0.22
124	Arg(Pmc)	Arg(Pmc)	NC	3
125	Trp-Arg(Pmc)	$CO(CH_2)_4CH_3$	NC	6
126	Arg(Pmc)	$CO(CH_2)_4CH_3$	NC	7
127	Trp-Arg(Pmc)	H	NC	9
128	Arg(Pmc)	Arg	NC	11
129	Trp-Arg(Pmc)	COCH3	NC	12
130	Trp-Arg	Trp-Arg	NC	14
131	Trp	Trp	C	19
132	Trp-Arg	$CO(CH_2)_4CH_3$	C	24
133	Arg(Pmc)	$COCH_3$	NC	69
134	Trp-Arg	$COCH_3$	C	69
135	Trp-Arg	H	C	83
136	Arg(Pmc)	H	NC	131
137	Trp	H	ND	>100
138	Trp	$COCH_3$	ND	>100
139	Trp	$CO(CH_2)_4CH_3$	ND	>100
140	Arg	H	ND	>100
141	Arg	$COCH_3$	ND	>100
142	Arg	$CO(CH_2)_4CH_3$	ND	>100

a: C: competitive, NC: not competitive, ND: not determined

inactivates the enzyme through covalent bonding [90]. Hamilton investigated various molecules related to lunarine, including products derived from cleavage of the polyamine bridge and the furan ring, and reduction products of the ketone and each and both vinyl groups (Fig. **6**). Many of these compounds were moderate inhibitors of TR, displaying time-dependent inhibition. Only three of them: (±)-**143**, (±)-**144** and (±)-**145** were active against TB *rhodesiense* trypomastigotes (IC_{50} = 65, 29, 56 μM respectively). The fact that *rac*-**144** showed more activity than the enantiopure natural product, reveals that the non-natural enantiomer may be a more suitable scaffold upon which thiophilic groups may be presented [91]. Partially reduced derivatives allowed to assess that the actual thiophilic Michael acceptor is the one stemming from the aromatic ring. The authors also proposed a partially reversible mode for this interaction.



143



144: R_1=R_2=-$NH(CH_2)_3NH(CH_2)_4NH_{2.2}$TFA
145: R_1=-$NH(CH_2)_3NH(CH_2)_4NH_{2.2}$TFA, R_2=OEt

Fig. (6). Lunarine and its simplified analogues.

Many naphthoquinones (NQs) are known to interact unspecifically with NAD(P)H-dependent disulfide reductases (TCTR, TCLipDH, HGR) promoting superoxide anion production, which leads to oxidative stress. They also inhibit the physiological disulfide reduction of TR [92], sometimes acting as subversive substrates or turncoat inhibitors and/or as weak reversible inhibitors. Subversive substrates mode of action is multifold since they not only compete with the physiological substrate for the enzyme but yield a reduction product that can be re-oxidized while generating ROS, imbalancing the redox status of the parasite (redox cycling). This prompted Salmon-Chemin *et al*. to prepare a library of 1,4-naphthoquinones with alkylamide chains as potential inhibitors of TCTR. The library of 1360 amides was screened using the crude compounds in an automated assay based on recombinant TCTR [93]. The screening revealed compounds **149-152** as the most active ones (Table **14**). In all series, the optimum spacer length was n = 4 (Fig. **7**). Concerning **146** and **147** series, 3-(dibutylamino)propylamine derivatives (**149-151**) displayed the highest inhibition percentage. The authors consider that these results were not surprising since this amine is a homologue of the 3-(diethylamino)propylamine side chain of the antiparasitic drug mepacrine, known for its competitive inhibitory activity of TCTR [76]. A drastic loss of activity was observed when the side-chain did not have an amine protonable at physiological pH, highlighting again the importance of cationic charge for TR recognition.

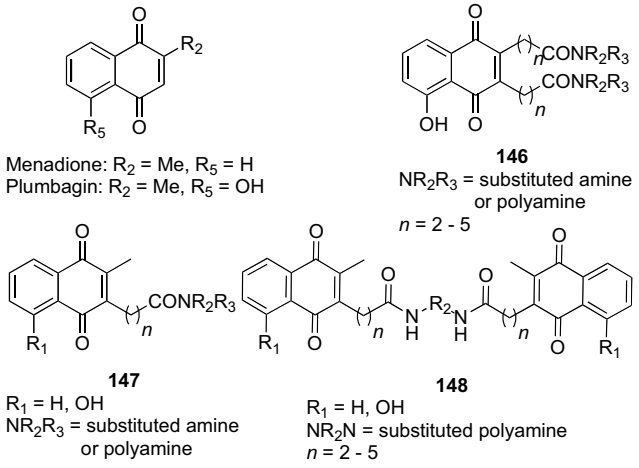


Fig. (7). Quinone-amine conjugates as putative TCTR inhibitors (Ref 76).

These four most potent inhibitors were re-synthesized and purified. IC_{50} values of the purified molecules were found to be significantly lower when compared to the parent molecules, menadione and plumbagin. In addition, these compounds showed also better selectivity. Compounds **149** and **152** were found to be uncompetitive inhibitors of TCTR.

The insight of the previous results, an improvement in NQs selectivity was attempted by designing polyamine derivatives of menadione, plumbagin and juglone. In order to obtain a variety of redox potentials in the cationic ligands, the three parent NQs were substituted in C2 and C3 with di or polyamine moieties including or not an alkyl spacer (Fig. **8**).

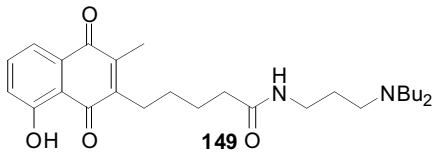
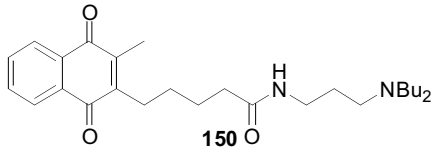
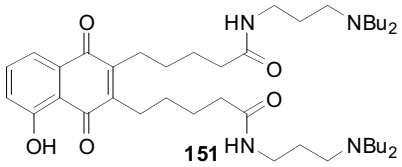
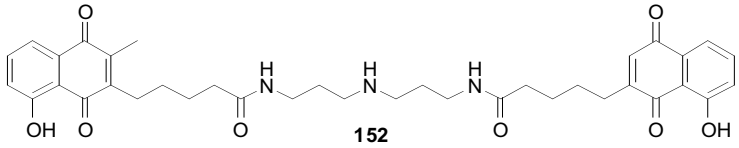
All the compounds were tested in a TS_2 reduction assay and the IC_{50} was determined for the most active ones. Compounds $\mathbf{153_{b\text{-}d}}$ and $\mathbf{156_{b\text{-}d}}$ were more potent than menadione (IC_{50} = 55 μM), but none of the plumbagin derivatives was more active than the parent compound (28 μM). In line with the requisite for substances interacting with TR active site to be cationic in nature, acids **157-159** did not inhibit TR, nor did the *N*-Boc derivatives **160-161**. Among amides, **162-163** showed little inhibition although amine **f** derivatives performed better that those of amine **g**, probably due to the presence of an additional protonable N atom. On the other hand, bisamides **164** and bisquinones **165-166** inhibited the enzyme in the low micromolar range (IC_{50}<10 μM), particularly when the chain length approached 4-5 methylene groups. The norspermidine derivative of compound **166** (n = 4) was the most potent of the series, with an IC_{50} value of 0.45 μM. When the inhibition type was determined for some of these compounds, **153b** showed a noncompetitive profile, while **166c** (n = 4) behaved as an uncompetitive inhibitor. Compounds of the **164-166** series showed selectivity towards TCTR as compared with HGR and human thiorredoxin reductase.

Compounds showing some TCTR inhibition were also tested as subversive substrates by measuring the rate of NADP production as compared with the enzyme intrinsic NADPH oxidase activity. Derivatives **153-156** showed poor redox cycling properties (2.0-6.4 fold) compared to the parent NQs. Some derivatives of **162-164** were as active as plumbagin. Compound $\mathbf{164_g}$ (n = 3) showed the highest reductase activity of its series (220 fold). Many derivatives from the **165-166** series showed redox cycling activities over 100 fold, the maximum value corresponding to $\mathbf{166_b}$ (n = 4), and were the most consistent group regarding this profile.

Compounds **153-156**, **157-159**, and **162-164** showed weak trypanocidal activity (in agreement with poor TCTR inhibition) or were cytotoxic. Some derivatives **158-166**, in contrast, were as active as nifurtimox as trypanocidals, and were also TCTR inhibitors, altough there was no linear correlation between both properties. Finally, the Boc protected amines **160-161** behaved as potent trypanocidals although ineffective as TC inhibitors. Taken together, these findings show that to be an effective trypanocydal, low K_i values must be accompanied by optimal physicochemical properties. In addition, there must be at least another target to account for the observed antitrypanosomal activities in compounds devoid of TCTR inhibiting properties [94].

In 2009, Cavalli *et al.* designed a series of inhibitors bearing in mind the topology of the active site of the enzyme [95]. They chose the aminoquinazoline nucleus since it is protonated at physiological pH and is not associated with poor oral bioavailability as aliphatic amidines and guanidines. Additionally, quinazoline is recognized as a privileged structure which should lead to drug-likeness in the synthetic products. Taking into account that 2,4-diamino-quinazolines have recognized activities on different targets including parasites [96], a library of 4-amino-2-piperazinyl-quinazolines was prepared with the aim of testing compounds with diverse net positive charges. Docking of the quinazoline core 1-(4-(4-amino-6,7-bis(2-(dimethylamino)-ethoxy)quinazolin-2- yl)piperazin-1-yl)ethanone showed a

Table 14. TCTR inhibition and selectivity for quinone-amine conjugates [93].

N°	ITCTR%[a]	IC_{50} TCTR[b] (μM)	IC_{50} HGR[b] (μM)
Menadione	-	55	63
Plumbagin	-	28	38
 **149**	88	0.3	41
 **150**	86	1.1	>50
 **151**	81	0.6	>50
 **152**	79	0.5	10

a: TCTR inhibition % at 25μM, values from primary screening of the crude compounds; b: Values obtained with purified compounds.

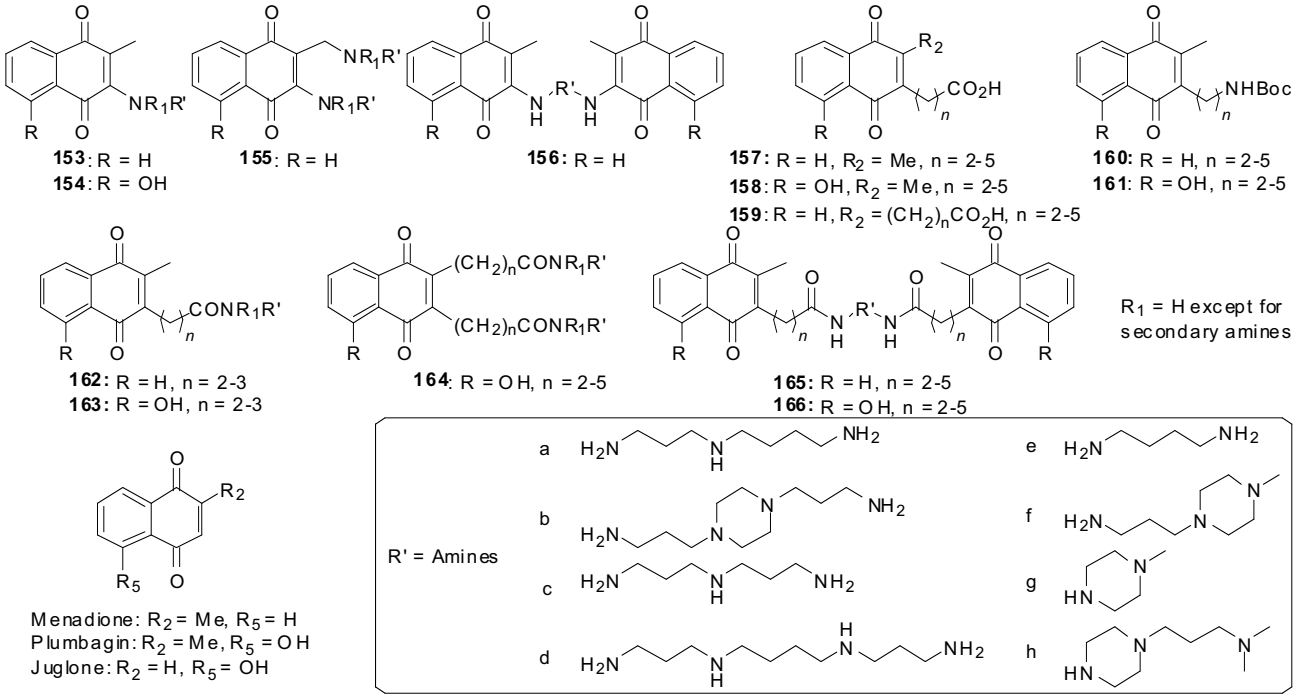
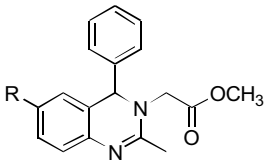


Fig. (8). Quinone-amine conjugates as putative TCTR inhibitors [76].

good interaction with TR active site. Then, seven compounds were designed choosing structural features of typical TR inhibitors, *i.e.* positive charge and extended hydrophobic regions [97]. The compounds were chosen in order to evaluate both different hydrophobic as well as redox-cycling active moieties.

Docking simulations did not allow to determine univocally the binding mode of these compounds. The authors suggest that they could bind at different sites, in accordance with a non-competitive or mixed inhibition mechanism. The compounds were experimentally tried as TCTR inhibitors (Table **15**). Tetrahydroisoquinoline and indole derivatives (**167, 168** and **169**,**172**, respectively) did not inhibit the enzyme, while compounds **170, 171** and **173** bearing quinone substituents, were active and therefore their inhibition constants were determined. The results were comparable with those obtained for other known TR inhibitors as mepacrine (K_i = 19 μM) [98] and clomipramine (K_i = 6.5 μM) [71a]. In addition, compounds **171** and **173** displayed good selectivity for TCTR. Trials against different parasite species showed once again poor correlation between the TCTR inhibition and antiparasitic activity and, in many cases, also poor selectivity relative to mammalian L6 cells.

3,4-Dihydroquinazolines **174** and **175** (Fig. **9**) were identified as TCTR low potency inhibitors after virtual screening of 100000 compounds.



174, R=Br
175, R=CH_3

Fig. (9). 3,4-Dihydroquinazolines reported in [95].

These two compounds were considered promising hits to treat stage 2 HAT, due to their low molecular weights, reasonable ligand efficiencies and low polar surface area (an important property for blood-brain barrier permeability). Kinetic analysis showed that compound **174** was a linear competitive inhibitor with good selectivity toward TCTR and devoid of toxicity against the mammalian cell line MRC5. On the other hand, as the dihydroquinazoline series underwent development, the mode of inhibition changed from competitive to mixed. This is potentially advantageous in order to overcome high intracellular concentration of trypanothione. In all development stages, compounds were tested for their activity against *T. brucei* TR and selected derivatives were then studied in bloodstream forms of TB *brucei* and in MRC5 cells.

As a first step, compounds, **174, 175** and 12 commercially available analogues were assayed against *T. brucei* TR. Although no analogues with improved potency were identified, it was possible to develop a preliminary SAR analysis. A hit expansion program was then initiated and new DHQs were synthesized. A general SAR concerning the different stages is presented in Fig. (**10**).

As a second approach, the X-ray crystal structure for the hit compound **174** in complex with *T. brucei* TR was solved and refined, being the first report of a high resolution noncovalent small molecule-protein X-ray crystal structure for TR. This complex revealed that the ligand binds in a region of the enzyme's active site cleft that would normally be occupied by the Gly-I and spermidine moieties of TS2 and is therefore consistent with the competitive inhibition model. The most interesting feature of the TR-**174** structure is that the enzyme undergoes an induced conformational change that generates a new hydrophobic subpocket within the active site, which accommodates the C4-phenyl of **174**.

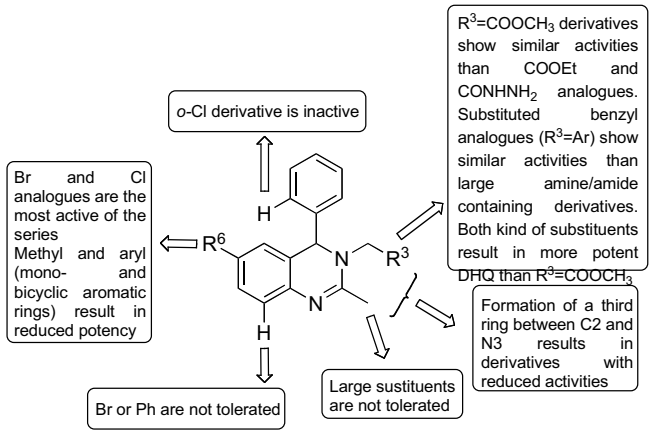


Fig. (10). SAR for 3,4-dihydroquinazolines.

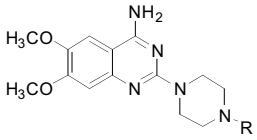
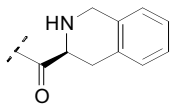
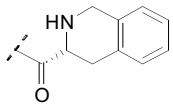
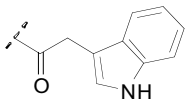
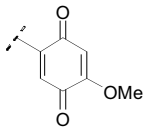
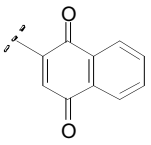
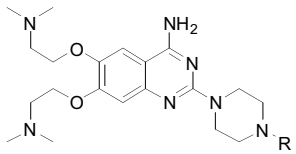
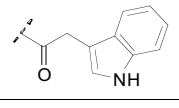
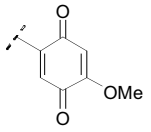
Previously, the active site of TR was believed to be rigid. [99]. In addition, the X-ray crystal structures of nonliganded TR, complexed with NADPH, and TR complexed with $T(SH)_2$ and NADPH were determined, as well as complexes with other selected compounds. On the basis of these results, the authors performed a structure-based inhibitor design (Fig. **11**) and synthesized a new DHQ series. As a result, it was possible to identify some trends from analysis of the cell screening data: the nature of the N3-substituent has a large effect upon selectivity, *i.e.* analogues with a basic N3- functionality have poor selectivity. In addition, compounds which vary only in their C6- halogen substituent, all display similar activities. Moreover, a compound with a C6 phenyl substituent is 10-fold more potent against *T. brucei* than against TR, suggesting this inhibitor works unselectively off-target. In fact, as seen in many cases, there is in general poor correlation between the TR IC_{50} and the *T. brucei* EC_{50}. This suggests that some analogues are not exerting all of their antitrypanosomal activity through inhibition of TR. The selectivity within this DHQ series is relatively low and varies from 1-20.

2.2. Polyamines and Related Compounds Aimed at Other Targets

2.2.1. Compounds Targeting the P2 Aminopurine Transporter

The use of the P2 transporter to selectively deliver cytotoxic compounds to the trypanosomes had been proposed by Carter and Fairlamb [100]. Considering that the benzylic polyamine **181** (MDL 27695, Fig. **12**) had shown

Table 15. TCTR and growth inhibition properties of substituted 2,4-diaminoquinazolines [97].

-	R	N°	Ki[a] (μM)	Inhibition type	IC_{50} (μM)				
					TC[e]	TBr[f]	LD[g]	PF[h]	L6
		**167**	nd[b]	-	14.3	1.81	>67	2.10	14.2
		**168**	nd[b]	-	27.6	5.37	>67	1.24	37.2
		**169**	nd[b]	-	10.2	2.21	42.2	1.35	19.6
		**170**	32[b]	Non competitive	35.6	1.98	45.7	3.01	52.5
		**171**	7.5[c]	Mixed type (Ki′ = 22 μM)	3.68	0.12	1.78	0.53	2.76
		**172**	nd[b]	-	>50	21.8	>54	2.19	>161
		**173**	11[d]	Non competitive	10.5	2.54	67.1	0.19	5.25
4,4'-bis(4-benzyloxy-3-methoxybenzimidoylamino)dicyclohexylmethane			2[a]	Mixed type (Ki′ = 16 μM)	-	-	-	-	-
Benznidazole		-	-	-	1.48	-	-	-	-
Melarsoprol		-	-	-	-	0.01	-	-	-
Miltefosine		-	-	-	-	-	0.34	-	-
Chloroquine		-	-	-	-	-	-	0.22	-
Podophyllotoxin		-	-	-	-	-	-	-	0.012

a: the assay was performed with recombinant TCTR; b: at 100 μM of the tested inhibitor; c: at 25 μM of the tested inhibitor; d:at 40 μM of the tested inhibitor; e: *T. cruzi* amastigotes in L6 cells; f: *T. brucei rhodesiense* bloodstream trypomastigotes; g: *L. donovani* axenic amastigotes; h: intra-erythrocytic form of *P. falciparum;* i: mammalian L6 cells (cytotoxicity).

activity against *Plasmodium falciparum*, the authors synthesized seven analogues hypothesizing that by attaching these polyamines to a substrate of the P2 transporter like a melamine group it should be possible to selectively deliver them to the trypanosome.

All the studied compounds possess a melamine moiety, attached either to the terminal nitrogens (**182**) or to the secondary amino group (**183**). The triazine group can be attached directly or via a spacer. These molecules should all be specifically recognised by the P2 transporter of trypanosomes and may, therefore, be selectively accumulated by the parasites.

The compounds were assayed for their ability to inhibit adenosine uptake through the P2 transporter of bloodstream TB *brucei* trypomastigotes, and there *in vitro* activity was also evaluated, but both parameters showed no correlation. It should be considered that adenosine uptake inhibition does not necessarily proof that the compounds were actually taken

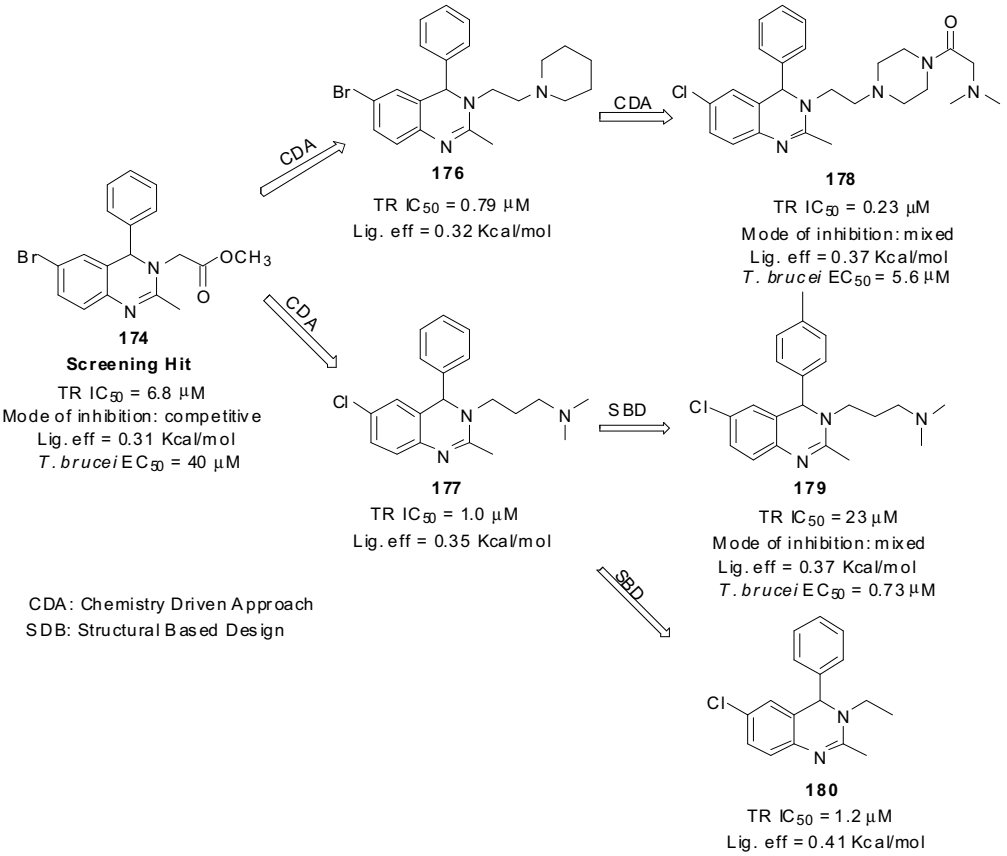


Fig. (11). Summary of the development of hit compound **174** by a chemistry-driven approach followed by a structure-based inhibitor design strategy.

up through the P2 transporter, nor does it rule out alternative routes of entry into the cell. The activities were in the µM range, compound **184** being the most active of the series (IC_{50}: 12 µM) [101].

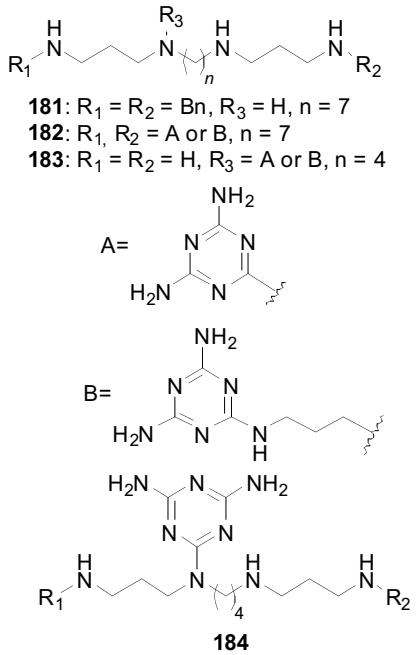


Fig. (12). Melamine derivatives of polyamines.

In a second related work, the same group designed and tested other 1,3,5-triazine substituted polyamines (Fig. **13**). In a first series (**185**), the influence of structural changes at the central core linker unit R was investigated. In a second stage (**186-188**), the effect the number of 1,3,5-triazine groups and additional methyl substituents $R_{1,2}$ was evaluated.

All compounds were assayed for their ability to inhibit adenosine uptake by the P2 transporter and *in vitro* toxicity against intact bloodstream TB *brucei* and TB *rhodesiense* trypomastigotes and also TC amastigotes. Table **16** summarizes the most active compounds. Since these parasites are intracellular, drugs cannot reach their plasma membranes without first permeating the host cell membrane, thus precluding the possibility of achieving selectivity through selective uptake. In fact, the most active compounds against *T. cruzi* amastigotes were also de most toxic ones for L-6 cells. In the first series **185**, the best results against TB *rhodesiense* were obtained with R = *n*-nonyl or *n*-dodecyl, while low/no activity was found against TB *brucei* and TC. A second series was designed by replacing the NH_2 groups on the triazine ring by $NHMe/NMe_2$ groups. Concerning the triazine moieties, methylamino or dimethylamino substituted triazines **186** and the elimination of a triazine unit increased the activity (**187**). A further improvement was observed when the triazine units were attached via an additional methylene spacer (**188**). These results demonstrate that the triazine moieties play a crucial role in the mode of action of this family of compounds. Here again, there is no obvious correlation

between the P2 transporter affinity and the antitrypanosomal activity.

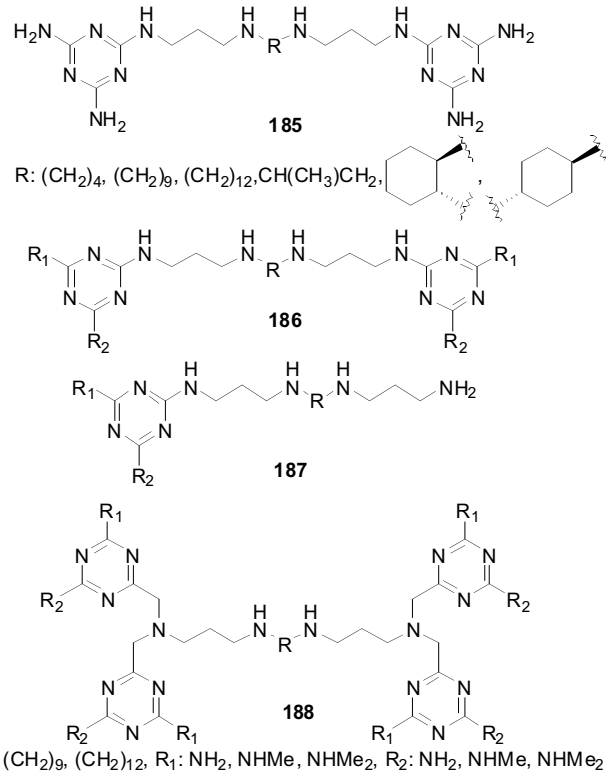


Fig. (13). Methylmelamine derivatives of polyamines.

Concerning P2 transporter affinity most new compounds displayed moderate affinity with apparent *Ki* values in the range of 3-70 μM. In unmethylated compounds **185**, an increase in the R length correlated with increased apparent affinity for the transporter. The introduction of methylamino and dimethylamino substituents on the triazine units was well tolerated and even increased the affinity in some cases, although a higher degree of methylation on the terminal triazine units was not beneficial.

Compound **191** (Table **16**) was the most active one with good parasite/host selectivity. Compounds **189-192** were tested for toxicity and anti-trypanosomal activity in mice. Unfortunately, 1 mg/kg intraperitoneal injection into mice was not curative, and concentrations higher than 10 mg/kg induced severe acute toxicity [102].

The same authors have developed nitroheterocycles linked to melamine moieties in order to direct them to the P2 transporter [103] or substituted benzene rings bound to a benzamidine group [104], but the compounds involved exceed the scope of this work.

Based on the hypothesis that polyamine analogues with structural changes altering their protonation state may disrupt parasitic polyamine metabolism the same group extended their investigation to polyaminoguanidines and polyaminobiguanides [105]. These compounds enter proliferating cells using the polyamine transport system and downregulate polyamine biosynthesis, but do not substitute for the natural polyamines in terms of cell growth and survival functions, leading to polyamine depletion and cell death. Substituted guanidines and biguanides are more basic than secondary amines, and the biguanide group appears in a number of important therapeutic agents, including chlorhexidine, metformin (antidiabetic drug) and the antimalarial agent proguanil (chlorguanide), among others [106]. It had previously been shown that non-polyamine dicationic fused ring systems with amidine and guanidine functions possess potent antitrypanosomal and antiplasmodial activity [107]. Also, the biguanide chlorhexidine acts as a TCTR inhibitor [97]. In order to test the hypothesis they synthesized a small series of substituted polyaminoguanidines and biguanides and evaluated them as TR and GR inhibitors, and for their ability to kill cultured blood forms of TB *brucei*. Relevant results showing selected IC_{50} values for TR and growth inhibition of TB *brucei* are collected in Table **17**.

All compounds, except for **197, 198** were effective inhibitors of TR, without any significant inhibition of HGR at concentrations up to 100 μM. They were also potent growth inhibitors, with IC_{50} values between 0.09 and 3.35 μM against TB *brucei*. In the guanidine series, compounds with a 3-7-3 carbon backbone **197-200** were the most effective agents against TB *brucei*, which is consistent with previous work of the authors on substituted polyamines [108, 109]. Among them, **197** and **198** were the most active ones showing again no correlation between TR inhibition and antitrypanosomal activity. The most potent biguanides **202** and **203** had a 3-3-3 or 3-4-3 carbon backbone respectively, although compounds **205** and **206** (3-7-3 backbone) also showed very promising results. The authors propose that differences in potency could be attributed to differential uptake by the P2 aminopurine transporter that, as mentioned before, is known to import pentamidine. This transporter selectively concentrates amidines and guanidines, causing lethal effects through accumulation in the mitochondrion and disruption of kinetoplast function [110].

Amidines were also studied by other authors. In particular, virtual screening of about one million commercially available chemicals was employed by Meiering *et al.* to identify new TCTR inhibitors [97].

A list of 25 *in silico* hits was subsequently subjected to kinetic analysis. Piperidine derivative **207** and chlorhexidine (Fig. **14**) proved to be strong TR inhibitors, yielding more than 90% inhibition at 100 μM inhibitor and substrate (TS_2). Both compounds share the typical TR inhibitors structural features: hydrophobic groups together with an overall positive charge, necessary for ligand binding to the disulfide substrate pocket of the enzyme. In addition, chlorhexidine behaved as a linear selective competitive inhibitor, and piperidine derivative **207** acted as a mixed selective inhibitor.

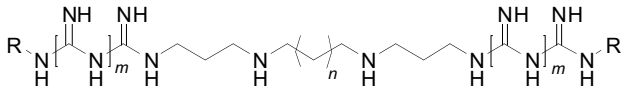
Based on chlorhexidine, and since several antitrypanosomal drugs such as pentamidine and berenil are diamidines or contain closely related structural elements [111], three different series of bisamides, bisamidines, and aromatic amidines were synthesized and studied as TCTR inhibitors (Fig. **15**). Additionally, and as mentioned before, the amidine group serves as a recognition motif for the uptake by the parasite purine transporter P2.

Table 16. Growth inhibition properties of methylmelamino derivatives of polyamines [102].

-				P2 *Ki* (μM)	IC_{50} (μM)			
N°	**R**	**R_1**	**R_2**		**TBr[a]**	**TBb[b]**	**TC[c]**	**L6[d]**
189	$(CH_2)_{12}$	NH_2	NHMe	0.135	9.8	2.0	116	Nd
190	$(CH_2)_9$	NH_2	NMe_2	17	0.265	3.0	54.8	177
191	$(CH_2)_9$	NHMe	NHMe	30	0.265	0.1	76.8	177
192	$(CH_2)_{12}$	NH_2	NMe_2	4.6	0.44	2.0	>77	105

a*: T. b. rhodesiense* trypomastigotes (strain STIB 900)
b: T. b. Brucei trypomastigotes (strain 427)
c: T. cruzi amastigotes (Tulahuen strain C2C4 containing the galactosidase (Lac Z gene)
d: L-6 rat myoblast cells

Table 17. Growth inhibition properties of polyaminobiguanides [105].



-				IC_{50} (μM)	
N°	***m***	***n***	**R**	**TCTR**	**TBb [a]**
193	0	1	3,3-diphenylpropyl	5.07	1.95
194	0	1	2,2-diphenylethyl	3.60	2.05
195	0	2	3,3-diphenylpropyl	2.97	2.4
196	0	2	2,2-diphenylethyl	4.56	3.35
197	0	5	cycloheptyl	17.68	0.18
198	0	5	benzyl	69.47	0.09
199	0	5	3,3-diphenylpropyl	2.24	0.61
200	0	5	2,2-diphenylethyl	4.72	0.5
201	1	1	3,3-diphenylpropyl	3.68	1.76
202	1	1	2,2-diphenylethyl	4.16	0.18
203	1	2	3,3-diphenylpropyl	2.96	0.19
204	1	2	2,2-diphenylethyl	2.74	1.05
205	1	5	3,3-diphenylpropyl	0.95	0.62
206	1	5	2,2-diphenylethyl	1.66	0.45

a: *T. b. brucei* EATRO 110 strain

None of the newly synthesized compounds performed better than chlorhexidine. Replacement of the two biguanide groups by amides or amidines strongly weakens the interaction with the enzyme, although amidines showed a slightly better inhibition profile than amides, as expected. The three most effective inhibitors were the symmetrical bisamidines **208**, **209**, and **210,** in which the *p*-chlorophenyl moieties of chlorhexidine had also been replaced (Table **18**).

Derivatives **208-210** possess the same bulky hydrophobic terminal substituents but differ in the nature of the linker connecting the two amidine moieties. Irrespective of the central substituent, **208-210** were mixed type inhibitors with very similar activities.

Dardonville *et al.* carried out an *in vitro* screening of 62 novel bisguanidines and bis-(2-aminoimidazolines) against TB *rhodesiense* trypomastigotes (STIB 900). Compounds were structurally related to the trypanocidal agent synthalin [112] and to norspermidine. The cytotoxicity in L-6 cells and the selectivity index were determined for the active compounds.

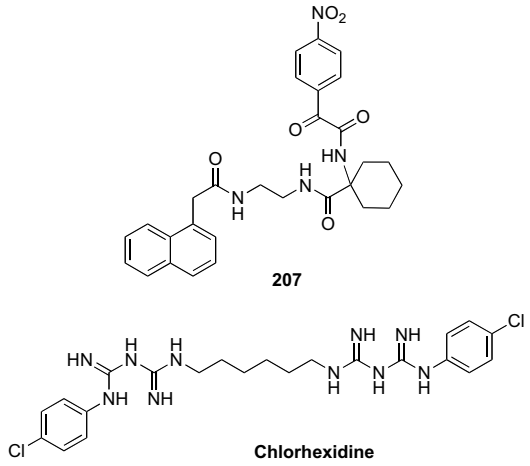


Fig. (14). Compound **207** and chlorhexidine.

In the alkyl and azaalkyl series (**211** and **212** respectively), guanidines were, in general, more active than 2-aminoimidazolines. Within alkyl derivatives **211**, activity increased with increasing chain length of the methylene spacer (*n*) in the order: 6 < 8 (ca. 30-fold) < 9 ~ 12, the nine-methylene derivative resulting more selective. Dicationic compounds were more active than the monocationic counterparts (R_1 = H). This behaviour was also observed for the azaalkyl compounds **212**. Among the latter, dicyclohexylguanidine was 9-fold more active than the guanidine analogue and was the most active compound of the series. This might reflect better pharmacokinetic properties of the more lipophilic derivative associated with enhanced biological membrane permeability. Azaalkyl compounds **212** presented only moderate activity as compared to the alkyl series **211**.

Within 3-aza-1,6-hexanediamines **213**, the dicyclohexylguanidine derivative displayed again the best activity and selectivity. In addition, substitution of the secondary amino group afforded molecules slightly more active than the parent compounds. The activity of cyclic analogues **214** was similar to the parent compound, but with lower selectivity. Among the diphenyl analogues **215**, the best activities were observed for compounds bearing a guanidinium group (3-10-fold with respect to the imidazoline analogues). However, selectivity followed an opposite trend, being 2-aminoimidazolines more selective than the guanidine counterparts. The effect of *N*-substitution of the imidazoline and guanidine moieties (*i.e.* Boc, $CH_2CH(OEt)_2$) is notable. *N*-

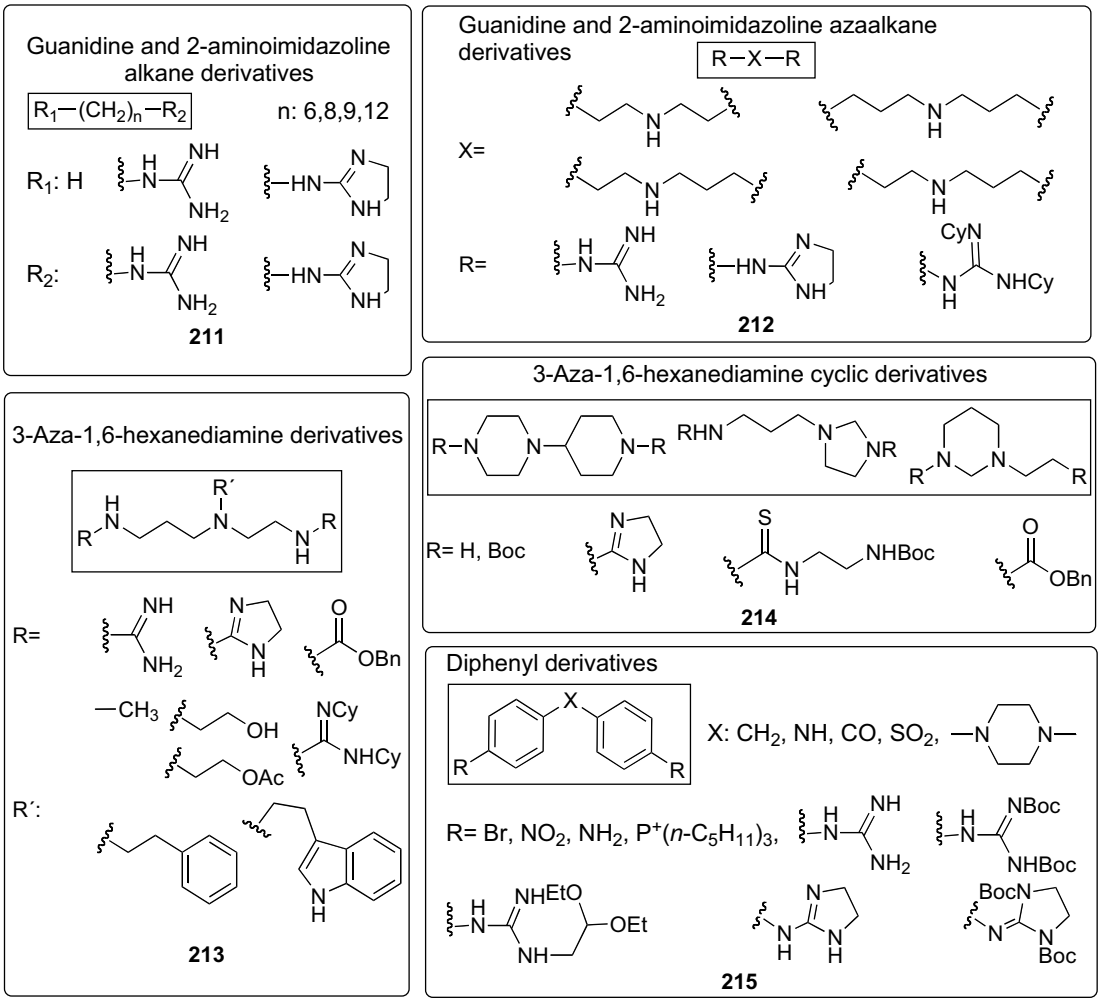
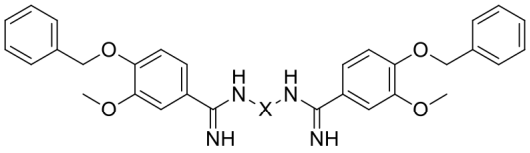
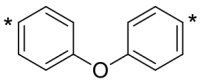
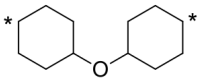


Fig. (15). Chemical structures of compounds reported in [111].

Table 18. Lipophilic bisamidines as P2 transporter inhibitors.



N°	X	% Inhibition[a]		Ki (μM)	Ki' (μM)
		110 μM [TS_2]	40 μM [TS_2]		
208		90	95	2	22
209		97	94	2	16
210	$(CH_2)_8$	85	92	5	47

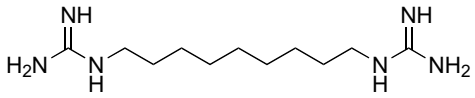
Boc protection afforded in general less active and selective compounds, while the 1,1-dimethoxyethane substituent caused a great increase in selectivity (26- 32-fold), with only a marginal loss in activity. Regarding the linker, the same trend was observed or the guanidinium and 2-aminoimidazolinium series, in which N, CH_2 > CO > SO_2.

The authors proposed that the most active guanidines reported in this work share the H_2N-C(R_1) = NR_2 motif and could, therefore, be transported into de parasite by the P2-aminopurine transporter. They identified four compounds with excellent *in vitro* activity in the low nanomolar range as well as high selectivity (Fig. **16**) [113].

2.2.2 Compounds Targeting Parasite SOD

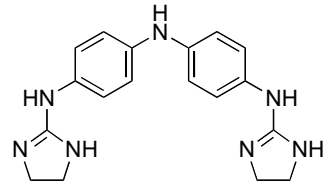
SOD activity had been demonstrated in protozoan parasites, and attributed to three different enzyme types, containing various transition metals (Cu/Zn, Fe and Mn) as the prosthetic group and aminoacid residues as the metal ligands. Among them, the homodimeric CuZnSOD, containing imidazolate bridged copper and zinc ions is widely distributed. Activity differences between E_2Zn_2SOD and E_2Cu_2SOD suggest that only the copper ion is essential for achieving catalysis. Since loss of the prosthetic group would impair the enzyme detoxification activity [114, 115], Rodríguez-Ciria *et al.* designed a series of metal-complexing phthalazines aimed at competing with SOD for copper, thus inactivating the enzyme [116].

Considering that 1,4-disubstituted phthalazines were studied for the complexation of metallic cations like Cu(II), Co(II), or Zn(II), the authors synthesized some new benzo[g]phthalazines with two flexible side chains containing nitrogen and oxygen atoms **220-222** (Table **19**). They hypothesized that pyridazine nitrogens should behave as donors for the metallic cations, and that the polyaminic pending groups with electronegative centres linked by a



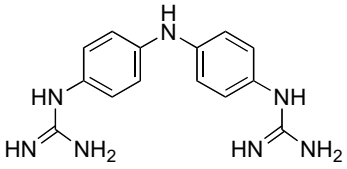
216

IC_{50}=0.049 μM; Selectivity >5294



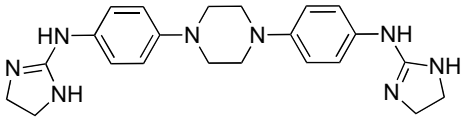
217

IC_{50}= 0.069 μM; Selectivity=3072



218

IC_{50}= 0.022 μM; Selectivity=29.5



219

IC_{50}= 0.118 μM; Selectivity=881

Fig. (16). Biguanide inhibitors of the P2 transporter.

short methylene chain should allow the simultaneous complexation with two ions. Variations in the nature of the complexation sites located at the end of the aliphatic chains were chosen so as to contain sp^2 (pyridine) and sp^3 (NH_2) nitrogens or OH groups. Physicochemical study of the complexes with Cu^{2+} supported this hypothesis: structural changes in

the donor sites at the side chains caused remarkable modifications in the geometry of complexation. Sp^3 or sp^2 nitrogens at the end of the alkylamino groups (**220, 221**) originate monopodal binuclear complexes that involve the side chains in coordination. However, substitution of nitrogen by oxygen in **222** leads to a tripodal binuclear complex in which the side chains do not participate. The authors postulate that these variations are probably responsible for the different biological activities of the podands.

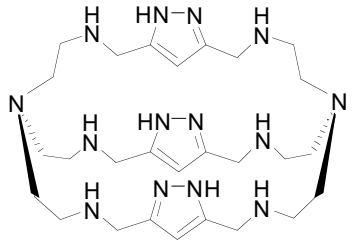
Firstly, the activity of podands **220-222** on TC epimastigotes was determined *in vitro*. The activity and toxicity of **220** were similar to those of the reference drug benznidazole. Compound **221** was also active, but less toxic against Vero cells. Additionally, **222** was significantly less active, which could be related to the different mode of complexation of metal ions previously discussed. In addition, the compounds (at 5 μM) halved the number of infected Vero cells and TC amastigotes and trypomastigotes in an 8 days propagation study.

In a second related work, the authors synthesized related benzo[g]phthalazine derivatives, functionalized with one or two imidazole rings at the end of the side-chains, linked through carbon or nitrogen (Figure on Table **20**) [117]. The imidazole motif was chosen considering both it's biological significance and its basicity. Following the same scheme as before, they studied the *in vitro* activity of **223-226** against *T. cruzi* epimastigotes (Table **20**). All compounds were more selective than benznidazole. In particular compound **224** was very active and presented the best overall profile.

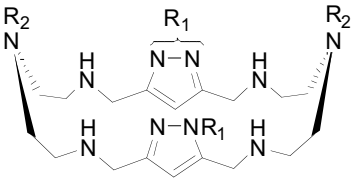
The authors then designed two new compounds **227** and **228** lacking the flexible side chains and replacing imidazole by pyrazole rings [118]. Pyrazoles are present in many pharmaceuticals with a wide range of biological activities, like celecoxib, dipyrone and pyrazofurin, among others [119]. In addition, pyrazoles can be considered robust bridging ligands since their conjugate bases bind metals in a variety of coordination modes [120]. The authors proposed that the increased system rigidity and the presence of a heterocyclic ring with well-known complexing abilities would favour Fe-SOD inhibition. Compounds **227** and **228** showed better *in vitro* selectivity than benznidazole when tested on epimastigotes, but did not improve the results obtained on imidazole derivatives **223-226**.

Derivatives **223-228** were also evaluated *in vivo* using a murine model in acute and chronic phases. All six compounds were able to reduce parasitemia in the acute phase. Monoalkylamino-substituted derivatives **224**, **226**, **228**, were more effective than disubstituted analogues. The order for *in vivo* activity could be established as: **226**> **224**≈ **228** > **223** ≈ **225** >**227** > BZN. The best results in the chronic phase were obtained again for monoamino derivatives **224**, **226**, **228**, which performed better than BNZ. In order to study the mechanism of action, inhibition experiments on the parasite Fe-SOD and human CuZn-SOD were performed. All the compounds were highly selective and once more **224**, **226**, **228** showed the best profile. These results were supported by docking experiments. Besides, compounds **223-228** induced a wide range of alterations on the ultrastructure of treated TC epimastigotes, and metabolic alterations related to the glycolytic pathway were also detected.

The same group studied than a set of macrocyclic pyrazole containing polyamines **229-232** (Fig. **17**). It is well-known that polyaminic macrocycles including pyrazole moieties act as powerful chelating agents [121] since pyrazole units can behave as monodentate ligands through their sp^2 nitrogens or as bridged bis(monodentate) η^1:η^1 ligands through deprotonation [120a, 122]. Firstly, they evaluated the *in vitro* activity of compounds **229-232** as hydrochlorides against TC epimastigotes and amastigotes and their selectivity indexes. Compounds **229** and **230** were the most active against both intra- and extracellular forms of the parasite, even surpassing the activity of the commercial drug benznidazole. The lipophilic polyamine **232** (R_2 = *N*-octyl) was the least active of the series. All the polyamines, in particular, **229-231**, showed less toxicity (and higher SI) than benznidazole. Since the less hindered compounds **229** and **230** displayed the best results, the authors propose that this would be a favourable structural feature for activity. Propagation assays showed a decrease in the infection rate of 74 and 80% for compounds **229** and **230**, respectively, during a 10-day treatment period, while benznidazole resulted only in a 23% decrease. The number of amastigotes and trypomastigotes was also considerably reduced by compounds **229, 230** during this study.



229



230, R_1= Me; R_2=H

231, R_1= Bn; R_2=H

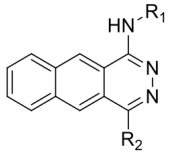
232, R_1= H; R_2=$(CH_2)_7CH_3$

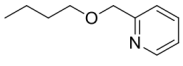
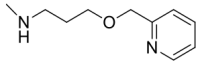
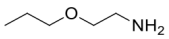
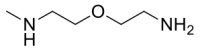
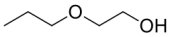
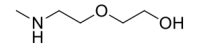
Fig. (17). Macrocyclic pyrazole SOD inhibitors.

All the compounds were highly selectivity for TC Fe-SOD as compared to human CuZn-SOD, but compound **230** showed the best results (IC_{50} Fe-SOD/ IC_{50}CuZn-SOD = 23). These results agree with the hypothesis of an interaction with the iron atom of SOD as a probable mechanism of action.

229-231 were studied *in vivo* on female BALB/c mice. Compound **229** showed the best results in both acute and chronic phases. Parasitemia was reduced by 53% on day 30 postinfection while benznidazole resulted in a 26% reduction. In the chronic phase, the reduction of specific antibodies levels followed the order **229**>**230**>>**331**>benznidazole. The authors suggest that these results could be related to the degree of protonation of the compounds at pH 7.4 (**229**>**230**>**331**).

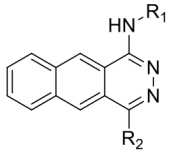
Table 19. Benzo[g]aminophthalazine-based inhibitors of SOD.

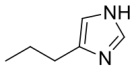
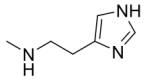
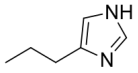
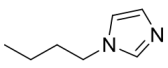
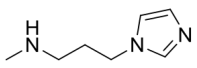
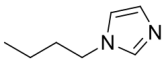
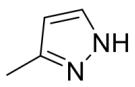
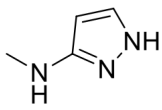
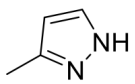


N°	R1	R2	TC IC_{50}[a] (μM)	Toxicity IC_{50}[b] (μM)	SI	%TC Fe-SOD inhibition[c]			%CuZn-SOD inhibition[d]		
						1 μg/mL	10 μg/mL	25 μg/mL	1 μg/mL	10 μg/mL	25 μg/mL
220			3.25	6.25	1.92	64	68	75	0	7	11
221			12.02	67.32	5.60	48	73	100	0	11	17
222			30.60	93.52	3.05	27	34	53	0	0	0
Rd	-	-	4.12	3.53	0,86	-			-		

Rd: BZD. *a: T. cruzi* epimastigotes (Maracay strain) at 72 h culture; *b:* Vero cells at 72 hs culture; *c:* Fe-SOD of *T. cruzi* epimastigotes: 23.36 ± 4.21 U/mg; *d*: CuZn-SOD of human erythrocytes: 23.36 ± 4.21 U/mg

Table 20. Azole substituted benzo[g]aminophthalazine inhibitors of SOD.



	-	-	IC_{50} (μM)		-	IC_{50} (μM)	
N°	R_1	R_2	TC [a]	Toxicity [b]	SI	TC Fe-SOD	CuZn-SOD[c]
223			14.8	88.7	6.2	29.6	365.8
224		Cl	<0.3	213.0	710.0	9.4	786.9
225			<0.2	69.3	346.5	28.3	67.9
226		Cl	13.7	145.8	10.6	1.3	129.3
227			32.8	98.3	3.0	22.9	96.6
228		Cl	17.5	132.6	7.6	17.2	103.3
Rd	-	-	15.8	13.6	0.9	-	-

Rd: BZD. a*: T. cruzi* epimastigote (Maracay strain) at 72 h culture; b*:* Vero cells at 72 hs culture; c*:* human erythrocytes

Table 21. Growth inhibition and selectivity of macrocyclic pyrazole SOD inhibitors.

-	IC_{50} (μM)				SI[c]		
	Epimastigote forms[a]	Axenic amastigotes[a]	Intracellular amastigotes[a]	Vero cells[b]	Epimastigote forms[a]	Axenic amastigotes[a]	Intracellular amastigotes[a]
229	1.3	7.2	6.2	149.1	114.7(143)	20.7(30)	24.0(40)
230	1.3	8.8	6.5	178.5	137.3(172)	20.3(29)	27.5(46)
231	16.5	10.0	13.8	195.4	11.8(15)	19.5(28)	14.2(24)
232	46.4	20.8	18.4	21.5	0.5(0.6)	1.0(1.4)	1.1(1.8)
BZN	15.9	18.9	23.3	13.6	0.8	0.7	0.6

a: *T. cruzi* SN3 strain of IRHOD/CO/ 2008/SN3
b: After 72 h of culture
c: In parentheses: the number of times that the compound SI exceeded the reference drug SI

A complementary histopathological analysis confirmed that the compounds tested were significantly less toxic to mammals than the reference drug and that **229** and **230** exhibited lower levels of damage than **231**.

2.3 Polyamines Derived from Antimalaric Agents

Based on previous information about antimalarial [123] and antileishmanial activity of *N*-benzyl polyamines [124], Bellevue *et al.* synthesized and tested a series of terminally alkyl-substituted polyamine analogues containing either a 3-3-3 or 3-7-3 polyamine backbone, taking **236** (Table **22**) as the lead structure. These analogues were evaluated *in vitro* as antitrypanosomal agents against one strain of *T. b. brucei* (LAB EATRO 110) and three strains of *T. b. rhodesiense* (KETRI 243, KETRI 243 As-10-3 and KETRI 269). Polyamines with a 3-3-3 carbon skeleton (**233-235**) had little antitrypanosomal activity, while those with a 3-7-3 carbon skeleton **236-238** showed potent antiparasitic activity. The analogue **238** was effective against all tested parasites, being superior to the lead compound with a submicromolar IC_{50} value [108]. These data prompted them to evaluate other alkylpolyamine analogues as high potency trypanocides. Therefore, the second generation of 3-7-3 polyamines was then developed and their *in vitro* and *in vivo* (mice) trypanocidal activities were evaluated. Analogues containing a 1,3-diaminopropane backbone showed no measurable activity (data not shown). Marginal antitrypanosomal activity was observed for conformationally restricted analogues **239-241**. Only compounds **242-244** displayed activity in the same order of magnitude than **238** (Table **22**). Compound **242** was as active as the reference drug, with the added benefit of being active against the melarsen oxide resistant K243 As-10-3 strain at submicromolar level [109]. Selected results are summarized in Table **22**.

Based on a potent antimalaric previously developed by Ellmann, [125] Dardonville prepared a series of 4-aminoalkyl-4-aminopiperidines structurally similar to bioactive polyamines and tested them against three kinetoplastids. Table **23** shows selected data from the 16 most potent drug candidates against TB *rhodesiense*, TC and L *donovani*. Compounds with 6-12 alkylene chains as linkers showed very good activity against TB and also good selectivity. Derivatives with unsubstituted 4-aminopiperidine moieties (R_1 = H) were the most active compounds of the series. Among them, the most potent was Boc amide **248** bearing an 8-carbon linker**,** which also displayed an excellent selectivity index. For every group of compounds, a general trend is evident and the longer the alkylene linker, the lower the IC_{50} value. Replacement of R_1 by a propionyl group decreased potency, and the authors suggest that an H bond donating group or a basic nitrogen atom in this position is crucial for anti-*T. brucei* activity. The lower activity observed against *L. donovani* amastigotes as compared with the *Trypanosome* strains was attributed to the lower pH of the culture media for the former. Although compounds **248-250**, **251**, **252**, **255**, and **260** were as active as the reference drug against TC amastigotes (IC_{50}<10 μM), their toxicity against myoblasts resulted in low selectivity indexes (data not shown).

Derivatives **248** and **259** were selected for *in vivo* testing on the basis of "hit criteria" of activity and selectivity as defined by the drug-screening network of TDR [126], but the compounds failed to extend the survival span of the infected animals [127].

Sitamaquine was first synthesized as part of the collaborative antimalarial program in the US (1944-1950) that led to the antimalarial Primaquine (Fig. **18**).

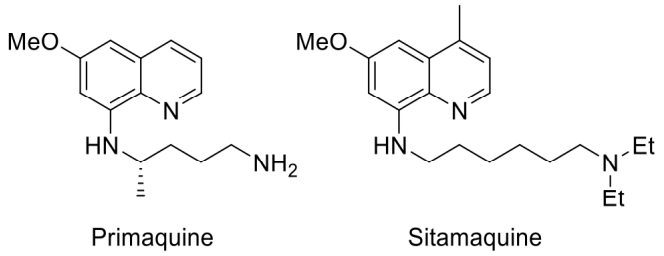
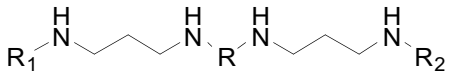
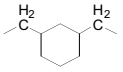


Fig. (18). Structure of Primaquine and Sitamaquine

Besides its antimalarial activity, Primaquine was also tested *in vivo* in a rodent model infected with *L. donovani* and pharmacomodulated in order to enhance its potency. The introduction of a 4-methyl group resulted in a 15-fold improvement on the antileishmanial activity. Further modifications such as increasing the size of the 4-substituent led to a decrease in potency, while 4-CF_3 and 4-MeO substituents did

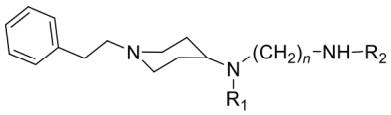
Table 22. Selected data from [110, 111].



-				IC_{50} (µM)	
Nº	**R**	**R_1**	**R_2**	**TBb[a]**	**TBr[b]**
233	-$(CH_2)_3$-	CH_3CH_2-		>100	>100
224	-$(CH_2)_3$-	CH_3CH_2-		>100	>100
235	-$(CH_2)_3$-	CH_3CH_2-		>100	>100
236	-$(CH_2)_7$-	Bn	Bn	14.5	13.0
237	-$(CH_2)_7$-	CH_3CH_2-		18.0	26.2
238	-$(CH_2)_7$-			0.125	0.78
239		Bn	Bn	4.05	56
240				73.0	-
241				3.25	-
242	-$(CH_2)_7$-	*S*-2-methyl butyl	*S*-2-methyl butyl	0.031	0.165
243		*S*-2-methyl butyl	CH_3CH_2-	0.31	0.79
244		3-Ph-Bn	3-Ph-Bn	0.24	0.20
245		3-MeO-Bn	3-MeO-Bn	22	5.5
Melarsen oxide				0.001	----

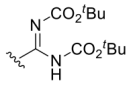
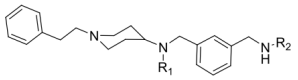
a: Lab 110 is a drug-sensitive *T. b. brucei* strain; b: K.243-As-10-3 is a pentamidine and melarsoprol resistant clinical *T. b. rhodesiense* isolate.

Table 23. Growth inhibition properties and selectivity of 4-aminoalkyl-4-aminopiperidines.



-				IC_{50} (µM) (SI)		
Nº	***n***	**R_1**	**R_2**	**TBr[a]**	**TC[b]**	**LD[c]**
246	6	H	$CO_2{}^tBu$	0.790 (36)	28.59	>74
247	7	H	$CO_2{}^tBu$	0.289 (41)	11.94	>72
248	8	H	$CO_2{}^tBu$	0.119 (130)	5.72	>69
249	9	H	$CO_2{}^tBu$	0.126 (48)	3.73	>67
250	12	H	$CO_2{}^tBu$	0.156 (52)	3.16	17.82
251	7	COEt	$CO_2{}^tBu$	3.742	9.93	19.28
252	12			4.070	8.07	6.20

(Table 23) contd…..

-				IC_{50} (μM) (SI)		
N°	***n***	**R_1**	**R_2**	**TBr[a]**	**TC[b]**	**LD[c]**
253	6	COEt		2.379	23.16	13.23
254	8			1.590	12.29	14.81
255	12			1.257	5.20	5.78
256	6	COEt		2.925	>47	39.66
257	7			14.068	>46	32.15
258	8			6.836	>45	18.74
259	9			3.748	>44	14.85
260	12			0.431 (24)	6.90	10.37
261				0.503 (219)	17.30	78.11
Reference drug [d]				0.0075	1.97	0.205

a: TBr: TB *rhodesiense* STIB900 trypomastigotes; *b:* TC: TC Tulahuen strain C2C4 trypomastigotes, axenically grown; *c:* LD: *L. donovani* strain MHOM/ET/67/L82 amastigotes; *d:* Reference drugs were: melarsoprol for TBr, benznidazole for TC and miltefosine for LD.

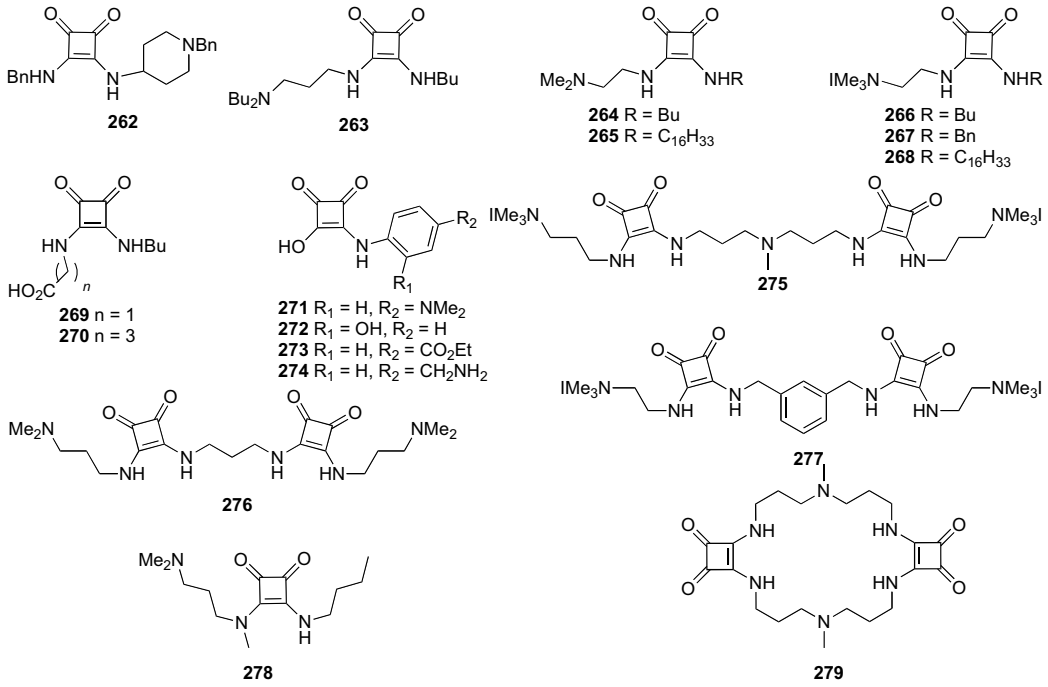


Fig. (19). Squaramide-based compounds with trypanocidal activity.

not modify the activity. In addition, modifications in the *N*-alkyl chain in a series of related 6-methoxy-4-methyl-8-aminoquinoline derivatives called lepidines led to Sitamaquine as the most active compound of the series.

In vitro assays against *L. donovani* amastigote showed an EC_{50} for Primaquine of 0.23 μM, 0.59 μM for Sitamaquine and 2.3 μM for the reference drug meglumine antimoniate. These differences between *in vitro* and *in vivo* results suggest that the activity displayed *in vivo* is due to one or more active metabolites. This hypothesis was reinforced by the fact that studies in different animal models displayed variable results, probably as a consequence of metabolic differences. In line with this, *in vitro* studies with *L. tropica* amastigotes revealed that the 6-hydroxy derivative was considerably more active than sitamaquine. Sitamaquine underwent phase II clinical trials with high efficacy at doses 1.5-3 mg/kg/day × 28 by oral route in India and Kenya [128].

On the basis of a previous report on squaramides with antimalarial activity and their own results on the antichagasic activity of cyclic polyamines [129], Olmo studied a group of Lipinski's rule of five (Ro5) compliant squaramides as candidates for treating Chagas disease. They explored a selected group of acidic, basic, neutral, and amphoteric squaramide-based compounds (Fig. **19**). The selection criteria empha-

Table 24. Selected data from [129].

	IC_{50} (μM)			SI	
Nº.	**TC-E[a]**	**TC-A[a]**	**Vero cell[b]**	**TC-E[a]**	**TC-A[a]**
262	96.9	34.7	337.5	3.5 (4)	9.7 (16)
263	21.1	6.0	147.8	13.3 (17)	24.6 (41)
264	10.5	9.3	260.2	24.8 (31)	28.0 (47)
265	49.8	22.4	90.1	1.8 (2)	3.4 (6)
266	107.2	38.5	57.6	0.5 (1)	1.5 (2)
267	60.8	39.2	74.2	1.2 (1)	1.9 (3)
268	44.9	26.3	151.4	3.4 (4)	5.7 (10)
269	35.7	23.8	54.8	1.5 (2)	2.3 (4)
270	17,3	66.9	21.6	1.2 (2)	0.3 (1)
271	15.9	10.1	12.9	0.8 (1)	1.3 (2)
272	50.8	44.0	228.3	4.5 (6)	5.2 (9)
273	26.3	18.4	21.5	0.8 (1)	1.2 (2)
274	89.1	72.6	36.6	0.4 (0)	0.5 (0)
275	18.5	17.1	300.7	16.2 (20)	17.5 (29)
276	96.2	46.0	11.4	0.1 (1)	0.2 (1)
277	118.9	43.9	73.1	0.6 (1)	1.7 (3)
278	9.4	8.5	453.1	48.2 (60)	53.3 (89)
279	21.8	31.2	110.6	5.1 (6)	3.5 (6)
BZN	15.9	23.3	13.6	0.8	0.6

a: TC SN3 strain of IRHOD/CO/2008/SN3. TC-E: Epimastigotes; TC-A: Amastigotes;
b: After 72 h of culture; c: In parentheses: the number of times that the compound SI exceeded the reference drug.

sized the molecular properties that determine druglikeness, but also the commercial availability of the starting reagents and the production process. These factors together with a short synthesis and simple purification steps are relevant economic issues for developing drugs. Concerning druglikeness, they computed the relevant molecular properties and selected the compounds according to Ro5 and with PSA<100 $Å^2$ to anticipate good oral bioavailability. The antichagasic properties and cellular toxicity were evaluated on TC epimastigotes and amastigotes. The study included infectivity assays on Vero cells with compounds with the higher activities. Using a murine model, they determined the *in vivo* trypanocidal activity on the acute and chronic phases of the Chagas disease. Analysis of the activity data (Table **24**) revealed that squaramides with positively charged tetraalkylammonium groups **266-268**, acidic squaramides **269,270** and also amidosquaric acids **271-274** had in general poor activity against both forms of TC and/or poor selectivity. In contrast, simple aminosquaramides displayed good activity. Derivatives **263**, **264**, **275** and **278** were the most promising compounds regarding both trypanocidal activity and selectivity indexes, performing even better than benznidazole. Among them, compound **278** demonstrated the best profile of the whole series.

It should be noted that squaramides, being vinylogous amides, display *E/Z* isomerism due to hindered rotation about the C = C-N bond. The more active compounds include a tertiary amino group separated from the squaramide unit by a short aliphatic chain. This arrangement allows for conformational transitions from extended (Z, Z) to folded (Z, E) forms, in which intramolecular hydrogen bonding offsets steric repulsion (Fig. **20**) [130]. This structural feature is lost after protonation but, since compound **278** has a pKa of 8.8, both acid and basic forms coexist in solution at physiological pH. Squaramide **278** also has distinctive properties: low molecular mass (267.4 Da), only one hydrogen bond donor and, remarkably, the smallest (36.3 $Å^2$) computed polar surface area within this group of compounds, exceeding the standard values for drug absorption and blood-brain barrier permeability [131]. Taken together, the high activity and low toxicity of **278** and other related aminosquaramides fulfill the criteria for investigating their antichagasic activity *in vivo*.

Compounds **263**, **264**, **275**, and **278** were also studied in a Vero cells propagation assay after a 10-day treatment. By day 10 the infection rate was lower in all cases with respect to the control. Again, compound **278** afforded the best results (67%), being much more effective than benznidazole

(20%). In the same line, the number of amastigotes per cell was significantly reduced to 52 and 15% for compound **278** and benznidazole, respectively. The number of trypomastigotes/mL was reduced by 75 and 21% for compound 278 *vs.* benznidazole. *In vivo* studies on female BALB/c mice in acute phase models performed in derivatives **263**, **264**, **275**, **278** showed a reduction in the number of trypomastigotes, but squaramide **278** was the most effective (67%) at day 40. Compound **278** was also included in chronic phase studies, suggesting a curative effect. Analysis of relevant biochemical parameters showed no significant toxicity for this compound. The authors conclude that amino squaramide **278** stops TC from settling down as a chronic disease because it is very effective against the intracellular parasite forms. In addition, its minimalist molecular structure and relatively simple synthesis are valuable features that all together make compound **278** a promising candidate as a preclinical drug [132]. In fact, a patent has already been filed for this family of compounds [133].

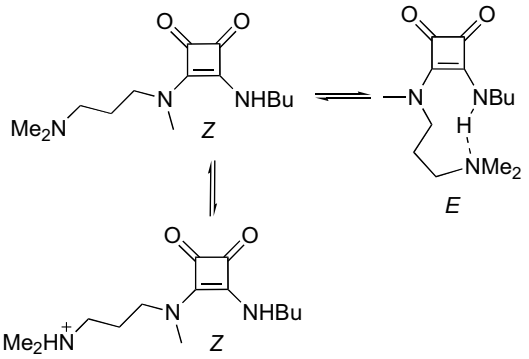


Fig. (20). E/Z isomerism of squaramides.

3. DIAMINES AND RELATED COMPOUNDS

3.1. Lipophilic Diamines, Aminoalcohols, Monoamines and Related Compounds

Under the assumption that polyamines play a key role in the metabolism of *Leishmania*, da Costa reported the synthesis and study of a series of long-chain *N*-monoalkylated diamines and two pyridinediamine derivatives, aimed at impairing the parasites metabolic pathways. The rationale for the design was the possible interaction between the hydrophobic chains and lipidic membranes, which would allow for the internalization of the drugs into the cytoplasm [134]. Compounds **280-297** were assayed against *L. amazonensis* and *L. chagasi* promastigotes. All the *N*-alkyl compounds tested showed some degree of growth inhibition, the better results were achieved with medium length chains (n = 7, 9, 11). *N*-methylpyridyl derivatives **296** and **297** were, on the other hand, inactive at the tested concentrations. (Table **25**). In the ethylenediamine series, except for derivative **280** which carries the less lipophilic chain, all the compounds were active against both strains. Compound **283** displayed the best activity, being >7 times more potent than the reference drug amphotericin B. Among propylenediamines (*m* = 3) the most active compounds were **286-290** for *L. chagasi* and **288-290** for *L. amazonensis*. Regarding butane and hexane diamines, longer lipophilic chains (*n* = 9) showed higher activities. Notably, optimum alkyl substituent length (*n*) varies according to the distance between both amino groups (*m*) [135].

In line with the previous results, Pinheiro *et. al.* described the synthesis and leishmanicidal activity of lipophilic diamines, expecting that the compounds would reach their target inside the host cell, *i. e.* in the parasitophorus vacuole.

The compounds were evaluated against *L. amazonensis* amastigotes (IFLA/BR/1967/PH8) in murine macrophages (Fig. **21**). A good correlation between clogP and potency was found, the *N*-Boc derivatives being in general more active [136].

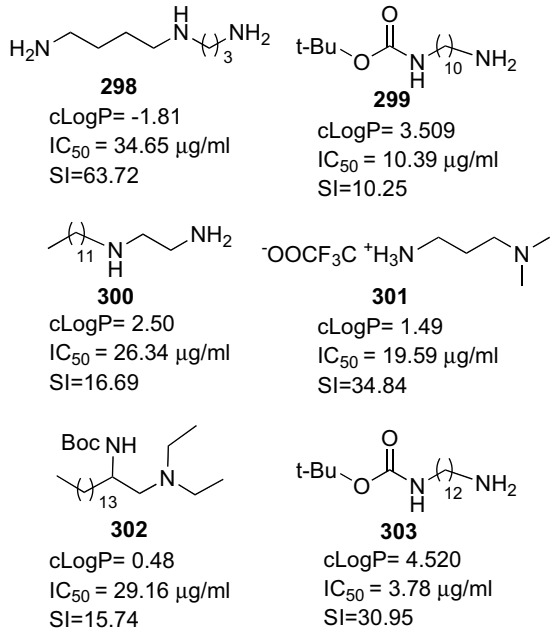
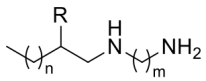
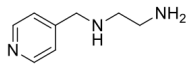
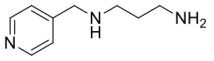


Fig. (21). Leishmanicidal activity of lipophilic diamine derivatives.

Yamanaka tested the antiparasitic effect of *N*-substituted lipophilic diamines on *L. braziliensis*, *L. chagasi*, and *T. cruzi* in both intra and extracellular forms. Compounds with no activity against the extracellular forms were also inactive on amastigotes (Table **26**). *Leishmania* strains were more sensitive to these diamines than TC. The hydrochlorides generally showed a slightly better activity than the free bases but were more cytotoxic. Amastigotes were, in general, more sensitive than the extracellular forms. The compounds showed poor TR inhibitory activities and they did not cause mitochondrial membrane depolarization, thus ruling out these mechanisms of action (data not shown). Compounds **305** and **310** were the most promising, showing high selective trypanocidal and leishmanicidal activities, respectively [137].

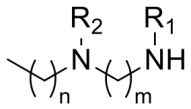
Long chain aminoalcohols and diamines can be considered as simplified analogues of sphingosine (Fig. **22**), a natural unsaturated C-18 aminodiol which is involved in intracellular homeostasis and is also proposed as an intracellular second messenger with the possibility of regulating signaling pathways related to cell survival [138]. Because of this, sphingosine and its synthetic derivatives have been regarded as potential drugs, displaying antimicrobial and antiparasitic activity [139].

Table 25. Growth inhibition properties of lipophilic diamines [135].

					
-				IC_{50} (µM)	
-	***m***	***n***	**R**	**LA**[a]	**LC**[b]
280	2	3	Et	25.10	27.90
281	2	5	H	7.20	2.10
282	2	7	H	3.90	3.40
283	2	9	H	0.94	0.26
284	2	11	H	4.90	5.00
285	2	13	H	13.20	3.20
286	3	3	Et	55.00	1.80
287	3	5	H	37.00	3.00
288	3	7	H	7.20	0.73
289	3	9	H	8.90	4.05
290	3	11	H	3.10	2.70
291	3	13	H	76.90	83.60
292	4	5	H	75.10	1.90
293	4	9	H	5.13	0.53
294	6	5	H	34.20	7.80
295	6	9	H	8.20	3.15
296			-	>227	>227
297			-	>227	>227
Rd[c]	-	-	-	0.9 (0.07)	1.9 (0.25)

a:L. amazoniensis; b:L. chagasi; c:Amphotericin B

Table 26. Growth inhibition properties and selectivity of *N*-substituted lipophilic diamines [137].

												
-					**Prom.**	**Epim.**	**Amastigotes**					
-					**LB**	**TC**	**LB**		**LC**		**TC**	
Nº	***n***	***m***	R_1	R_2	IC_{50} (µM)	IC_{50} (µM)	IC_{50} (µM)	**SI**	IC_{50} (µM)	**SI**	IC_{50} (µM)	**SI**
304	9	4	H	H	13.6	14.5	8.5	26.1	8.0	27.5	23.6	9.4
305	9	6	H	H	15.4	1.6	8.2	37.9	7.1	43.8	1.6	194.1
306	11	2	$CH_3(CH_2)_{11}$	H	NA	NA	NA	–	NA	–	NA	–

(Table 26) contd….

-					Prom.	Epim.	Amastigotes					
-					LB	TC	LB		LC		TC	
Nº	***n***	***m***	**R_1**	**R_2**	**IC_{50} (μM)**	**IC_{50} (μM)**	**IC_{50} (μM)**	**SI**	**IC_{50} (μM)**	**SI**	**IC_{50} (μM)**	**SI**
307	11	4	H	H	9.8	11.9	5.3	31.6	5.6	30.2	NA	–
308	11	4	$CH_3(CH_2)_{11}$	H	15.1	NA	7.4	30.7	14.0	16.3	NA	–
309	11	6	H	H	18.3	NA	11.7	18.5	9.1	23.8	NA	–
310	13	4	H	H	12.5	14.8	2.6	118.8	3.0	101.9	9.2	33.4
311	13	6	H	H	11.3	14.6	5.3	42.0	7.1	31.2	NA	–
312	15	4	H	H	12.2	13.5	28.2	7.7	15.5	14.0	NA	–
313	15	4	$CH_3(CH_2)_{15}$	H	NA	NA	NA	–	NA	–	NA	–
314	15	6	H	H	NA	NA	NA	–	NA	–	NA	–
315	9	4	H.HCl	H.HCl	14.2	14.1	5.2	52.1	6.6	41.2	5.9	45.9
316	9	6	H.HCl	H.HCl	12.3	14.3	4.6	52.1	6.2	38.6	8.7	27.6
317	11	6	H.HCl	H.HCl	11.7	NA	4.3	34.6	12.5	11.9	NA	–
318	13	4	H.HCl	H.HCl	9.4	14.1	CT	–	CT	–	CT	–
319	13	6	H.HCl	H.HCl	9.0	15.1	4.4	39.2	4.3	40.4	5.2	33.3
320	15	6	H.HCl	H.HCl	NA	NA	NA	–	NA	–	NA	–
Rd1	–	–	–	–	0.13	--	0.06	–	0.07	–	–	–
Rd2	–	–	–	–	–	31.2	–	–	–	–	10.8	–

Rd1: Amphotericin B; Rd2: Benznidazole. LB: *L. braziliensis* (MHOM/BR/96/LSC96-H3); LC: *L. chagasi* (MHOM/BR/08/LSC08-D2); TC: *T.cruzi* (MHOM/BR/00/Y)

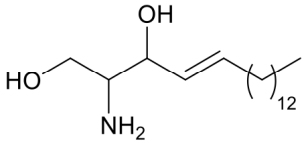


Fig. (22). Sphingosine.

Del Olmo *et al*. developed a number of aliphatic 1,2-diamines, 1-amino-2-alkanols and 2-amino-1-alkanols as sphingosine pseudo-analogues, and evaluated them against a bloodstream human-infective strain of TB *rhodesiense* (Table **27**). All three series of compounds displayed activity in the nanomolar range, although none of them was more potent than the reference drug (pentamidine).

The N^2-ethyl derivative **322** was the most potent within the alkanediamine series. Introduction of either bulkier or polar substituents led to a decrease in activity (data not shown). In the 2-amino-1-alkanol series **323-327**, *N*-ethyl diamines **323-325,327** showed a correlation between chain length (n) and potency, being the dihydrosphingosine analog **325** (n = 15) the most active. The presence of an *N*-hemiglutaroyl substituent decreased the activity (data not shown). In the 1-amino-2-alkanol series **328-333** (inverted dihydrosphingosin analogues), *N*-isopropyl/4-methyl-piperazinyl/cyclohexyl substitution (**329**, **331**, **328**, respectively) led to the three most potent compounds.

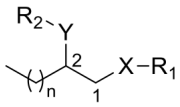
Derivatives showing satisfactory activities and selectivities according to the DNDI recommendations (EC50 < 1 μM and SI > 10) were selected for further studies against TB *gambiense* and TB *rhodesiense* amastigotes (Table **28**).

Additionally, clogP values of 6.3, 5.8 and 5.3, respectively, suggest the ability of compounds **325**, **329** and **331** to cross the blood-brain barrier and support their potential use during the neurological phase of chronic HAT induced by TB *gambiense*. Microscopy images of the parasites after 24 hours treatment with compound **325** showed substantial morphological changes denoting endocytosis inhibition leading to an enlargement of the flagellar-pocket membrane [140].

In the same context, Legarda-Ceballos synthesized a series of aminoalcohols (**334-340**, Table **29**) and diamines (**341-348**, Table **30**) derived from 2-aminolauric, 2-aminopalmitic and 2-aminostearic acids with different substitution patterns. The compounds were tested against TC epimastigotes and amastigotes, and their cytotoxicity was determined on macrophages.

Among aminoalcohols, the palmityl series **335, 338-340** (n = 13) included the most promising compound **339**. The results showed that R_2 = Bu is the optimal substituent length for the tested strains regarding both potency and selectivity index. Amino group dialkylation or -OH benzylation decreased tripanocidal activity (data not shown). *β*-

Table 27. Trypanocidal properties of lipophilic diamines and aminoalcohols [140].



Nº	*n*	X	R_1	Y	R_2	TBr[a] EC_{50} (μM)
321	13	NH	H	NH	Boc	0.961
322	13	NH	Et	NH	Boc	0.704
323	9	O	H	NH	Et	1.087
324	13	O	H	NH	Et	1.139
325	15	O	H	NH	Et	0.584
326	13	O	H	NH	n-Bu	0.817
327	13	O	H	NEt	Et	0.722
328	13	NH	Cyclohexyl	O	H	0.790
329	13	NH	2-Propyl	O	H	0.436
330	13	NEt	Et	O	H	2.001
331	13	NH	4-Me-piperazin-1-yl	O	H	0.524
332	13	NH	4-Bu-piperazin-1-yl	O	H	1.781
333	13	NH	Morpholin-1-yl	O	H	>2.500
Pentamidine						0.0037
Suramin						0.1853

a: T. b. rhodesiense EATRO3

Table 28. Trypanocidal profile of selected diamines and aminoalcohols [140].

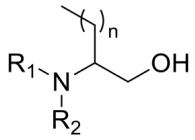
	EC_{50} (nM)		SI	
Nº	TBr[a]	TBg[b]	TBr[a]	TBg[b]
321	**961**	ND	17.0	-
322	**704**	ND	38.8	-
324	1139	ND	39.1	-
325	**584**	**451**	27.7	35.9
329	**436**	**329**	27.1	35.8
330	2001	ND	19.9	-
331	**524**	**431**	23.7	28.8

a: T. b. rhodesiense; b: T. b. gambiense;
ND: not determined

Aminopalmitols **335, 338** and **339** were selected for further studies because of their PNFX and/or SI indexes.

On the other hand, diamine derivatives were less potent and selective than the corresponding aminoalcohols. N^{2-} mono- or dialkylation (R_1, $R_2 \neq H$) decreased biological activity. The most potent and selective compounds of the series were the *N*-Boc derivatives **341,342** which were selected for further studies. All selected compounds were more potent than Nfx against epimastigotes and displayed equal or less activity against amastigotes. Comparatively, the

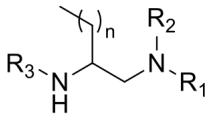
Table 29. Trypanocidal properties of fatty aminoalcohols [141].



-				MG Strain		JEM Strain		Epimastigotes CL-B5		Amastigotes CL-B5	
Nº	**R_1**	**R_2**	**n**	**IC_{50} (μM)**	**PNFX**	**IC_{50} (μM)**	**PNFX**	**IC_{50} (μM)**	**PNFX**	**IC_{50} (μM)**	**PNFX**
334	H	H	9	14.5 (1.0)	1.5	33.0 (0.4)	0.4	–	–	–	–
335	H	H	13	23.7 (3.3)	0.9	3.0 (26)	4.9	3.5 (7.2)	3.0	1.8 (14.0)	0.3
336	H	H	15	46.2 (0.1)	0.5	9.9 (0.3)	1.5	–	–	–	–
337	H	Hex	9	21.1 (1.7)	1.0	37.2 (1.0)	0.4	–	–	–	–
338	H	Et	13	21.6 (2.0)	1.0	19.3 (2.3)	0.8	2.4 (10.6)	4.3	0.6 (54.0)	1.0
339	H	Bu	13	3.2 (20)	6.6	2.8 (23)	5.3	4.2 (11.8)	2.5	2.0 (47.8)	0.3
340	H	Hex	13	6.1 (1.3)	3.5	NA	–	–	–	–	–
NFX	–	–	–	21.2 (2.3)	1.0	14.8 (3.3)	1.0	10.4 (6.7)	1.0	0.6 (116.0)	1.0

MG strain: MHOM/CO/04/MG; JEM strain: MHOM/CO/05/JEM.
PNFX (potency relative to NFX) = IC50-NFX/IC50-compound. NA: Not active (IC_{50}>70 μM).

Table 30. Trypanocidal properties of fatty diamines [141].

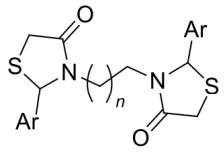


-					MG Strain		JEM Strain		Epimastigotes CL-B5		Amastigotes CL-B5	
Nº	**R_1**	**R_2**	**R_3**	**n**	**IC_{50} (μM)**	**PNFX**	**IC_{50} (μM)**	**PNFX**	**IC_{50} (μM)**	**PNFX**	**IC_{50} (μM)**	**PNFX**
341	H	H	Boc	9	10.8 (4.4)	2.0	14.5 (3.3)	1.0	2.5 (11.8)	4.2	3.0 (9.9)	0.2
342	H	H	Boc	13	12.0 (4.0)	1.8	20.4 (2.4)	0.7	2.4 (10.6)	4.3	2.6 (9.8)	0.2
343	H	H	Boc	15	13.9 (1.7)	1.5	6.7 (3.6)	2.2	-	-	-	-
344	Hex	H	Boc	13	26.7 (0.2)	0.8	30.6 (0.1)	0.5	-	-	-	-
345	Hex	Hex	Boc	13	NA	–	–	–	-	-	-	-
346	H	H	H	13	19.2 (4.1)	1.1	26.6 (2.9)	0.6	-	-	-	-
347	Hex	H	H	13	32.9 (0.2)	0.6	48.8 (0.1)	0.3	-	-	-	-
348	Et	Et	H	13	42.2 (0.5)	0.5	NA	-	-	-	-	-
NFX					21.2 (2.3)	1.0	14.8 (3.3)	1.0	10.4 (6.7)	1.0	0.6 (116.0)	1.0

selected diamines were more potent against the extracellular forms, while aminoalcohol **338** was the most active, selective and thus promising compound against the intracellular parasitic form [141].

Da Silva *et al.* studied another group of fatty aminoalkanols as potential antileishmanial. Selected results are shown in Table **31**. In this case, the tested compounds were less active than the reference drug, but derivatives **351, 354, 358, 359, 361, 364** and **349-354, 357, 363** displayed IC_{50} values below 10 μM against *L. amazonensis* and *L. chagasi*, respectively. Structures containing alkyl chains with 10 and 12 carbon atoms were the most active against both species. Diaminoalcohol **365** showed the best antiproliferative activity against *L. chagasi* promastigotes (IC_{50} = 1.26 μM), surpassing the reference drug [142].

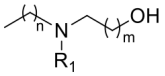
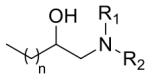
The same group explored in a related publication a series of aminoalcohols derived from 3-phenetylamino-propylenglycol and 3-phenethoxy-1-amino-2-propanol as antileishmanials (Table **32**). Phenethylamine derivatives were inactive against all tested strains (data not shown). Within the *N*-hydroxyalkyl series, compounds displaying good activity on *L. major* **366** and **368** are both formally derived from monoethanolamine. The more lipophilic 1,1-dimethyl-2-hydroxyethylamine derivative **368** was more active than the unbranched compound **366**. These compounds did not impair the growth of the other two *Leishmania* strains. In the alkylamidoethyl series, the optimum activity against both *L. major* and *L. amazonensis* was obtained with an intermediate length acyl chain (n = 8), being the lower and higher homologues less active. *N*-Alkyl derivatives included the most active compound of the series against *L. major* (**374**), with IC_{50} in the same order of magnitude as the reference drug. Also in this case, a longer alkyl chain (from n = 5 to n = 7) resulted in a more active compound [143].



375

Fig. (23). Alkylene-bisthiazolidinones.

Table 31. Leishmanicidal properties of fatty aminoalcohols [142].

-					IC_{50} (μM)	
	N°	***n***	***m***	**R_1**	***LA*[a]**	***LC*[b]**
	349	7	1	H	76.38	9.65
	350	9	1	H	11.74	5.50
	351	11	1	H	8.57	5.42
	352	11	1	2-hydroxyethyl	14.82	4.90
	353	11	2	H	25.14	3.20
	354	13	1	2-hydroxyethyl	4.90	9.22
	355	15	1	H	16.92	19.60
		n	R_1	R_2		
	356	9	H	H	26.00	14.58
	357	9	H	2-hydroxyethyl	11.78	2.17
	358	9	H	3-hydroxypropyl	5.09	ND
	359	9	2-hydroxyethyl	2-hydroxyethyl	9.16	11.28
	360	9	H	1,1-dimethyl-2-hydroxyethyl	12.30	16.38
	361	9	H	2-aminoethyl	2.82	Nd
	362	9	H	2-aminopropyl	4.09	1.26
	363	11	H	3-hydroxypropyl	>227	7.70
	364	11	2-hydroxyethyl	2-hydroxyethyl	8.07	16.40
	365	11	H	1,1-dimethyl-2-hydroxyethyl	>227	45.60
Rd					0.90	1.90

a: L. *amazonensis* (MHOM/ Br/75/ Josefa: isolated from a patient with diffuse cutaneous leishmaniasis) promastigotes; *b:* L *chagasi* (MHOM/ Br/74/ PP75: isolated from a patient with visceral leishmaniasis) promastigotes; Rd: Reference drug: Amphotericyn B.

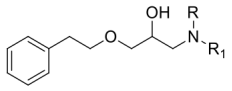
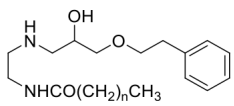
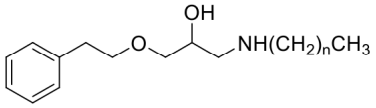
In line with synthetic *N,N'*-disubstituted diamines acting as antiprotozoan agents, Leal *et al*. prepared three families of compounds: alkylene-bisthiazolidinones **375** (Fig. **23**), symmetrically substituted *N,N'*-bis(arylmethyl)-alkylenediamines **376-383** and symmetrically substituted *N,N'*-bis(chloroacetyl)alkylendiamines **374-387**, and tested them against *L. infantum* promastigotes and TC epimastigotes (Table **33**). *Bis*-thiazolidinones (**375**, n = 1-3) were inactive against both parasites. Regarding their leishmanicidal effect, none of the compounds was more effective than the reference drug, although **377** and **387** displayed good activity. The authors also tested the compounds on *L. infantum, L. panamensis* and *L. amazonensis* amastigotes, but IC_{50} values were above 10 μM and their selectivity was low. Compounds **376, 379, 380,385** and **387** were more active than the reference drug against TC epimastigotes. Although compounds **377** and **379** displayed very good selectivity indexes, no activity was found on TC amastigotes [144].

Labadie *et al.* developed a solid phase synthesis of *N, N*-disubstituted diaminopropanes **388-398** and diaminobutanes **399-409** by means of two sequential reductive aminations of resin-bound diamines with variouslyly substituted benzaldehydes. Compounds were tested in an *L. donovani* promastigote growth inhibition assay. In all cases, the activity was enhanced by bulky substituents in the benzyl group **390-393** and **401-404** and abolished by the presence of hydroxy groups **394-396** and **405-407**, regardless of the diamine chain length (Table **34**) [145].

On the basis of their previous results, the same group prepared afterward a series of *N,N'*-di (substituted benzyl) 1,*n*-alkanediamines (n = 3, 4, 6, 8, 10) by reductive amination of the parent diamines with benzaldehyde and oxybenzaldehydes (Table **35**). The resulting compounds were tested against TB trypomastigotes, *L. donovani* promastigotes and the less sensitive TC epimastigotes. Hydroxybenzyl substituted diamines were, accordingly with the previous work, generally not effective against TC (data not shown), while unsubstituted or methoxy derivatives had activities under 10 μM only for the longer chain diamines. On the other hand, all benzyloxy derivatives showed good activity. In particular, the 3-OMe-4-OBn series **425-429** had submicromolar IC_{50} values regardless of the chain length *n* for all three parasites, even surpassing the activity of the reference drugs. Taken together, these results indicate that lipophilicity plays, again, an important role in the absorption of the compounds by the protozoa, in their interaction with the biological target or in both. The authors pointed out that the homogeneously dissimilar response of the three species towards the tested substances may reflect differences in polyamines absorption and metabolism between the parasites [146].

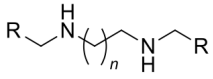
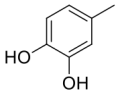
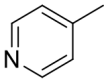
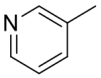
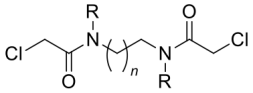
Since imidazolidines possess a variety of biocidal activities, such as antifungal, antibacterial and antiviral activities, Caterina *et al.* studied a series of *N,N'*-disubstituted derivatives as proliferation inhibitors of TC epimastigotes. A first preliminary growth inhibition screening at 25 μM revealed over 30 compounds deserving further examination, and IC_{50} determination was undertaken (Table **36**). Hydrolytic reversal from the imidazolidine to the parent diamine is an actual possibility, and taking into account that many of the most potent substances were derived from *N,N'*-4-chlorobenzyl and *N,N'*-4-methoxybenzyl ethylenediamine, IC_{50} was also determined for these compounds, revealing values of 2.0 and 16.2 μM, respectively. Structure-activity relationships showed that the lipophilicity of the compounds is closely related with their bioactivity. Some preliminary metabolite studies suggested that these compounds target the mitochondrial function [147].

Table 32. Leishmanicidal activity of selected aminopropyleneglycol phenethyl derivatives [143].

-	-			IC_{50} (μM)		
	Nº	**R**	**R_1**	**LA**[a]	**LM**[b]	**LC**[c]
	366	H	$(CH_2)_2OH$	>87.0	13.6	>87.0
	367	H	$(CH_2)_3OH$	>87.0	>87.0	>87.0
	368	H	$C(CH_3)_2CH_2OH$	>87.0	4.6	>87.0
	369	CH_2CH_2OH	CH_2CH_2OH	>87.0	>87.0	>87.0
	-	*n*	-	-	-	-
	370	6	-	49.0	14.4	N.T.*
	371	8	-	18.6	5.2	>87.0
	372	10	-	46.4	19.7	>87.0
	373	5	-	37.3	11.4	27.5
	374	7	-	18.5	0.72	30.0
	RD[d]	-	-	0.4	0.32	1.9

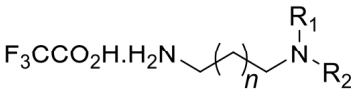
a:(MHOM/Br/74/PP75) Promastigotes; *b:*(IFLA/Br/67/PH8) Promastigotes; *c:*(MRHO/SU/59/P) Promastigotes; *d:*Amphotericyn B; N.T.: Not tested.

Table 33. Growth inhibition properties of diamines and N,N'-bis(chloroacetyl)alkylendiamines [144].

-	-			LI[a] promastigotes		TC[b] epimastigotes	
	R	**Nº**	**n**	**IC_{50} (μM)**	**SI**	**IC_{50} (μM)**	**SI**
	Ph	376	1	12.19	19,58	2.54	29,62
		377	2	1.96	86,29	5.35	64,2
		378	3	11.23	12,75	112.00	0,53
		379	1	40.24	>7.48	0.88	>300
		380	3	>250	>0.89	1.22	>200
		381	1	180.54	>2.28	>250	>0.86
		382	3	28.83	>12.82	>250	>0.35
		383	3	44.19	>8.36	>250	>1
	H	384	1	176.02	>2.66	65.94	0,38
	H	385	2	76.43	2,07	1.01	32,35
	H	386	3	132.17	2,76	54.21	0,68
	Bn	387	3	3.91	6,4	0.02	>11.87
Rd1[c]	–	–	–	0.014	948,57	ND	ND
Rd2 [d]	–	–	–	ND	ND	2.71	28,23

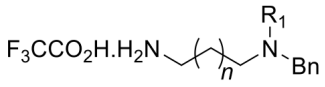
a: L. infantum; b: T. cruzi; c: Amphotericyn B; d: Nifurtimox.

Table 34. Leishmanicidal activity of *N, N*-disubstituted diamines [145].



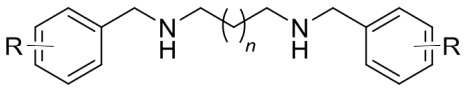
Nº	**$R_1 = R_2$**	***n***	**LD[a] IC_{50} (μM)**	**Nº**	**LD IC_{50} (μM)**
388	Benzyl	1	24.17	2 **399**	26.15
389	4-methoxy-benzyl	1	10.04	2 **400**	10.62
390	4-*i*-propylbenzyl	1	2.65	2 **401**	3.00
391	4-benzyloxy-benzyl	1	1.12	2 **402**	1.85
392	3-MeO-4-BnO-benzyl	1	0.67	2 **403**	1.83
393	2,4-dibenzyloxy-benzyl	1	1.64	2 **404**	1.86
394	4-hydroxy-benzyl	1	NA	2 **405**	NA
395	3-methoxy-4-hydroxy-benzyl	1	NA	2 **406**	NA
396	4-methoxy-3-hydroxy-benzyl	1	NA	2 **407**	NA

(Table 34) contd….

Nº	$R_1 = R_2$	*n*	LD[a] IC_{50} (μM)	Nº	LD IC_{50} (μM)
					
Nº	**R_1**	***n***	***LD*** IC_{50} **(μM)**	**N**	***LD***
397	4-benzyloxy-benzyl	1	1.37	2 **408**	0.37
398	4-*i*-propyl-benzyl	1	1.58	2 **409**	1.88

a: L. donovani

Table 35. Antiparasitic activity of *N*,*N´*-dibenzyl 1,*n*-diamines.

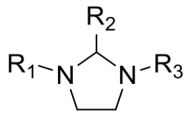
								
-			**TC[a]**		**TB[b]**		**LD[c]**	
Nº	***n***	**R**	IC_{50} **(μM)**	**SI**	IC_{50} **(μM)**	**SI**	IC_{50} **(μM)**	**SI**
410	1	H	>10	—	6.45	>3	11.01	>27
411	2	H	>10	—	7.08	>3	18.63	>1
412	4	H	>10	—	2.03	>8	13.83	>1
413	6	H	>10	—	1.18	>128	10.17	>1
414	8	H	8.17	>3	0.61	>22	1.75	>8
415	1	4-OMe	>10	—	2.04	>7	1.24	>12
416	2	4-OMe	>10	—	3.20	>5	8.52	>2
417	4	4-OMe	>10	—	0.22	60	6.73	2
418	6	4-OMe	>10	—	5.50	>2	1.56	>5
419	8	4-OMe	2.30	>5	>10	—	1.45	>8
420	1	4-OBn	0.78	8	0.062	97	0.26	23
421	2	4-OBn	0.76	8	0.097	60	0.031	187
422	4	4-OBn	1.83	>5	0.14	>67	0.24	>40
423	6	4-OBn	0.99	>9	0.20	>45	1.14	>8
424	8	4-OBn	>10	—	0.62	>14	4.60	>2
425	1	3-OMe-4-OBn	0.81	8	0.19	35	0.23	29
426	2	3-OMe-4-OBn	0.73	12	0.17	52	0.24	37
427	4	3-OMe-4-OBn	0.74	>11	0.19	>44	0.21	>40

(Table 35) contd….

–			TC[a]		TB[b]		LD[c]	
Nº	***n***	**R**	**IC_{50} (μM)**	**SI**	**IC_{50} (μM)**	**SI**	**IC_{50} (μM)**	**SI**
428	6	3-OMe-4-OBn	0.82	6	0.19	24	0.22	21
429	8	3-OMe-4-OBn	0.80	>10	0.11	>67	1.20	>6
Rd1	-	-	-	-	-	-	0.32	-
Rd2	-	-	-	-	-	-	6.20	-
Rd3	-	-	2.10	-	1.70	-	-	-

a: T. cruzi; *b: T. brucei* MITat 427 strain, clone 221a;
c: L. donovani Strain S1; *d:* Amphotericin B;
e: Pentamidine; *f:* Nifurtimox

Table 36. Trypanocidal activity of *N,N'*-disubstituted imidazolidines.

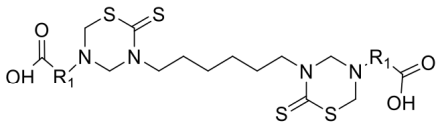


Nº	**R_1**	**R_2**	**R_3**	**PGI%**	**IC_{50} (μM)**
430	4-$ClC_6H_4CH_2$	H	4-$ClC_6H_4CH_2$	100.0	10.3
431	3-$ClC_6H_4CH_2$	H	3-$ClC_6H_4CH_2$	100.0	9.7
432	3,4-$Cl_2C_6H_3CH_2$	H	3,4-$Cl_2C_6H_3CH_2$	100.0	15.1
433	4-$ClC_6H_4CH_2$	CH_3	4-$ClC_6H_4CH_2$	40.0	37.0
434	4-$CH_3OC_6H_4CH_2$	C_6H_5	4-$CH_3OC_6H_4CH_2$	75.3	1.1
435	4-$CH_3C_6H_4CH_2$	C_6H_5	4-$CH_3C_6H_4CH_2$	100.0	12.5
436	4-$ClC_6H_4CH_2$	C_6H_5	4-$ClC_6H_4CH_2$	94.5	5.0
437	3-$ClC_6H_4CH_2$	C_6H_5	3-$ClC_6H_4CH_2$	100.0	14.7
438	3,4-$Cl_2C_6H_3CH_2$	C_6H_5	3,4-$Cl_2C_6H_3CH_2$	100.0	6.4
439	4-$ClC_6H_4CH_2$	2-Furyl	4-$ClC_6H_4CH_2$	89.4	4.3
440	3,4-$Cl_2C_6H_3CH_2$	2-Furyl	3,4-$Cl_2C_6H_3CH_2$	93.9	4.1
441	4-$CH_3OC_6H_4CH_2$	–CH = CHC_6H_5	4-$CH_3OC_6H_4CH_2$	98.6	10.0
442	4-$ClC_6H_4CH_2$	–CH = CHC_6H_5	4-$ClC_6H_4CH_2$	100.0	10.2
443	4-$ClC_6H_4CH_2$	4-$CH_3OC_6H_4$	4-$ClC_6H_4CH_2$	100.0	5.0
444	4-$ClC_6H_4CH_2$	3-OHC_6H_4	4-$ClC_6H_4CH_2$	97.4	4.6
445	4-$ClC_6H_4CH_2$	2-OHC_6H_4	4-$ClC_6H_4CH_2$	86.8	4.3
446	4-$CH_3OC_6H_4CH_2$	4-ClC_6H_4	4-$CH_3OC_6H_4CH_2$	100.0	14.9
447	4-$CH_3C_6H_4CH_2$	4-ClC_6H_4	4-$CH_3C_6H_4CH_2$	100.0	9.0
448	$C_6H_5CH_2$	4-ClC_6H_4	$C_6H_5CH_2$	60.0	23.5
449	4-$ClC_6H_4CH_2$	4-ClC_6H_4	4-$ClC_6H_4CH_2$	100.0	8.9

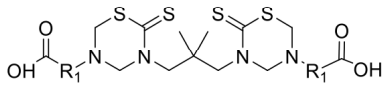
(Table 36) contd….

Nº	R_1	R_2	R_3	PGI%	IC_{50} (μM)
450	3,4-$Cl_2C_6H_3CH_2$	4-ClC_6H_4	3,4-$Cl_2C_6H_3CH_2$	50.0	25.0
451	4-$CH_3OC_6H_4CH_2$	3-ClC_6H_4	4-$CH_3OC_6H_4CH_2$	95.0	14.0
452	4-$CH_3C_6H_4CH_2$	3-ClC_6H_4	4-$CH_3C_6H_4CH_2$	99.0	13.4
453	4-$ClC_6H_4CH_2$	3-ClC_6H_4	4-$ClC_6H_4CH_2$	100.0	4.7
454	4-$CH_3OC_6H_4CH_2$	2-ClC_6H_4	4-$CH_3OC_6H_4CH_2$	49.0	25.3
455	4-$ClC_6H_4CH_2$	2-ClC_6H_4	4-$ClC_6H_4CH_2$	100.0	11.0
456	4-$CH_3OC_6H_4CH_2$	3,4-$Cl_2C_6H_3$	4-$CH_3OC_6H_4CH_2$	91.0	16.0
457	4-$ClC_6H_4CH_2$	3,4-$Cl_2C_6H_3$	4-$ClC_6H_4CH_2$	92.0	9.0
458	4-$CH_3OC_6H_4CH_2$	4-$NO_2C_6H_5$	4-$CH_3OC_6H_4CH_2$	83.0	16.5
459	4-$ClC_6H_4CH_2$	4-$NO_2C_6H_5$	4-$ClC_6H_4CH_2$	100.0	14.5
460	4-$CH_3OC_6H_4CH_2$	3-$NO_2C_6H_5$	4-$CH_3OC_6H_4CH_2$	93.0	23.0
Nfx				100.0	7.7

As a part of the development of new antiprotozoals with the tetrahydro-(2*H*)-1,3,5-thiadiazine-2-thione (bis-THTT) scaffold, Coro synthesized two series of related compounds and tested them in *L. donovani* amastigotes, TB *rhodesiense* and *P. falciparum*. (Fig. **24**).



461



462

L. donovani IC_{50} 3.53-7.27 μM (SI = 0.3-1.1)
T. b. rhodesiense IC_{50} 0.37-2.8 μM (SI = 1.7-3.4)

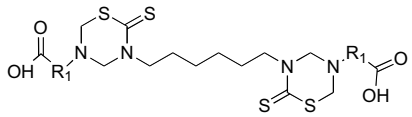
Fig. (24). Bis-THTT derivatives.

Series **461** comprised 10 compounds, with IC_{50} values in the range 2.18-30 μM against *L. donovani* and poor therapeutic index. Best activity and selectivity results were obtained against TB *rhodesiense*, with compounds **463-466** (Table **37**).

Series **462** consisted of four compounds with good trypanocidal activity but higher cytotoxic than series **461** analogues. The authors suggested that the connective methylenic chain could account for this behaviour. *In vivo* assays were hampered due to low solubility [148].

In 1999 Kelly reported that TB and, to a lesser extent, TC and *L. major* were sensitive to the anti-influenza drugs rimantadine and amantadine (Fig. **25**) [149]. In the case of Influenza A, the drugs target is the ion channel protein M2, which is essential for viral replication by regulation of proton conductance. The authors hypothesized that an analogous mechanism would be operative in protozoans, where a membrane-localized H^+-ATPase was identified as responsi-

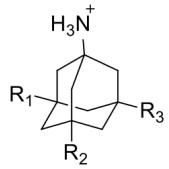
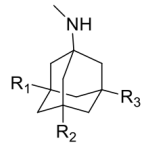
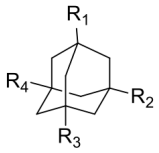
Table 37. Growth inhibition properties of bis-THTT derivatives.



-		IC_{50} (μM) (SI)	
Nº	R_1	LD[a]	TBr[b]
463	$CH_3S(CH_2)_2CH$	7.26(7.3)	2.30(41.2)
471 464	-$(CH_2)_4$-	6.74(2.4)	0.36(44.4)
465	-$(CH_2)_2$-	2.18(5.6)	0.56(21.8)
466	-$(CH_2)_3$-	24.33(3.5)	4.27(19.9)

a*: L. donovani* amastigote form; b*: T. b. rhodesiense* bloodstream form

Table 38. Anti *T. brucei* activity of adamantlyamines [151].

-	-	R_1	R_2	R_3		IC_{50} (μM)	IC_{90} (μM)
	**467**	H	H	*i*-Pr		4.91	8.07
	468	H	H	Ph		2.77	3.75
	469	H	H	*c*-C_6H_{11}		0.52	0.70
	470	H	*c*-C_6H_{11}	*c*-C_6H_{11}		0.37	0.51
	471	CH_3	CH_3	CH_2CH_3		5.25	7.58
		R_1	R_2	R_3		IC_{50}	IC_{90}
	**472**	H	H	*i*-Pr		6.48	11.39
	473	H	H	*n*-Bu		1.55	2.52
		R_1	R_2	R_3	R_4	IC_{50}	IC_{90}
	**474**	CH_3	-$(CH_2)_2NH_3Cl$	H	H	2.14	3.64
	475	H	-$(CH_2)_3NH_3Cl$	H	H	1.22	1.48
	476	H	-$(CH_2)_2NH_3Cl$	CH_3	CH_3	1.15	1.56
	477	*c*-$C_6H_{10}NH_3Cl$	H	H	H	0.33	0.41
	478	Ph	-$(CH_2)_2NH_3Cl$	H	H	0.62	0.92

ble for pH homeostasis [150]. Rimantadine was thus an interesting candidate for drug repurposing as it is inexpensive, can be administrated orally, has few side effects and crosses the blood/brain barrier, which is a necessary feature in the advanced HAT. When rimantadine was tested on the bloodstream form of TB (strain 427), the compound showed pH-dependent trypanocidal activity. Amantadine was also active, but at higher concentrations. Analogous results were obtained against TC and *L. major*. The response of TC cells to rimantadine treatment was characterized by swelling and loss of typical epimastigote morphology, including the flagellum.

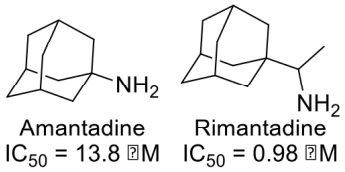


Fig. (25). The potency of Amantadine and Rimantadine against *T. brucei.*

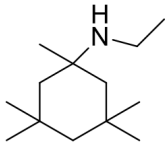
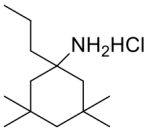
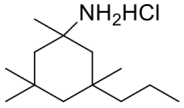
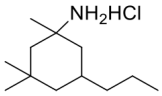
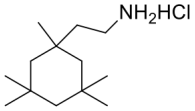
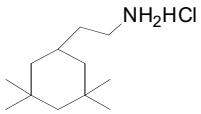
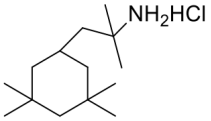
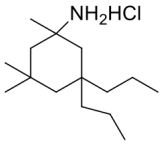
The fact that rimantadine and amantadine, which are structurally related, display very different potency against *T. brucei* (Fig. **25**) encouraged the development of new adamantane analogues. Consequently, in 2001, Miles investigated a series of aminoadamantanes, cyclohexylamines and related compounds as growth inhibitors of *T. brucei* (Table **38**) [151].

Aminoadamantanes monosubstituted at the bridgehead positions **467-469** showed increasing activity with increasing bulkiness of the substituent. Disubstitution (compound **470**) resulted in a further increase in activity, but trisubstitution as in **471** did not, probably reflecting some size or shape constraint in the target. *N*-methyl substitution actually decreased potency. Aminoalkyl adamantanes were, in general, more potent than the first groups and derivative **477** was the most active compound of this series. A certain relationship between hydrophobicity and potency was also evident within this group, and derivatives bearing polar substituents were devoid of trypanocidal properties (data not shown).

When the adamantane nucleus was replaced by polyalkylated cyclohexyl groups, a similarly narrow range of activities was observed (Table **39**). In this group of compounds, the spatial distribution of the lipophilic and cationic portions of the molecules seemed to have a considerable effect on potency.

Finally, adamantamines containing hydroxy substituents were synthesized by Zoidis *et al.* and their anti-influenza A and anti TB activity were investigated [152]. This design combines the aminoalcohol motif with the adamantane ring lipophilic properties. Structure-activity analysis showed that the optimum potency was achieved when both polar groups were separated by 4-5 carbons (*i.e.* compound **490**). These results show that the hydrophobic chain present in the sphingosine analogues previously discussed can be replaced by the adamantane moiety (Fig. **26**).

Table 39. Trypanocidal activity of cyclohexylamine analogues [151].

–		TB[a]	
	N°	**IC_{50} (μM)**	**IC_{90} (μM)**
	**479**	5.86	8.38
	**480**	6.11	7.99
	**481**	2.35	3.76
	**482**	2.59	3.50
	**483**	1.03	1.62
	**484**	1.05	1.41
	**485**	0.89	1.17
	**486**	0.95	1.11

T. brucei strain 427.

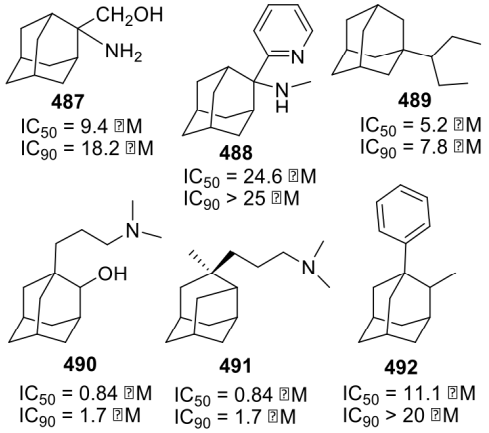


Fig. (26). Anti *T. brucei* activity of selected adamantyl amines**.**

CONCLUDING REMARKS

Synthetic polyamines and related organic cationic compounds have been widely studied as possible chemotherapeutics in the treatment of kinetoplastid diseases during the last years. The literature includes compounds with a considerable structural diversity and involves several functional groups. Besides the primal rationalization of the possibility that polyamines should be useful in the regulation of the unique, kinetoplastid specific, metabolite trypanothione, other functions that could be modulated by this class of compounds have emerged. Among these target- specific activities, some TR and SOD inhibitors, as well as aminopurine transporter ligands proved highly effective in enzymatic assays *in vitro*, showing submicromolar inhibitory constants and affinities. However, these promising results generally correlated poorly with cell culture trials involving

different parasite stages. These findings reinforce the need for continuous research concerning polyamines transport and metabolism, in order to get a better insight into this potential drug family. On the other hand, pharmacomodulation of compounds with antiparasitic activity (*i.e.* antimalarials) showed more promising chemotherapeutic profiles. Finally, aminoalcohols derived from sphingosine, lipophilic diamines and monoamines, were studied by several groups with the purpose of designing structures with improved membrane permeability. In this way, compounds could reach the parasite cytoplasm or penetrate the host intracellular parasitophorous vacuole and exert their activity. This group includes some of the most powerful compounds considered in this work, being more effective on *Leishmania spp*. These data suggest that for any compound to be effective against trypanosomatids, it should not only be a specific modulator of the kinetoplastid biochemistry but it must also be capable of interacting efficiently within the frame of the host cell.

Additionally, the medicinal chemistry attempts to modulate the parasites polyamine biochemistry, together with knowledge arising from other fields will provide a deeper insight into their ultimate functions and regulation, which are still poorly understood.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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