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REVIEW

Detection and treatment of *Trypanosoma cruzi*: a patent review (2011-2015)

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ABSTRACT

Introduction: *Trypanosoma cruzi* is the etiologic agent of American trypanosomiasis (Chagas disease), which is one of the important parasitic diseases worldwide. The number of infected people with *T. cruzi* diminished from 18 million in 1991 to 6 million in 2010, but it is still the most prevalent parasitic disease in the Americas. The existing chemotherapy is still deficient and based on two drugs: nifurtimox and benznidazole, which are not FDA-approved in the United States.

Areas covered: This review covers the current and future directions of Chagas disease chemotherapy based on drugs that interfere with relevant metabolic pathways. This article also illustrates the challenges of diagnosis, which in recent infections, is only detected when the parasitemia is high (direct detection); whereas, in the chronic phase is reached after multiple serological tests.

Expert opinion: The current chemotherapy is associated with long term treatments and severe side effects. Nifurtimox and benznidazole are able to cure at least 50% of recent infections. Nevertheless, they suffer from major drawbacks: selective drug sensitivity on different *T. cruzi* strains and serious side effects. The aim of this review is focused on presenting an up-to-date status of the chemotherapy and diagnosis.

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Trypanosoma cruzi; Chagas disease; drug discovery; drug treatment; antiparasitic agents; diagnosis

1. Introduction

American trypanosomiasis or Chagas disease, first described by Carlos Chagas, is a chronic parasitosis caused by the kinetoplastid parasite *Trypanosoma cruzi* [1,2]. As a consequence of public policy on the control of Chagas diseases vectors, the number of infected people with *T. cruzi* infection declined from 18 million in 1991 to 6 million in 2010, yet, Chagas disease is still the most prevalent parasitic disease in the American continent [3]. Like other trypanosomatids, *T. cruzi* has a complex life cycle that involves blood-sucking activity between reduviid insects and mammals [4]. The parasite proliferates in the insect gut as an epimastigote form and is spread as a nondividing metacyclic trypomastigote from the insect feces by contamination of intact mucosa or wounds produced by the blood-sucking activity of the vector. In the mammalian host, *T. cruzi* multiplies intracellularly in the amastigote form and is subsequently released into the bloodstream as a nondividing trypomastigote (Figure 1) [4]. Transmission of Chagas disease could also occur through the placenta or by blood transfusion. This latter mechanism is responsible for the occurrence of Chagas disease in areas where this disease is not endemic due to increasing travel and immigration of infected people from other areas. As the amastigote form is the dividing clinically more relevant form of *T. cruzi*, this form is more recommended for biological evaluation of new compounds against different discrete typing units of the parasite.

At the present time, there are no vaccines to prevent infection of *T. cruzi* [5]. The current chemotherapy relies on two empirically discovered drugs: nifurtimox (Lampit®, Bayer – El Salvador, **1**) and benznidazole (Abarax®, Elea – Argentina, **2**), which have been shown to cure at least 50% of recent infections [1,6]. Both drugs produce serious side effects including vomiting, anorexia, peripheral neuropathy, and allergic dermopathy, and as a consequence, are not FDA-approved drugs. In the United States, they are available only from CDC under investigational protocols. Since 2012, benznidazole is being produced by Laboratorios ELEA and Maprimed, Argentina, and it is commercialized as Abarax® [7]. Both compounds are not suitable for pregnant women [8]. Their toxic effects can be attributed to their corresponding mode of action by oxidative stress, most effective in *T. cruzi* cells than mammalian cells [9,10]. Lastly, the main weakness of these two drugs is their modest antiparasitic activity in the chronic phase of the disease (Figure 2).

Other drugs available for the treatment of Chagas disease are itraconazole (**3**) and allopurinol (**4**). However, none of these compounds offers satisfactory treatments in the chronic and acute stages of the disease [11,12].

The dye gentian violet (**5**) is the only currently available chemoprophylactic agent to prevent blood transmission of Chagas disease. Unfortunately, this drug gives a purple color to the blood and stains the skin. In addition, safety concerns have been raised, as it was shown to be carcinogenic in animal models (Figure 2) [13].

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Article highlights

- Chagas disease is a parasitic illness produced by *Trypanosoma cruzi*, endemic from southern United States to southern South America.
- The current treatment for Chagas disease involves the use of two empirically discovered drugs: nifurtimox and benznidazole, which present severe side effects and are not FDA approved.
- The development of putative drugs exploits the metabolic differences between the parasite and the host.
- Drugs have been developed targeting several metabolic pathways, with many key enzymes present: $\Delta^{24(25)}$-sterol methyltransferase, 14α-demethylase (CYP51), squalene epoxidase, squalene synthase, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, farnesyl pyrophosphate synthase, cysteine proteinases, trypanothione biosynthesis, Fe-superoxide dismutase, protein kinases and trans-sialidase.
- Some drugs have non-identified targets such as those producing oxygen active species, analogues of ML341 and lychnopholide.
- Currently, only two drugs have progressed into clinical trials: posaconazole and the prodrug of ravuconazole E1224.
- Patents filed regarding the diagnosis of the disease utilize biochemical tools such as antigenic polypeptides, oligonucleotide sequences and the detection of *T. cruzi* antibodies.

This box summarizes key points contained in the article.

The growing knowledge of the biochemistry and physiology of *T. cruzi* has led to the finding of new and valid targets for drug design. Based on unique aspects of parasites' biochemistry and physiology of the parasite, and taking into account the metabolic differences between the mammalian host and the parasite, we have selected the more promising molecular targets for drug design. At the present time, several metabolic pathways are being targeted such as sterol biosynthesis [14–16], pyrophosphate metabolism [17,18], trypanothione biosynthesis [19], cysteine proteinases [20], metacaspases [21], metallopeptidases [22], protein prenyltransferases [23], and others [24–29].

2. Isoprenoid biosynthetic pathway

Isoprenoids are essential metabolites for the cellular machinery of all organisms because of their roles in a variety of biological processes. Certainly, this biosynthetic pathway in *T. cruzi* contains a number of enzymes that are involved in the synthesis of sterols [30], in farnesyl diphosphate formation [31], and in protein prenylation [32]. These enzymes can be considered as relevant molecular targets against trypanosomatids.

Sterol biosynthesis in parasites differs from that in mammalian hosts, as it leads to ergosterol rather than cholesterol, the main sterol present in the mammals. Depletion of endogenous sterols such as ergosterol or 24-ethylcholesta-5,7,22-trien-3β-ol elicits inhibition of multiplication of *T. cruzi* [14–16]. The isoprenoid biosynthetic pathway is illustrated in Scheme 1 [14–16]. The red bars crossing the arrows indicate the availability of inhibitors for each particular enzyme.

3. Inhibitors of $\Delta^{24(25)}$-sterol methyltransferase

Azasterols are known inhibitors of $\Delta^{24(25)}$-sterol methyltransferase. This type of drugs exhibited selective antiproliferative effects against trypanosomatid parasites [33,34]. For example, 22,26-azasterol (**6**) is a potent inhibitor of the enzymatic activity of $\Delta^{24(25)}$-sterol methyltransferase. This enzymatic activity is associated with an efficient antiproliferative action against *T. cruzi* cells both *in vitro* and *in vivo* [33,34]. In addition, the synthetic compound 24(*R,S*),25-epiminolanosterol (**7**) [35] has also exhibited growth inhibitory effect against *T. cruzi* cells producing ultrastructural alterations as evidenced by the appearance of electron-dense granules, mitochondrial swelling, and vacuolization leading to cell lysis [36]. The use of this compound at lower concentrations but in combination with ketoconazole produces a synergic antiproliferative effect

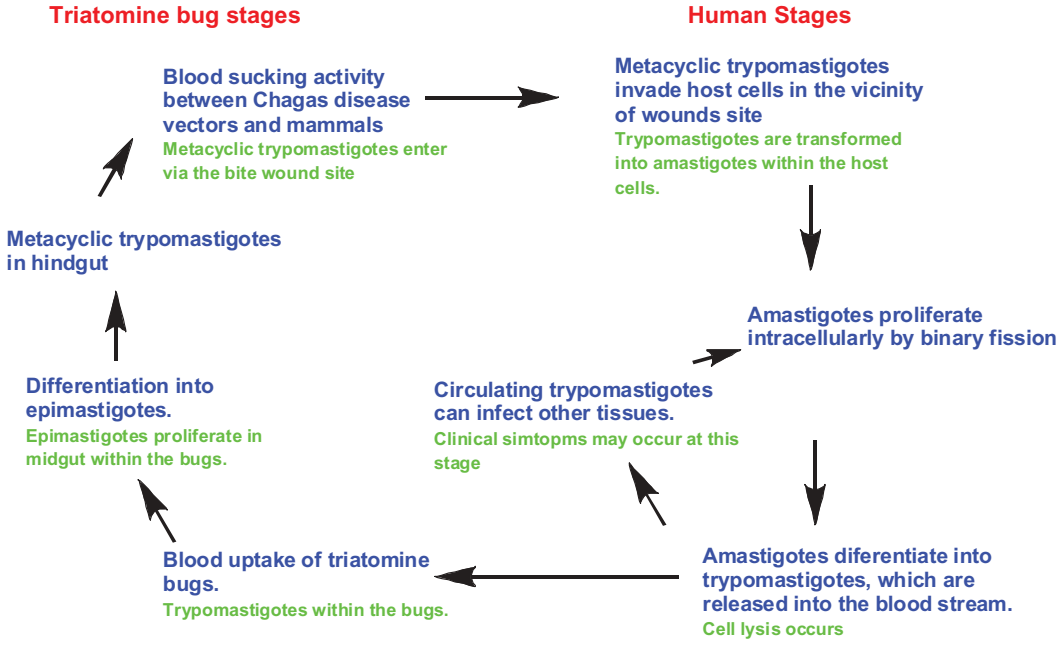


Figure 1. Life cycle of *Trypanosoma cruzi*.

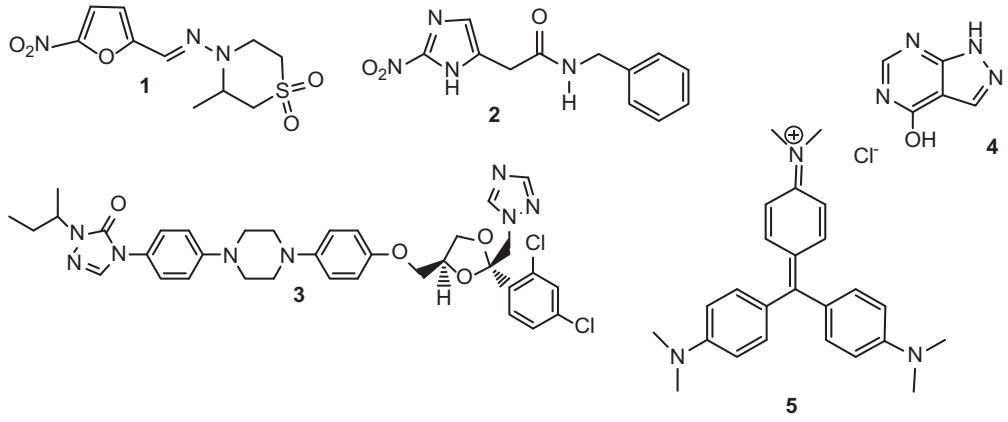


Figure 2. Chemical structures of the existing drugs for the treatment of Chagas disease.

against *T. cruzi* resulting in the same ultrastructural alterations (Figure 3).

4. Inhibitors of the 14α-demethylase (CYP51) activity

This target offers great potential, as it takes advantage of drugs currently in use in the clinic. As the sterol biosynthesis pathway in fungi is similar to the corresponding one in trypanosomatids, drugs currently used as broad-spectrum antifungals can be repurposed as antiparasitic agents such as imidazole and triazole derivatives [37]. *T. cruzi*, as well as fungi and yeasts, requires specific endogenous sterols for cell viability and growth. Most of the clinically employed sterol biosynthesis inhibitors are not able to induce complete parasitological cure in Chagas disease and animal models [14,38], but some interesting examples are very efficient as will be discussed later. A relevant broad-spectrum antifungal agent is ketoconazole (**8**), an imidazole derivative that behaves as a potent inhibitor of *T. cruzi* proliferation targeting 14α-demethylase [39].

The *R*–(+)-enantiomer of bis-triazole derivative ICI 195,739 (**9**) [40], known as D0870, is an interesting example of an antifungal agent to control *T. cruzi* proliferation. This compound is able to achieve complete parasitological cure in murine models of the acute and chronic stages of this disease [37].

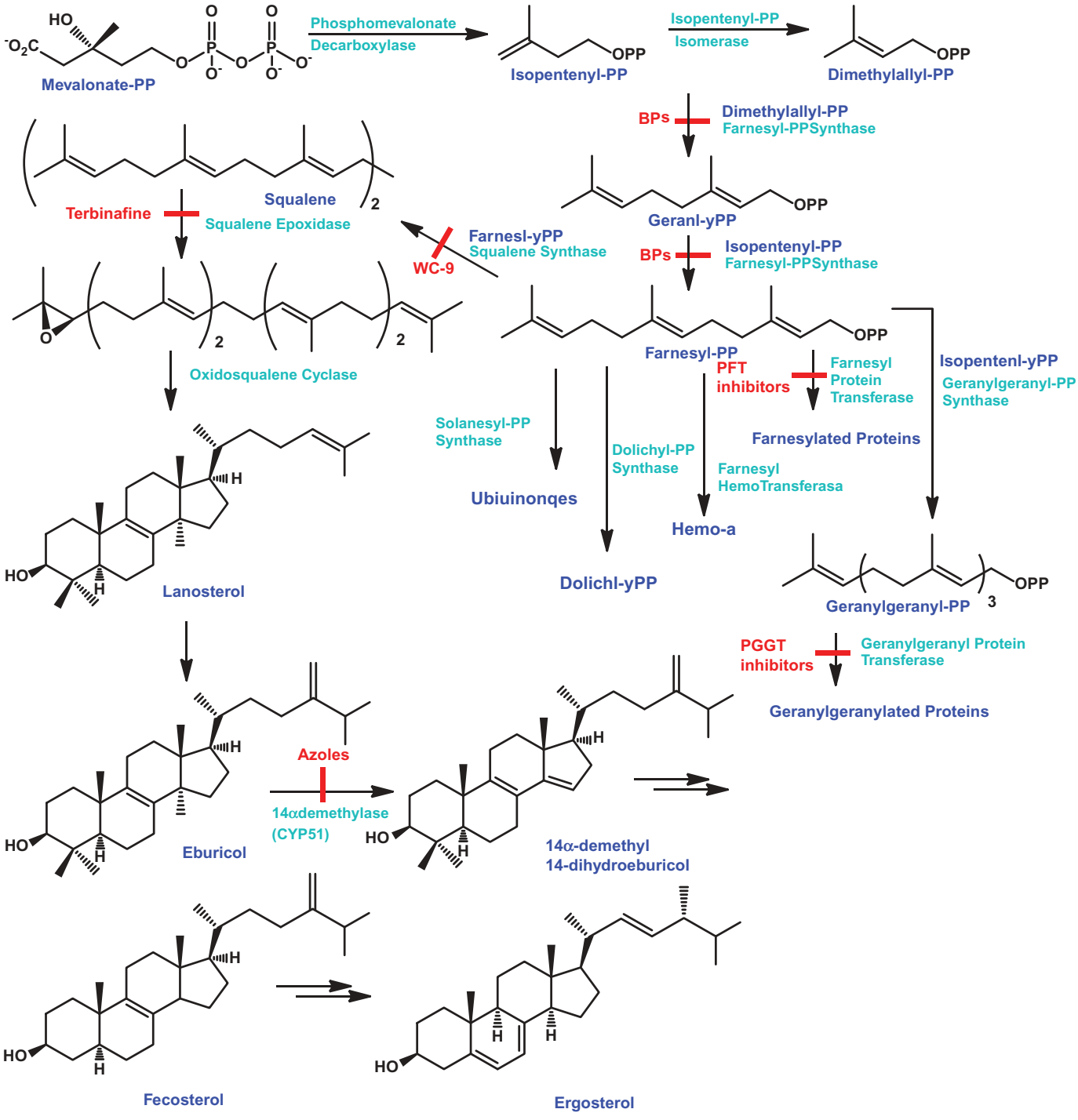
Posaconazole (**10**) is a triazole derivative that targets 24α-demethylase [14,41], exhibiting a broad-spectrum antifungal activity and an extremely potent inhibitory action against *T. cruzi* proliferation by blocking ergosterol biosynthesis [42]. This drug shows potent antiparasitic activity against a variety of *T. cruzi* strains resistant to benznidazole, nifurtimox, and ketoconazole [42] (Figure 4).

Ravuconazole (**11**) is another potent and broad-spectrum antifungal agent possessing a potent inhibitory action against *T. cruzi* proliferation. This drug blocks ergosterol biosynthesis at the level of cytochrome P-450-dependent sterol C14α demethylase [14,43]. Since 2012, posaconazole and ravuconazole are in clinical trials in Spain, Bolivia, and Argentina [14,44]. A very interesting prodrug of ravuconazole is the phosphonooxymethyl derivative **12** with improved water solubility [45]. This prodrug is expected to be hydrolyzed by an alkaline phosphatase to produce the corresponding hemiacetal derivative, which is a very labile derivative that spontaneously hydrolyzes to afford free ravuconazole [45]. Of particular interest is the monoester of lysine of **12** (E1224), which has excellent prospects as a promising candidate for the potential treatment of Chagas disease [14,46]. This drug is administered once a week orally and has good absorption and tolerability profiles besides being reasonably inexpensive to manufacture. An encapsulated formulation of this compound has been recently patented [47]. TAK-187 (**13**) is another antifungal and a potent growth inhibitor of *T. cruzi* amastigotes, inhibiting the parasite CYP51 at nanomolar concentrations [48]. Recently, simpler and cheaper compounds compared to posaconazole chemical structure have been envisioned giving rise to potent inhibitors of *T. cruzi* proliferation such as **14** acting at the very low nanomolar range [49]. Further optimization and simplification of this structure lead to **15**–**17**, which are extremely efficient growth inhibitors having EC_{50} values at the subnanomolar level [50].

The antitumor agent tipifarnib (**18**) is a potent growth inhibitor of intracellular *T. cruzi* [51]. This cellular activity is associated with an inhibition of the enzymatic activity of CYP51 [51]. The tipifarnib analog **19** is more potent than **18** against amastigotes and shows a good level of protection in *in vivo* mouse models but to a lesser extent than posaconazole [52].

The imidazole-containing compounds **20** and **21**, known as VNI and VNF, respectively, are potent growth inhibitors of *T. cruzi* targeting CYP51 [53]. Particularly, **20** is able to cure both acute and chronic experimental Chagas disease models [54]. Closely related analogs have been recently developed, and structural studies have been conducted in the search of an improved molecular recognition [55]. In this context, **22** is a representative member of imidazole-containing derivatives, which exhibits a very potent EC_{50} value against amastigotes (ED_{50} = 1.2 nM) [55].

In summary, the sterol 14α demethylase constitutes a valid target for drug design in Chagas disease chemotherapy. Therefore, posaconazole and ravuconazole are promising candidates, and they are currently in clinical trials for Chagas disease [56]. One limitation of posaconazole is its extremely high cost that might restrict its use in areas where Chagas disease is endemic [56].



Scheme 1. Overview of the isoprenoid biosynthesis in trypanosomatids. Red bars indicate the availability of inhibitors targeting each individual enzyme. Full color available online.

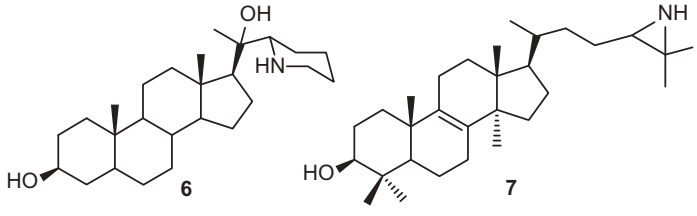


Figure 3. Chemical structures of 22,26-azasterol and 24,25-(*R,S*)-epiminolanosterol.

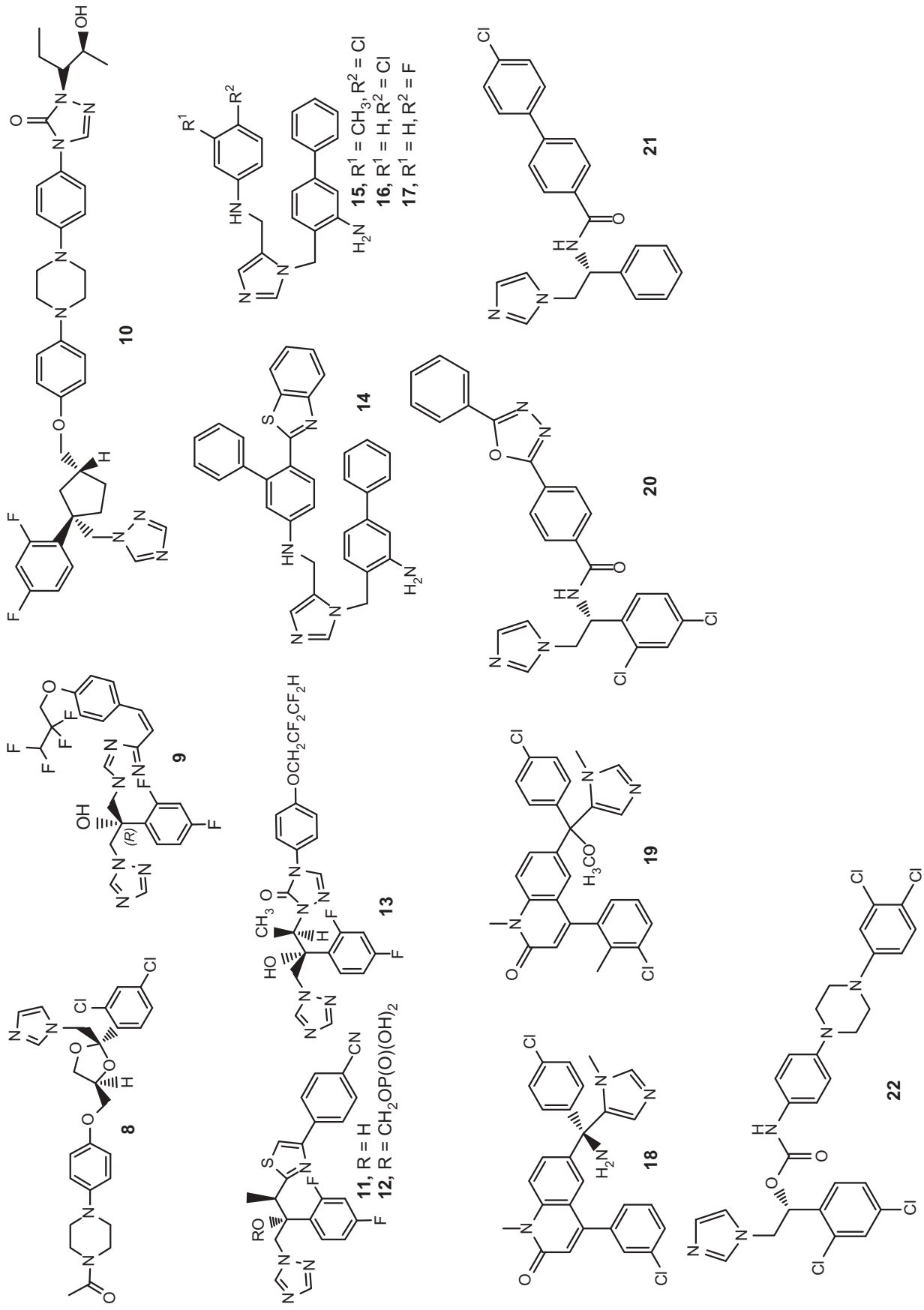


Figure 4. Chemical structures of representative 24α demethylase inhibitors having potent action as inhibitors of *T. cruzi* proliferation.

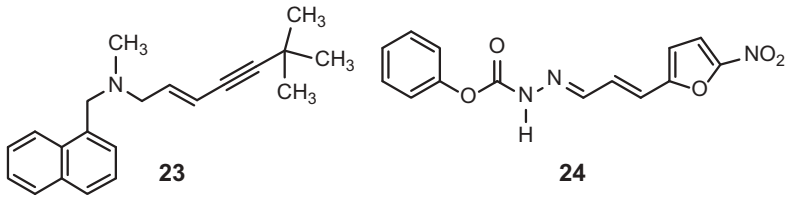


Figure 5. Chemical structures of terbinafine, a representative antifungal agent targeting squalene epoxidase and compound **24.**

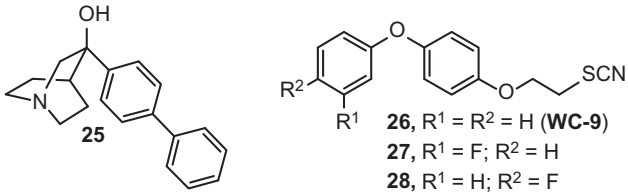


Figure 6. Chemical structures of inhibitors of the enzymatic activity of squalene synthase.

5. Inhibitors of squalene epoxidase

Terbinafine (**23**) is a broad-spectrum antifungal agent that exhibits potent inhibitory action against amastigotes [57] targeting squalene epoxidase [58]. Although terbinafine is less effective than ketoconazole, it has a significant synergistic effect, as these drugs act at different points of the ergosterol biosynthesis [57]. Based on the nifurtimox and terbinafine chemical structures, 5-nitrofuranes such as **24** has been conceived [57], which behaves as a potent inhibitor of amastigotes at the submicromolar range acting with a dual mechanism of action: inhibition of squalene epoxidase and oxidative stress [59] (Figure 5).

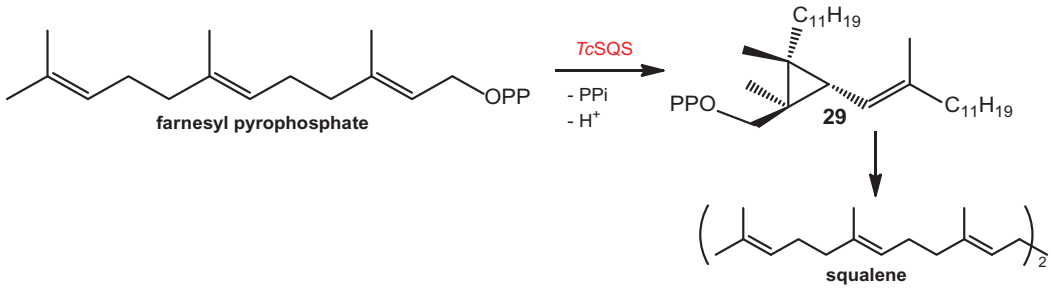
6. Squalene synthase

Squalene synthase (SQS) is a valid target against trypanosomatids [60]. SQS catalyzes the first committed step in sterol biosynthesis: a reductive dimerization of two molecules of farnesyl pyrophosphate to yield squalene. The human counterpart of SQS has been studied as a cholesterol-lowering target, and the crystal structure of this enzyme is available [61]. It is worthy to point out that the quinuclidine derivative **25**, a known inhibitor of human SQS [62], exhibits cellular activity against epimastigotes [60], which is associated with a noncompetitive nanomolar inhibition of the enzymatic activity of SQS [60]. **WC-9** (**26**) is an interesting compound, which presents ED_{50} values at the low nanomolar range against intracellular *T. cruzi* even more potent than nifurtimox and ketoconazole when assayed at the same conditions [63] (Figure 6). **WC-9** is a potent noncompetitive inhibitor of both glycosomal and mitochondrial *T. cruzi* SQS, with IC_{50} values at the low nanomolar range [64]. A rigorous SAR study has been conducted on the **WC-9** chemical structure concluding that the phenoxyethyl thiocyanate unit is the pharmacophore [63,65,66]. The introduction of electron withdrawing atoms such as fluorine at the terminal phenyl ring of **WC-9** has proven to be quite beneficial for biological activity [65]. Certainly, fluoro derivatives **27** and **28** are much more potent than **WC-9** against *T. cruzi* (amastigotes) proliferation [65]. Recently, different **WC-9** analogs have been synthesized and biologically evaluated, but their potency was found to be slightly below than that of the lead structure **WC-9** [66]. The crystal structure of the **WC-9** –*Tc*SQS complex is not available yet. However, the X-ray crystallographic structure of **WC-9** bound to dehydrosqualene synthase (CrtM) from *Staphylococcus aureus* has been recently published [67]. It has been postulated that **WC-9** might bind into the same hydrophobic S2 pocket in *Tc*SQS as it does in CrtM keeping the same polar interactions with the thiocyanate group of **WC-9** [67]. Bearing in mind the relevance of **WC-9**, crystallization of this compound bound to its target enzyme *Tc*SQS has been attempted but, unfortunately, without positive results [68]. Interestingly, the crystal structure of the **WC-9**–human SQS complex has been recently published [68], but no inhibition data against the enzymatic activity are available [68]. This information can be used to facilitate rational drug design to optimize the **WC-9** chemical structure. Despite the great projection of **WC-9**, there is no patent application involving its structure or other closely related compounds.

Even though there are no crystal data at hand in *Tc*SQS, based on the charge distribution of **WC-9**, it is reasonable to suggest that the electrophilic carbon atom of the thiocyanate group acts by mimicking the carbocationic transition state of the reaction that leads to the formation of the cyclopropylcarbinyl intermediate presqualene-diphosphate **29**, a precursor of squalene (Scheme 2).

Our lead structure **WC-9** originated while working on the design and preparation of juvenile hormone analogs (JHAs) of insects, which are crucial metabolites to maintain larval stages [69]. Fascinatingly, Chagas disease vectors such as *Rhodnius prolixus* or *Triatoma infestans*, when treated with JHA, are less susceptible to get natural infections with *T. cruzi* than non-treated vectors [70]. In addition, it has been found that JHAs, which act on non-Chagas disease vectors, also show moderate inhibitory action against *T. cruzi* multiplication [71]. This dual action of JHAs on Chagas disease vectors and also on *T. cruzi* cells led to modification of the chemical structure of the insect growth regulator fenoxycarb (**30**), which has the 4-phenoxyphenoxy moiety in its structure [72] (Figure 7). Structure optimization of **30** yielded **WC-9** and other relevant analogs.

E5700 (**31**) and ER-119884 (**32**) are two novel active SQS inhibitors that have been designed as cholesterol-lowering and triglyceride-lowering agents for humans (Figure 8). These compounds are potent inhibitors of *T. cruzi* proliferation. This cellular activity can be attributed to an efficient noncompetitive (E5700) or mixed (ER-119884) inhibition of



Scheme 2. Reaction calalyzed by SQS, converting FPP into squalene with of cyclopropylcarbinyl presqualene-diphosphate **29** as intermediate.

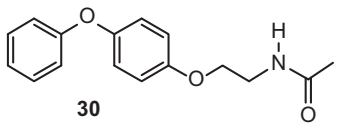


Figure 7. Chemical structure of the well-known insect growth regulator fenoxycarb.

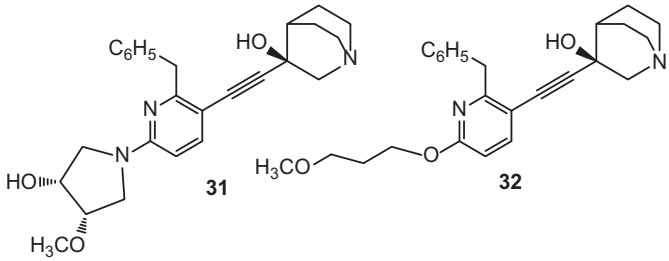


Figure 8. Chemical structure of E5700 and ER-119884, two potent growth inhibitors of *T. cruzi* growth targeting squalene synthase.

the enzymatic activity of *Tc*SQS at the subnanomolar level [73]. **31** is more efficient than **32** in *in vivo* assays, while **31** shows a dose-dependent effect on parasitemia and survival at 50 mg/kg/day; its analog only induces a 50% survival at the same dose [73].

SQ109 (**33**) is a relatively new drug against tuberculosis [74] and at the present time is in advanced (Phase II) clinical trials for tuberculosis (Figure 9) [75]. The target of SQ109 is MmpL3, an acid transporter, which is crucial for the incorporation of mycolic acid into bacterial cell wall [75]. Based on previous studies, **33** and closely related analogs such as **34**–**36** were found to behave as antibacterial agents toward different species including *Mycobacterium tuberculosis* and *Plasmodium falciparum* and also against fungi [76]. Interestingly, SQ109 acts against the three main morphologies of *T. cruzi* [77]. **33** acts at the nanomolar range against trypomastigotes (IC_{50} = 0.050 μM) and is practically devoid of action on blood cell hemolysis rendering a selectivity index greater than 1600 [77]. SQ109 also inhibits amastigote proliferation (ED_{50} = 1.2 μM), whereas its analogs **34**–**36** present a comparable efficacy toward this form of *T. cruzi* [78]. In addition, SQ109 acts synergistically with posaconazole suggesting that *Tc*SQS might be its molecular target, but SQ109 modestly inhibits *Tc*SQS [77]. The authors have considered that SQ109 functions as an uncoupler producing ultrastructural cell alterations in all the stages of the parasite [77]. Very recently, these and other closely related compounds have been patented based on their potential as antibacterial and antiparasitic agents [78].

7. 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase

Lovastatin (**37**) is a cholesterol-lowering agent for humans targeting 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) [79]. The parasitic counterpart is a glycosomal enzyme and is a valid target for drug design [80]. In fact, lovastatin has a synergistic effect when used in combination with ketoconazole against intracellular *T. cruzi* [81], but when used alone, lovastatin exhibits a vanishing inhibitory action against amastigotes. Fluconazole (**38**), itraconazole (**39**), and miconazole (**40**) are other examples of antifungal agents that behave as antiparasitic agents as well [82] but to a lesser extent than those drugs previously discussed (Figure 10).

8. Farnesyl diphosphate synthase

Farnesyl diphosphate synthase (FPPS) is a key enzyme of isoprenoid biosynthesis that catalyzes the consecutive condensation of isopentenyl pyrophosphate first with dimethylallyl pyrophosphate and then with geranyl diphosphate to form farnesyl diphosphate. In protozoan parasites, the FPPS gene has been cloned from *T. cruzi* [83]. So far, all the FPPSs that have been characterized are homodimeric enzymes and

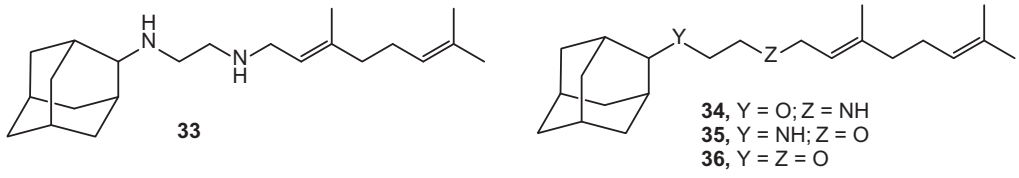


Figure 9. Chemical structure of antituberculosis agent SQ109, which also shows activity as becomes an antiparasitic agent, and other structurally related compounds.

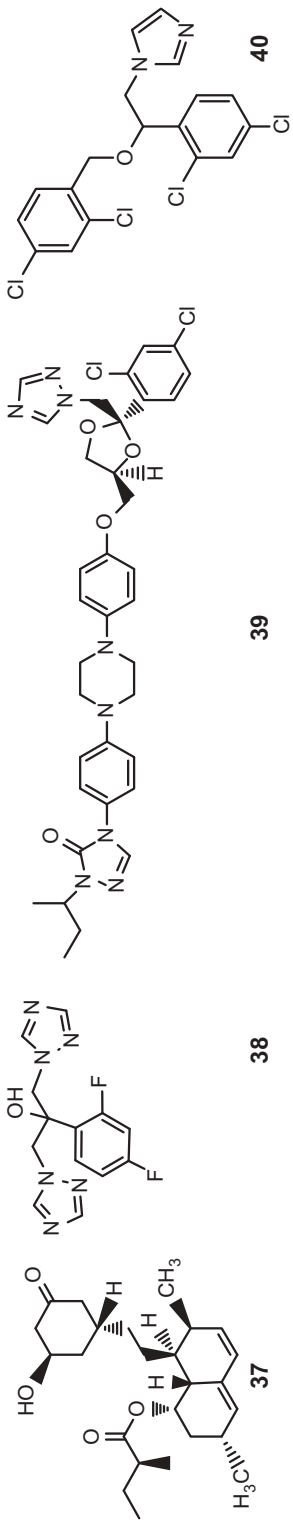


Figure 10. Chemical structures of lovastatin, fluconazole, itraconazole, and miconazole.

require divalent metal ions such as Mg^{2+} or Mn^{2+} for activity [84].

The replacement of the oxygen atom bridge of pyrophosphate (**41**) with substituted methylene groups gives rise to metabolically stable compounds known as bisphosphonates (**42**), initially designed to mimic the chemical structure of pyrophosphate [85]. Several bisphosphonates such as etidronate (**43**), clodronate (**44**), pamidronate (**45**), alendronate (**46**), risedronate (**47**), tiludronate (**48**), ibandronate (**49**), zoledronate (**50**), and incadronate (**51**) are in clinical use for the treatment and prevention of osteoclast-mediated bone resorption associated with various bone disorders (Figure 11) [86].

Besides their clinical use in long-term treatment of bone disorders, bisphosphonates exhibit a broad scope of biological activities such as antibacterial agents, antitumor agents, as selective inhibitors of the enzymatic activity of acid sphingomyelinase, and, of particular interest in this article, as antiparasitic agents against trypanosomatids [17,84,87].

Most of the bisphosphonates of pharmacological importance are nitrogen-containing bisphosphonates, from which some of them are effective inhibitors of *T. cruzi in vitro* and *in vivo* without toxicity to the host cells [87]. For example, risedronate is able to significantly increase the survival of mice infected by *T. cruzi* [88], indicating that bisphosphonates possess great prospects to be candidate drugs to treat infections by *T. cruzi*.

Of special interest are linear bisphosphonates such as 2-alkyl(amino)ethyl derivatives. For example, **52**–**54** are representative members of this class of bisphosphonates, and they behave as potent growth inhibitors of amastigotes and possess IC_{50} values at the nanomolar range against the target enzyme [89,90]. The crystal structures of *T. cruzi* FPPS with some of these compounds have been recently solved [91]. With these data at hand, it is possible to design new compounds with the aid of molecular modeling studies. Unexpectedly, the corresponding bisphosphonate derivatives bearing a hydroxyl group at the C-1 position are devoid of activity against both *T. cruzi* cells and the target enzyme [92]. Taking **55** as an example, which is free of antichagasic activity, but it is a selective and efficient inhibitor of *T. gondii* cells and the enzymatic activity of *Tg*FPPS (Figure 12) [92].

1-Hydroxy-, 1-alkyl-, and 1-amino-derivatives such as **56**–**59** are bisphosphonates that turn out to be a relevant starting point in an attempt to establish a rigorous structure activity relationship as antiparasitic agents [93–95]. **56** is an interesting example of a compound that has no functionality in its structure but the bisphosphonate unit possessing a moderate inhibitory action against *T. cruzi* [93] targeting FPPS [95].

Unexpectedly, linear α-fluoro-1,1-bisphosphonates such as **60** and **61** are not efficient antiparasitic agents against either trypanosomatids or their target enzyme FPPS [96]. Sulfur-containing bisphosphonates **62**–**65** are interesting compounds exhibiting inhibitory action against trypanosomatids [97]. For example, the thioether derivative **62** is moderately potent against *T. cruzi* (amastigotes). This cellular activity is associated with a potent inhibition of the enzymatic activity of *Tc*FPPS enzyme at the nanomolar range [97]. Oxidation of the

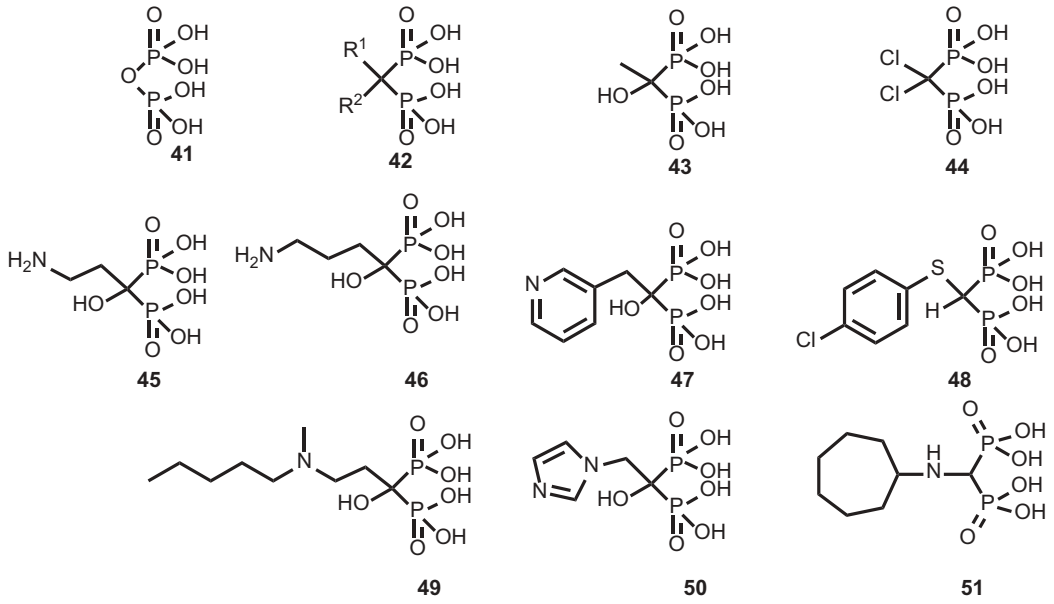
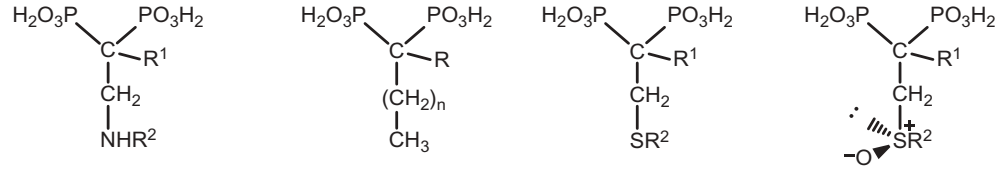


Figure 11. Chemical structures of representative FDA-approved bisphosphonates clinically employed for the treatment of different bone disorders.



52, R^1 = H; R^1 = *n*-hexyl
53, R^1 = H; R^2 = *n*-heptyl
54, R^1 = H; R^2 = *n*-dodecyl
55, R^1 = OH; R^2 = *n*-hexyl

56, n = 5; R = OH
57, n = 9; R = OH
58, n = 5; R = H
59, n = 4; R = NH_2
60, n = 7; R = F
61, n = 8; R = F

62, R^1 = H; R^2 = *n*-hexyl
63, R^1 = H; R^2 = *n*-dodecyl

64, R^1 = H; R^2 = *n*-heptyl
65, R^1 = H; R^2 = *n*-octyl

Figure 12. Chemical structures of representative members of bisphosphonic acids derived from linear carboxylic acids.

thioether function to form the corresponding sulfoxide gives rise to bisphosphonate that are devoid of anti-*T. cruzi* activity, but they show potent action against both *T. gondii* cells and *Tg*FPPS. **64** exhibits high selectivity toward *T. gondii* and is practically inactive against *T. cruzi* [97].

A recent patent confirms and shows the great potentiality that bisphosphonates have as antiparasitic agents with characterized mechanisms of action involving the blockade of FPPS, geranylgeranyl diphosphate synthase, and decaprenyl diphosphate synthase [98]. The compound of formula **66** could potentially be used as antiparasitic agent (Figure 13). M could be a cation or an alkyl group; Y could be a hydrogen atom, a hydroxyl group, or a halogen atom; n varies from 1 to 3; R^1, R^2 could be substituted amines or mercaptans; Z could be substituted phenyl groups or five- or six-membered heterocyclic aromatic rings [98].

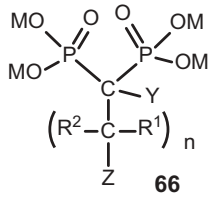


Figure 13. Chemical structure of generic bisphosphonates.

In conclusion, bisphosphonates present good perspectives for being good candidates to control parasitic diseases caused by trypanosomatids, particularly, to control American trypanosomiasis based on the fact that bisphosphonates are straightforwardly synthesized, they are FDA-approved drugs, and many of them are currently in use for long-term treatments for bone disorders.

9. Cysteine proteinases

Cysteine proteinases can also be considered as valid targets for Chagas disease chemotherapy where cruzipain, also known as cruzain, is the major cysteine protease of *T. cruzi* [20,21,99]. This enzyme is expressed in all the stages of the parasite and is crucial for *T. cruzi* life cycle including immunoevasion, nutrient uptake, and parasite differentiation [99]. Several compounds have been conceived as inhibitors of its enzymatic activity, and as a consequence of these studies, there are crystal structures of several target enzyme-inhibitor complexes [100]. **67** and **68** are irreversible inhibitors of cruzipain known as K11777 and WRR-483, respectively. They have great potentiality to be used clinically as very potent trypanocidal agents,

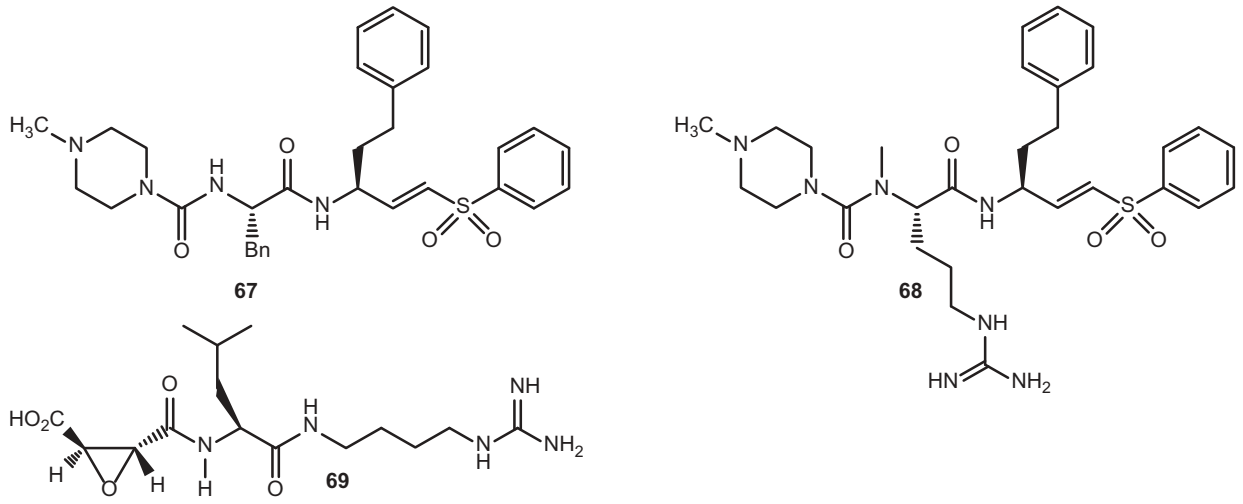


Figure 14. Chemical structures of the inhibitors of the enzymatic activity of cruzipain known as K11777 (**67**), WRR-483 (**68**), and E-64 (**69**) .

as they have shown activity *in vitro* and *in vivo* [101], whereas **69** (E-64) is one of the first irreversible inhibitors described against cysteine proteases [102] (Figure 14). However, the main drawback of designing irreversible inhibitors is that they can also inhibit host proteases producing undesired side effects due to their off-target activity and further irreversible covalent binding.

Certainly, there are a number of compounds with diverse structure that have proven to be anti-*T. cruzi* agents targeting cruzipain, particularly nonpeptidic derivatives such as **70**, which has a potent inhibitory action against the target enzyme at the low nanomolar range and an associated cellular activity against amastigotes [103]. **71**–**73** are also relevant nonpeptidic derivatives targeting cruzipain [103,104] (Figure 15).

As mentioned earlier, there are several compounds of diverse chemical structures with anti-*T. cruzi* activity targeting cruzipain that have been patented. For example, compounds bearing the 2,2-dioxidoimidazo[4,5-*c*][1,2,6]thiadiazine moiety in their structure have been depicted as growth inhibitors of *T. cruzi* whose mode of action is the blockade of cruzipain [105]. Taking **74** and **75** as representative examples, they exhibit a moderate antiparasitic activity against epimastigotes and some inhibitory action toward cruzipain [105] (Figure 16).

Nitrile-containing compounds such as **76** [106] and **77** [107] are reversible inhibitors of the enzymatic activity of cruzipain at the very low nanomolar range. Both of these drugs are very efficient orally administered agents in murine models of acute Chagas disease with a 90% of protection at a dose as low as 3 mg/kg [108]. The structures of these drugs and other closely related compounds have been recently patented [109] (Figure 17).

There are other examples of compounds that inhibit proliferation of trypanosomatids, particularly of *T. cruzi*, which act by inhibiting the enzymatic activity of cruzipain [110]. For instance, **78**–**81** show some degree of activity against *T. cruzi* cells [110]. Recently, a number of closely related analogs of **78**–**81** have been synthesized and evaluated against *T. cruzi,* with **82** emerging as a representative member of this class of compounds [111]. However, there is no discussion on whether **82** and its analogs still target cruzipain [111] (Figure 18).

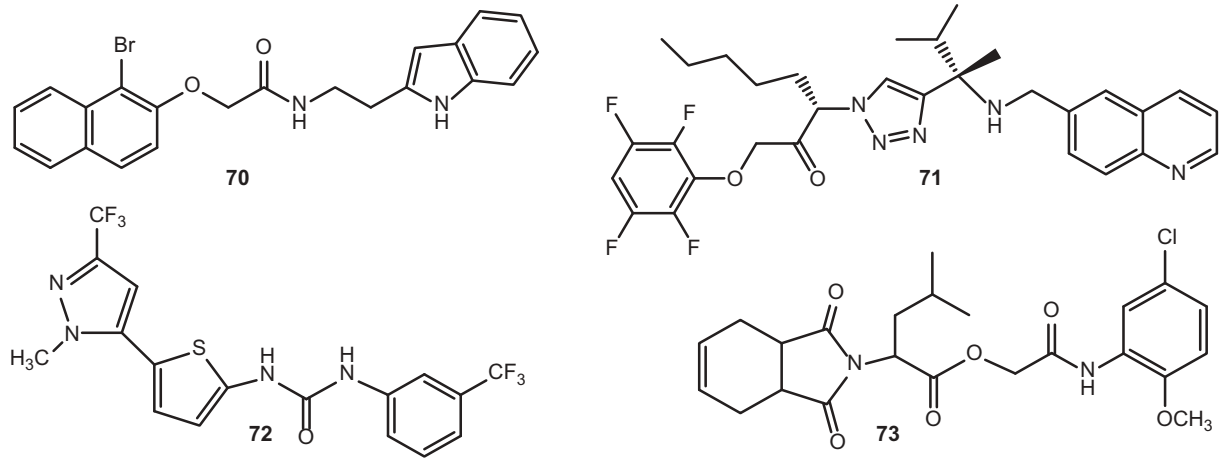


Figure 15. Chemical structures of the non peptidic inhibitors of the enzymatic activity of cruzipain.

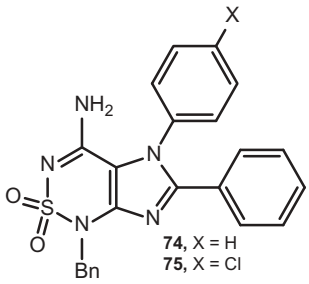


Figure 16. Chemical structure of relevant 2,2-dioxidoimidazo[4,5-*c*][1,2,6]thiadiazine derivatives.

10. Trypanothione biosynthesis

Trypanothione (**83**) is a crucial metabolite in trypanosomatids for two reasons: (i) it is responsible for maintaining the cell redox equilibrium as glutathione does in the mammalian host; (ii) these microorganisms do not have an alternate mechanism of protection against the oxidative stress [112]. Both cysteine residues present in trypanothione take part in the interconversion between its oxidized [**83a**, $T(S)_2$] and its reduced forms [**83b**, $T(SH)_2$] by breaking and restoring the corresponding intramolecular disulfide bond (Figure 19). The enzyme that catalyzes the reduction of trypanothione is trypanothione reductase (TryR), which is highly specific for trypanothione and does not recognize glutathione as a substrate [113].

Trypanosomatids are exposed to highly reactive oxygen species, which can cause lethal damage by reacting with DNA or cellular membrane lipids [114]. The defense system maintains low levels of H_2O_2 and O^{2-}. Glutathione reductase activity is not present in trypanosomatids, but there exists the trypanothione–trypanothione reductase system instead. Trypanothione synthase (TryS) emerges as an interesting molecular target, as it has no counterpart in mammals [1] (Scheme 3). TryS is a flavoenzyme ATP-dependent C:N ligase, requiring Mg^{2+} as cofactor, and they catalyze trypanothione formation through a condensation reaction of spermidine with two molecules of glutathione [115].

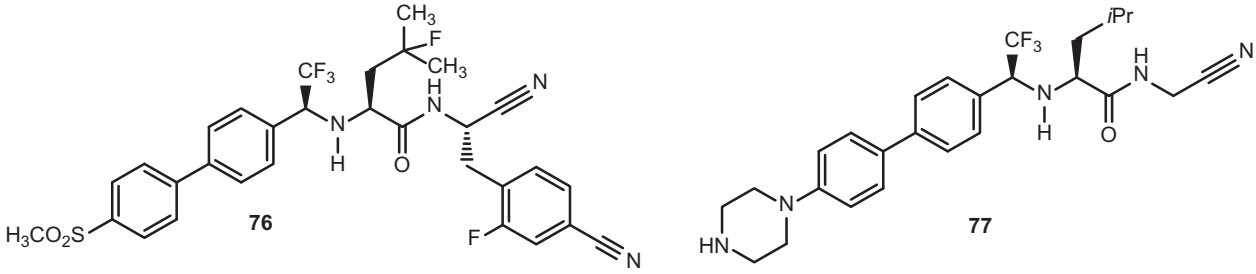


Figure 17. Chemical structures of reversible inhibitors of the enzymatic activity of cruzipain bearing nitrile groups.

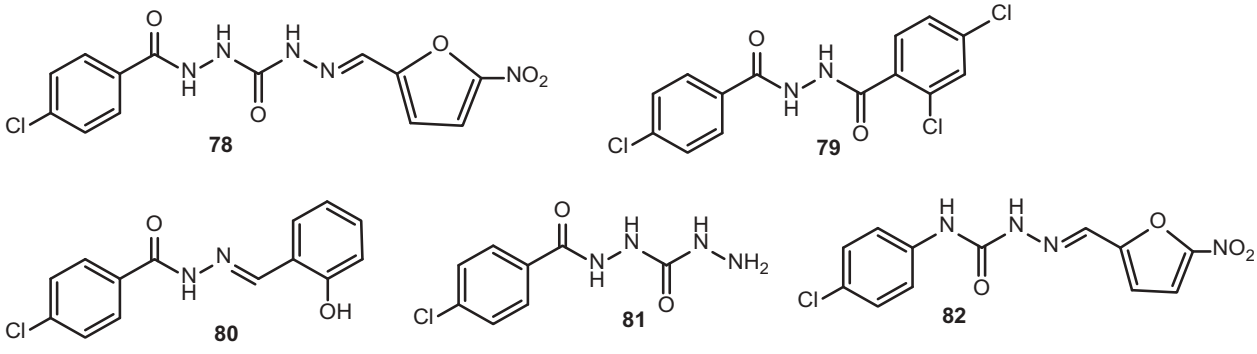


Figure 18. Chemical structures of hidrazide-*N*-acylhydrazone derivatives targeting cruzipain.

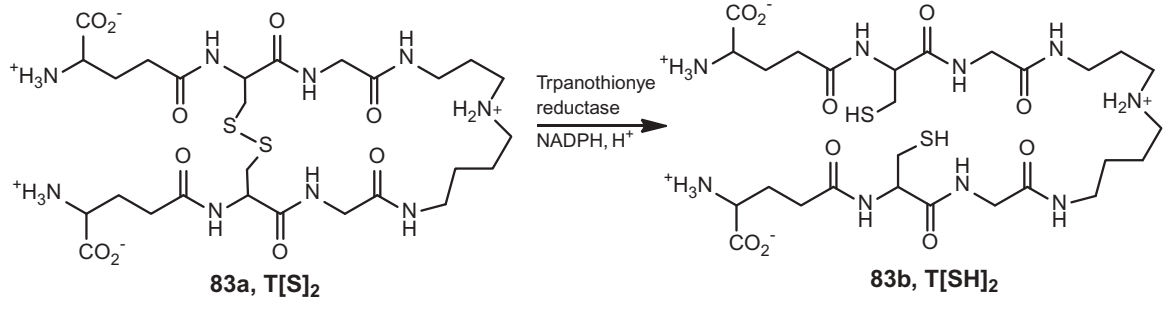
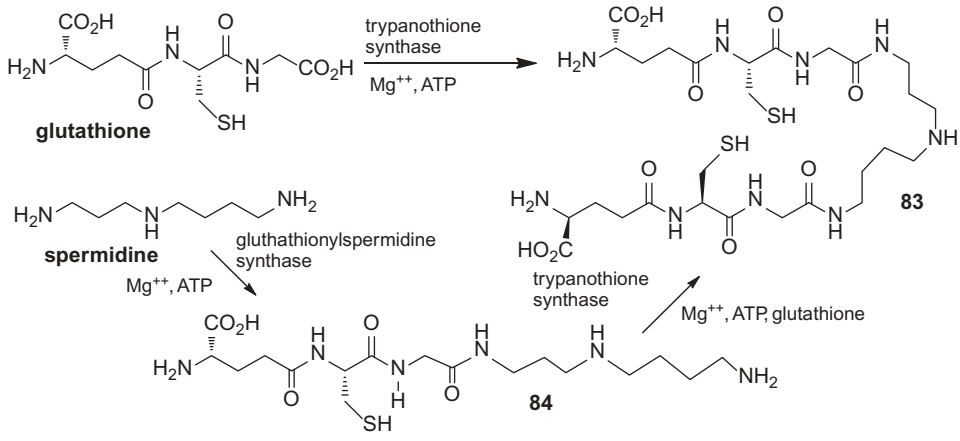


Figure 19. Chemical structure of the oxidized (**83a**) and reduced (**83b**) forms of trypanothione.



Scheme 3. Overview of trypanothione biosynthesis.

TryR is essential in trypanosomatids, but it has its corresponding counterpart in mammals in gluthatione reductase [116]. This target has been carefully studied, and the crystal structures of the enzyme–inhibitors complexes are available. At the present time, many inhibitors have been designed, with different mechanisms of action. They either function as covalent, competitive, or suicide inhibitors of this enzyme [1,116].

Quinone derivatives are representative examples of potent inhibitors of the enzymatic activity of TryR in *T. cruzi* [117]. In this sense, a series of hybrids of two quinines such as 2-phenoxy-anthraquinone (**85**) and 2-phenoxy-naphthoquinone (**86**), which, in turn, show potent inhibitory action toward TryR from *T. b. rhodesiense* [118], with different polyamines such as putrescine, cadaverine, spermidine, and spermine, has been conceived as conjugate structures of formula **87**–**94** [119]. **85** and **86** show a moderate action against *T. cruzi* cells; however, the selectivity index values are disappointing. In addition, the conjugate products **87**–**94** exhibit vanishing antiparasitic action against *T. cruzi* cells [119] (Figure 20).

As mentioned earlier, TryS is a unique enzyme in trypanosomatids with no counterpart in mammals. An interesting example of inhibitors that mimic the tetrahedral transition state of this C:N ligase is a series of phosphinopeptides structurally related to glutathione. Of this family of compounds, **95** and **96** are the most representative examples being potent growth inhibitors of amastigotes [9]. The efficacy for these drugs is comparable to **WC-9** [9]. The simple phosphinopeptide structure found as a pharmacophore constitutes a starting point for the development of optimized drugs. Phosphonates or phosphinates are interesting examples of compounds to be used to mimic the tetrahedral transition states of other C:N ligases, giving rise to inhibitors of Ala-D-Ala ligase, glutamine synthase, and glutathione synthase [120]. The design of the simplified structures **95** and **96** has been based on the relatively complex structures of the slow tight-binding inhibitors of glutathionyl spermidine synthase from *Crithidia fasciculata* **97** and **98** [121]. Then, as their synthesis is straightforward, the core structure of phosphinopeptides would facilitate further optimization (Figure 21).

As previously discussed, trypanothione biosynthesis is a potentially selective target given the differences in oxidative stress responses in *T. cruzi* versus the respective host cells. However, at the present time, numerous published studies have not succeeded in finding effective inhibitors of the enzymatic activity of TryS that can be moved forward into drug candidates.

11. Compounds producing oxygen active species

Quinoxaline *N,N'*-dioxides are interesting chemical structures that exhibit antiparasitic activity particularly against *T. cruzi* without toxicity for the host cells [122]. The mode of action of this family of compounds could be attributed to the ability of these molecules to produce reactive oxygen species such as HO• which interfere with the corresponding redox metabolism, particularly, by inhibiting mitochondrial activity [123]. **99**

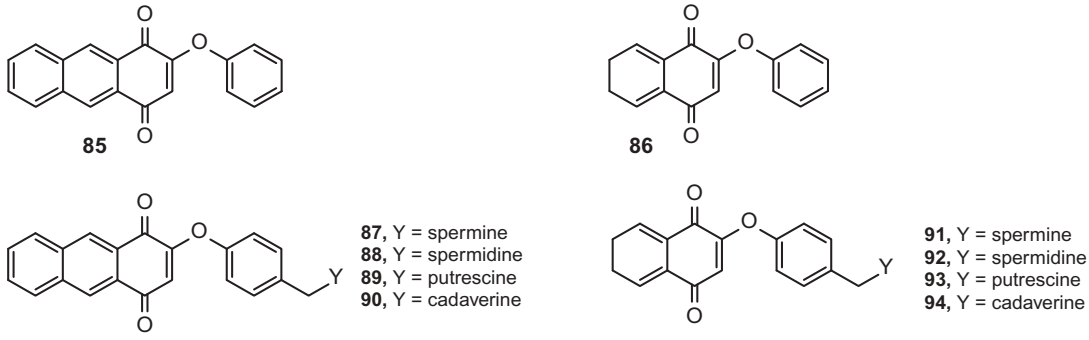


Figure 20. Chemical structures of relevant quinines and hybrid quinoline-polyamine derivatives targeting trypanothione reductase.

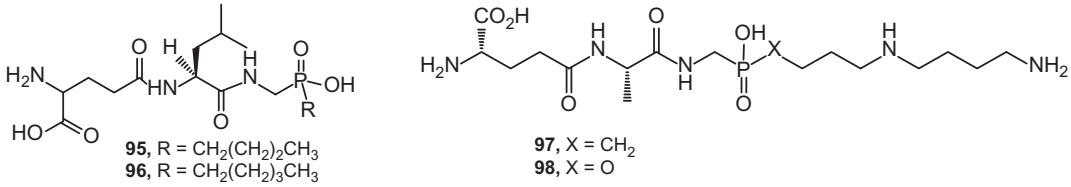


Figure 21. Chemical structures of phosphinopeptides targeting trypanothione synthase.

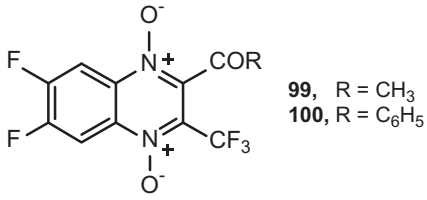


Figure 22. Chemical structure of representative quinoxaline *N,N'*-dioxides.

and **100** are representative members of this class of molecules that behave as trypanocidal agents against trypomastigotes [123]. Preliminary *in vivo* assays show good prospects for these compounds [123]. The use of this type of compounds has been recently patented against *T. cruzi* [124] (Figure 22).

12. Fe-superoxide dismutase

Fe-superoxide dismutase (Fe-SOD) is other crucial enzyme in the defense mechanism against oxidative stress in trypanosomatids that has no counterpart in mammals, which possess manganese and copper/zinc superoxide dismutases instead [125]. Very recently, a macrocyclic polyamine bearing a pyridiphane core has been designed and evaluated against trypanosomatids [126]. These inhibitors of the Fe-SOD activity are named scorpiand ligands due to the ability of the side chain toward the macrocyclic skeleton [126]. Representative members of this class of compounds such as **101**–**104** are shown in Figure 23. The title compounds exhibit biological activity against the three main morphological forms of *T. cruzi* [126]. For example, **101**–**104** exhibit potent inhibitory action against amastigotes possessing IC_{50} values of 6.0, 4.2, 2.8, and 5.2 μM, respectively [126]. These scorpiand-like ligands were originally designed as intercalating agents of DNA that affect cell viability [127]. The use of these scorpiand azamacrocycles as antiparasitic agents against *T. cruzi* has been recently patented [128].

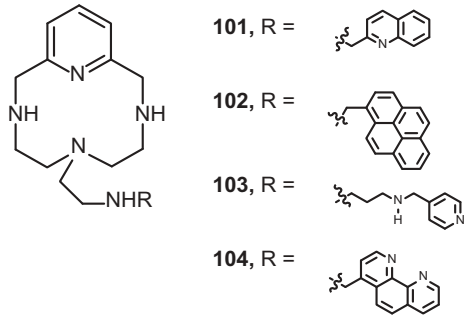


Figure 23. Chemical structures of relevant scorpiand ligands that function as anti-*T. cruzi* agents.

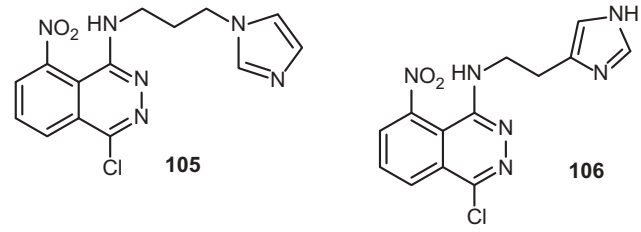


Figure 24. Chemical structures of relevant scorpiand ligands that behave as anti-*T. cruzi* agents.

In addition, imidazole derivatives containing a nitrophthalazine moiety turn out to be growth inhibitors of *T. cruzi* at the low micromolar range [129]. Taking derivatives **105** and **106** as representative examples, these molecules possess ED_{50} values of 8.8 and 4.0 μM against intracellular *T. cruzi* [129]. Although these compounds present inhibitory action against the enzymatic activity of Fe-SOD, it is too modest to be considered as the primary target of these compounds [129] (Figure 24).

Squaramides are interesting structures that are potent inhibitors of *T. cruzi* proliferation [130]. Although many members of this class of compounds have been evaluated, most of them present low selectivity index values, but **107** behaves as a potent growth inhibitor against intracellular *T. cruzi* (ED_{50} = 8.5 μM) and shows an excellent selectivity index (toxicity in Vero cell, ED_{50} = 453.1 μM, SI = 53) [130]. **107** is also very efficient in *in vivo* assays toward the chronic and the acute stages of the disease [130]. **107** produces ultrastructural alterations of epimastigotes. In fact, parasites treated with **107** suffer from a complete breakdown of their cell structures leading to mitochondrial swelling with rupture of the membrane [130]. Although the precise mode of action of these compounds has not been resolved yet [130], their use against *T. cruzi* infections has been recently patented [131] (Figure 25).

13. ML341 and related compounds

108 constitutes an interesting example of a potent inhibitor of intracellular *T. cruzi* at the very low nanomolar range [132,133]. This lead compound is a representative structure that has

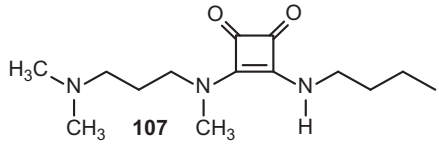


Figure 25. Chemical structure of a representative member of squaramine derivatives that behave as a potent inhibitor of *T. cruzi* growth.

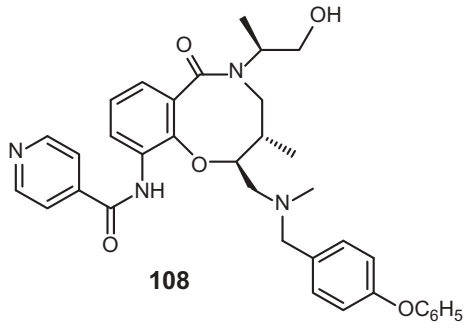


Figure 26. Chemical structure of compound **108** also known as **ML341.**

emerged as a result of a high-throughput screening of about 100,000 compounds obtained from diversity-oriented synthesis [132,133]. **ML341** has three stereogenic centers in its structure where the optimal combination turns out to be all in the *S* configuration (*S,S,S*) [132]. **108** is an extremely potent growth inhibitor of amastigotes possessing an ED_{50} value of 0.016 μM [132]. Bearing in mind the potentiality of **ML341** and other structurally related compounds, these molecules and their applications have been recently patented [133]. To the best of our knowledge, the mode of action of **108**, also described as **ML341**, is still unknown (Figure 26).

14. The sesquiterpene lactone known as lychnopholide

The sesquiterpene lactone lychnopholide (**109**), isolated from *Lychonophora trichocarpha*, is an interesting natural product that had previously exhibited potent action against trypomastigotes [134]. **109** is a hydrophobic molecule, but if loaded in polymeric nanocapsules, it could be useful as a potential antiparasitic agent [135,136]. In fact, encapsulation of **109** in nanocapsules enhances the efficacy exhibiting an *in vivo* effect in the acute models of Chagas disease [137] (Figure 27).

15. The phosphoinositide-3-kinase pathway

An interesting molecular target is the phosphoinositide-3-kinase (PI3-kinase) pathway, which has been previously employed for cancer chemotherapy. Protein kinases are involved in regulating cellular pathways that are associated with cell growth, cell survival, energy homeostasis, and stress resistance [138]. There are at least 12 PI3-kinases [139] and the downstream enzyme mammalian target of rapamycin (mTOR), which belongs to the family of PI3K-related protein kinases

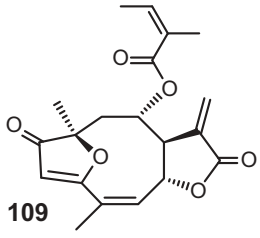


Figure 27. Chemical structure of the sesquiterpene lychnopholide isolated from *Lyconophora trichocarpha.*

[140]. Some kinases of *Trypanosoma brucei* TOR such as *Tb*TOR1 and *Tb*TOR2 have been identified [141]. In addition, two other putative kinases have been found in *T. brucei: Tb*TOR-like 1 and *Tb*TOR-like 2 [142]. *Tb*TOR-like 1 would modulate acidocalcisome and polyphosphate metabolism [142]. In this context employing the concept of drug repurposing strategy [139], it was considered that the trypanosomal PI3-kinase pathway was an interesting target to develop antiparasitic compounds [143]. In fact, there are many antitumor and anti-inflammatory agents available whose target is mTOR or PI3K [144]. Some representative human mTOR/PI3K inhibitors such as **110**–**117** have shown *in vitro* and *in vivo* antiparasitic action against trypanosomatids [143] (Figure 28). Only **110** was effective against *T. b. brucei* [143]. **113, 114,** and **117** were devoid of antiparasitic activity, whereas **111, 112, 115,** and **116** exhibited potent action against trypanosomatids [143], particularly **116**, which exhibited trypanocidal action against *T. cruzi* (trypomastigotes), showing an IC_{50} value of 0.12 μM [142]. *In vivo* assays indicated that **116** was also effective against *T. brucei rhodesiense* but proved not to be efficacious against *T. cruzi* [143]. The use of **110**–**117** and other closely related molecules as antiparasitic agents targeting mTOR/PI3K has been recently patented [145].

16. Lapatinib analogs

Continuing with the target repurposing concept [146], and based on the structure of the antitumor agent lapatinib (**118**) that had previously exhibited action on protein kinases of African trypanosomes [147], several compounds structurally related to **118** were synthesized and evaluated against trypanosomatids given their close phylogenetic relationship [147,148]. Taking **119** as a representative lapatinib analog, this molecule was very effective against *T. brucei* (ED_{50} = 0.042 μM) [147], but also exhibited inhibitory action against *T. cruzi* (ED_{50} = 1.8 μM) [148]. These results were very encouraging leading to further structural optimization by synthesizing many compounds bearing quinoline, isoquinoline, cinnoline, phthalazine, and 3-cyanoquinoline moieties in their chemical structures, with **120** emerging as the most potent compound in this family of lapatinib analogs [148]. **120** proved to be a very effective growth inhibitor of amastigotes exhibiting a low ED_{50} value of 0.09 μM [148] (Figure 29). The use of these compounds as antiparasitic agents has been recently patented [149]. In addition, a number of 5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine derivatives targeting PI3K have been recently patented. **121** arose as a relevant member of this class of drugs inhibiting the target enzyme at a low nanomolar concentration [150].

17. ß-Lapachone analogs

Naphthoquinone derivatives are interesting low-molecular-weight compounds exhibiting a variety of biological activities. Of particular interest are those structurally related to the natural product ß-lapachone (**122**), which behaved as growth inhibitors of *T. cruzi* [151]. Imido-substituted 1,4-naphthoquinone derivatives such as **123**–**125** also showed antiparasitic activity against epimastigotes at the low micromolar range

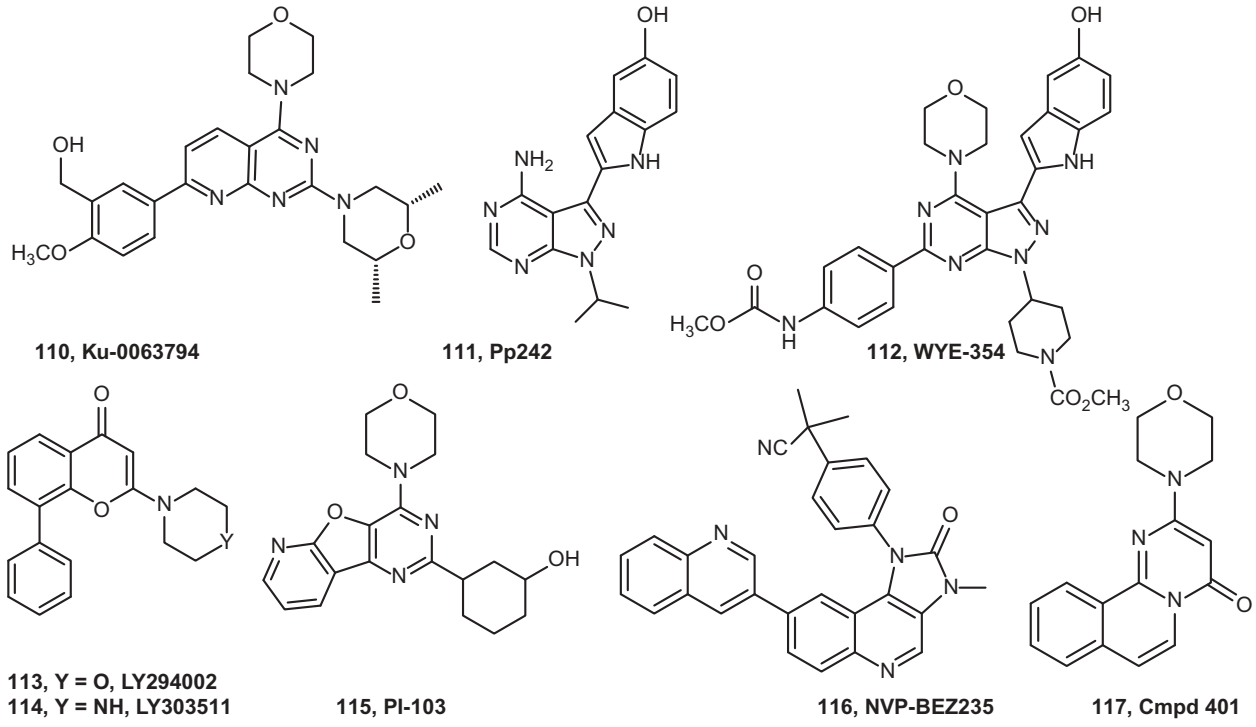


Figure 28. Chemical structures of inhibitors of human mTOR/PI3 K enzymes acting in *T. cruzi* cells.

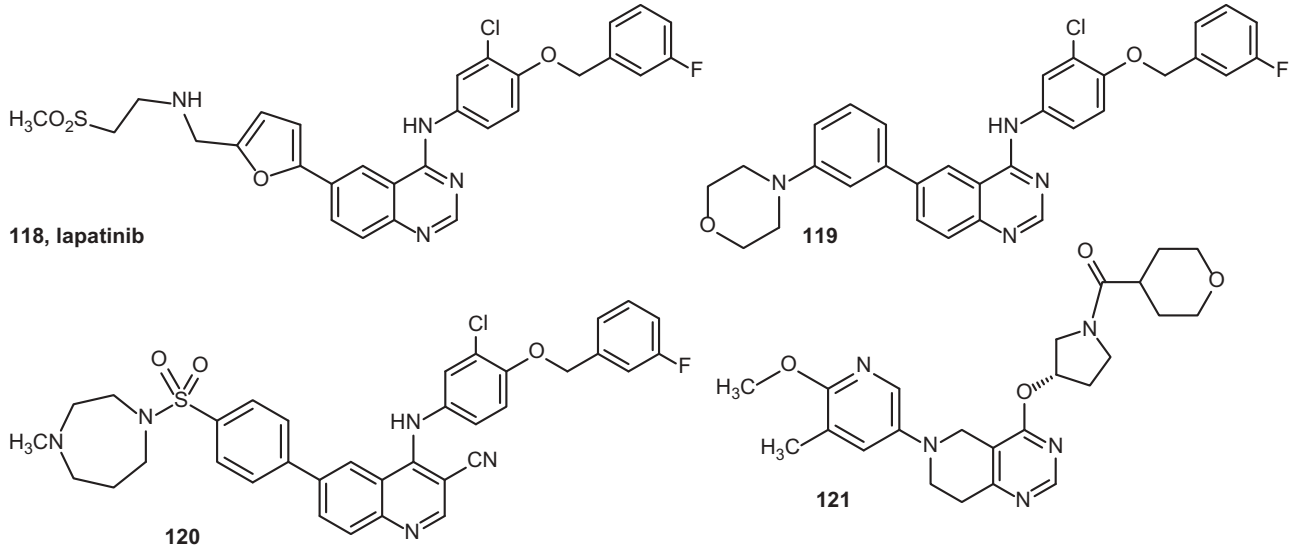


Figure 29. Chemical structures of lapatinabid and other structurally related compounds.

[152]. **126**, designed as an antitumor agent [153], arises as a relevant member of this type of compounds. The authors suggested that **126** might act by inhibiting tubulin polymerization in *T. cruzi* [154] (Figure 30).

18. *trans*-Sialidase

trans-Sialidase is a distinctive glycosylphosphatidylinositol (GPI)-anchored enzyme that is associated with the invasion process of trypomastigotes to the host cells [155]. Sialic acid (**127**) is an important metabolite that protects cells from invasion of pathogenic microorganisms by blocking the corresponding binding sites of host galactose residues. This enzyme transfers sialic acid from host sialic acid-containing glycoconjugates to mucins (*O*-glycan-type glycoproteins) on the parasite surface [156]. The crystal structure of *T. cruzi* *trans*-sialidase is available, which provides valuable information for drug design [157]. The Tyr342 residue plays a key role in the transferring process of sialic acid by forming a covalent bond at the C-1 position [158]. Based on these data, the fluorine-containing derivative **128** was conceived, which turned out to be an effective inhibitor of the enzymatic activity of *trans*-sialidase [158]. Structure optimization of **128** led to more efficient inhibitors such as **129** and **130**

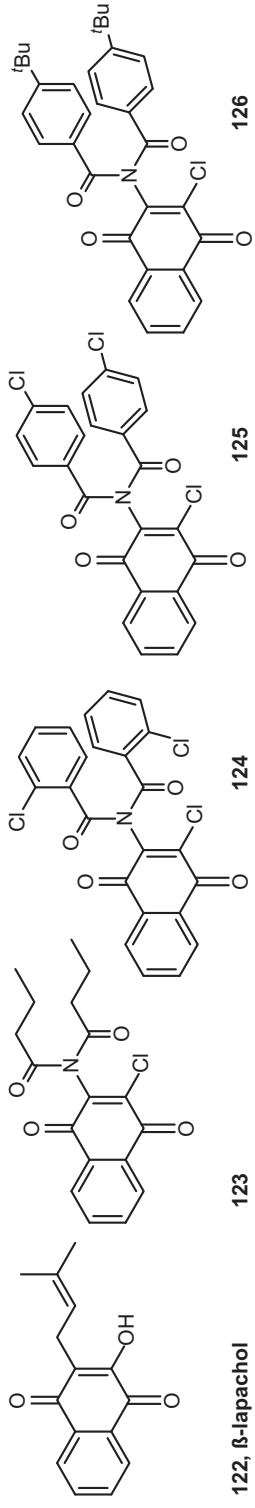


Figure 30. Chemical structures of ß-lapachone and other structurally related compounds.

[159]. Interestingly, sialic acid analogs and precursors such as 2-deoxy-*N*-acylmannosamines proved to be inhibitors of *T. cruzi* invasion to mammalian cells [160]. In this sense, 2-deoxy-*N*-propionylmannosamine (**131**) was the most relevant sialic acid precursor to prevent cell invasion [160]. The use of 2-deoxy-*N*-acylmannosamines as potential protecting agents against *T. cruzi* invasion has been recently patented [161] (Figure 31).

19. Boron-containing compounds

Recently, a new class of boron-containing compounds has been developed as potential antiparasitic agents caused by trypanosomatids. These compounds known as benzoxaboroles were evaluated as antiparasitic agents but were initially conceived as antibacterial and anti-inflammatory agents [162,163]. Benzoxaboroles **132** and **133** resulted to be potent inhibitors of *T. cruzi* growth with an ED_{50} value of 1.15 and 0.49 µM, respectively, and promising *in vivo* activity [162,163]. The precise mode of action of these compounds is still unknown, but the authors have hypothesized that the presence of the boron atom having an empty p-orbital would interact with their actual target [162]. The potential use of benzoxaborole derivatives as antiparasitic agents has been patented [164] (Figure 32).

20. Diagnosis

At the present time, diagnosis of infection by *T. cruzi* is still a challenge in spite of having several methods at hand ranging from the wet smear preparation to immunoenzymatic assays with recombinant antigens. In recent infections, unless parasitemia is elevated and acute symptoms awfully severe, *T. cruzi* is seldom detected [5,165]. Certainly, in the acute phase of the disease, direct detection of *T. cruzi* trypomastigotes in the bloodstream can be done through parasitological fresh-blood test, and also through smear and thick blood test, the former being more sensitive than the latter one [166]. If the diagnosis is negative but classical symptoms are observed such as inflammation at the wounds produced by the vector parasite known as chagoma or the characteristic cellulitis of the eyelid (Romaña sign) [167], further evaluation should be conducted [166]. In this case, concentration tests such as the microhematocrit or Strout test should be employed [166]. Individuals who are suspected of having acute Chagas disease that obtained negative results from direct blood testing are encouraged to go through concentration test. As Chagas disease is transmitted through the placenta, it is very important to have a precise diagnosis in newborns from potentially infected mothers especially taking into account the high rate of cure in children with the current chemotherapy. Children having negative results concerning direct detection of trypomastigotes should be checked for specific IgG antibodies against *T. cruzi* within 9 months. In the chronic stage, the number of circulating trypomastigotes diminishes below the capacity of microscopic detection or by the presence of IgG antibodies directed toward antigens of *T. cruzi* [5,165,166]. Diagnosis at this stage should be based on conventional serology, indirect immunofluorescence test, indirect

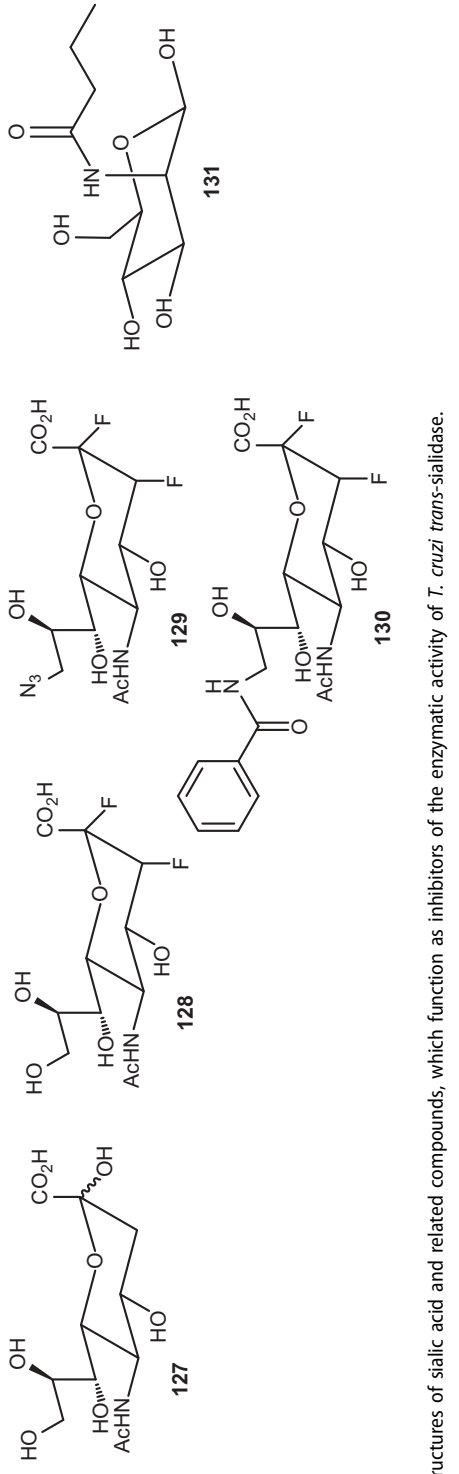


Figure 31. Chemical structures of sialic acid and related compounds, which function as inhibitors of the enzymatic activity of *T. cruzi trans*-sialidase.

hemagglutination test, enzyme-linked immunosorbent assay, or on indirect parasitological methods such as xenodiagnosis samples or hemoculture [168]. These indirect methods are specific, but their major drawbacks are the lack of sensitivity [166,168]. A reliable diagnosis is very relevant to avoid spread of this disease through blood transfusion and organ transplantation even in areas where this disease is not endemic.

In the acute phase of Chagas disease, there is a high rate of cure after treatment either with nifurtimox or with benznidazole. In this case, a period of 2 years is required to consider a patient cured under the conventional serology assay. On the other hand, in the chronic phase of the disease, given that negative sero-conversion is slow, many years of surveillance are required to be sure that a patient is cured [166,169]. The complement-mediated lysis test is employed to detect antibodies against trypomastigotes [170]. This test has been employed as an additional assay to search for an active infection [171]. In summary, diagnosis at an early stage, when parasites may be directly detected, is not frequently sought. In most cases, this disease evolves without diagnosis into the chronic phase where the number of circulating trypomastigotes diminishes to an undetectable threshold, from which direct detection is no longer possible. As discussed previously, a definite diagnosis requires several serological tests together with epidemiological data and clinical symptoms [165,166]. Recently, an interesting review article has appeared indicating a challenge for an effective chemotherapy monitoring the chronic and the acute disease in all stages [172]. Certainly, the lack of a proper biomarker has been stated not only for Chagas disease, but also for many other parasitic diseases [172]. In summary, adult patients present the major challenges for diagnosis for a number of reasons: (i) evaluation of the clinical response to a special treatment requires many years of surveillance; (ii) negativization of parasitemia is poorly evaluated by conventional serology, its reaction is slower as the time of the original infection increases; (iii) in the chronic phase, the level of bloodstream trypomastigotes is below the detection threshold of direct assay, requiring polymerase chain reaction (PCR) instead [173].

As discussed previously, most of the methods available are based on indirect methods and are used during the chronic stage of the disease. In the acute phase, symptoms are scarce and dwell of fever, the characteristic Romaña sign, or inflammations around the wounds caused by the vector. There are new patents available in which innovative tools for the detection of this disease are described. For example, *T. cruzi* antigenic polypeptides proved to be a valuable resource in diagnosis, and there is a patent describing the use of *T. cruzi* polypeptides immobilized onto a surface to diagnose for *T. cruzi* infection [174]. A further invention takes advantage of using oligonucleotide sequences as detection probes followed by a nucleic acid amplification as a method to diagnose *T. cruzi* infections [175]. It is worth mentioning a patent which describes a method to diagnose the Chagas disease by monitoring the presence of antibodies to *T. cruzi* peptides from the membrane protein. The method is able to differentiate all the stages of the disease and is particularly useful for monitoring the treatment in chronic patients [176]. Furthermore, a method has been patented to diagnose a *T. cruzi* infection

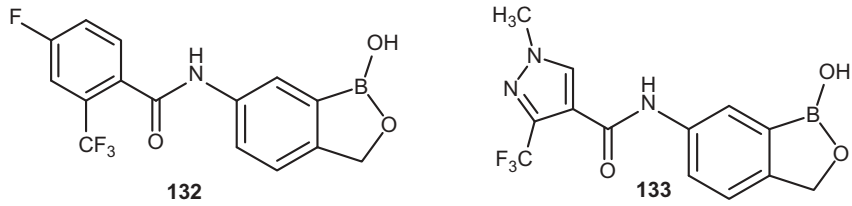


Figure 32. Chemical structure of representative oxaborole derivatives, which show antiparasitic activity against *T. cruzi*.

using loop-mediated isothermal amplification, in which the Tc24 is amplified and detected [177]. Finally, there is a patent available describing the detection of antibodies for sequences of collapsin response mediator protein 5 from *T. cruzi* as a means to diagnose the disease [178].

21. Conclusion

In summary, the aim of this review was given attention to presenting a wide-ranging panorama of some relevant targets for drug design to treat American trypanosomiasis or Chagas disease. This health problem can be judged as a neglected parasitic disease, which does not have the consideration from pharmaceutical companies to carry out a serious investment program due to the lack of financial incentives. At the present, Chagas disease still represents a major health problem associated with important cause of morbidity and mortality mostly in developing countries where this illness is endemic. It is worth mentioning that its occurrence is strongly associated with poverty and bad housing quality that allows the vector of the disease to interact with humans. Although there are other potentially valuable targets, only the more illustrative ones were discussed here. Finally, with the appropriate knowledge of the biochemistry and physiology of *T. cruzi* will be possible the full suppression of this parasitic disease.

In spite of many compounds that act on a variety of molecular targets, only two of them have been developed since the appearance of nifurtimox and benznidazole. These drugs are the antifungal agent posaconazole and E1224, a prodrug of ravuconazole. Different treatments based on combinations with existing chemotherapies have been essayed in last years [179].

22. Expert opinion

The aim of this review was focused at presenting a broad scope of several relevant targets for rational drug design for the treatment and surveillance of American trypanosomiasis or Chagas disease. These drugs exploit metabolic differences between the etiologic agent of this disease, the hemoflagellated protozoan *T. cruzi*, and the mammalian host. One of the most important challenges of Chagas disease chemotherapy is the development of a compound that is able to cross the corresponding infected cell membrane and to make a journey through the complex environment present in the cell cytoplasm to finally cross the membrane of the multiplying intracellular parasites (amastigotes, the most clinically relevant dividing form of the parasite). Finally, this molecule must arrive at its target for the pharmacological action to occur.

Chagas disease can be classified as a neglected disease for presenting insignificant profits for pharmaceutical companies to carry out a serious research and developmental program for drug design. As a result, most of the research, including the patent applications, has been carried out by Academia. In spite of the reduction in the number of infected people observed in the last decade, this disease is still an important cause of mortality and deterioration in the quality of life, particularly, in developing countries where it is endemic. In fact, its appearance is clearly associated with poverty and bad housing quality, which facilitate the spreading of the vectors. In countries where Chagas disease is not endemic, its transmission is congenital or through transfusion of infected blood from people moving from other areas. In addition, in the recent years, a reactivation of the disease has been observed in patients with AIDS.

Currently, only two drugs are available for the treatment of this chronic zoonosis: nifurtimox and benznidazole. However, they present serious drawbacks: both are not effective against some of the *T. cruzi* strains, are associated to long-term treatments presenting considerable side effects, are not effective in the chronic phase of the disease, and are not FDA approved. Evidently, there is an urgent need for new and safe medicines to treat and monitor this disease, and the concept of rational drug design can aid in the discovery of new compounds.

In our opinion, and as evidence by the new compounds discovered in the recent decades, several metabolic pathways are available that can provide effective drug targets. Of particular interest is ergosterol biosynthesis, since this compound is a crucial metabolite for the parasite as it is a key component of the cell membrane. Many enzymes in this pathway are interesting molecular targets: $\Delta^{24(25)}$-sterol methyltransferase, 14α-demethylase (CYP51), squalene epoxidase, and SQS. From these enzymes, 14α-demethylase is a very promising target because there are many potent inhibitors developed so far. In fact, posaconazole and the prodrug of ravuconazole E1224 act toward this enzyme at the low nanomolar range. Posaconazole and ravuconazole are known antifungal drugs but have the disadvantage of being too expensive from the point of view of manufacturing. However, in spite of having this potent inhibitory action, clinical evaluation of posaconazole and E1224 on chronic Chagas disease indicated that these drugs had vanishing effectiveness. These results suggest the urgent need for developing better screening processes for new approaches to control Chagas disease. In addition, farnesyl pyrophosphate synthase also has great prospective bearing in mind that there are abundant structural data available on the binding of their inhibitors, novel compounds bearing a bisphosphonate group. 4-Phenoxyphenoxyethyl thiocyanate

(**WC-9**) constitutes one of the few examples of a lead drug possessing a covalently bonded thiocyanate group. This compound targets SQS and has great prospects due to its drug-like character. Trypanothione reductase is a unique enzyme in *T. cruzi* which is not present in the mammalian host, having glutathione reductase as its counterpart. Therefore, it has great potential to be utilized as a target for the design of selective inhibitors. *trans*-Sialidase is an interesting enzyme that could be potentially employed to control the invasion process especially during the acute phase of the disease.

In conclusion, the appropriate knowledge of the biochemistry and physiology of this parasite will allow the control and surveillance of this parasitic disease.

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Declaration of interest

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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