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Review article

Current advances in drug discovery for Chagas disease

Cauê Benito Scarim [a, *, 1], Daniela Hartmann Jornada [a], Rafael Consolin Chelucci [a], Leticia de Almeida [b], Jean Leandro dos Santos [a], Chung Man Chin[a]

[a] Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista "Júlio de Mesquita Filho", UNESP, Araraquara, SP, Brazil
[b] Departamento de Biologia Celular e Molecular, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, USP, Brazil

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ABSTRACT

Chagas disease, also known as American trypanosomiasis, is one of the 17 neglected tropical diseases (NTDs) according to World Health Organization. It is estimated that 8–10 million people are infected worldwide, mainly in Latin America. Chagas disease is caused by the parasite *Trypanosoma cruzi* and is characterized by two phases: acute and chronic. The current therapy for Chagas disease is limited to drugs such as nifurtimox and benznidazole, which are effective in treating only the acute phase of the disease. In addition, several side effects ranging from hypersensitivity to bone marrow depression and peripheral polyneuropathy have been associated with these drugs. Therefore, the current challenge is to find new effective and safe drugs against this NTD. The aim of this review is to describe the advances in the medicinal chemistry of new anti-chagasic compounds reported in the literature in the last five years. We report promising prototypes for drug discovery identified through target-based and phenotype-based strategies and present some important targets for the development of new synthetic compounds.
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Contents

Abbreviations: NTD, neglected tropical disease; *T. cruzi*, *Trypanosoma cruzi*; TDD, target-based drug discovery; PDD, phenotypic-based drug discover; NFX, nifurtimox; BZN, benznidazole; SI, selective index; CC_{50}, concentration to inhibit grow of 50% control/human cells; IC_{50}, concentration to inhibit grow of 50% of *T. cruzi*; MDR-TB, Multidrug-resistant-*Mycobacterium tuberculosis*; TcTIM, glycolytic enzyme triosephosphate isomerase; NTRs, Nitroreductases; FMN, flavin mononucleotide; FAD, flavin adenine dinucleotide; ATPs, adenosine triphosphate synthesis; SOD, Superoxide dismutase; ^{1}H NMR, ^{1}H- Magnetic Nuclear Resonance; Fe-SOD, Iron superoxide dismutase; TR, Trypanothione reductase; SQS, enzyme squalene synthase; FPPS, Farnesyl Diphosphate Synthase; TcTIM, glycolytic enzyme triosephosphate isomerase; FPP, farnesyl-pyrophosphate; USP, University of São Paulo.

* Corresponding author.
E-mail address: cauebenitos@gmail.com (C.B. Scarim).
[1] Present address: Rodovia Araraquara-Jaú km-1 s/n, campus ville, 14800-903, Araraquara - SP, Brazil.

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1. Introduction

Chagas disease, also known as American trypanosomiasis, is one of the 17 neglected tropical diseases (NTDs) listed by WHO. NTDs prevail in tropical and subtropical countries and since they usually affect people with lower socio-economic status, they are not a profitable venture for the pharmaceutical industry [1–4]. The disease was discovered 109 years ago by the Brazilian sanitary physician Carlos Chagas, and is caused by parasite *Trypanosoma cruzi (T. cruzi)*, a hemoflagellate protozoan of the order *Kinetoplastida* and family *Trypanosomatidae* [1,5,6].

Clinically, Chagas disease has two phases: an acute early stage (acute phase) lasting a few weeks which is characterized by fever and many circulating parasites in bloodstream, and the chronic phase which can be classified into the indeterminate and determinate forms. The indeterminate form is characterized by a febrile state and few parasites in the bloodstream, and the determinate form manifests with significant cardiac (chagasic cardiopathy), digestive (megacolon and megaesophagus) and neurological changes [7,8].

Chagas disease is endemic in 21 Latin American countries. Due to globalization, this parasite has spread across European countries like Austria, Belgium, France, Germany, Italy, Netherlands, Portugal, Spain, Sweden, Switzerland and the United Kingdom, as well as Australia, Japan, Canada and the southern United States bordering with Mexico [9,10]. The World Health Organization (WHO) estimates that 8 million people are infected with *T. cruzi* and more than 10,000 die every year on account of the infection [11]. Furthermore, an increase in the number of HIV-positive patients and indiscriminate use of corticosteroids, has led to the reactivation of asymptomatic Chagas disease indicating that *T. cruzi* is also an opportunist parasite [12,13].

Current chemotherapy for Chagas disease is restricted to only two nitroheterocyclic drugs: nifurtimox (NFX) (**1**) and benznidazole (BZN) (**2**) (Fig. 1), discovered in the 1960s and 70s, respectively [14,15]. The likely mechanism of action of both drugs is the formation and accumulation of free radicals from the nitro groups which overwhelm the antioxidant capabilities of *T. cruzi* and eventually kill the parasite [16]. NFZ and BZN are more effective in the acute phase and the treatment response varies according to the strains of *T. cruzi*. Both drugs show limited activity in the indeterminate form of chronic phase. In addition, NFX and BZN show several side effects such as anorexia, psychic alterations, digestive manifestations, bone marrow depression, peripheral polyneuropathy, hypersensitivity, lymphadenopathy, thrombocytopenic purpura, and agranulocytosis [17–26].

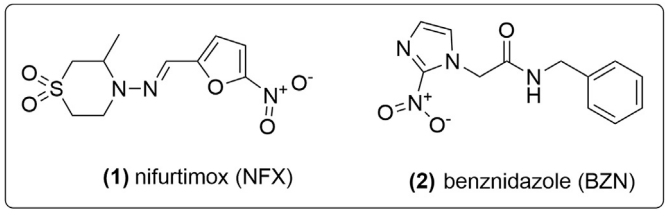

Fig. 1. NFX and BZN structures. Standard drugs against Chagas disease.

Drug discovery for NTDs has been suffering from the lack of interest by pharmaceutical companies due to low income of the majority of the affected people. Chagas disease remains a major challenge and all current drug discovery programs are aimed at identifying drugs or vaccines to eliminate *T. cruzi* from the host [27]. In drug development, two different approaches are typically undertaken to identify compounds of interest within a specific program: target-based drug discovery (TDD) or phenotype-based drug discovery (PDD) [28].

In the past three decades, TDD has been a dominant approach in identifying new drugs for inadequately treated medical concerns [29,30]. However, the interest in PDD approaches has been revived in recent years. PDD involves evaluating different chemicals against the pathogen's phenotype in a biological system such as an animal model or a cell line [30]. In this review, we have discussed the different drug discovery approaches used for Chagas disease, as well as some drug targets explored in the last few years. In this context, we have presented the current advances in the medicinal chemistry of new antichagasic compounds reported in the literature in the last five years. We have focused on the most active analogs with selective index (SI) superior or equal to 10 that have been reported in studies obtained from the PubMed, Web of Science, and Scopus databases. The SI values were calculated as a ratio of the CC_{50} (concentration to inhibit the growth of 50% control/human cells) and IC_{50} (concentration to inhibit the growth of 50% of *T. cruzi*) to compare the molecules and discuss the development of novel antichagasic lead compounds.

2. Phenotype-based screening

Phenotypic screens have the advantage of the whole organism and, therefore, all the targets and biological pathways of the organism are exposed to the compound [31]. PDD approaches, thus, do not rely on previous knowledge of a specific drug target or a hypothesis regarding its role in the disease [30]. Some examples of compounds discovered through phenotypic assays against *T. cruzi* are discussed below.

Thiazoles are an important class of compounds that are able to inhibit *T. cruzi* growth. Alvarez et al. synthesized 82 new thiazoles derivatives and discovered three compounds (**3**, **4** and **5**) showing activity against epimastigotes (Tulahuen 2 strain) and amastigotes (Sylvio X-10 strain) (Fig. 2). Their results showed that the IC_{90} values of analogs **3, 4** and **5** against amastigote forms were 1.30 μM, 0.31 μM and 1.4 μM, respectively. All of them presented an SI above 1000, about five times higher than NFX. In addition, they did not show any nonspecific toxicity or mutagenic activity. Compounds **4** and **5** were evaluated in BAL/c mice during the acute phase of Chagas disease, and both showed toxicity against Trypomastigotes (Y strain). They were able to decrease parasitemia at the doses of 50, 100 and 200 mg/kg/day, with the highest dose showing results similar to that of BZN [32].

The same research group has synthesized a series of new thiazole analogs that are more hydrophilic than compound **4**. Compound **6** (Fig. 2), the most promising analog, was very active in *in vitro* assays against the epimastigote (Tulahuen 2 strain), trypomastigote (Y strain) and amastigote forms (Sylvio X-10 strain). At

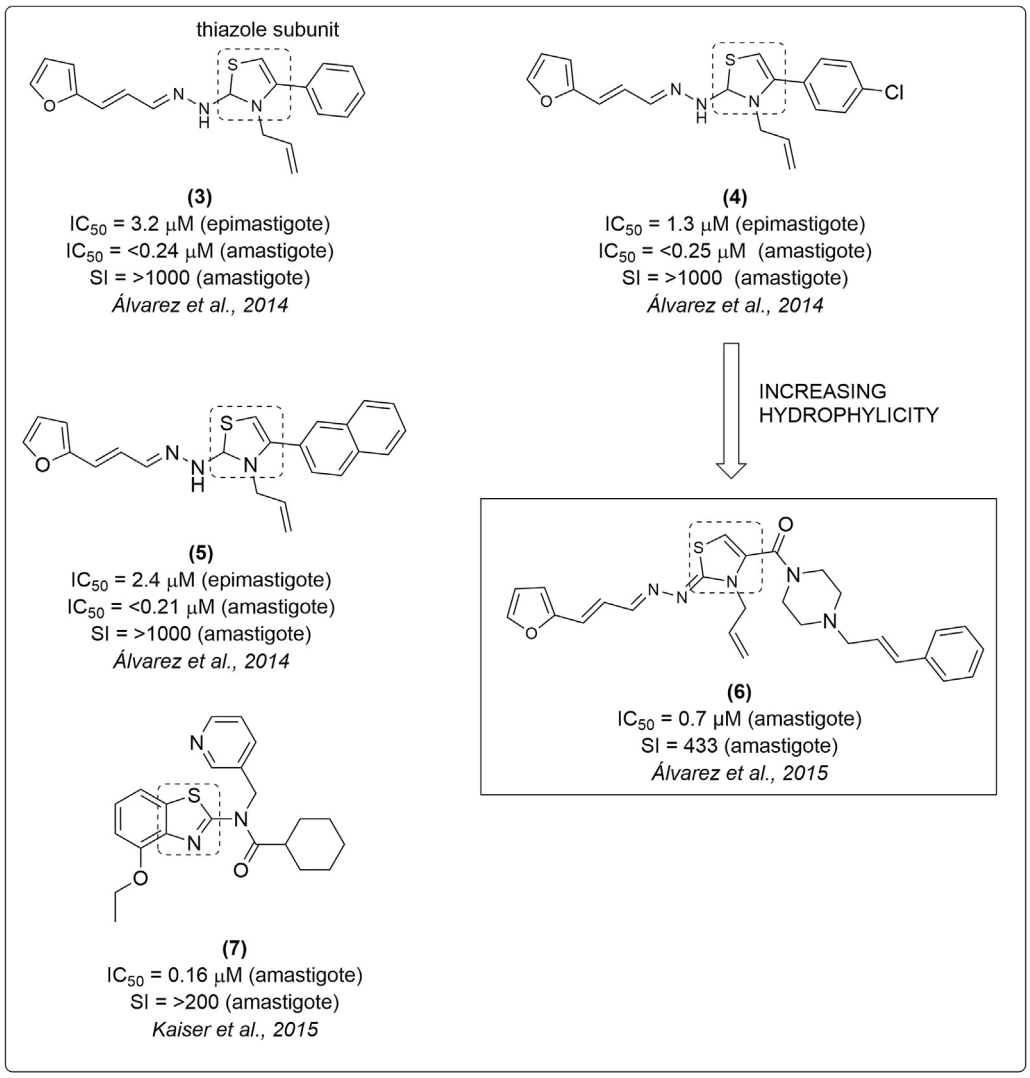

Fig. 2. Thiazoles analogs structures. Novel thiazole derivatives actives against *T. cruzi* strains.

0.7 μM, it reduced 50% of *T. cruzi* amastigotes and showed SI of 433. Compound **6** also suppressed the parasitemia in mice and was stable under hydrolysis in rat microsomal hepatocytes. In addition, compound **6** was not mutagenic *in vitro*, non-clastogenic *in vivo* and exhibited low acute toxicity in the mice. Although it was not more active than BZN in the animal model of acute disease when administered along with a vehicle (oil microemulsion), compound **6** reduced parasitemia, mortality, and anti-*T. cruzi* IgG antibody levels [33].

Other active prototypes containing thiazole subunit have been discovered through the 'repurposing strategy', which aims to discover novel activities for already described drugs [34]. In this case, the researchers performed a repurposing screening in Malaria Box. They started with 400 compounds and then selected 21 of the most active compounds against amastigotes of *T. cruzi* (Tulahuen CL2 strain) with IC_{50} values lower than 5.0 μM and SI superior to 10. On the basis of Lipinski's rule of five– cluster analysis, the absence of toxicophores, novelty and frequent hitters, drug-likeness and number of hits for cluster– compound **7** (Fig. 2) was identified. However, in spite of its high IC_{90}, this compound had suboptimal plasma drug concentration and therefore was not considered for further development [35].

Imidazothiazole derivatives were also synthesized and assayed against *T. cruzi* strains. Their IC_{50} values ranged between 0.045 and 52 μM. The best compounds, **8** ($IC_{50} = 0.04$ μM/SI = 1600) **9** ($IC_{50} = 0.035$ μM/SI = 1828) and **10** (Fig. 3), also showed high SI values ranging from 1828 to 1663. Compound **10**, a bio-isostere of delamanid (**11**), was the most promising analog [36].

BZN derivatives have been developed in order to optimize the antichagasic effect of this drug. With this aim, new 1, 2, 3-triazole analogs were synthesized and evaluated, and three promising compounds of this series showed better activity against the *T. cruzi* Tulahuen strain compared to the reference drug. The best compound was *N*-benzyl-2-[4-(4-nitrophenyl)-1*H*-1,2,3-triazol-1^1-yl] acetamide (**12**) (Fig. 4), which showed a potent trypanocidal activity ($IC_{50} = 7.0$ μM), and was also active against trypomastigotes of *T. cruzi* (Y strain). Compound **12** was capable of crossing the macrophage membrane to exert its effect and showed low cytotoxicity to the host (SI = 114.3). Therefore, this analog was selected for the 'proof of concept' of using *T. cruzi* trypomastigotes (Y strain) infected mice as the model for Chagas disease. However, compound **12** showed poor water solubility resulting in pharmacokinetic issues [37].

Another study developed a series of 5-nitroindazole derivatives against *T. cruzi*. The researchers obtained 13 compounds that were more selective than BZN, showing SI ranging between 18.10 and

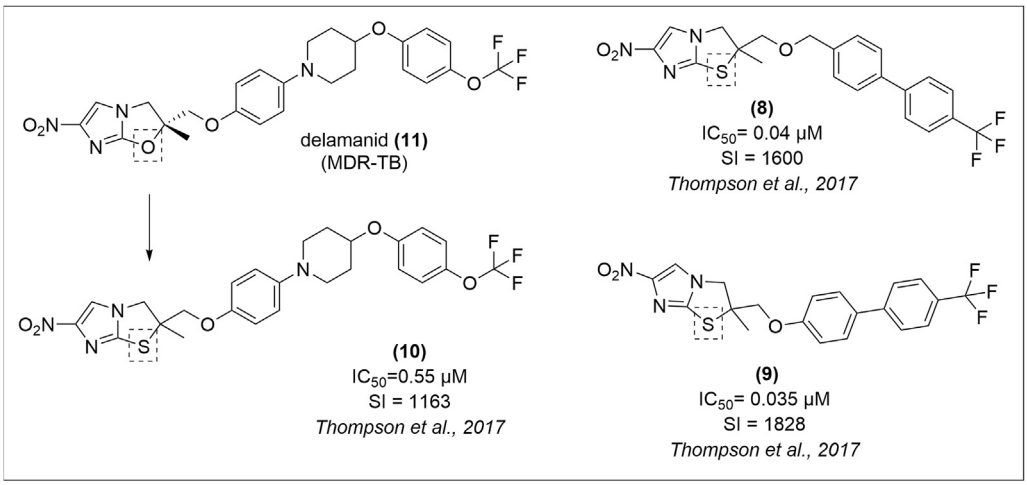

Fig. 3. Structures of imidazothiazoles derivatives. Novel Thiazoles analogs based on delamanid chemical structure.

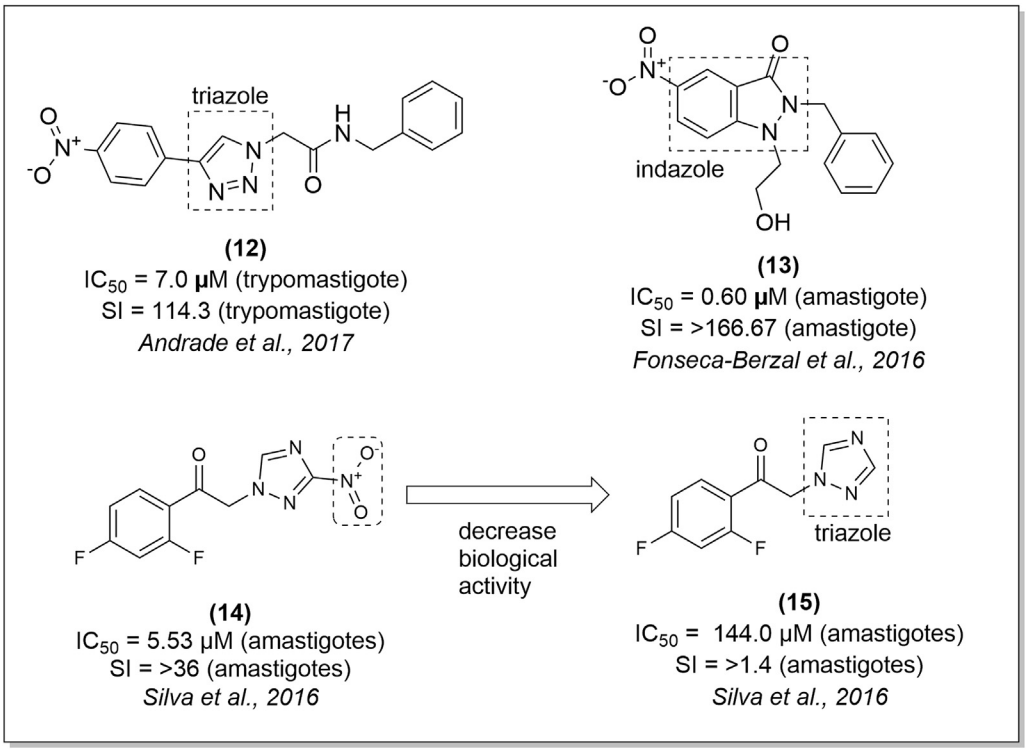

Fig. 4. Structures of triazoles and indazole analogs. The activity of triazoles and indazole derivatives against *T. cruzi*.

162.02 against the epimastigotes. In addition, when tested against amastigotes, four compounds were more selective than BZN. The SI values obtained ranged from 474.07 to 1163.64. The most active compound against both forms of *T. cruzi* CL Brener strain (DTU TcVI) was compound **13** (Fig. 4), which was also very active against *T. cruzi* Y strain (IC_{50} = 0.60 μM and SI = 166.67 against amastigotes forms), with a 3-fold higher selectivity than the reference drug BZN [38].

Bio-isosteric replacement was used to obtain new 1,2,4-triazolic derivatives against Y strain of *T. cruzi*. The most active compound **14** (BZN bio-isosteric) showed an IC_{50} of 5.53 μM and SI values above 36. However, this compound was less efficient than the parental drug. The researchers observed that the presence of a nitro group was extremely relevant and its absence, as observed for compound **15**, reduced its anti *T. cruzi* activity by 26-fold (Fig. 4). Finally, they concluded that the BZN bio-isosterics can be used as parental drugs for the development of new antichagasic drugs [39].

Lapatinib (**16**) and its derivative NEU-617 (**17**) are used to treat lung cancer (Fig. 5). Interestingly, these compounds are also active against *Trypanosoma brucei*, the etiologic agent of Human African trypanosomiasis (HAT). One group screened 66 new derivatives of lapatinib (**16**) and NEU-617 (**17**) against the amastigote forms of *T. cruzi*. The researchers found 32 active compounds that were capable of inhibiting cell proliferation by over 65%. The best analog was a homopiperazinyl sulfonamide-substituted derivative, compound **18** (Fig. 5), that presented an EC_{50} = 0.51 μM and an

SI = 85.6. To expand the screening, the researchers incorporated a molecular modification in the quinazoline scaffold and tested other bicyclic aromatic derivatives. They isolated compound **19** (((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-6-(4-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)quinoline-3-carbonitrile) (Fig. 5) as a potent analog (IC_{50} = 0.09 μM), which is approximately 2-fold more selective than compound **18**. They finally concluded that quinazoline moiety is a promising scaffold to develop novel lead compounds through cross-parasite screening [40].

In another study, the researchers synthesized a series of alpha-pyrone derivatives and evaluated their efficacy against *T. cruzi*. The two best compounds, **20** and **21** (Fig. 6), have shown high efficacy against the trypomastigotes forms of *T. cruzi* (Y strain), with IC_{50} values of 1.3 and 2.6 μM, respectively. Although the compounds had SI values higher than 70, both failed to adequately eliminate the intracellular amastigotes of *T. cruzi* (Y strain). Nevertheless, the pyrone derivative **21** was evaluated in an acute-phase experimental model (BALB/c mice) of Chagas disease and compared to BZN. Five days post-infection, compound **21** was administered intraperitoneally at 30 mg/kg/day and parasitemia was followed from the 8^{th}–14th days post infection. The results showed that compound **21** was able to reduce 55% of the parasite burden [41].

A series of N^1,N^2-dibenzylethane-1,2-diamine hydrochlorides (benzyl diamines) and N^1-benzyl,N^2-methyferrocenylethane-1,2-diamine hydrochlorides (ferrocenyl diamines) were found to be active against epimastigotes of *T. cruzi* (Y strain). Velásques et al. (2014) have shown that the ferrocenyl group linked into benzyl diamines increases its anti-*T. cruzi* activity. Compound **22** (Fig. 7) (IC_{50}=2.21 μM; SI = 14.6) was found to be the most potent trypanocidal agent [42].

Through another random screening, one group obtained compound **23** ((*E*)-5-(4-hydroxyphenyl)-3-[2-oxo-1-(2-oxo-2-naphthylethylthio)-2-naphthylethylidene]-3H- [1,2]dithiole), a 3H- [1,2]dithiole derivative with a highly activity against the epimastigotes form of *T. cruzi* (Tulahuen 2 strain). This compound exhibited IC_{50} = 4.9 μM and SI = 12.8, but the mechanistic studies were not able to correlate its activity with TcTIM (glycolytic enzyme triosephosphate isomerase), cruzipain or membrane sterol biosynthesis (Fig. 8) [43].

3. Target-based screening

For the target-based approach, the starting point is the identification of putative molecular targets that play important roles in the disease [29]. Small-molecules are then designed to disrupt specific targets that (a) are essential for parasite survival, and (b) are either absent from, or structurally dissimilar to those occurring in the host [28].

Several targets extracted from *T. cruzi* genome data analysis have been investigated for the development of high throughput target-based drug screening assays [44]. These screens notably included enzymes belonging to the ergosterol biosynthesis pathway and several proteases (such as cysteine protease cruzain). Many additional *T. cruzi* putative drug targets including intermediates of pathways of glycolysis (e.g., glucose-6-phosphate dehydrogenase), thiol-dependent redox metabolism (e.g., trypanothione synthetase) and polyisoprenoids biosynthesis, have been identified, validated and used in various drug discovery programs [28].

3.1. Nitroreductases

Nitroreductases (NTRs) catalyze the nitroreduction process and are classified into two types on the basis of their oxygen sensitivity [45]. Type I NTRs, a class of flavin mononucleotide-binding proteins commonly found in bacteria, are oxygen-insensitive and function via a series of two-electron reduction of the nitro-group, leading to the formation of DNA damaging moieties [46]. Type II NTRs are ubiquitous oxygen-sensitive proteins that act as flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) as cofactors. They catalyze the one-electron reduction of a substrate, forming a nitro anion radical. In the presence of oxygen, this radical

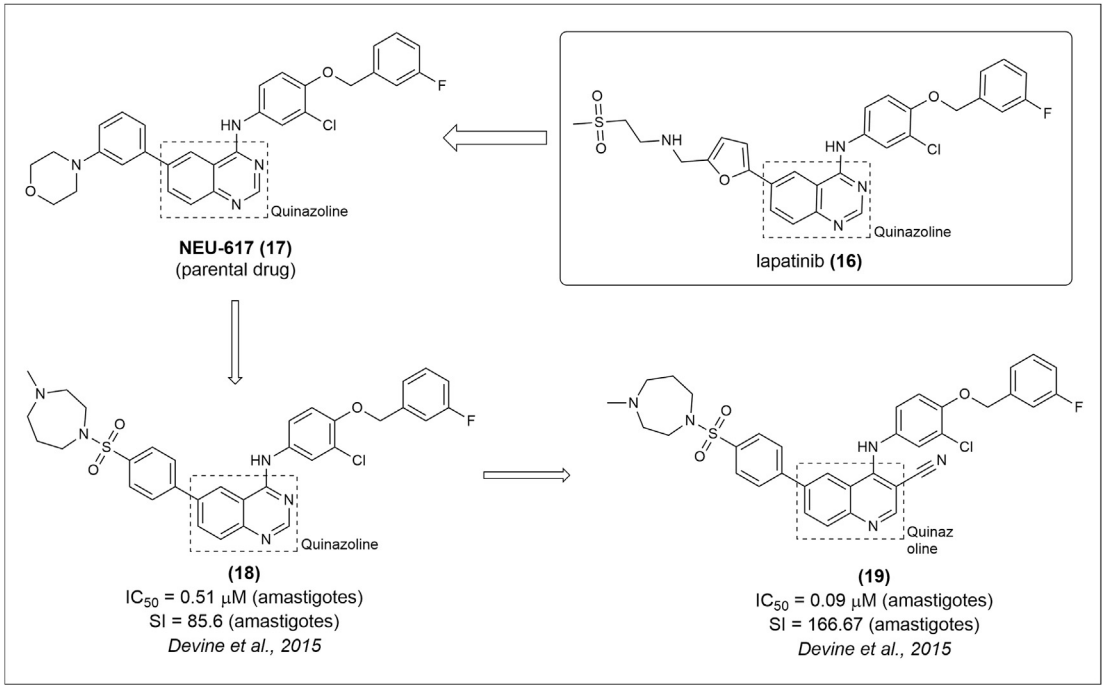

Fig. 5. Structures of lapatinib analogs (NEU-617). Novel lapatinib derivatives with antichagasic activity.

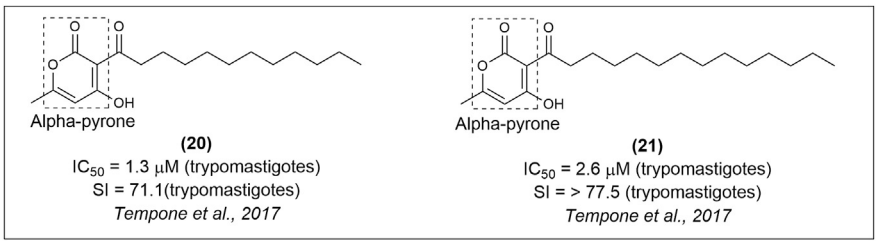

Fig. 6. Structure alpha-pyrone analogs. Alpha-pyrone derivatives active against trypomastigotes of *T. cruzi*.

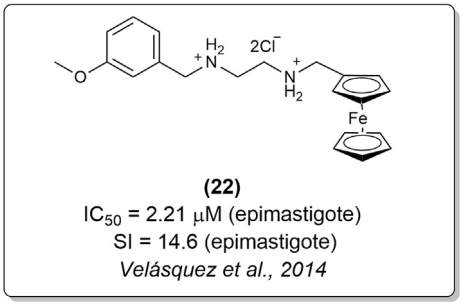

Fig. 7. Structures of ferrocenyl analogs. The most potent ferrocenyl derivative against *T. cruzi*.

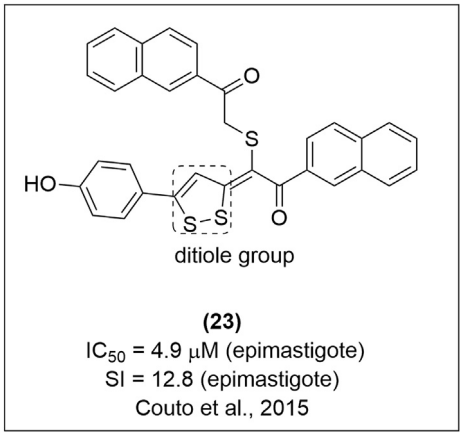

Fig. 8. Structure of dithiole analogs. Dithiole derivatives very effective against epimastigotes form of *T. cruzi* (Tulahuen 2 strain).

undergoes futile cycling, resulting in the production of superoxide anions [45]. Hall et al. described the role of trypanosomal type I NTRs in NFX activation [45], following which they exploited the drug target potential of NTRs by nitroheterocyclic agents [47–49]. The studies on some of the derivatives are presented in this section.

Triazole derivatives exhibit activity against the amastigotes forms of *T. cruzi* (Tulahuen C4 strains), with IC_{50} lower than 0.5 μM. The most actives analogs compounds **24** and **25** (Fig. 9) have an SI of 1194 and 2797, respectively. Earlier studies on their structural and functional relationships revealed that a chlorine atom in the *para* position is capable of increasing the biological activity. This effect was validated by the studies on the polar surface area [50]. *In vivo* evaluation using a murine model infected by *T. cruzi* showed that compounds **24** and **25** were able to reduce the parasitemic sanguineous concentration to sub-detectable levels. ADMET studies revealed that compound **24** crossed the plasma membrane of Caco-2 cells and was stable in the presence of murine or human microsomes. Furthermore, compounds **24** and **25** were also evaluated against *T. cruzi* nitrorreductase, but no relevant antichagasic activity was seen [50].

A later study by the same group described another, more potent 3-nitrotriazole derivative. Compounds **26** and **27** (Fig. 9) have nanomolar IC_{50} values for the Tulahuen C4 strain of amastigotes (8 nM and 36 nM respectively) and high SI values of 3615 and 1954, respectively. Compound **26** was 269-fold and 4.5 fold more active than BZN and analog **27,** respectively, making it the most active derivative. As with their previous study, the researchers observed that *T. cruzi* NTR enzymatic activity was not a determinant of antichagasic activity. All these compounds were capable of reducing the parasite load to undetectable levels in murine models infected with *T. cruzi*, indicating that these compounds are promising new antichagasic drugs [51].

3.2. Cruzain

Cruzain is a lysosomal cysteine protease of *T. cruzi* and a key etiological factor of Chagas disease [52,53]. Cruzain is expressed in all life cycle stages of the parasite and performs various biological functions [52]. Studies have shown the involvement of this enzyme in intracellular replication and differentiation of the epimastigotes to the infectious metacyclic form, immune evasion and host-parasite interactions [54].

Therefore, cruzain is a highly relevant therapeutic target and cruzain inhibitors have been developed by various research groups. A research group designed and synthesized several analogs of 8-chloro-*N*-(3-morpholinopropyl)-5*H*-pyrimido [5,4-b]indol-4-amine and screened them for antichagasic activity. The analogs consisted of indole, pyrimidine, quinoline, aniline, and pyrrole derivatives. The 4-aminoquinoline analogs showed the most potent anti-cruzain activity with IC_{50} values ranging from 15 to 125 μM, and were also selective for the parasitic proteases. Compound **28** (IC_{50} = 15 μM) (Fig. 10), the best anti-cruzain compound, was also active against the trypomastigote form of *T. cruzi* (Tulahuen strain) (IC_{50} = 67.7 μM) demonstrating a high correlation between enzyme inhibition and anti-parasitic activity, but showed a low selectivity index (SI = 1.8). The pyrimidine analog **29** (Fig. 10) was the most active and selective compound (IC_{50} = 3.1 μM; SI = 128) against the parasite, but was inactive against cruzain; therefore, requiring further studies to elucidate the mechanism of its action along with *in vivo* studies [55].

Another group identified a new class of imidazoles through computational screening, followed by the synthesis and biological evaluation. The most active compound **30** (Fig. 10) showed a high trypanocidal activity against the epimastigote form of *T. cruzi* of three different strains (Tulahuen 2, CL-clone B5 and Y), with IC_{50} values of 4.5 μM, 3.6 μM, and 16.9 μM respectively. Furthermore, the compound **30** was also active against the amastigotes of *T. cruzi* CL-clone B5 strain with an IC_{50} of 19.9 μM. In addition, the compound did not show any mutagenic activity in Ames' test. The

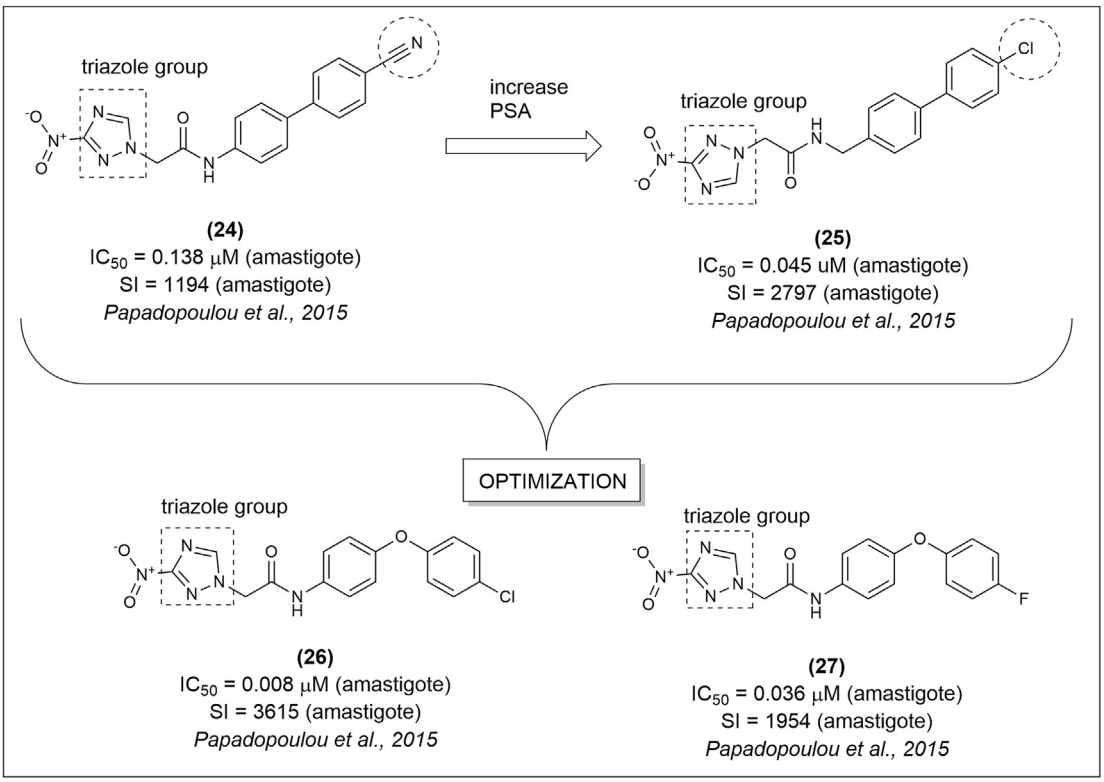

Fig. 9. Structures of 3-nitrotriazole analogs. The best nitrotriazole derivatives active against amastigotes form of *T. cruzi*.

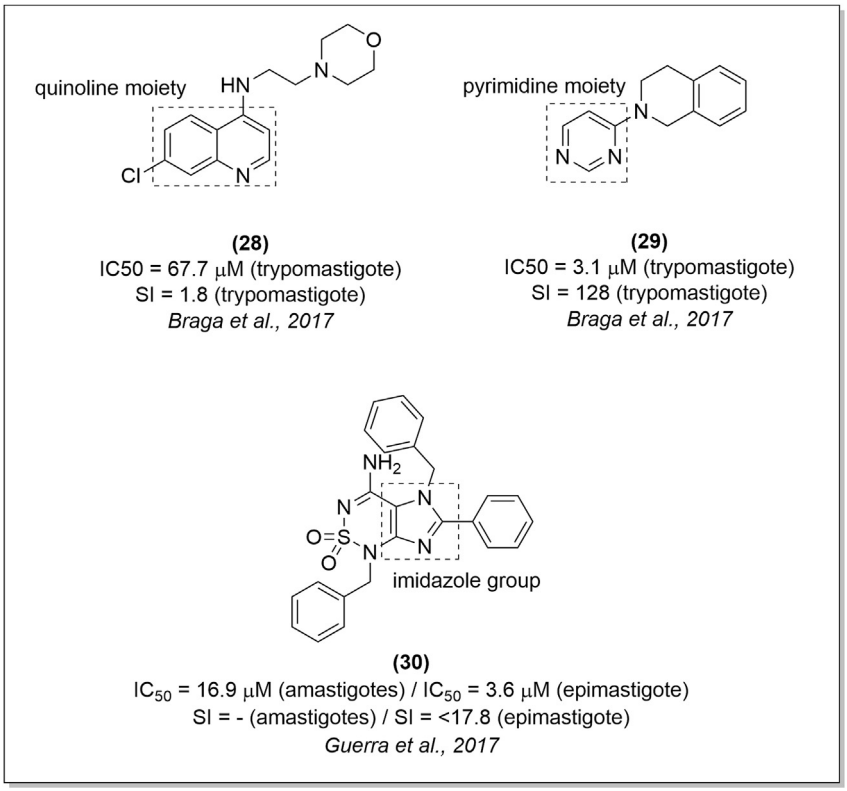

Fig. 10. Structures of quinoline, pyrimidine, and imidazole analogs. Quinoline, pyrimidine and imidazole derivatives active against *T. cruzi*.

cruzipain inhibitory assay was also evaluated ($IC_{50} = 81 \pm 4\ \mu M$), indicating a possible mechanism of action. *In vivo* assay with *T. cruzi* Y strain showed that compound **30** was also a potent trypanocidal analog during the acute phase of Chagas disease [56].

3.3. Mitochondrial alterations

Mitochondria are important cellular organelles involved in cellular redox, ATP synthesis, calcium homeostasis, nutrient oxidation, and apoptosis [57,58]. Hydrogen peroxide is the main oxidative species produced in mitochondria and is related to cytosol signaling in oxidative processes. Mitochondrial deregulation can lead to an increase in reactive oxygen species (ROS), resulting in cytotoxicity and death [59,60]. *T. cruzi* has unique mitochondria that contain kinetoplasts and highly compact networks of mitochondrial DNA that account for approximately 30% of the total genome [61]. This feature makes the mitochondria an important target in the development of new antichagasic agents.

One study evaluated the *in vitro* trypanocidal activity of 4-nitrobenzaldehyde thiosemicarbazone (**31**) (Fig. 11), an *S*-(−)-limonene derivative. The researchers discovered a potent activity of this compound against the trypomastigote ($IC_{50} = 1.43\ \mu M$; $SI = 26.2$) and amastigote ($IC_{50} = 11.84\ \mu M$; $SI = 11.6$) forms of *T cruzi* (Y strain). Compound **31** induced mitochondrial alterations like cytoplasmic vacuolization, loss of membrane potential, and increase in free radical formation in the epimastigotes and trypomastigotes [62].

In another study, the antichagasic activity of four C-4 functionalized azalactone derivatives was evaluated. The most potent compound was **32** (Fig. 11), with an $IC_{50} = 33\ \mu M$ and $SI = 6.74$ against the epimastigote form of *T. cruzi*, and a 3-fold higher potency against *T. cruzi* amastigotes ($IC_{50} = 2.34\ \mu M$ and $SI = 95.04$) compared to BZN. Flow cytometry and electron microscopy revealed a significant decrease in mitochondrial membrane potential and in the size of epimastigotes, without any damage to the plasma membrane. Based on their findings, the researchers recommended the molecular modifications of this analog in developing new antichagasic lead compounds [63].

New antichagasic agents were obtained by linking gallic acid derivatives with lipophilic properties and the triphenylphosphonium (TPP+) moiety to target the small molecules on *T. cruzi* mitochondria. The researchers obtained two highly active compounds against trypomastigotes (Y strain), **33** ($EC_{50} = 1\ \mu M$) and **34** ($EC_{50} = 1\ \mu M$) (Fig. 12), which were more active than NFX. Compounds **33** and **34** were also more selective and were capable of modifying the mitochondrial membrane potential that leads to pore formation, indicating their potential in the development of new antichagasic analogs [64].

3.4. Heme peroxidation

Heme, or ferroprotoporphyrin, is involved in many biological processes such as respiration, detoxification, oxygen transport, cellular signaling, antioxidant gene expression, metabolism, and energy production. Heme breakdown produces free radicals that damage lipids, DNA, and proteins. *T. cruzi* requires heme from the host's blood for epimastigote multiplication through the redox mechanism, and blocking this pathway can potentially inhibit the epimastigotes [65,66].

Quinoline derivatives are nitrogen heterocyclic compounds that form complexes with heme, and are known for their activity against *Plasmodium*, *Leishmania*, *T. cruzi*, bacteria, and even in cancer [67–71]. Researchers have evaluated the activity of a series of 4-arylaminoquinoline-3-carbonitrile derivatives against all forms of *T. cruzi*, and obtained compound **35** (Fig. 13) that was active against epimastigotes when combined with hemin, with higher potency than BZN ($IC_{50} = <1\ \mu M$, $SI = >111.1$). In addition, 10 μM of this compound inhibited 65% of amastigotes growth after 72 h [72]. High-performance liquid chromatography analysis showed that heme is enzymatically converted to biliverdin, thus favoring heme oxygenase activity in the parasite. The authors also suggested that the formation of heme-quinoline **35** complex could be attributed to

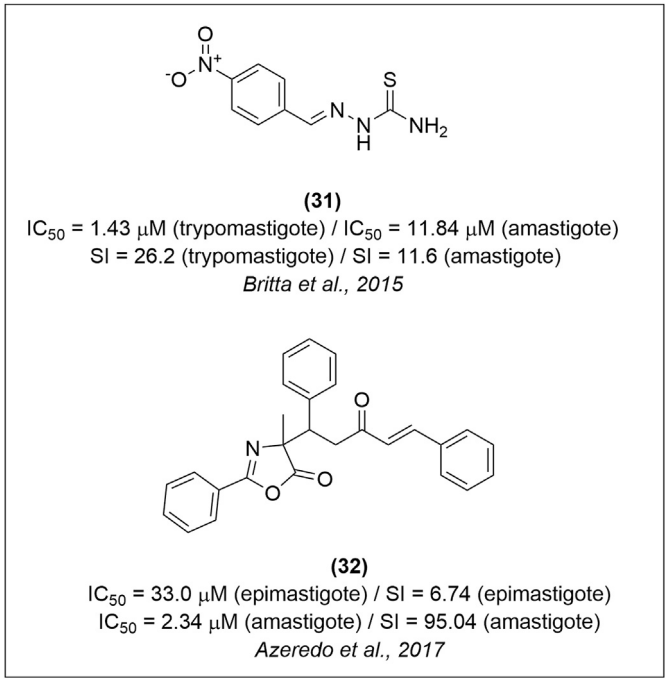

Fig. 11. Structures of nitrobenzaldehyde thiosemicarbazone and azalactone derivatives. Some compounds are described as possible mitochondrion target analogs.

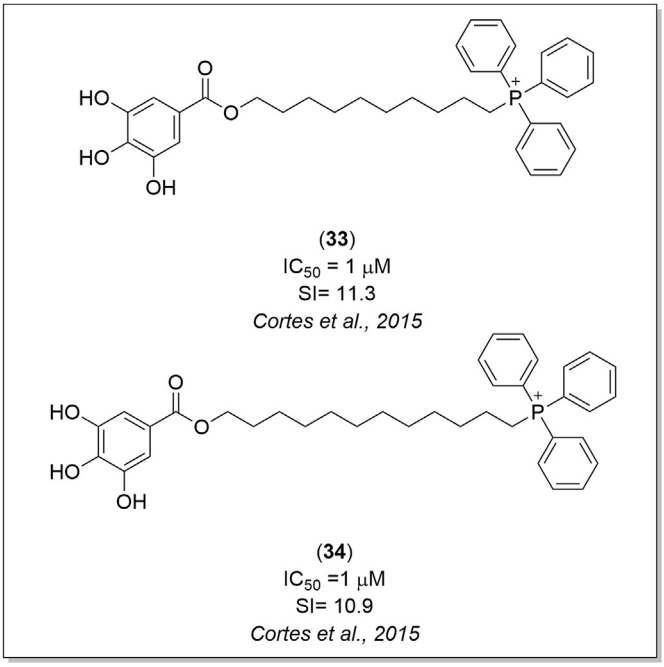

Fig. 12. Structures of triphenylphosphonium analogs. This is a class that provoke alterations in *T. cruzi* mitochondrion.

the decrease in available heme levels and increase in the production of reactive oxygen species, leading to parasite death [72].

3.5. Iron superoxide dismutase (Fe-SOD)

Superoxide dismutase (SOD) catalyzes the transfer of superoxide anion through dismutation to oxygen or hydrogen peroxide, and is a key enzyme that neutralizes the toxicity of free oxygen radicals [73]. *T. cruzi* has four Fe-SODs that are produced in the mitochondrion (TcSODA and C), cytosol (TcSODB1), and glycosomes (TcSODB1-2) [74]. Since *T. cruzi* SODs are different from their human counterparts, they are often the targets for drug discovery [75].

By repurposing existing screening libraries, some researchers synthesized and evaluated a series of antichagasic arylaminoketone derivatives. Their study was based on previous ^{1}H- Magnetic Nuclear Resonance (^{1}H NMR) results that indicated these analogs exerted their effects via Fe-SOD. The compounds **36, 37, 38** and **39** (Fig. 14) showed higher antichagasic activity and less *in vitro* cytotoxicity when compared to BZN in three different *T. cruzi* strains (SN3, Arequipa, and Tulahuen) against all three parasitic forms (Fig. 14), with SI over 17. These compounds were selected and evaluated *in vivo* during the acute phase of the disease, and only compound **38** did not result in the reactivation of blood parasitemia during the immunosuppression cycles [76], in addition to inhibiting Fe-SOD *in vitro*. The authors hypothesized that the fluorine atoms and nitro group of the compound interacted with the Lys35, Gln68 and Asn117 of the enzyme docking site through hydrophobic interactions. The compounds do no interact with the metal of binding site, which can inhibit the molecule to have access to the active site of Fe-SOD [76].

3.6. Trypanothione reductase

Trypanothione reductase (TR) is an attractive chemotherapeutic target for developing new anti-trypanosome drugs since it is unique to the *Trypanosomatidae* family and absent from mammalian cells [77–79]. TR is a flavoprotein consisting of a spermidine moiety linked to two glutathione molecules. It catalyzes the NADPH dependent reduction of trypanothione disulfide to trypanothione, and acts as an important antioxidant defending the parasite from oxidative stress [78–81].

Some researchers have designed and synthesized, through the microwave-irradiated synthesis, several isoxazole analogs based on the structure of the natural lignans, grandisin, and veraguensin, which have shown potent action against trypomastigotes and intracellular amastigotes of *T. cruzi* (Tulahuen strain). The main

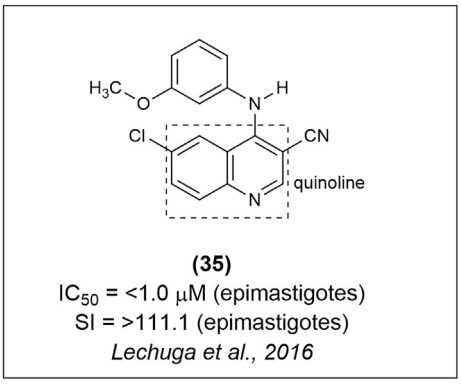

Fig. 13. Structures of quinoline derivatives. Analog **35** described as an interferent of heme peroxidation of *T. cruzi*.

derivatives were compounds **40**, **41** and **42** (Fig. 15) that showed low inhibitory concentrations for the amastigote forms of *T. cruzi* with $IC_{50} = 5.26\ \mu M$ (SI = 13.3), 1.74 μM (SI = 46.7) and 1.13 μM (SI = 160.9), respectively, and none of them were active against the trypomastigotes form. The enzymatic assay results suggested that the isoxazoles can act by the mechanisms independent of trypanothione reductase [82].

3.7. Squalene synthase (SQS)

T. cruzi requires endogenous sterols like ergosterol and its analogs for growth and survival [83]. Ergosterol is essential for the maintenance of plasma membrane and performs functions analogous to that of cholesterol in human cells [84]. Ergosterol biosynthesis requires squalene synthase (SQS) which catalyzes the reductive dimerization of two farnesyl-pyrophosphate (FPP) molecules in a two-step process to form squalene [83].

Some researchers synthesized new isosteric analogs based on compound **43** (4-phenoxyphenoxyethyl thiocyanate) that was previously described as a good non-competitive allosteric inhibitor of *T. cruzi* SQS [83,85], in order to understand the effect of different atoms on the activity of this inhibitor [86]. The activity of these analogs were tested against *T. cruzi* amastigote (CL strain) overexpressing a tdTomato red fluorescent protein, and their toxicity and SI were tested in Vero cells. The compounds with selenocyanate moiety, especially derivative **44** (Fig. 16), were extremely potent inhibitors of *T. cruzi* proliferation, almost 50 times more potent and 100 times more selective than **43**. Compound **45** (Fig. 16), also a selenocyanate derivative with a fluorophenoxy group, was also a very effective inhibitor of the parasite with very low toxicity [86].

Compounds **43** and **44** were selected and subjected to in silico analyses with crystallography data from the Cambridge Structural Database (CSD). The results indicated that it was the presence of Se and not S that increased the interaction between the compounds and SQS [86].

3.8. Farnesyl diphosphate synthase (FPPS)

Another enzyme that has been considered as a valid target against various parasitic diseases is farnesyl diphosphate synthase (FPPS) [87]. It is involved in the mevalonate pathway [88]. FPPS catalyzes the condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) to form geranyl diphosphate (GPP), and subsequently farnesyl diphosphate (FPP) [89]. FPP is the substrate for enzymes catalyzing the first committed step in the biosynthesis of sterols, ubiquinones, dolichols, heme group, and prenylated proteins [87]. FPP is an important branching point in the mevalonate pathway leading to protein farnesylation, and biosynthesis of dolichols and sterols such as cholesterol and ergosterol [89]. Inhibition of FPPS blocks farnesyl pyrophosphate and geranylgeranyl pyrophosphate synthesis [90].

Bisphosphonates are potent inhibitors of bone resorption and are used to treat several bone disorders such as osteoporosis, Paget's disease, hypercalcemia, and tumor bone metastases. The selective antiparasitic action of bisphosphonates is likely due to the presence of acidocalcisomes, which facilitate the accumulation of bone minerals on account of similar composition [87–92].

Researchers have synthesized and evaluated 2-alkylmercaptoethyl-1,1-bisphosphonate derivatives against *T. cruzi* in the presence of FPPS. The results showed that sulfur-containing bisphosphonic acids with long aliphatic chains were effective against the amastigote form of *T. cruzi* (CL strain) overexpressing a tdTomato RFP, with potent activity against *Tc*FPPS. The most active compounds **46** and **47** (Fig. 17) were also the most potent inhibitors

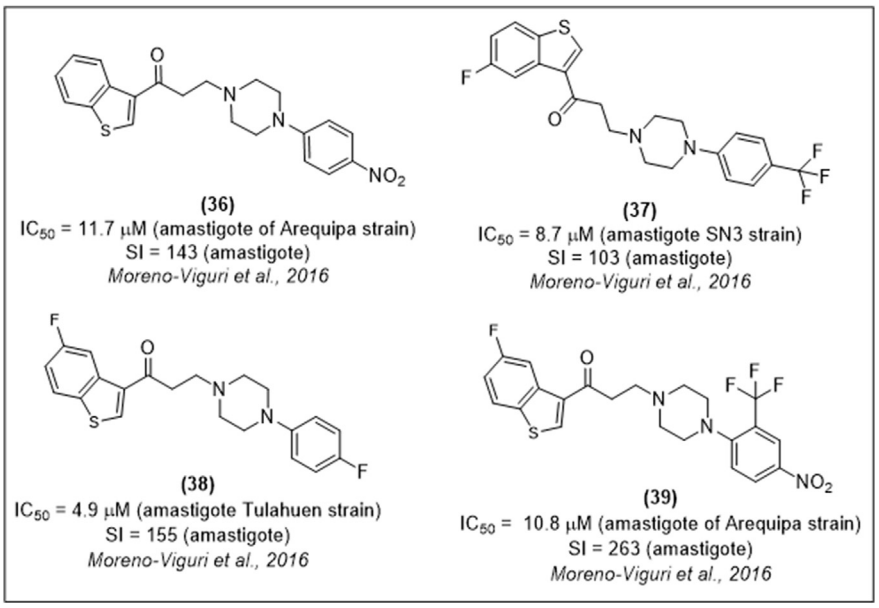

Fig. 14. Structures of arylaminoketones derivatives. The best arylaminoketones analogs were described by Moreno-Viguri et al. (2016).

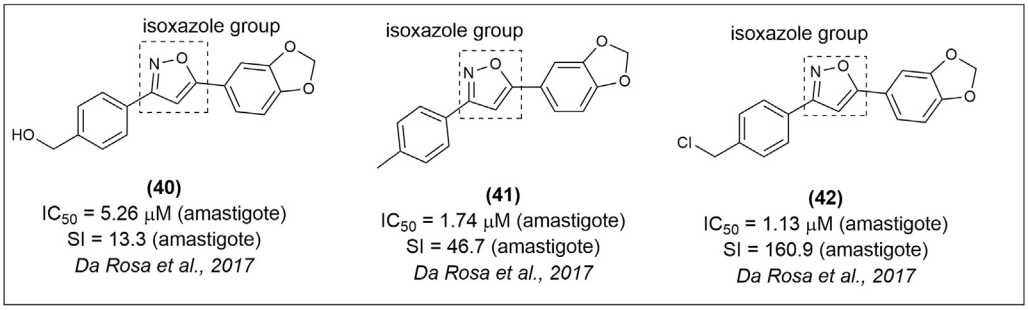

Fig. 15. Structures of isoxazoles derivatives. Isoxazoles analogs active against amastigotes form of *T. cruzi* (Tulahuen strain).

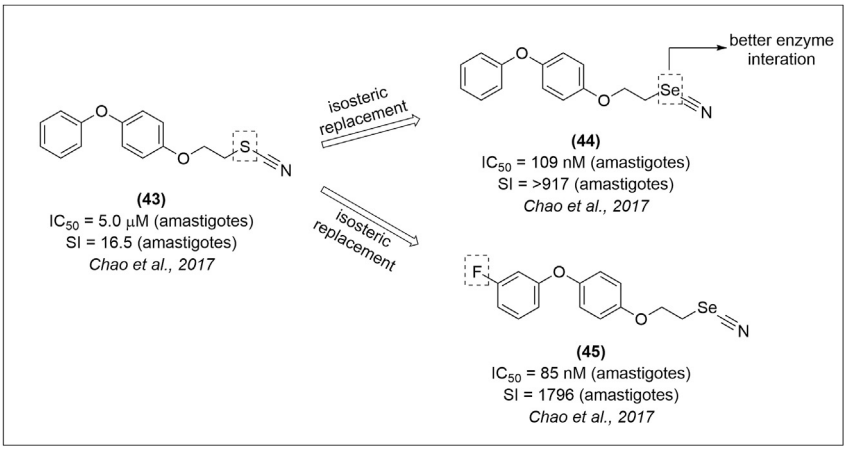

Fig. 16. Structures of thiocyanate derivatives. The isosteres of compound **43** active against *T. cruzi*.

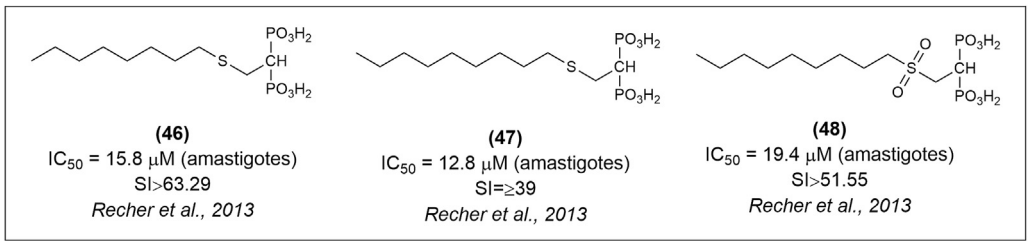

Fig. 17. Structures of bisphosphonate analogs. The best bisphosphonate derivatives described for Recher et al., 2013.

of FPPS. Analog **48** also showed good activity against the amastigotes but was not a good inhibitor of the enzyme (above 10 μM could promote 50% inhibition) [93].

3.9. Physico-chemical properties and selective indices of the most active compounds

Since the physicochemical properties of a compound are correlated with its biological activity [94–99], we have summarized some properties of the most active compounds such as lipophilicity, number of hydrogen bond acceptors and donors, number of rotatable bonds, molecular weight and surface area (Table S1. supplementary material).

Lipophilicity (cLog*P*) is a vital property in the context of drug discovery and development since it affects the solubility, permeability, and bioavailability of compounds [100–104]. In general, all of the active compounds reviewed here exhibit lipophilicity and molecular weight values range from 0.7 to 9.1 and 211.2 to 656.2, respectively. Fig. 18 describes the correlation between the SI values and cLog*P* for the most active compounds. Nine compounds exhibited SI values over 900 and cLog*P* values from 2.7 to 5.68 when tested against different *T. cruzi* strains like Sylvio X-10 (3), Tulahuen C4 (4), and CL (2).

4. Current drugs under clinical trials and perspectives

Azole analogs such as ketoconazole, itraconazole, fluconazole, posaconazole, and ravuconazole are the main inhibitors of sterol 14-α demethylase (CYP51). Crystallographic studies (PDB code: 3K1O) have shown the highest enzyme inhibitory effect and affinity of Posaconazole (**49**) (Fig. 19). This compound is effective against both the epimastigote and amastigote forms of *T. cruzi*, and also shows good activity against the drug-resistant strains like Y and Colombia [105–110]. In 2015, a study reported its limited capacity for "curing" Chagas disease through highly sensitive *in vivo* bioluminescence imaging assay. Clinical studies in Argentina, Bolivia, and Spain compared the effects of posaconazole and BZN in adults with chronic Chagas disease [111], and showed lower activity of this new drug relative to BZN [112]. A clinical trial investigating the effects of posaconazole and BZN co-administration is currently being conducted by Merck.

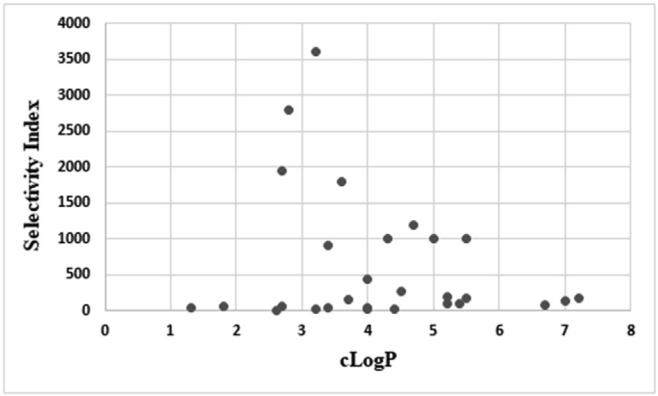

Fig. 18. Correlation between SI (IC_{50}/CC_{50}) values and cLog*P* for the compounds. cLog*P*: calculated partition coefficient.

Ravuconazole (**50**) and its prodrug E−1224 (**51**) (Fig. 18) have also demonstrated high *in vitro* efficacy against *T. cruzi* [113,114] but the low bioavailability and half-life of the former discouraged further evaluation. Therefore, only the prodrug was selected for clinical trials [113–115], and the results showed that compared to the standard drug, this compound did not remain effective after one year of treatment [116]. Adverse effects were also observed at high doses, leading to the cessation of the treatment in phase II.

K777 (**52**) (Fig. 20) is a vinyl sulfone cysteine protease inhibitor, which has shown potent inhibition of cruzain [117–119], and has a complex structure as per crystallography (PDB code: 2OZ2). In addition, it is effective against the epimastigote and amastigote forms of *T. cruzi* and reduced blood parasitemia in animal models, thereby prolonging their survival [120]. However, K777d did not proceed to clinical trials due to its high hepatotoxicity.

Hydroxymethylnitrofurazone (NFOH) (**53**) (Fig. 20) is a new drug candidate that has shown high *in vitro* and *in vivo* trypanocidal activity in both trypomastigotes and amastigotes, along with a lower mortality rate in the acute phase compared to BZN [121–123]. Similar concentrations of nitrofurazone and NFOH, respectively, inhibited 30% and 60% cruzain (PDB code: 1ME4) [124]. Davies and co-workers (2014) evaluated the short-term (21 days) and long-term (60 days) hepatoxicity of NFOH (150 mg/kg/day) in an acute phase animal model treated for five days post-infection, and noted lower liver infiltration compared to BZN treatment but no significant alteration in other hepatic markers [125]. Using transgenic *T. cruzi* Brazil (Luc strains) expressing the firefly luciferase, researchers have reported 78.5% of the trypanocidal activity after four days of NFOH treatment (50 mg/kg) [126]. These encouraging studies indicate that NFOH can be a potential candidate for clinical studies.

Intensive research on the discovery of new analogs for the treatment of Chagas disease has been undertaken. The data from clinical trials indicate that most of these drugs are new diagnostic methodologies or new formulations with standard treatment drugs for children including old (known) and new (unknown) drugs. The treatment to Chagas disease is still a challenge in medicinal chemistry. Among the 58 clinical trials on Chagas disease, only 40 are interventional studies at different stages of completion: completed (17), unknown (9), recruiting (6), not yet recruiting (3), active not recruiting (2), terminated (2), and enrolling by invitation (1) (Fig. 21, Table S2. Supplementary material).

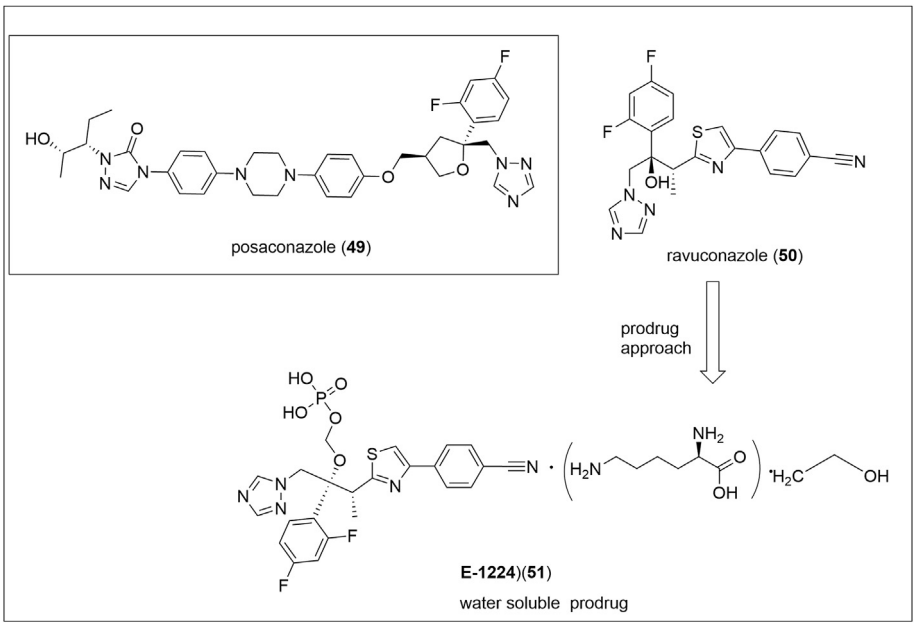

Fig. 19. Structures of azole analogs: Azole derivatives active against *T. cruzi.*

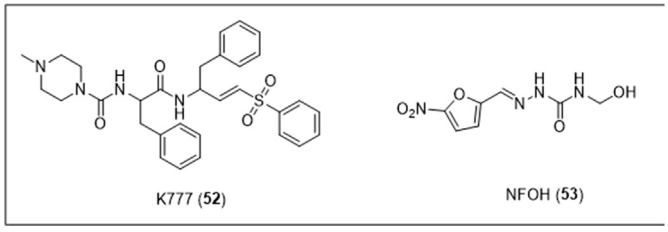

Fig. 20. Structures of K777 (52) and hydroxymethylnitrofurazone (NFOH) (53). Novel candidates against Chagas disease.

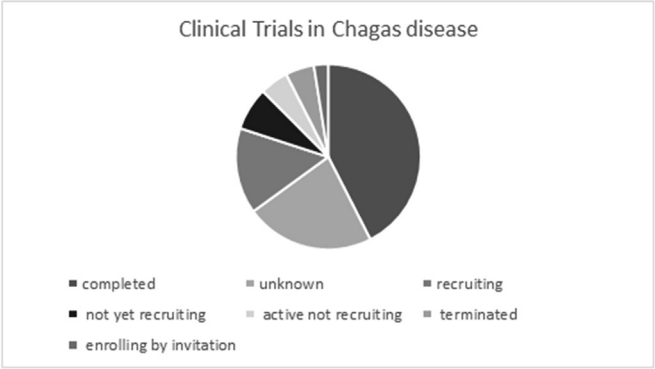

Fig. 21. The ongoing clinical trial on Chagas disease: 40 studies are interventional (number of studies): completed (17), unknown (9), recruiting (6), not yet recruiting (3), active not recruiting (2), terminated (2), and enrolling by invitation (1).

5. Conclusions

Despite the large number of new molecules that have been identified and synthesized and a greater insight on the molecular biology of *T. cruzi*, which has further increased the pool of potential targets, the development of novel antichagasic drugs remains a difficult task. This is largely due to the lack of interest by the pharmaceutical companies, failure of pre-clinical and clinical trials, and the socio-economic disparities in developing countries. Strategies aiming to develop a partnership between the industry, academia and non-profit organizations could help accelerate the drug development process. However, in spite of this bleak scenario, several promising antichagasic compounds have been described in recent years. The different chemotypes described in this review can provide the necessary prototypes for further development of new drugs against *T. cruzi*.

Authors' contribution

All authors contributed equally for the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2018.06.040.

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