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REVIEW

Review on Experimental Treatment Strategies Against *Trypanosoma cruzi*

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Abstract: Chagas disease is a neglected tropical disease caused by the protozoan *Trypanosoma cruzi*. Currently, only nitroheterocyclic nifurtimox (NFX) and benznidazole (BNZ) are available for the treatment of Chagas disease, with limitations such as variable efficacy, long treatment regimens and toxicity. Different strategies have been used to discover new active molecules for the treatment of Chagas disease. Target-based and phenotypic screening led to thousands of compounds with anti-*T. cruzi* activity, notably the nitroheterocyclic compounds, fexinidazole and its metabolites. In addition, drug repurposing, drug combinations, re-dosing regimens and the development of new formulations have been evaluated. The CYP51 antifungal azoles, as posaconazole, ravuconazole and its prodrug fosravuconazole presented promising results in experimental Chagas disease. Drug combinations of nitroheterocyclic and azoles were able to induce cure in murine infection. New treatment schemes using BNZ showed efficacy in the experimental chronic stage, including against dormant forms of *T. cruzi*. And finally, sesquiterpene lactone formulated in nanocarriers displayed outstanding efficacy against different strains of *T. cruzi*, susceptible or resistant to BNZ, the reference drug. These pre-clinical results are encouraging and provide interesting evidence to improve the treatment of patients with Chagas disease.

Keywords: *Trypanosoma cruzi*, drug discovery, new chemical entities, repurposing, drug combination, nanomedicine

Introduction

Chagas disease (American trypanosomiasis) was discovered and described by Carlos Chagas in 1909.[1] The disease is caused by the protozoan *Trypanosoma cruzi* and affects 6–7 million people worldwide, with an estimated 75 million people at risk of infection.[1,2] It is a neglected disease and related to poverty in tropical and subtropical countries, especially in Latin America.[3] The affected population lives in rural and peri-urban areas in inappropriate buildings and in vulnerable socioeconomic conditions.[4] Recently, Chagas disease has spread to non-endemic areas in Europe, United States and Japan, and it has become a globalized public health and medical problem.[3,4]

T. cruzi infection is transmitted mainly via insect vector, where trypomastigotes of *T. cruzi* in the excreta of contaminated blood-sucking triatomines can penetrate sites of lesioned skin or mucosa in humans.[5] Other transmission routes have been also reported, such as congenital infection,[6,7] blood transfusions[8] or organ transplants, laboratory accidents,[9] and oral contamination.[10,11] Sustained efforts to control the vector have resulted in a decrease in the incidence of Chagas disease in several countries of Southern Cone in Latin America.[12] In recent years, the

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epidemiological relevance of oral transmission has been increased, especially in countries of the Amazon region.[13–15] Several outbreaks associated with orally transmitted acute Chagas disease have been related to the intake of contaminated foods/juices.[16]

The acute phase of Chagas disease persists for 4–8 weeks after infection and it is asymptomatic in most cases.[17] Some symptoms, such as fever, malaise, lymph nodes and subcutaneous edema, hepatosplenomegaly and electrocardiographic or neurological disorders eventually appear.[17] The acute severe symptoms, as myocarditis, pericardial effusion, and meningoencephalitis affect 1–5% of patients,[18] mainly children and immunosuppressed patients.[19–21] In the absence of an effective etiologic treatment, the chronic phase gradually sets in.[17,18] Most of chronic patients are asymptomatic, characterized by the undetermined clinical form of the disease, and may remain so for an indefinite period. In these cases, the patients have detectable anti-*T. cruzi* antibodies, absence of clinical signs and symptoms of cardiac and digestive clinical forms of the disease.[22] However, after 10–30 years of asymptomatic period, 30–40% of chronically infected individuals may progress to symptomatic forms of Chagas disease.[17,18] About 14–45% of them develop cardiac abnormalities and dysfunctions (cardiac form), and 10–21% present intestinal involvement, as megaviscera, specially megaesophagus and megacolon (digestive form).[17,18,22,23]

Clinical manifestations of Chagas disease, specially chronic Chagas cardiomyopathy, are responsible for high morbidity and mortality in economically productive young adults, and result in progressive inability to continue working and consequent burden of the health system.[24,25] Experimental and clinical studies demonstrate that the presence of the parasites and their DNA in tissues is closely associated with the pathogenesis of the disease which reinforces the hypothesis that the etiological treatment improves patient outcome.[26–31]

In this review, we attempted to provide an overview of the most impressive experimental preclinical results concerning new chemical entities and therapeutic strategies tested in experimental *T. cruzi* infection. PubMed, Scopus, and Web of Science databases were accessed concerning chemotherapeutic options that have been tested. As a plethora of new chemical compounds have already been tested in vitro, only in vivo data were selected.

Chagas Disease Chemotherapy

Several new compounds were tested for the treatment of Chagas disease until the 1960s but without promising results.[27] Afterwards, a more effective class of anti-*T. cruzi* agents were introduced - the nitrofurans,[32–34] among which nifurtimox (NFX) stood out for its superior efficacy.[32] In addition, 2-nitroimidazole derivative benznidazole (BNZ) was discovered and included for the treatment of Chagas disease.[35] NFX and BNZ are nitroheterocyclic class of compounds with the nitro groups linked to furan or imidazole rings, respectively.[36] Currently, BNZ and NFX are standards of care in clinical chemotherapy of Chagas disease, recommended by WHO.[2] Their mechanism of action has not yet been fully elucidated, but both act as prodrugs and must be activated by nitro-reductases present in *T. cruzi* to exert their cytotoxic effects.[36] NFX action on *T. cruzi* involves redox cycling and radical species that results in damage to the parasites, i.e. causing marked reduction on the level of intracellular thiols, with evidence of DNA damage and lipid peroxidation.[39] Differently, the reduction of BNZ occurs through various free radical intermediates and electrophilic metabolites that alkylate macromolecules such as DNA, lipids, and proteins, as recently reviewed by Patterson and Fairlamb.[37] There is also the hypothesis that BNZ may act via immune system control producing trypanosome death through interferon-γ that is likely to be increased due to inflammation caused by macromolecule damage.[38] Furthermore, it has been shown that the DNA of parasites affected by BNZ could undergo extensive unpacking with overexpression of DNA repair proteins. These findings support the idea that DNA damage could contribute to the mechanism of action of BNZ.[39]

In 2017, BNZ obtained accelerated first treatment approval for the treatment of Chagas disease in children aged 2 to 12 years by the U.S. Food and Drug Administration (FDA) in the United States.[40] The treatment regimen with NFX or BNZ is long, and many adverse effects can occur and compromise the continuity of the treatment. Common adverse events in NFX treatment include gastrointestinal symptoms (nausea, vomiting and anorexia), symptoms of central nervous system toxicity (insomnia, irritability, and disorientation) and occasionally headache, rash, myalgia, arthralgia, dizziness or vertigo and mood changes.[17,18] Less commonly, but more severe adverse effects may appear, such as polyneuropathy, paraesthesia and peripheral neuritis.[17,18] The common adverse effects of BNZ are allergic dermatitis, nausea, vomiting, anorexia, weight loss, insomnia, loss of taste, onycholysis and dose-dependent peripheral sensitive neuropathy may also appear.[17,18] Rare serious events include neuropathy and depression of bone marrow.[17,18] Although the treatment of recent stages of

infection is efficacious using both nitroheterocyclic drugs, their benefits are limited in the chronic phase, with variation of efficacy following geographical locations.[18,41–43] In addition to the decrease in treatment effectiveness with the time of infection, the drug side effects are more frequent in patients of advanced ages.[2]

In order to search for new treatment alternatives, preclinical studies have been identified promising new candidates in specific pharmacological classes. Particularly, the C14-α-demethylase (CYP51) inhibitors were tested in clinical trials, such as posaconazole and fosravuconazole (or E-1224 a prodrug of ravuconazole), but both failed to induce cure and were less effective than the treatment using BNZ.[44,45]

New Drug Candidates for Chagas Disease

Some pharmacological classes are specially promising to treat Chagas disease: nitroheterocyclic compounds, inhibitors of sterol biosynthesis, cruzipain inhibitors, aromatic amides, trypanothione reductase inhibitors, ruthenium complexes carrying trypanocidal molecules, oxaboroles and nucleoside derivatives. Table 1 summarizes the main data on investigational compounds. In the class of nitroheterocyclic compounds, fexinidazole demonstrated potential against kinetoplastid diseases, and particularly in *T. cruzi* infections.[46–48] Fexinidazole (Figure 1) was more efficacious than BNZ in murine model, promoted the reduction of parasitemia and induced cure in mice infected by BNZ-resistant strains upon treatment with 300 mg/kg/day for 20 days.[46] Similarly, the treatment of infected mice (Y strain) with sulfone and sulfoxide metabolites of fexinidazole induced 100% cure in acute phase with 100 mg/kg/day, indicating that these metabolites are more active than BNZ and the parent drug (fexinidazole).[49] The mechanism of action of these compounds has not yet been elucidated, but indirect evidence indicates that they are also metabolized by *T. cruzi* nitro-reductases.[37] Other nitroheterocyclic compounds have been extensively investigated for their activity against *T. cruzi* (see Table 1 and Figure 1),[50–58] although toxicity potential of this class of molecules can limit their use. Thereby, a thorough experimental study on the toxic potential of new compounds must be performed in the drug discovery process.

Another hit class for the treatment of *T. cruzi* infections includes the inhibitors of sterol biosynthesis pathway (Figure 2), in particular C14-α-demethylase (CYP51) inhibitors, used originally as antifungals. In the repurposing strategy, these compounds can block *T. cruzi* ergosterol biosynthesis, and compromise the parasite survival. For example, treatment with non-azole VNI (Figure 3) led to the cure of animals in acute and chronic phases of infection with Tulahuen strain.[59] In addition, VNI promoted suppression of parasitemia and protection against mortality, but did not cure murine infections by Y and Colombian strains.[60–62] Similarly, the treatment with VFV, a VNI structure-based fluoro-analog, resulted in complete parasitemia suppression and mortality protection. VFV was more potent than VNI (Figure 3).[60] Moreover, fenarimol (Figure 3), a CYP51 inhibitor, showed a potent anti-*T. cruzi* activity, and two derivatives were able to induce cure in infected mice, with efficacy comparable to posaconazole and superior to BNZ.[57,63,64] Other classes of ergosterol biosynthesis inhibitors have been found to impact *T. cruzi* infections (Figure 3 and 4).[65–67]

In view of the excellent activities presented by nitroheterocyclic and azole compounds, new nitrotriazole-based compounds were developed as bifunctional compounds against *T. cruzi*, acting on CYP51 enzyme and acting as substrates of nitroreductases. Nitro-triazoles displayed in vitro anti-*T. cruzi* activity and induced the reduction of parasitemia in acute model of infection.[68–76]

An important drug target for Chagas disease treatment is cruzipain (also named cruzain), an essential cysteine protease of *T. cruzi* responsible for the proteolytic activity in all stages of parasite life [77]. K777, a vinyl sulfone cruzipain inhibitor, exhibited anti-*T. cruzi* activity and induced the cure in experimental murine models (Figure 3).[77,78] In infected dogs, the treatment with K777 reduced the myocardial damage caused by the parasite, though it was not able to induce the parasitological cure.[79] In addition, Cz007 and Cz008 cruzipain inhibitors presented potent activity in vitro, reduced parasitemia, and show cure in *T. cruzi*-infected mice in acute phase.[80] Nonpeptidic tetrafluorophenoxymethyl ketone reduced parasitemia of infected mice with no apparent toxicity.[81] Thiazole compounds showed in vitro activity and promoted reduction of parasitemia and mortality possibly by acting as cruzipain inhibitors (Figure 3).[82–85]

Amidine-containing compounds represent a versatile class of drugs, with potential antiprotozoal treatment, acting through multiple mechanisms against *T. cruzi*.[86] Many compounds presented in vitro and in vivo activity against *T. cruzi* (see Table 1). For example, treatment with DB766 reduced parasite load in blood and heart, and prevented mortality of infected

Table 1 Classes of Promising New Chemical Entities for Experimental Treatment of Chagas Disease

Mechanism/ Target	Chemical Class/Compound	Outcome	Ref.
DNA and kinetoplast-DNA targeting and topoisomerase inhibitors and unknow targets	Amide-containing thiazoles	Safe compounds that had in vitro and in vivo anti-*T. cruzi* activities.	[93]
	Arylimidamide derivatives	18SAB075 exhibited best selective index; and slightly reduced parasitemia and mortality in mice infection.	[92]
	Amidines and analogues Bis-arylimidamides	Bis-arylimidamides displayed in vitro activity. 28SMB032 reduced parasitemia, but without mortality protection. DB1957, DB1959 and DB1890B reduced parasitemia levels and/or mortality.	[91,94]
	DB1831 and its mesylate salt derivative (DB1965)	Compounds presented in vitro and in vivo activity. DB1965 produced no parasitological cure, but reduced parasite burden and protected against mortality	[90]
	DB766 and analogues	DB766 displayed potent in vitro activity. In murine infection, the drug reduced parasite load in blood and heart; reduced hepatic and cardiac lesions; prevented electrocardiographic alterations and prevented mortality.	[87–89]
Antimicrobial peptides	AS-48 Bacteriocin	AS-48 was active in vitro and in acute stage of mice infection it reduced parasitemia and in chronic stage the parasitic load.	[98,99]
Copper complexes	Ternary copper (II) complexes	Compounds with higher selectivity index were able to reduce parasitemia.	[100]
Cysteine protease inhibitors and Cruzipain inhibitors	Fluoromethyl ketone–derivatized pseudopeptides	K777 is an irreversible peptidyl inhibitor of cruzipain and it was able to reduce parasitemia and mortality, and induce cure in murine model. In infected dogs, it reduced cardiac damage, but without cure. Nonpeptidic tetrafluorophenoxymethyl ketone reduce parasitemia in acutely infected mice with no apparent toxicity.	[77–79] [81]
	Reversible cruzipain inhibitors containing a nitrile "warhead"	Cz007 and Cz008 presented potent in vitro activity and displayed parasitemia reduction and cure in mice acutely infected.	[80]
	Thiazole compounds	Thiazole compounds showed in vitro activity and promoted reduction of parasitemia and mortality in infected mice. These compounds inhibited cruzipain.	[82–85]
Ergosterol biosynthesis inhibitors	4-aminopyridyl-based lead compounds	Compounds showed anti-*T. cruzi* effects in acute and chronically infected mice, but without cure.	[67]
	Fenarimol analogues	Fenarimol analogues were active in vitro and in vivo against *T. cruzi* infected mice	[57,63,64]
	Non-azole LP-10	LP-10 was able to reduce parasitemia and to induce cure in infected mice.	[65]
	Ravuconazole and E1224 (fosravuconazole)	Ravuconazole presented effective control of infection in mice and dogs. E1224 or fosravuconazole, a prodrug of ravuconazole were well tolerated and effective in suppressing parasitemia. Cure was detected in mice infected by Y strain, but no cure was observed in Colombian infection in mice.	[116,117] [118]
	VFV	VFV was more potent than VNI in reducing parasitemia and presented effects on mortality protection.	[60,119]
	VNI	VNI presented promising anti-*T. cruzi* activity in vitro and in vivo. The drug promoted reduction of parasitemia in mice infected by different strains. Cure was detected in animals acute and chronically infected by Tulahuen strain.	[59–62]
	VT-1161	VT-1161 is a potent *T. cruzi* CYP51 inhibitor and revealed in vitro and in vivo activity, as well as suppression of parasitemia peak in mice infection by Y strain.	[66]

(*Continued*)

Table 1 (Continued).

Mechanism/ Target	Chemical Class/Compound	Outcome	Ref.
Nitro-heterocyclic compounds and ROS inducers	5-Nitroindazole derivatives	Megazol, a nitroimidazole-thiadiazole derivative, demonstrated a curative action in infected mice. The mechanism of action is related to the impairment of protein synthesis. Megazol derivatives showed in vitro activity. In infected mice, S2 led to parasitemia reduction, and S3 to mortality reduction.	[50,53] [54]
	Nitrotriazoles	Nitrotriazole-based compounds exhibited in vitro and in vivo anti-*T.cruzi* activity. Some compounds are potential inhibitors of *T. cruzi* CYP51 and substrates for the type I nitroreductase. Triazole-based analogues of BNZ showed in vitro activity. In infected mice, the analogues exhibited lower potency and higher toxic than BNZ.	[68–76] [58]
	Fexinidazole	Fexinidazole was more effective than BNZ, with cure rate greater than 70% in animals infected by different strains and treated during the acute or chronic phases.	[46–48]
	Fexinidazole metabolites	Fexinidazole sulfone and fexinidazole sulfoxide induced higher cure rates than fexinidazole.	[47,49]
	Imidazole Derivatives	Phthalazine derivatives containing imidazole rings showed in vitro and in vivo anti-*T. cruzi* activity.	[55]
	MK-436 (2,5-nitroimidazole)	MK-436 was highly efficacious against acute and chronic infection by different strains, and induced cure in acute stage.	[51,52]
	Ruthenium complexes	The ruthenium compounds exhibited potent in vitro and in vivo trypanocidal activities by acting on active cysteine (cys166) site or releasing nitric oxide. NO donor compound led to parasitological cure.	[101–104]
Purine Salvage Pathway	Nucleoside derivatives	Nucleoside analogues presented in vitro activity and the most potent showed suppression of parasitemia and mortality in acutely infected mice.	[106,107]
	Xanthine analogs	Xanthine analogs showed in vitro activity and were able to reduce parasitemia in CL-infected mice.	[105]
Oxamates	Benzyl ester of N-propyl oxamate; ethyl esters of N-propyl and N-isopropyl oxamates; and N-isopropyl oxamate	Compounds exhibited trypanocidal activity in vitro and in vivo. They acted as possible inhibitors of T. *cruzi* α-hydroxy acid dehydrogenase (HADH)-isozyme II	[108–110]
Oxaboroles multiple cellular targets	Oxaboroles	AN4169 showed broad efficacy in vitro against strains of *T. cruzi* and was a fast-acting trypanocidal. The drug induced cure in mouse model.	[56,57]
Proteasome inhibitor	Triazolopyrimidine	GNF6702 cure mice in chronic phase of infection with *T. cruzi*.	[114,115]
Quinolines	Quinolines	Tested quinolines were more active in vitro than BNZ. DB2186 reached 70% reduction of the parasitemia load in mice infected by Y strain.	[111]
Sphingosine kinase inhibitor	N,N-dimethylsphingosine (DMS)	DMS blocked sphingosine-1-phosphate production, a cell mediator during inflammatory responses, and exhibited anti-parasitic activity in vitro and in vivo and immunomodulatory actions in chronically infected mice.	[112]
Squaramides	Squaramides	New long-chain squaramides displayed in vitro and in vivo activity with low toxicity	[55,113]
Terpene and terpenoid derivatives	Terpenoid derivates	Synthesized terpenoid derivates displayed in vitro anti-*T. cruzi* activity and nontoxic effects upon host cells. In infected mice, these derivatives reduced parasitic load and anti-*T. cruzi* antibodies during chronic stage.	[55]

(*Continued*)

Table 1 (Continued).

Mechanism/ Target	Chemical Class/Compound	Outcome	Ref.
Polyamine surface transporters and metabolism Trypanothione metabolism inhibitors	Thiazolidines	LPSF SF29 displayed trypomastigote lysis and amastigote death, probably by interfering with polyamine biosynthesis and consequently trypanothione biosynthesis, leading to increased sensitivity to oxidative metabolism.	[96]
	Tetradentated polyamine complexes	Tetraamines presented anti-*T. cruzi* activity in vitro and in vivo with low toxicity.	[97]

Abbreviations: BNZ, benznidazole; NO, nitric oxide; ROS, reactive oxygen species.

mice; the drug reduced hepatic and cardiac lesions and prevented electrocardiographic abnormalities induced by the parasite infection.[87–89] In addition, DB766 was found in DNA-enriched compartments and induced considerable damage to the mitochondria.[88] Amide-containing thiazole derivatives, arylimidamide derivatives, and other amidines have been found to be active in vitro and in vivo.[90–94]

Trypanosomatids, unlike humans, have a unique redox metabolism based on thiol and relying on trypanothione reductase. This trypanothione enzyme acts in defense against oxidative damage, redox homeostasis and replication, significantly supporting infectivity and survival of the parasite in the host system.[95] The inhibition of trypanothione reductase metabolism increases the parasite susceptibility to drugs and/or oxidative

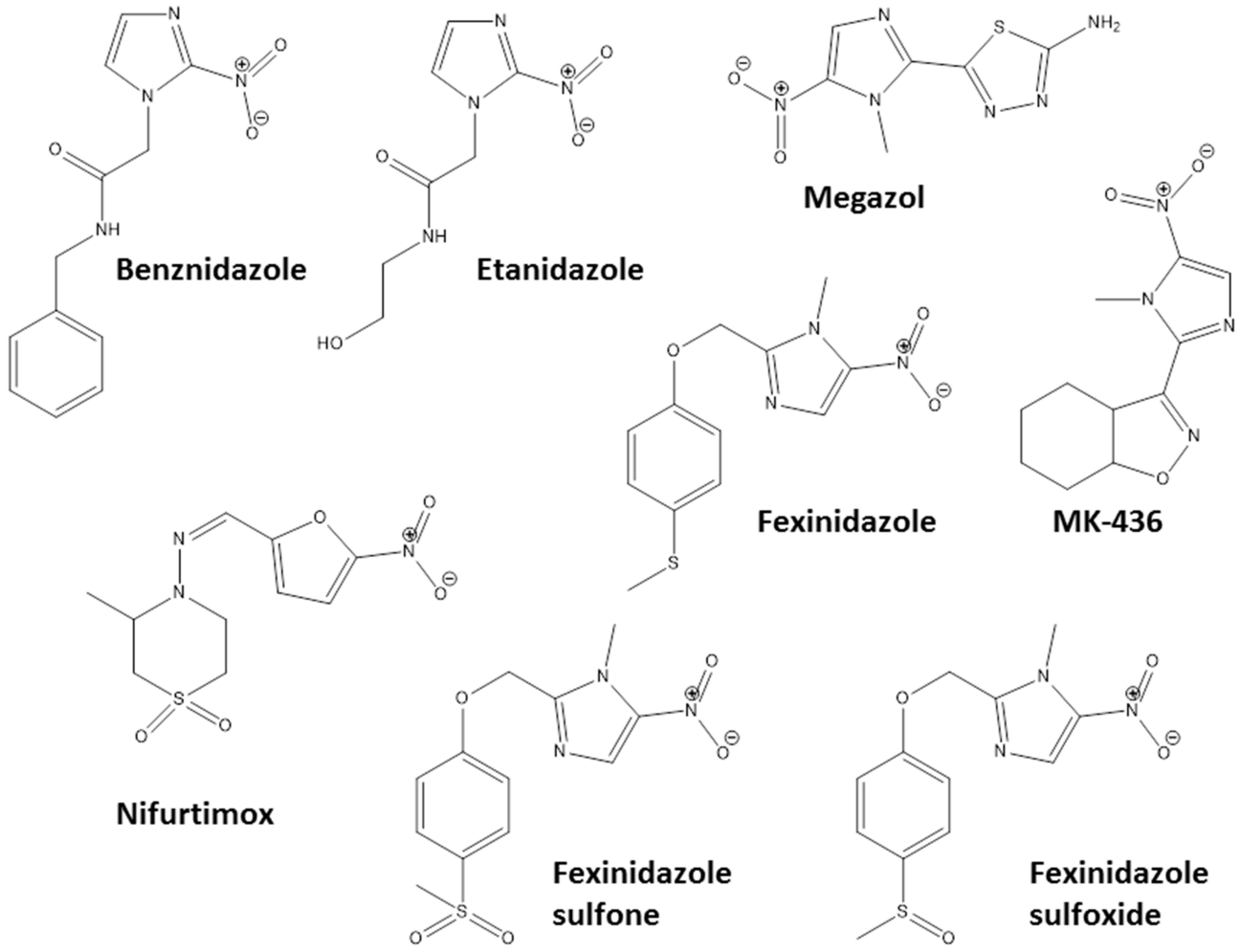

Figure 1 Chemical structures of the most promising nitro-heterocyclic compounds that induce cure with parasite elimination in mice.

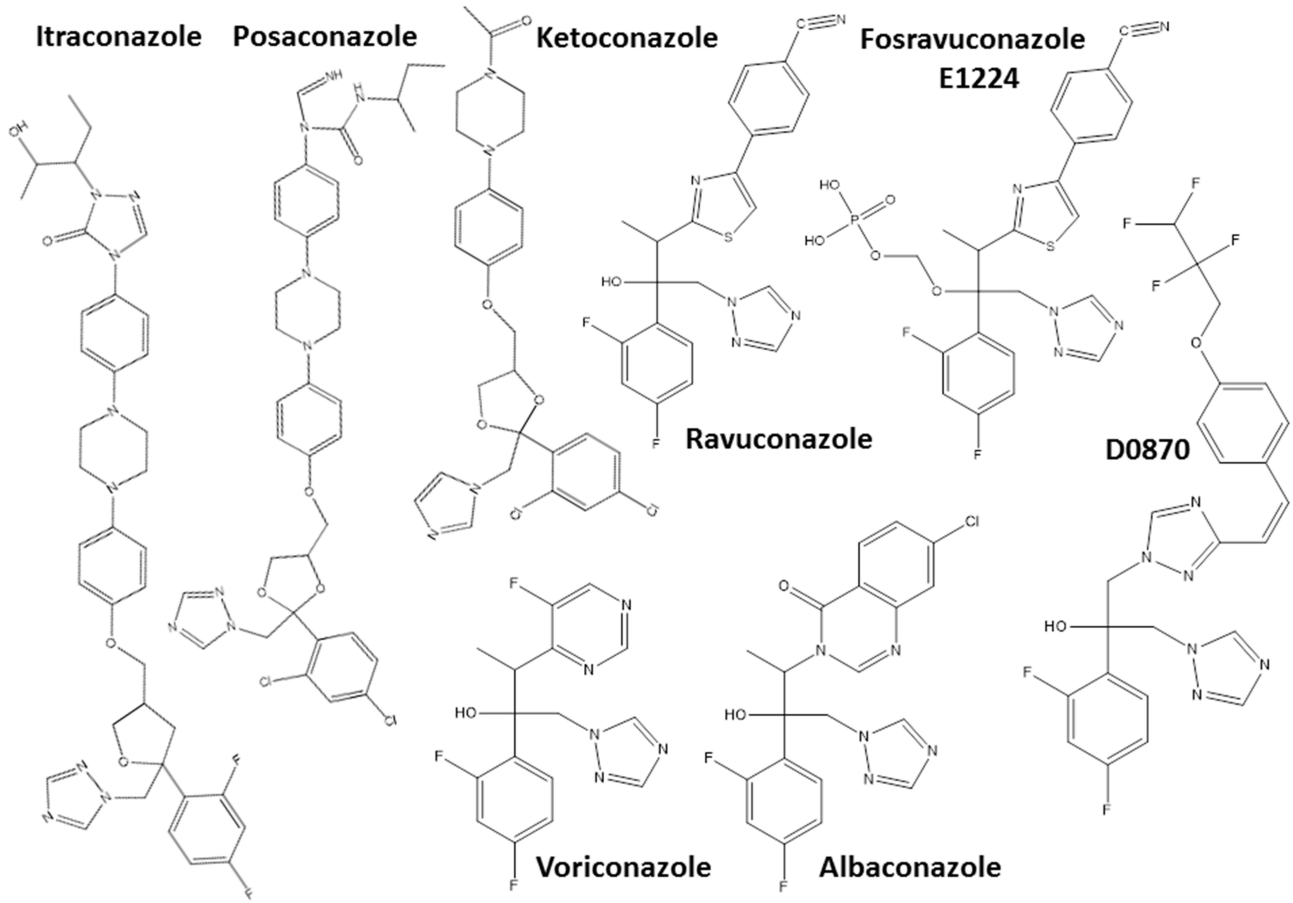

Figure 2 Chemical structures of the most promising azole derivatives that induced cure with parasite elimination in mice.

stress induced by host defense.[95] Thiazolidines LPSF and SF29 promoted trypomastigote lysis and amastigote death, probably by interfering with polyamine biosynthesis and consequently trypanothione biosynthesis, leading to increased sensitivity of the parasite to the oxidative metabolism.[96] In addition, tetraamines were able to inhibit iron superoxide dismutase and trypanothione reductase of *T. cruzi* and presented activity in vitro and in vivo with low toxicity.[97]

Other classes as antimicrobial peptides;[98,99] copper[100] or ruthenium[101–104] complexes, compounds that impact on purine salvage pathway,[105–107] oxaboroles,[56,57] oxamates,[108–110] quinolines,[111] sphingosine kinase inhibitor,[112] squaramides,[55,113] terpene and terpenoid derivatives[55] and proteasome inhibitors,[114,115] revealed interesting results, impacting the evolution of *T. cruzi* infection (Table 1), but the mechanisms are not well known. Figure 4 shows schematic representation of the main drug targets identified in *T. cruzi*.

Natural Products

In the search for new alternatives for the therapy of Chagas disease, natural diversity can provide a wide range of bioactive agents or *lead* compounds. Natural products are the source for structural chemical backbone that could be used to inspire synthesis of new active molecules. Natural products isolated from different botanical sources exhibited activity against *T. cruzi* (see Table 2).[120–132] Several approaches have been developed focusing on identification and isolation of plant-based products with anti-*T. cruzi* activity[133,134] (Table 2). In this sense, extracts from different plants as *Salvia, Valeriana, Hypericum, Silybum, Arnica*, and *Curcuma* showed activity against *T. cruzi*.[135] Sesquiterpene lactones have been demonstrating outstanding anti-*T. cruzi* activity.[136–145] They are isolated from a variety of species, mainly from the Asteraceae family. Lychnopholide[144] and goyazenzolide[145] are examples of potent sesquiterpene lactones, which showed high efficacy in *T. cruzi*-infected mice. Unfortunately, preclinical research

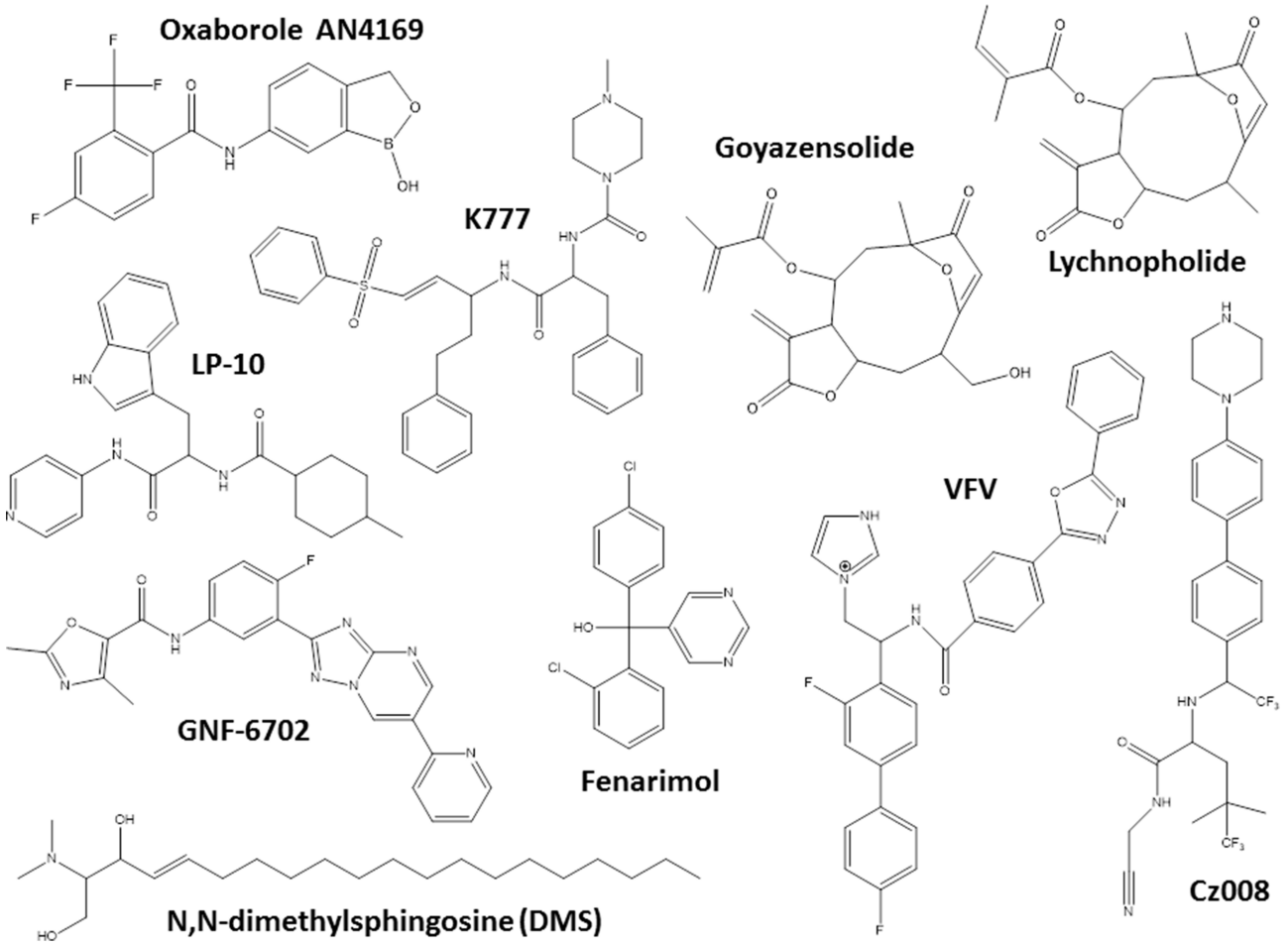

Figure 3 Chemical structures of the miscellaneous classes of most promising compounds with different mechanisms of action that induced cure with parasite elimination in mice.

involving natural products faces obstacles to translate their findings to clinical phases due to difficulties in isolating the active compound and dimensioning the batches, as well as difficulties in the standardization of the plant extracts following the different seasons.[134]

Drug Repurposing

Drug repurposing is a source of alternative chemotherapies for several diseases, especially those neglected, since the time and cost of the process required for clinical approval can be shortened. Anti-*T. cruzi* activity of compounds from different pharmacological classes has been tested in experimental infection models (Table 3). Particularly, the antifungals albaconazole, itraconazole, ketoconazole, posaconazole, ravuconazole and its prodrug fosravuconazole, which are CYP51 inhibitors, have demonstrated anti-*T. cruzi* activity by inhibiting ergosterol biosynthesis of the parasite.[116–118,146–157] The effectiveness of this class of compounds has been demonstrated in several experimental models, and high cure rates has been detected in mice or dog infections.[116–118,146,147,151,153] In addition, the anticancer drug tipifarnib can also inhibit CYP51 and showed potent suppressive activity on parasitemia in infected mice.[158–160]

The antihypertensive benidipine and antibiotic clofazimine reduced parasite load and inflammatory process in cardiac and skeletal muscle of chronically infected mice.[161,162] The likely mechanism of action of both compounds would be the inhibition of cruzipain[161,162] or disruption of calcium homeostasis for benidipine.[163,164] Differently, the anti-cancer imatinib was moderately active against different strains and forms of *T. cruzi*.[165] Antidepressants sertraline and fluoxetine showed in vitro anti-*T. cruzi* activity, while fluoxetine treatment displayed insufficient parasitemia reduction in infected mice.[166,167] Antihyperuricemic allopurinol was evaluated for Chagas disease treatment and showed controversial results. This drug is a hypoxanthine analogue and acts as an alternative substrate of

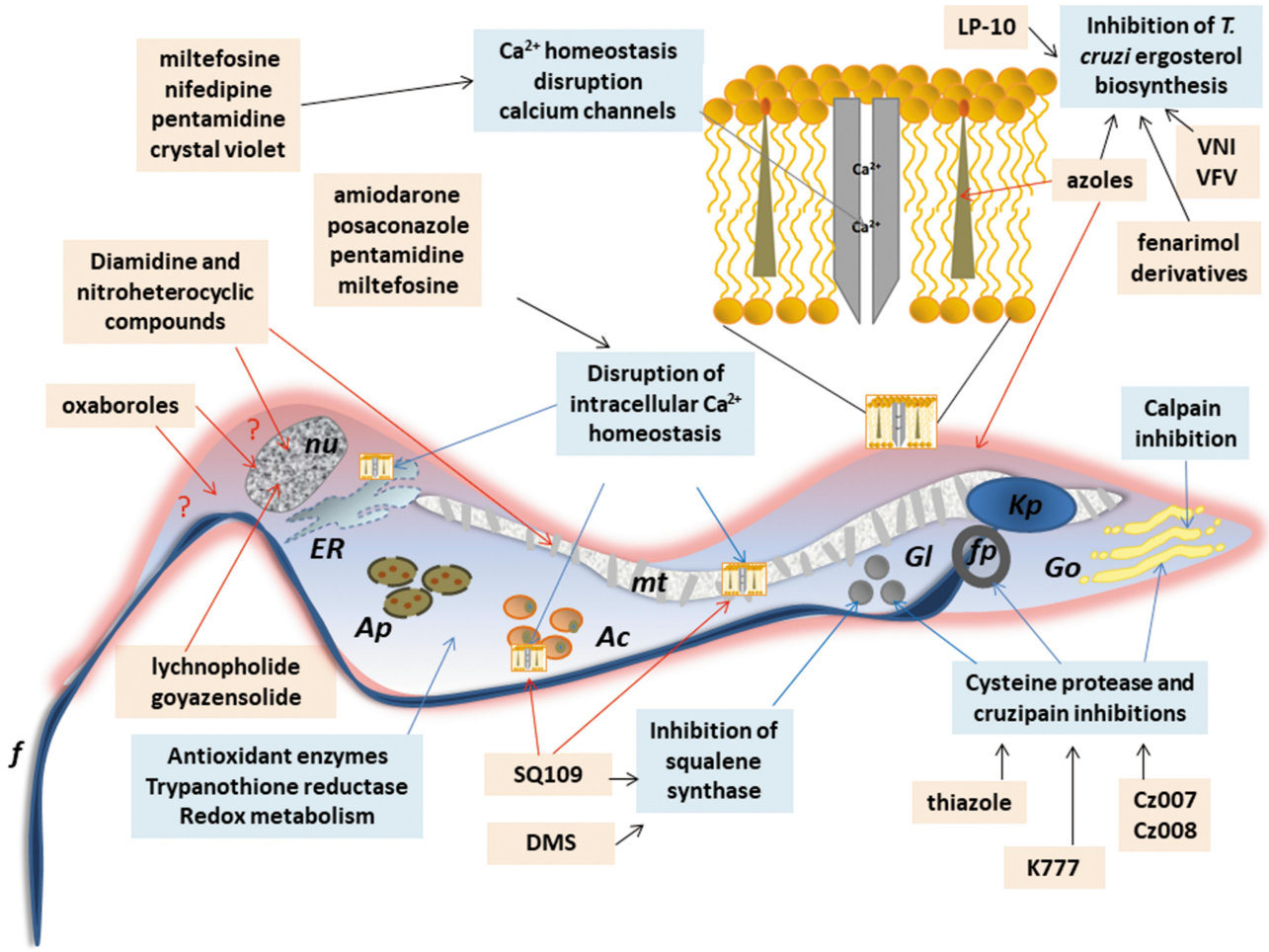

Figure 4 Schematic representation of a trypomastigote of *Trypanosome cruzi* and the main cellular targets of the investigational compounds in the pre-clinical phase of development.

Notes: The selected targets are in blue boxes and compounds are in rose boxes. Organelles: Kp: kinetoplast, f: flagellum, fp: flagellar pocket, mt: mitochondrion, nu: nucleus, ER: endoplasmic reticulum, Go: golgi apparatus, Ac: acidocalcisomes, Gl: glycosomes, Ap: autophagosomes. The drawing is based on data from Benaim et al and Vannier-Santos et al.[163,228]

the *T. cruzi* hypoxanthine-guanine phosphoribosyl transferase.[168] The enzyme can incorporate allopurinol into parasite RNA, creating a nonfunctional nucleotide, blocking *de novo* synthesis of purines, affecting protein synthesis, and inducing parasite death.[168] Studies have been demonstrated the beneficial results of allopurinol treatment in reducing parasitemia and/or modifying the evolution of acute and chronic murine infection.[169–171] Differently, Mazzeti et al demonstrated that this drug did not affect the evolution of Y acute infection in mice.[172]

Other drug tested using repositioning strategy are the antiarrhythmic amiodarone,[154] auranofin[173] and clomipramine,[174,175] which were also active against *T. cruzi* in preclinical experimental infections (see Table 3). Amiodarone and miltefosine have mechanisms of action related to disruption of calcium homeostasis in *T. cruzi*.[163] Taken together, the preclinical results of drug repositioning strategy indicate that most of them were active but failed to induce sterile cure in mice.

Drug Combination Therapy

Drug associations concomitantly or sequentially can improve the effectiveness of Chagas disease treatment as well as interfere in the duration of treatment and/or drug dose. Combination therapy using distinct pharmacological classes has been evaluated in experimental *T. cruzi* infection (Table 4). The use of suboptimal doses or treatment length of BNZ in association with CYP51 inhibitors may maintain or increase the effectiveness of treatment by the synergistic or additive effect of compounds with different mechanisms of action or cellular targets.[56,118,119,150,155–157,176–180] Similarly, allopurinol combined with low dose of BNZ had a positive interaction

Table 2 Promising Natural Products in Experimental Treatment of *Trypanosoma cruzi* Infections

Plant	Natural Product	Outcome	References
Aristeguietia glutinosa	Secondary metabolites isolated from the hydro-ethanolic extract of the aerial parts	The substances displayed low in vitro toxicity and anti- *T. cruzi* effect in infected mice by inhibiting parasite mitochondrial dehydrogenases and biosynthesis of membrane sterols.	[128]
Arrabidaea brachypoda	Dimeric flavonoids from roots	Flavonoids were active against *T. cruzi* in vitro and in infected mice.	[129]
Carica papaya	Crude seed extracts	Extracts were able to reduce parasitemia, but not the cardiac amastigotes nests of infected mice.	[127]
Delphinium staphisagria	Flavonoids from aerial parts	Flavonoids were active against T. cruzi in vitro and in infected mice.	[124]
Artemisia annua	artemisinin (sesquiterpene lactone)	Artemisinin presented in vitro activity and inhibited calcium-dependent ATPase activity in *T. cruzi* membranes.	[136]
Lychnophora passerina	goyazensolide (Sesquiterpene lactone from the aerial parts)	Goyazensolide was active in vitro and led to parasitemia reduction and negativation in parasitological tests in mice infection by Y and CL strains.	[145]
Lychnophora trichocarpha	Lychnopholide (LYC) and LYC-loaded nanocapsules (sesquiterpene lactone)	Nanocapsules containing lychnopholide reduced parasitemia and mortality in murine infection and induced cure in mice infected with *T. cruzi* strains resistant and sensitive to BNZ in acute and chronic phases of infection. LYC-nanocapsules formulation prevented cardiotoxicity in long term treatment.	[138–140], [144]
Mikania species	Deoxymikanolide and other sesquiterpene lactones	Deoxymikanolide showed the highest selectivity index and induced parasitemia and mortality reduction in infected mice.	[141]
Tithonia diversifolia	Tagitinin C a sesquiterpene lactone isolated from leaves	Tagitinin C presented in vitro and in vivo activity alone and combined with BNZ.	[143]
Physalis angulata	Physalins (secosteroids) and concentrated ethanolic extract	Physalins reduced the invasion process in vitro, and intracellular parasite load, alone or in combination with BNZ. Concentrated ethanolic extract showed in vitro and in vivo activity alone and associated with BNZ.	[126,130]
Piper jericoense	Furofuran lignan	Furofuran lignan was active in vitro and reduced parasitemia levels in infected mice. The compound affected the parasite structure without altering the energetic metabolism.	[132]
Salvia gilliesii	Isolated diterpene	Compound reduced parasite load and increased survival of infected mice.	[131]
Senna villosa	(8-hydroxymethylen)-trieicosanyl acetate	Isolated compound showed in vitro and in vivo activity against *T. cruzi*.	[122,125]
Zanthoxylum chiloperone	Canthin-6-one alkaloids and leaves extract	Canthin-6-one alkaloids exhibited trypanocidal activity in vitro and in mouse model of acute or chronic infection. Leaves extract reduced parasitaemia in vivo.	[121,123]
Zanthoxylum naranjillo	Lignans isolated from the leaves	Lignans displayed in vitro and in vivo activities.	[120]

Abbreviation: BNZ, benznidazole.

Table 3 Drugs Repositioned in Experimental Treatment of Chagas Disease

Compound	Original Use	Outcome	References
Albaconazole	Antifungal	Albaconazole suppressed the parasite proliferation, prevented the death of infected dogs, and induced cure in animals infected by Y strain, but not in those infected by Berenice-78.	[153]
Allopurinol	Antihyperuricemic	Allopurinol was effective in reducing parasitemia and/or modifying the evolution of acute and chronic murine infection. Allopurinol derivatives displayed similar in vitro activity than pattern drug and good bioavailability properties for oral absorption.	[169,170] [171]
Amiodarone	Antiarrhythmic	Amiodarone showed *in vitro* activity against epimastigote and amastigote. In acute treatment of infected mice, it reduced parasitemia and increased survival.	[154]
Auranofin	Antirheumatic	Auranofin showed activity against *T. cruzi* in vitro and reduced parasitemia and mortality in infected mice.	[173]
Benidipine	Antihypertensive	Benidipine reduced parasite load and inflammatory process in cardiac and skeletal muscle of chronically infected mice by probably acting as cruzipain inhibitor.	[161,162]
Clofazimine	Antibiotic	Clofazimine reduced parasite load and inflammatory process in cardiac and skeletal muscle of chronically infected mice, likely acting as cruzipain inhibitor.	[161,162]
Clomipramine	Antidepressant	Clomipramine reduce parasitemia and electrocardiographic changes and preventing myocardial structural damage in murine infection. Clomipramine improved survival by reducing the parasitic tissue load and preventing progression of cardiac damage in chronically infected mice	[174] [175]
Imatinib	Anti-cancer	Imatinib was moderately active against different strains and forms of *T. cruzi*	[165]
Itraconazole	Antifungal	Itraconazole led to parasitemia reduction and mortality protection of infected mice. Itraconazole treatment promoted significant parasitemia reduction in infected dog.	[146,152,157] [167]
Ketoconazole	Antifungal	Ketoconazole showed anti-*T. cruzi* in vitro activity. The drug displayed in vivo activity in infected mice.	[148,149] [147,150]
Posaconazole	Antifungal	Posaconazole showed potent in vitro activity and in vivo trypanocidal activity, even against multiresistant *T. cruzi* strains.	[151], [154–156]
Ravuconazole	Antifungal	Ravuconazole presented efficacy to control the infection in mice and dogs.	[116,117]
Fosravuconazole (E1224) prodrug of ravuconazole	Antifungal	E1224 was well tolerated and effective in suppressing parasitemia with cure in Y strain-infected mice.	[118]
Tipifarnib	Anticancer	Tipifarnib showed potent in vitro activity due to CYP51 *T. cruzi* inhibition. Tipifarnib analog had potent suppressive activity on parasitemia in infected mice.	[159] [158,160]

in *T. cruzi* infection outcome.[172,181] Sequential treatment with allopurinol and BNZ was able to reduce parasitemia and attenuate tissue damage in infected mice.[182] Generally, drug association allows to decrease the duration of the treatment limiting adverse effects related to time-dependent drug accumulation.[155] Table 4 shows other promising drug associations for the treatment of experimental *T. cruzi* infection.[87,90,154,164,183–194]

Benznidazole Re-Dosing Regimens

Current results of clinical trials have highlighted the need to reassess BNZ treatment regimens to achieve efficacy

Table 4 Promising Drug Combinations in Experimental Treatment of Chagas Disease

Combination	Outcome	References
BNZ/VFV	Combination presented promising results on parasitic blood load and cure levels in infected mice.	[119]
BNZ/itraconazole	Combination improved parasitemia reduction and total survival, reduced heart qPCR positivity and cardiac damage in VL-10-infected dogs. Itraconazole improved plasma concentration of BNZ. Combination led to an improvement in parasitemia reduction, and cardiac damage in infected mice.	[176,178] [177] [157]
BNZ/ketoconazole	Combination induced a synergic effect in mice infected with CL and Y *T. cruzi* strains, but no differences were observed in Colombian strain-infected animals.	[150]
BNZ/fosravuconazole	In vitro interaction was positive and in infected mice. Early treatment induced 100% of cure and beneficial effect on well-established infection.	[118]
BNZ/posaconazole	Shorter duration of the treatment with combination induced cure in acutely or chronically Tulahen-infected mice but did not improve the curative effect of posaconazole in Y strain infection. Sequential and combined treatments were beneficial in murine infection, decreasing the time or dose of the BNZ treatment. Combination showed same efficacy of each drug alone in Colombian strain-infected mice and improved efficacy in Brazil strain-infected mice. Treatment of acute murine infection with the combination was more effective in reducing parasitemia and myocardial injury, compared to monotherapies.	[155] [156] [56] [179]
BNZ/voriconazole	In vitro results indicated an additive interaction. In vivo, all treatments were well tolerated, and combination elicited no additional benefits over BNZ alone.	[180]
BNZ/arylimidamides	Trypanocidal activity was improved by combination therapies (BNZ plus DB766, DB289 or DB1965) in infected mice.	[87,90]
BNZ/allopurinol	Allopurinol combined with low dose of BNZ had a positive interaction on serology and pathology of infected mice. Sequential treatment exhibited beneficial effects on acute and chronic infection in mice. Allopurinol plus NFX or BNZ showed in vitro synergic effect. The combinations increased the cure rate compared to BNZ alone in murine infection.	[181] [182] [172]
BNZ/ascorbic acid	Ascorbic acid combined with a low dose of BNZ improved its trypanocidal activity and attenuated the toxic effects of BNZ. Combination also reduced cardiac inflammation and hepatic damage.	[188]
BNZ/acetyl salicylic acid	Aspirin combined with lower dosages of BNZ showed better therapeutic effect in infected mice.	[193]
BNZ/clomipramine	Clomipramine improved BNZ activity in vitro and in vivo with no cell toxicity and fewer side effects	[184,189]
BNZ/fenofibrate	Fenofibrate plus a low dose of BNZ attenuated cardiac dysfunction and promoted parasite clearance in mice sequentially infected with two strains of different genetic backgrounds.	[185]
BNZ/levamisole	Monotherapies of levamisole did not decrease parasitemia nor mortality rates. In combinations with a low dose of BNZ, it led to a slight improvement in the effectiveness of monotherapy	[192]
BNZ/NFX	Combining shorter treatments could cure mice, but the association led to the behavioral alterations of treated mice.	[155]
BNZ/simvastatin	Simvastatin improved BNZ activity in murine model of Chagas heart disease	[186]
BNZ/clofazimine or BNZ/benidipine	Reduced dose of 30mg/kg of BNZ associated with both drugs had the same efficacy as single 75mg/kg of BNZ in chronic model of Chagas disease in mice with no cure.	[164]
Itraconazole/ amiodarone	The combination was benefic in infected dogs compared to non-treated control. The combinations were more effective in vitro *T. cruzi*-infection than monotherapy or BNZ; without affecting host cell metabolism and better preserving the integrity of infected cells.	[190] [191]

(*Continued*)

Table 4 (Continued).

Combination	Outcome	References
Posaconazole/ amiodarone	Amiodarone has direct in vitro and in vivo activity against *T. cruzi* and showed synergic effect with posaconazole. In vitro association improved IC_{50} and lead alterations in the parasite.	[154] [183]
Ravuconazole/ amlodipine	In vitro assays showed an additive effect for the combination. Amlodipine improved anti-*T. cruzi* activity of ravuconazole in infected mice.	[194]
NFX/dipyridamole	Dipyridamole potentiated the in vitro effect of NFX and improved efficacy of low dose of NFX in murine model.	[187]

Abbreviations: BNZ, benznidazole; NFX, nifurtimox.

and reduce the incidence of side effects in Chagas disease patients.[195] In addition, considering that the treatment protocol with BNZ for experimental murine infection was defined empirically 30 years ago,[196] new experimental studies are evaluating other therapeutic regimens. In this sense, intermittent treatment with BNZ was as effective as a continuous scheme in chronically infected mice.[197] Similarly, low-doses of BNZ in experimental chronic stage of mice infection promoted absence of parasitism in blood, heart and colon.[198] Bustamante et al proposed new once-a-week regimens of BNZ administration at 2.5 to 5 times higher than standard daily dose. This intermittent regimen rapidly eliminated actively replicating parasites and ultimately eradicated the residual, transiently dormant parasite population in mice.[199]

Molina and co-authors reviewed the outcomes of BNZ treatment regimens in murine infection and found that the dose, either daily or the cumulative dose, had the greatest impact on effectiveness.[200] Clearly, the data showed the higher the dose or exposure to the BNZ, the greater the likelihood of cure.[200] Mazzeti et al showed time and concentration-dependent trypanocidal effect of BNZ in acute murine model. Furthermore, it was demonstrated that extended treatment for 40 days led to increased levels of cure in mice infected with Y or Colombian strain.[201] Efficacy associated with pharmacokinetic (PK/PD) studies can give support in determining the most appropriate therapeutic regimens.

New Drug Delivery Systems

The standard of care and the majority of chemotherapeutic candidates for the treatment of *Trypanosoma cruzi* infections are poorly water-soluble molecules and their efficacy may be limited by their biopharmaceutical profile (Figures 1-3). Thus, the design of new drug delivery systems can improve drug stability, increase dissolution and absorption rate in the gut, reduce drug efflux and increase intestinal permeability, improving drug bioavailability and biodistribution profile.[202] Nanostructured delivery systems can even circumvent multidrug resistance in some cases. In this sense, few attempts were reported concerning the use of classical formulations containing anti-*T. cruzi* drugs in preclinical studies.[203,204] Among them, a solid dispersion and a solution containing co-solvents were used in order to improve BNZ dissolution rate as shown in Table 5.

Nanotechnology-Based Formulations

Many formulations based on the nanotechnology were tested in vivo in mice model of experimental infection with *T. cruzi* (Table 5). Different types of liposomes, nanoemulsions/microemulsions and self-emulsifying delivery systems were developed. Yardley and Croft tested commercially available Amphotericin B lipid nanoformulations by intravenous and oral routes in mice.[205] Amphotericin B was active in vitro and reduced parasite burden in *T. cruzi*-infected mice (Y/Tulahuen strains). Among them, AmBisome® was the most active formulation of amphotericin B, although no formulations cleared parasites from blood as effectively as BNZ. Cencig et al evaluated AmBisome® in Tulahuen infected mice in acute and chronic phases and observed that intraperitoneal treatment with Amphotericin B failed to cure mice from infection and to eliminate parasites.[155,206] Similar results were obtained by Clemons et al.[207] Taken together these studies demonstrated that amphotericin B was active against *T. cruzi* infection, but it was unable to produce cure and to eliminate parasites from tissues in pre-clinical models.

Table 5 Drug Delivery and Nanomedicine-Based Strategies Evaluated Against *Trypanosoma cruzi* in Experimental Pre-Clinical Studies

Formulation	Drug/Active Molecule	Route	Outcome	Ref.
Classical formulations				
Solid dispersion in poloxamer 407	BNZ	oral	15–60 mg/kg/day solid dispersion compared with classical 50 mg mg/kg/day. Same efficacy in infected mice with lower side effects in acute and chronic phases, and reduced hepatotoxicity in mice.	[203]
Solutions with co-solvents: PEG_{400}/water and CMC/water	BNZ	oral	20–60 mg/kg/day solutions compared with classical BNZ doses produced the same efficacy in infected mice (Tulahuen strain)	[204]
Nanostructured lipid-based formulations				
Nanostructured Lipid formulations	Amphotericin B	i.v. oral	Fungizone® deoxycholate, AmBisome® liposome, Amphocil® colloidal dispersion, Abelcet® lipid complex. AmBisome® was the most effective formulation with the lowest host toxicity, but less than BNZ and NFX. Different protocols: BALB/c mice infected *T. cruzi* Y/Tulahuen strains BNZ or NFX (controls) cleared blood trypomastigote more rapidly than amphotericin B (1 week × 3 weeks). AmBisome was the most effective formulation with the lowest host toxicity.	[205]
Liposome (Ambisome®)	Amphotericin B	i.p.	Efficacy in acute and chronic phases of mice infected with *T. cruzi* Tulahuen strain. The formulation showed efficacy in acute and chronic phases of mice infected with *T. cruzi* Tulahuen strain.	[206]
Liposome (Ambisome®)	Amphotericin B	i.v.	AmBisome® prolonged survival, without cure. Amastigote nests found in tissues of all mice treated, heart and brain in histopathological analysis in acute and chronic mice models (Y and CL strains). AmBisome® prolonged survival without cure of infection in repeated dose regimen.	[207]
pH-sensitive liposomes (DOPE:CHEMS)	Etanidazole	i.v.	Reduced parasitemia in infected mice with liposome formulation at lower doses versus no effect of free drug. Liposome showed in vitro activity against amastigote and reduced parasitemia of infected mice.	[209]
Nanoarchaeosomes	Imiquimod		Infected mice (RA strain) treated with the formulation in acute phase had improved survival and showed parasitemia reduction. However, efficacy was lower than BNZ classical treatment with 100mg/kg/day.	[218]
Self-emulsifying drug delivery system (SEDDS)	BNZ	oral	Toxicity and efficacy similar to free-BNZ. Practical and personalized orally administered liquid dosage form.	[212]
Self-emulsifying drug delivery system (SEDDS)	BNZ	oral	Formulation was safe for mice. No additional drug toxicity in infected mice (Y strain) in 20 days at doses of 100 mg/kg/day was observed. Oral BNZ-SEDDS increased BNZ AUC.	[210]
Self-emulsifying drug delivery system (SEDDS)	Ravuconazole	oral	SEDDS demonstrated low in vitro and in vivo toxicity and improved ravuconazole activity in vitro and reduced toxicity in vivo. No formulation and drug toxicity was observed in mice.	[211]
Polymeric-based nanomedicines				

(*Continued*)

Table 5 (Continued).

Formulation	Drug/Active Molecule	Route	Outcome	Ref.
Poly-aggregated Amphotericin B in albumin microspheres	Amphotericin B	oral	*T. cruzi*-infected mice (Y strain) treated for 10 days (10–15 mg/kg/day) reduced 75% parasitemia and prolonged survival. Unable to cure mice.	[217]
Nanospheres of polyethylene glycol-polylactide	Bis-triazole D0870	i.v.	*T. cruzi*-infected mice treated for 30 days (3 mg/kg/day) had cure rate of 90% for CL strain and 60% for Y strain in the acute phase of infection	[216]
Nanocapsules of polyethylene glycol-polylactide	Lychnopholide (LYC) (sesquiterpene lactone)	i.v. oral	Formulation induced high cure levels in acute or chronic mice infection, including drug resistant strains and displayed minimal cardiotoxicity. Potent in vivo anti-*T. cruzi* activity. LYC-NC reduced cardiotoxicity compared with free-LYC. In vivo LYC-NC effects include: cure rates of 100% by PCR analysis in tissue; it induced cure in mice infected with Colombian strain and in mice in chronic phase of infection; nanocapsules increased AUC of LYC in 12-fold; high cure rates and reduced inflammation in heart of mice infected by Y, CL, VL10 and Colombian strains.	[138–140], [144]
Micelles of polyoxyethylene-polyoxypropylene block copolymer (poloxamer 188)	Benznidazole	oral	Acute treatment of infected mice decreased heart inflammation and anti-*T. cruzi* specific antibodies levels. Intermittent treatments of mice with chronic infection were as effective as daily treatment. Nicaragua strain-infected mice (C57BL/6) treated (25–75mg/kg/day) during the chronic phase: low doses of BNZ-nanoparticles treatment (25 mg/kg/day) resulted in 40% negative PCR in the immunosuppressed mice. Continuous 30 consecutive days and intermittent regime once time week during 13 weeks for 7 days showed similar reduction of parasitemia compared to classical regime.	[197,213]
Nanoparticles of poly-epsilon-caprolactone	Ursolic acid	i.v.	In vivo anti-*T. cruzi* activity led to reduction of parasitemia levels in*T. cruzi*-infected mice (C57BL/6), Y strain. Mice treated during the acute phase during 7 days with ursolic acid-nanoparticles showed similar reduction of parasitemia compared to BNZ. Reduced hepatotoxicity for nanoparticles.	[214]
Microparticles of poly(*D,L*-lactide-*co*-glycolide)	(-)-Hinokinin	s.c.	*T. cruzi* infected mice (clone CLB5) treated 20 days at 40mg/kg with Hinokinin-nanoparticles and 20 mg/kg/day with free-Hinokinin. Blood parasitemia was slightly reduced with Hinokinin-nanoparticles by fresh blood examination. No mice cure was reported.	[215]

Abbreviations: i.v, intravenous; i.p., intraperitoneal; s.c., subcutaneous; BNZ, benznidazole; NFX, nifurtimox.

BNZ lipid formulations were also developed. They failed to improve efficacy compared with classical BNZ treatment.[208] Etanidazole, which is more soluble than BNZ was encapsulated in pH-sensitive DOPE/CHEMS liposomes and showed high improvement of the activity toward amastigotes of *T. cruzi* compared with free-drug. The study demonstrated that pH-sensitive liposomes provide a pathway to reach more efficiently the parasites in the bloodstream and in the macrophages during the acute phase of infection.[209] Lipid-based nanocarriers dispersed in aqueous media showed disadvantages due to their susceptibility to oxidation and poor stability, depending on the composition.

In this sense, self-emulsifying drug delivery systems (SEDDS) are simple lipid formulations and more versatile to associate drugs for Chagas disease treatment. These

anhydrous systems form nano or microemulsion droplets spontaneously after reaching aqueous fluids in gastrointestinal tract. They are stable under storage with good ability to dissolve drugs with poor water solubility. BNZ was loaded in SEDDS and an improvement of the extent of BNZ absorption and body exposure after oral administration were observed in mice, with an increase of 25% in bioavailability.[210] No adverse effects were observed in *T. cruzi*-infected mice after 20 oral doses of BNZ at 100 mg/kg/day.[210] Spósito et al using the same strategy incorporated ravuconazole in SEDDS type IIIA lipid formulation. An increased activity (1.8-fold) of ravuconazole-SEDDS against intracellular amastigotes was observed in host cardiomyocyte cell line compared with free-ravuconazole, without additional drug toxicity.[211] Furthermore, an increase in efficacy was achieved with 20 mg/kg ravuconazole-SEDDS in *T. cruzi*-infected mice (Y strain) compared to free-ravuconazole in 30 day-treatment (unpublished personal results). Thus, SEDDS are promising formulations for use in neglected diseases, including Chagas disease, because of their low cost, high stability, and ease of preparation.[212]

Polymer-Based Nanomedicines

An interesting work associated BNZ with non-ionic surfactant (poloxamer 188), which produced micelles with mean particle size of 63 nm.[197,213] Treated mice had reduced inflammatory cardiomyopathy and fibrosis in a dose-dependent manner, with doses as lower as 25 mg/kg/day, which resulted in 40% negative Polymerase Chain Reaction (PCR) tests in immunosuppressed mice. Thus, BNZ polymeric nanoformulations have potential to be used in experimental therapy. By contrast, polylactides, polyglycolides, polycaprolactone, and their copolymers were used to prepare polymeric nanospheres and nanocapsules (Table 5). Poly-Ɛ-caprolactone nanoparticles containing ursolic acid with sizes lower than 200 nm were prepared by nanoprecipitation method and exhibited no in vitro toxicity toward LLC-MK2 fibroblasts. In *T. cruzi*-infected mice (Y strain) treated during 7 days with ursolic acid-nanoparticles, the parasitemia levels were reduced similarly to BNZ classical treatment.[214] The same group prepared poly(*D,L*-lactide-co-glycolide) microparticles encapsulating (-)-hinokinin (HNK), which induces only a slight reduction of parasitemia peak compared with HNK-free molecule.[215] The nanospheres of polyethylene glycol-*block*-polylactide were prepared with bis-triazole D0870 and tested in mice infected with CL and Y strains of *T. cruzi*. Mice were treated daily by the intravenous route with 3 mg/kg/day of D0870-loaded nanospheres and a significant cure rate of 60% for Y and 90% CL strains in acute mice model was observed.[216]

Lychnopholide (LYC), a sesquiterpene lactone encapsulated in polyethylene glycol-polylactide and in poly-ε-caprolactone nanocapsules (LYC-NC) showed one of the most promising nanotechnological approaches investigated up to date. In acute and chronic phases of *T. cruzi* experimental infection with strains sensitive and resistant to BNZ, LYC-NC administered by oral and intravenous routes showed the highest rates of cure compared with BNZ, free-LYC and other drugs tested in vivo.[138–140,144] The treatment promoted complete elimination of amastigote parasites from heart tissue in mice infected with cardiomyotropic VL10 strain, using oral doses of 12 mg/kg/day during 20 days.[138–140,144] Nanoencapsulation promoted in this case an outstanding improvement of pharmacokinetic properties of LYC and dramatic reduction of cardiotoxicity.[140] This study attests the potential of nanotechnological approaches to the therapy of Chagas disease. Other studies have also shown the potential use of nanotechnology approaches in experimental Chagas disease.[197,213,216–218]

Final Comments and Future Perspectives

Chagas disease was discovered more than a hundred years ago, but current treatment is based on two old nitroheterocyclic drugs, BNZ and NFX. Knowledge about *T. cruzi* and the disease has expanded, but the complexity of the parasite, the pathogenesis and the immunology of the infection defy the scientific community and hinder the drug discovery process.[200] Notwithstanding, target-based and mainly phenotypic screening approaches have been widely applied. Technological advances have positively influenced the development of new compounds and approaches against *T. cruzi* infection. In this sense, many challenges on Chagas disease drug discovery pipeline must be overcome. The natural resistance to BNZ and NFX, verified in vitro and in vivo is an intrinsic characteristic of *T. cruzi* strains. The parasite stocks have shown different susceptibility profiles, a factor that can trigger some treatment failures.[196,219–221] The components involved in drug resistance are not yet fully understood,[36,39,220–222] but may include alternative activation of enzymes by drugs,[223] increased oxidative defense[224] or DNA repair pathways,[39] induction of drug efflux transporters[222] and glutamine metabolism.[225] In

addition, dormant forms of *T. cruzi* have been evidenced and increased drug tolerance was demonstrated.[226] Thousands of compounds have been experimentally tested in vitro, however, very few achievements in terms of translation were accomplished and among them, very few produced sterile cure in mice. Additionally, we highlight the lack of predictive and harmonized models in vitro and in vivo,[227] sensitive and accurate tests to determine therapeutic efficacy, mainly in chronic stage and proper determination of toxicity and pharmacokinetics of compounds.

There are no vaccines available for Chagas disease and, considering the immunological complexity and the long duration of the infection, advances in this area are still incipient. Apart from BNZ standard of care, in front of this plethora of chemical drug classes and the preclinical efficacy results discussed in this review, sesquiterpene lactone class of natural substances associated to nanocapsules seems to be the most promising chemical entities for further investigation for Chagas disease chemotherapy. Lychnopholide and goiazensolide showed outstanding efficacy against different strains of *T. cruzi* with variable sensitivity to BNZ. Additionally, fexinidazole advanced to new clinical trial with *T. cruzi* infection (FEX12-NCT03587766). Fexinidazole metabolites seem to be an encouraging approach for further clinical trials. Among new chemical entities and repositioned drugs, nitroheterocyclic compounds and CYP51 inhibitors showed potent activity against *T. cruzi*. However, a careful evaluation of toxicity and more effective regimens must be established. Additionally, associations of drugs that share or not share the same mechanism of action can lead to superior therapeutic efficacy.

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