

Chagas Disease: Drug Development and Parasite Targets



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Abstract Chagas disease (CD) is a neglected disease that is endemic to Central and South America and is caused by the protozoan parasite *Trypanosoma cruzi*. The discovery of new drugs against CD has not made any significant progress, as the same two drugs have been in use since the 1960s. Benznidazole (BZN), the first-line treatment and Nifurtimox (NFX), the second-line treatment are both nitro-heterocyclic derivatives. Significant problems of resistance have emerged with both drugs. Although new drugs and new *Trypanosoma cruzi* targets are the focus of studies worldwide, their development and release onto the market remain unresolved. This chapter aims to review current drugs for Chagas disease and their targets, as well as to discuss the challenges that exist in the discovery of new drugs. Furthermore, the evidence that points to the need to strengthen a collaborative network between institutions is emphasized along with the importance of multi-omic studies to support the development of new drugs for Chagas disease.

Keywords Chagas disease, Drug discovery, Drug target, Omics platforms, *Trypanosoma cruzi*

1 Introduction

1.1 Chagas Disease: A Problem for Public Health

Chagas disease (CD) from among the group of neglected tropical diseases (NTDs) is still an important disease worldwide with a high morbimortality of about 50,000 deaths each year. CD is endemic to Central and South American countries but currently, non-endemic places such as Canada, USA, Europe, Australia, and Japan have been affected due to the increasing global migration from endemic countries to non-endemic areas [1, 2]. The prevalence is variable depending on the location but is highest in Bolivia and Argentina. In the United States, it is estimated that more than 30,000 Latin American immigrants are currently infected with Chagas disease [3, 4]. The major route is vector-based transmission, but other transmission routes have been found (Fig. 1), including sexual transmission. In summary, the spread of CD is related to the migration of individuals with Chagas disease to previously non-endemic countries [1, 5, 6].

CD is caused by infection with the protozoan *Trypanosoma cruzi* which has a complex life cycle with insect vectors of the subfamily Triatominae (Hemiptera: Reduviidae). *T. cruzi* diverged from other trypanosomatids about 200 million years ago; and it circulates in 120 species of mammals, including humans [7]. Historically, it has been established that Chagas' disease existed as early as 7050 B.C. Exhumed mummies from archaeological sites in both Peru and Chile showed positive for *T. cruzi*'s kinetoplast DNA by polymerase chain reaction (PCR) [5, 8]; Approximately seven million people are infected by *Trypanosoma cruzi*, and 1.8–2.4 million of these infected people will develop severe clinical manifestations [9, 10]. In its life cycle, the parasite presents two evolutive forms: the first is the circulating infective but not replicative form known as trypomastigotes, which is the longest phase, and

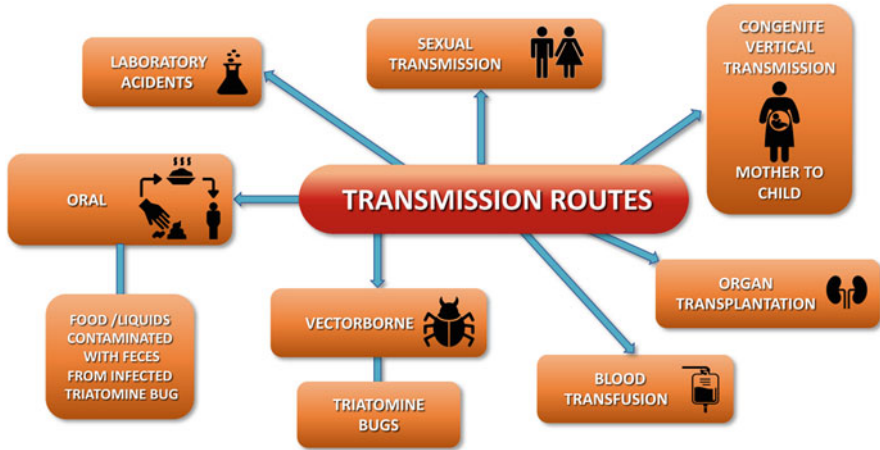


Fig. 1 Main transmission routes for Chagas disease

the second is the replicative and intracellular forms, known as amastigotes. The extracellular amastigotes have also been shown to be infective [11]. The metacyclic trypomastigotes and epimastigotes forms are found only in the insect vector. The former is infective and although epimastigotes were considered non-infective, some differentiated epimastigotes may be infective to mammalian hosts [12]. Besides the complex life cycle there is a genetic polymorphism in the parasite populations showing multiple genotypes and phenotypes. Currently the species is subdivided into seven genetically discrete typing units (DTUs): TcI to TcVI and TcB, an additional clade associated with bats [13, 14]. *T. cruzi* I is the DTU with the broadest geographical distribution and associated with severe cardiomyopathies. Whole genome sequencing results of several TcI isolates and the genetic subdivisions within TcI may be needed in the future [15]. Different *Trypanosoma cruzi* strains have been isolated from patients and it has been suggested that these parasite strains, regardless of the clinical presentation, reflect the principal DTU circulating in a particular region. However in several orally transmitted outbreaks, sylvatic strains are implicated [13]. All these factors regarding this genotypic and the phenotypic differences of *T. cruzi* strains and the different geographic distribution of DTUs increase the complexity making standardization of serological tests [16].

There are two distinct phases in the infection: (1) The acute phase – after vector-borne *T. cruzi* exposure. The acute phase begins after an incubation period of 1–2 weeks, and is characterized by a high parasitemia and circulating trypomastigotes are detectable by microscopy in fresh blood. This stage could be asymptomatic (90%) or the patient could present symptoms (10%) such as fever, anorexia, and tachycardia. These symptoms disappear in 90% of the cases, the mortality rate of the acute phases is 5%. (2) The chronic phase – 8 to 12 weeks after infection, parasitemia levels become undetectable by microscopy and, in the absence of treatment, some infected patients progress to the chronic phase – 60–70% of the infected individuals do not develop symptoms and are identified as a group with the indeterminate form of the disease. Up to 30% of the chronically infected

people develop cardiac alterations and up to 10% develop digestive (megacolon or megaesophagus), neurological, or mixed alterations which may also require specific treatment [17]. The chronic chagasic cardiomyopathy is the most serious clinical manifestation of the disease [1, 2, 18]. The infection in an individual with immunodeficiency (HIV infection, organ transplantation, autoimmune disease, or oncological treatments) can be reactivated and generate great morbidity and mortality [19]. Another distinct difference between the two phases is that during the acute phase of CD the trypomastigotes are abundant in the peripheral blood, and during the chronic phase, the amastigotes are abundant in various tissues [12].

Recently in a study by McCall et al., the authors presented results that demonstrate that infection by *T. cruzi* modulates the fecal microbiome, suggesting host-microbe interaction in the CD [20]. In this work the authors applied metabolomics and sequencing of 16S rRNA during the acute and chronic stages of infection using a murine model of CD. Consequently, they were able to verify the microbial and chemical disturbances associated with the *T. cruzi* infection, highlighting the importance of multi-“omics” and poly-microbial studies in the area of parasitic diseases in general, and in CD in particular [20]. However, as there are no well-validated targets for CD, phenotypic screening of various compound collections is still considered the most useful and economical strategy to identify new leads or starting points [21].

At last, it is important to consider that Chagas disease and other neglected diseases are part of a group of illnesses for which a set of measures are being developed to prevent, control, and treat until the eradication of these diseases.

The World Health Organization established a current road map – WHO (2021–2030) coordinated by the public, private, the not-for-profit research Drugs for Neglected Disease Initiative (DNDi), and humanitarian medical organizations.

The Wellcome Trust, the Bill and Melinda Gates Foundation, and the NIH contribute with the research in this area, as well as world Research institutions, pharmaceutical companies such as GlaxoSmithKline, Tres Cantos Open Lab Foundation (TCOLF), Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, The Novartis Institute for Tropical Disease (NITD), in cooperation with the Singapore Economic Development Board. All these efforts together bring a considerable development in the research of new drugs against these diseases [22–24].

2 Targets for the Treatment of Chagas Disease

Until this moment, there are no effective treatments against *Trypanosoma cruzi*. Currently, the research strategy is based on the biological differences between the parasite (*Trypanosoma cruzi*) and host cells (mammals) [25]. Intracellular parasites have high proteolytic activity, participating in various physiological and pathological events such as colonization, invasion, replication, differentiation, nutrition, dissemination, and evasion of the host’s immune system [26–28].

In the last 20 years, new strategies and tools had been used in the field of drug discovery, including new computational methodologies for omics analysis and medicinal chemistry, screening of new drugs, the study of the relation of three-dimensional structures and functions of biological molecules [16, 29]. Furthermore, studies into the basic biochemistry of the parasite have identified metabolic pathways in *T. cruzi* that provide or could provide novel targets for chemotherapy [30].

The inhibition of specific enzymes, metabolic pathways, or organelles exclusive to the parasite is an interesting strategy, called *Target-Based Drug Discovery*. Proteasomes is one of the major targets not only for Chagas disease but also for Leishmaniasis and sleeping sickness caused *leishmania* spp. and *Trypanosoma brucei*, respectively [31].

Other enzymes such as α -carbonic anhydrases from *T. cruzi* have also been studied [16, 32]. Several essential pathways, such as the glycolysis, pentose phosphate, and the Redox metabolism present in *T. cruzi*, have also been identified. Studies of the isoprenoid pathway have resulted in drugs that are being used in clinical trials. The toxicity and selectivity of the protozoan organelles is another crucial factor, since few compounds show such features. The characteristics of acidocalcisomes make these organelles potential targets for trypanocidal drugs [30]. Some of these targets are abandoned and are no longer of interest.

Despite the increase and urgency of research for new trypanocidal compounds against the infectious forms of *T. cruzi*, there is still no effective cure for Chagas disease. Most of these compounds being researched end up as inadequate due to the high toxicity to host cells. The selection of the therapeutic target is all-important, as it favors the rational search for compounds that induce a specific therapeutic response against *T. cruzi* [16, 30, 33–35]. An improved differentiation of the potential targets in the parasites that are absent in humans will help fight this neglected disease. Numerous questions arise related to the research of new drugs, and some of them were reported by [16, 23]. One recognized problem is the lack of standard methodology, different strains, different detection methods, and biomarkers to evaluate responses to therapy, diagnosis, and monitoring drug efficacy. In addition, according to Chatelain [36] the absence of standardization of animal models designed for Chagas disease drug discovery is directly involved in the translational process failure. All these factors constitute barriers in the development of new drugs.

In the next section the most promising and consolidated *Trypanosoma cruzi* targets are discussed.

2.1 Drug Targets

2.1.1 Cysteine Peptidase-Cruzipain

Some peptidases have been identified as possible targets for the development of new drugs, such as cysteine, serine, metallo- and threonine-peptidases. In general,

peptidases are hydrolytic enzymes that have the capacity to break the peptide bonds of proteins and protein fragments with selectivity and specificity [37, 38].

Among the peptidases of *T. cruzi*, we can highlight cruzipain, a cysteine peptidase, which has a prominent proteolytic activity in all the developmental stages of *T. cruzi*. Thus, cruzipain is a therapeutic target that has been intensely studied for the treatment of Chagas disease [39, 40]. Also, according to Cazzulo [39], recombinant enzymes have been developed, and different drugs are being studied that specifically inhibit cysteine peptidase in vitro, blocking the proliferation of epimastigotes, amastigotes, and metacyclogenesis of the parasite.

Cruzipain inhibitors cause the parasite to die in vitro, probably due to the accumulation of proteins within the Golgi complex, which results in an osmotic shock within the endoplasmic reticulum of the parasite [41]. This enzyme is present in organelles related to lysosomes and is associated with the plasma membrane [42, 43]. The K777 is a peptide derivative of vinyl sulfone that inhibits cruzipain irreversibly [44]. It is worth mentioning that many of these inhibitors have shown a lack of selectivity and low bioavailability in the in vivo assays. In addition, some compounds are potent cruzipain inhibitors but with slight effectiveness against *T. cruzi* in cell cultures. Concerted efforts are being made to overcome these difficulties and obtain an inhibitor with the selectivity and safety required to treat Chagas disease [44, 45]. This project to perform the preclinical studies demonstrating safety pharmacology and toxicology was started in 2010 by DNDI. Still, it was stopped in 2013 due to poor tolerability findings at low doses in primates and dogs [46]. Another derivative, the Neq0682, a reversible covalent inhibitor, of cysteine peptidases obtained by replacing the K777 vinyl sulfone group with a nitrile moiety was synthesized and is under study [47]. Other inhibitors against cruzipain are being studied such as quinoline [48], thiophen-2-iminothiazolidine derivatives, and thiophene–thiazolidine hybrids [49].

2.1.2 Kinetoplast Proteasome

The proteasome is an enzymatic complex formed by threonine peptidases. They are responsible for numerous biological functions in eukaryotic cells, such as the turnover of short-lived, abnormal/damaged proteins, cell cycle regulation, cell differentiation, signal transduction pathways, stress signaling, inflammatory responses, and apoptosis among others [50]. The 26S proteasome was identified in the epimastigote stage of *Trypanosoma* as a high molecular weight complex involved in parasite cell differentiation. This effect is mediated by the 20S proteasomal degradation of oxidized proteins through an ATP/ubiquitin-independent mechanism [51, 52]. In this context, it has been demonstrated that inhibition is associated with significant defects in parasite proliferation, turning on the proteasome as an attractive target for Chagas disease and other trypanosomatids and *Plasmodium falciparum* [53]. The Genomics Institute of the Novartis Research Foundation (GNF) has identified an azabenzoxazole compound series (GNF5343 and the optimized GNF6702) [54, 55]. In addition, GlaxoSmithKline (GSK) and

Dundee Drug Discovery have also identified a similar azabenzoxazole (GSK3494245). Azabenzoxazole (GNF6702), a non-competitive inhibitor of proteasome chymotrypsin-like activity, is a promising candidate for preclinical evaluation against neglected tropical diseases [22, 56]. This inhibitor was effective when tested against *Trypanosoma cruzi* as well as *T. brucei* and *Leishmania donovani*. Besides this, it does not inhibit the mammalian proteasome or growth of mammalian cells and is well tolerated in mice [16].

2.1.3 Carbonic Anhydrase

Carbonic anhydrases (CA, EC 4.2.1.1) are metalloenzymes with various physiological functions in all areas of life. They are involved in the pathological processes of human and prokaryotic/eukaryotic microorganisms such as bacteria, fungi, and protozoa. CAs catalyze the CO_2 reversible hydration: $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+$, which are involved in several physiological and pathological processes. CO_2 homeostasis, biosynthetic reactions, calcification, and tumorigenicity are some examples, among others. In addition, these enzymes are related to the growth and virulence of microbial pathogens. In researching new medicines, this target was established to develop anticonvulsants, anti-obesity, anticancer, and anti-infective drugs. In this context, CAs belonging to the α - and β -class were recently identified, cloned, and recognized their potential as new enzymatic targets in *T. cruzi* (α TcCA) and from *L. donovani chagasi* (LdccCA), respectively [57, 58].

In *Trypanosoma cruzi*, an α -CA was identified, cloned, and characterized by Pan et al. [58]. The α -TcCA has a high catalytic activity for the CO_2 hydration reaction. Inhibitors such as anions, sulfonamides, sulfamates, thiols, and hydroxamates were effective in low nanomolar in vitro tests [32, 59, 60]. One of the best inhibitors was hydroxamates which inhibited the growth of all three evolutive forms of the parasite at low concentrations (IC50 values from 7.0 μM to $<1 \mu\text{M}$) [61, 62]. Synthetic inhibitors such as sulfonamides have been tested against TcCA from *T. cruzi* with success. Bonardi et al. demonstrated that *N*-nitrosulfonamides and their salts inhibited the growth of the epimastigotes of *T. cruzi*, based on CA inhibition and are promising lead compounds for rational optimization of innovative agents for the treatment of Chagas disease [63]. All these results demonstrated the potential of the α -TcCA as a target yet underexplored for Chagas disease drugs [59]. The Cas is not a validated target for Chagas disease yet but is a possibility and further studies need to be performed to confirm this hypothesis.

2.1.4 Sirtuins

Silent-information regulator 2 (SIR2) proteins, or sirtuins, are a family of enzymes evolutionarily conserved and present in all kingdoms of life, from bacteria to higher eukaryotes [64]. According to Matutino Bastos et al., the inhibition of *T. cruzi* sirtuins by nicotinamide can cause growth arrest and morphological alterations in

the parasite, thus being a possible candidate for a drug against CD [65]. In the search for new inhibitors, these same authors [65] characterized human sirtuin inhibitors against *T. cruzi* sirtuins. As a result, they reported seven inhibitors of sirtuins, where all compounds prevented the proliferation of *T. cruzi* in mammalian cells.

2.1.5 Cyclophilin

Cyclophilins are enzymes that perform several biological functions such as protein folding, where Cyclophilin D (CypD) is a mitochondrial isoform with a crucial role in opening the pores for mitochondrial permeability [66]. It is known that this enzyme activity is inhibited by the immunosuppressant Cyclosporin A. In a study by Búa et al., the authors demonstrated anti-*T. cruzi* activity with Cyclosporin A through the inhibition of Cyclophilins, suggesting that this may be a molecular target [67]. According to Jha et al., Cyclophilin 19 is an enzyme present in all stages of life of *T. cruzi* participating in several functions, among them the generation of ROS that increases the growth of the parasite. In the study carried out by the authors, a mutant knock-out parasite of Cyp19 was generated, which was unable to replicate in cell cultures or in immuno-competent mice [68]. The authors also performed repeated inoculation of knock-out parasites where they observed specific antibodies and T-cell responses. According to the authors, these results demonstrate a 100% effective immunization in preventing Chagas disease. This study generated a patent entitled “Live attenuated parasitic vaccine” (US20200147148A1) [69].

2.1.6 *N*-myristoyltransferase

N-Myristoyltransferase (NMT, EC 2.3.1.97) is an enzyme that catalyzes the co- and posttranslational addition of myristic acid (C14: 0) onto the N-terminal glycine of specific proteins [70]. Studies have shown that NMT is both essential and druggable in *T. cruzi* [71], where it was shown that the inhibitor DDD8564630 caused a reduction in parasite proliferation in the epimastigote stage [72]. In a study by Herrera et al., the authors demonstrated the effectiveness of inhibitors as antiproliferative agents, presenting very low toxicity against mammalian cells. Where, according to the authors, it was possible to demonstrate its specificity and validation of NMT as a drug target using inhibitors with potential for future explorations such as anti-CD [73].

2.1.7 Pentose Phosphate Pathway: Glucose-6-Phosphate Dehydrogenase and Trypanothione Reductase

Glucose is metabolized through two major pathways: glycolytic and pentose phosphate (PPP). The PPP produces the ribose 5-phosphate (R5P) required for nucleotide synthesis and reducing power in the form of NADPH. One branch of the pathway is

the oxidative branch, involving glucose 6-phosphate dehydrogenase (G6PDH), among other enzymes. G6PDH is of central importance because it often has a high control coefficient for the PPP and can be considered a potential target for developing drugs for CD [74].

The studies with the PPP in *Trypanosoma* were scarce, and most of the pathway's enzymes, their properties, and subcellular localization were unknown until 2004 [74, 75]. PPP is important for parasites, considering that oxidative burst forming reactive oxygen is the first line of host cell defense against infection. Maugeri and coworkers [76] demonstrated an increased flow of the PPP pathway in *Trypanosoma cruzi* in response to oxidative stress. The parasite is highly sensitive to oxidative stress. The primary protection against reactive oxygen (ROS) is the Trypanothione, which is kept reduced by trypanothione reductase, using NADPH as a cofactor [75]. In addition to the cytosolic localization, PPP is present in the organelle glycosomes from trypanosomatids. Dehydroepiandrosterone (DHEA), epiandrosterone (EA), and derived 16-bromoepiandrosterone (16BrEA) are known to be uncompetitive inhibitors of mammalian and trypanosome G6PDH [77]. Trypanothione reductase is an attractive target for antitrypanosomal drug. Some compounds such as Quinoxaline and Clomipramine showed activity against *T. cruzi* TR [78, 79] and quebrachamine, cephalotaxine, cryptolepine, tomatidine, solanidine, and solasodine were detected as potent inhibitors [80].

In a study by Fredo Naciuk et al. [81], the authors synthesized and evaluated 26 steroid derivatives of epiandrosterone (nonselective inhibitors of G6PDH) in enzymatic assays. As a result, compounds 40, 15, 39, and 6 showed a certain degree of selectivity in cell assays was achieved, at least in terms of toxicity in the system used (intracellular *T. cruzi* forms and rat cardiomyocytes). Although these results are promising, further studies are necessary to get more selective inhibitors, which increases the knowledge about the interactive action mechanisms of the inhibitors with the host cell/parasite.

Ortiz et al. [82] demonstrated in *Trypanosoma cruzi* using immunofluorescence a cytoplasmic distribution of G6PDH and the absence of signal in major organelles. In addition cytochemical assays confirmed that parasitic G6PDH is the molecular target of derivative epiandrosterone. These results together demonstrated that glucose 6-phosphate dehydrogenase shows a potential for a drug target for Chagas disease and other diseases caused by trypanosomatids as already shown for the African *Trypanosoma brucei* [82].

2.1.8 Ergosterol Biosynthesis Inhibitors

As ergosterol is essential for *T. cruzi*, enzyme inhibitors of its biosynthesis pathway have become common targets in studies [83]. Several studies have focused on the trypanosome ergosterol biosynthesis pathway (Fig. 2) due to the availability of existing drugs (posaconazole or ravuconazole) capable of inhibiting sterol 14 α -demethylase (CYP51). CYP51 is an essential enzyme for this pathway and

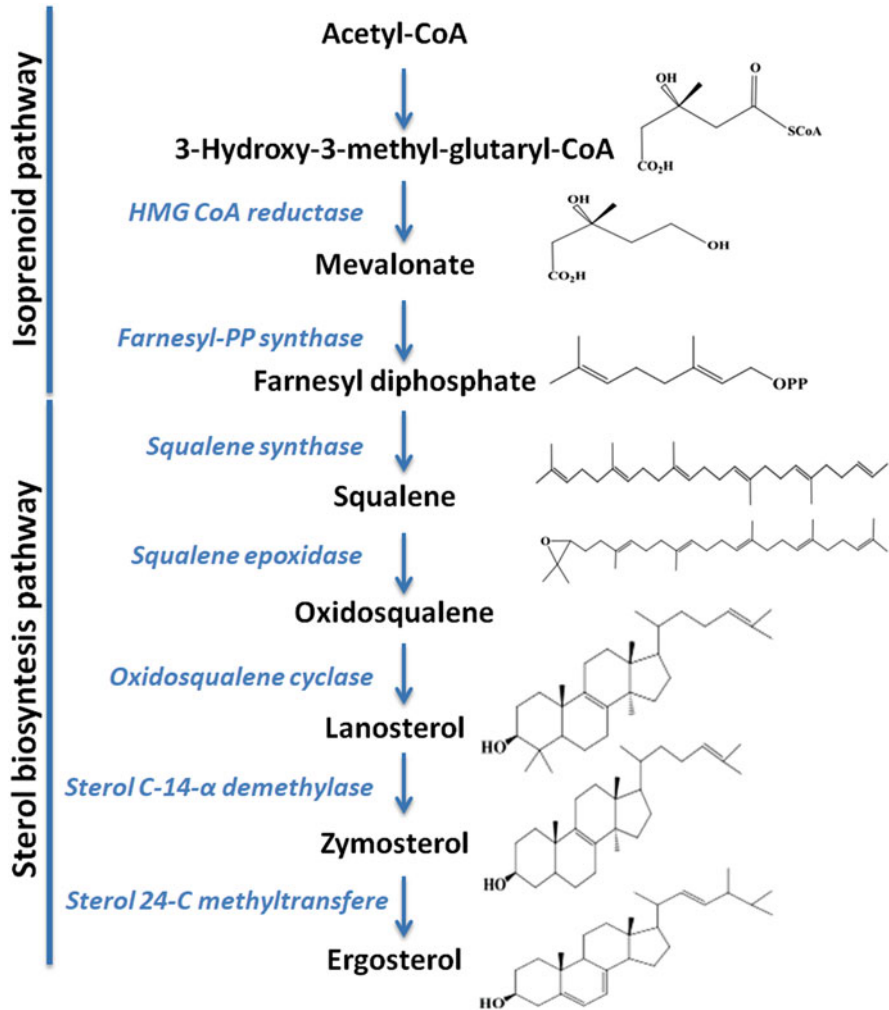


Fig. 2 Simplified ergosterol biosynthesis pathway in *T. cruzi* epimastigotes. Critical enzymes (blue color) of the ergosterol pathway. Adapted from [84]

its inhibition reduces the capacity for invasion of heart cells by trypomastigotes and inhibits the multiplication of amastigotes [85, 86]. Posaconazole is not yet a viable alternative as it has a high cost and has a structure that is difficult to interact with *T. cruzi* CYP51 [86]. According to Osorio-Méndez and Cevallos [83], the evidence suggests that these inhibitors effectively fight infection in vitro and murine models; however, they have failed in clinical trials [23]. It is worth noting that the failure of CYP51 as a drug target is due to the cytostatic consequence of inhibition [23]. Consequently, assays have been developed to filter out compounds identified in phenotypic screens with this undesirable mode of action [87]. However, it may be possible

to rescue posaconazole by combination therapy described recently by Rocha-Hasler et al. [88] demonstrating that Tomatidine improves the potency of posaconazole as antitrypanosomal agent.

2.1.9 Sphingolipids

Sphingolipids are biological molecules found in eukaryotes and prokaryotes. Their structure is composed by a sphingoid base with a fatty acid attached through an amide bond, forming the ceramide. A variable polar head-group is present (phosphocholine, inositol phosphate, or carbohydrates). In epimastigotes from *Trypanosoma cruzi* glycosphingolipids were detected [89]. The parasite synthesizes inositol phosphorylceramide (IPC) and sphingomyelin (SM) and its expression is modulated during parasite development. The IPC synthase enzyme catalyzes the transfer of inositol phosphate to ceramide moiety. Several glycoconjugates are attached to membranes by a glycosylphosphatidylinositol (GPI) anchor and in this context *T. cruzi* have different surface molecules including the glycoinositolphospholipids (GIPLs, in epimastigotes), trans-sialidase and Tc-85 glycoprotein in trypomastigotes, mucins in epimastigotes and metacyclic forms and complex GPI-anchored glycopeptide called NETNES in epimastigotes. All these molecules have important biological function on parasite related to the antigenicity, pathogenesis, cellular survival, and programmed cell death (PCD) and in cellular survival [90–92].

Landoni et al. [93] studied the effect of tamoxifen (TAM) on the sphingolipid pathway of *T. cruzi*. TAM is an anti-estrogen used for the treatment of breast cancer. This drug is involved in apoptosis mechanisms. Although there are few studies about the effect of TAM in the sphingolipid pathway, the authors tested it in *Trypanosoma cruzi*. The results demonstrated a dose-dependent inhibition according to the evolutive stage of the parasite. Lipid extracts from epimastigotes were analyzed by MALDI-TOF and HPLC-ESI mass spectrometry. The results showed that after TAM treatment, a high and discrete increase in the level of ceramide/ceramide-1P and sphingosine, respectively, indicates the involvement of TAM in the breakdown of ceramide. Microscopy analysis and flow cytometry of the treated parasite demonstrated an apoptotic-like death process. Previous studies of Miguel et al. [94] showed that TAM was ineffective in the treatment of the acute phase of Chagas disease in mice. These results suggest that the sphingolipid pathway could be a target for drug development in Chagas disease. More studies are needed, including tests with new concentrations of the drug and evaluation of toxicity in host cells.

2.1.10 Intracellular Calcium Homeostasis

Ca^{2+} is an essential signaling messenger in eukaryotic cells, including the *Trypanosoma cruzi* and other trypanosomatids [95–97]. The function of Ca^{2+} as a signaling messenger in trypanosomatids is well documented in several biological

functions like flagellar movements, cellular differentiation, invasion of the host cell, osmoregulation, and nitric oxide transduction pathway [95, 98–108]. Ca^{2+} concentration in *T. cruzi* is regulated by mechanisms present in the plasma membrane and by intracellular organelles such as mitochondria, endoplasmic reticulum, and acidocalcisomes. The maintenance of calcium homeostasis is very important considering that Ca^{2+} is involved in the apoptotic process [109, 110]. In this context the disruption of intracellular calcium homeostasis has been proposed by Benaim et al. as a possible therapeutic target for drug development in Chagas disease [25]. The amiodarone and derivatives, for instance, an antiarrhythmic drug showed trypanocidal effects through disrupting the parasite Ca^{2+} .

2.1.11 Acidocalcisomes

Acidocalcisomes are organelles that play an important role in osmoregulation which are rich in polyphosphate bound to calcium and different cations such as magnesium, calcium, sodium, and zinc [111, 112]. The matrix contains enzymes related to poly P metabolism and the membrane of the acidocalcisomes has several pumps and transporters including a Ca^{2+} -ATPase for Ca^{2+} uptake. The Ca^{2+} release is controlled by the inositol 1,4,5-trisphosphate receptor (IP3R) that is located in acidocalcisomes [95, 113]. Chiurillo et al. demonstrated that the removal of the IP3R gene by CRISPR/Cas9 genome editing inhibits host cell invasion by trypomastigotes [114]. In contrast, TcIP3R overexpressing parasites showed decreased metacyclogenesis, trypomastigote host cell invasion, and intracellular amastigote replication. Summarizing Ca^{2+} signaling is important for the *T. cruzi* differentiation for host cell invasion and to maintain cellular bioenergetics [95, 114]. Based on these facts it had been suggested that the acidocalcisomes and their components could be potential targets for chemotherapy [115, 116].

2.1.12 Kinetoplast

Trypanosomes have a single mitochondria that has the peculiar characteristic of having a circular DNA network known as kinetoplast or kDNA [117, 118]. The kDNA is composed of circular and interconnected DNA molecules forming a single network, therefore, knowledge of this topological architecture is fundamental for understanding the replication and segregation of the kDNA circles [119]. In a study by Zuma et al. [120] the authors analyzed the effects of Berenil on the ultrastructure and replication of *T. cruzi* kDNA. As a result, the authors demonstrated that Berenil caused significant changes in the kDNA arrangement and a reduction in the growth of *T. cruzi*, but cell viability was not affected. The authors suggest that Berenil mainly affects kDNA topology and replication, demonstrating, according to them, that Kinetoplast represents a potential target against trypanosomatids [120].

According to Cavalcanti and de Souza [119], previous studies used atomic force microscopy to analyze the effect of acriflavine, an intercalating drug [121] in the

T. cruzi kDNA network. As a result, it was possible to evaluate the structure of kDNA and investigate the topology changes caused by drugs, and the authors proposed that kDNA can be an interesting target because it is affected by DNA-binding drugs, intercalating agents, and topoisomerase inhibitors [119].

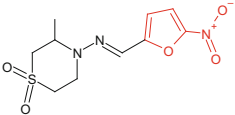
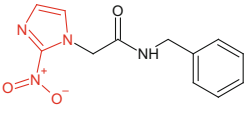
3 Current Treatments for Chagas Disease

Only two drugs have been used to treat CD, nifurtimox or (RS)-3-methyl-*N*-[(1E)-(5-nitro-2-furyl)methylene] thiomorpholin-4-amine 1,1-dioxide (NFX – Tables 1, 1) and benznidazole or *N*-benzyl-2-(2-nitro-1H-imidazol-1-yl)acetamide, which is a 2-nitroimidazole derivative (BZN – Tables 1, 2). Both were discovered more than 50 years ago, showing that there is a continued lack of investment in research and development (R&D). The justification for this is that the predominance of CD is in emerging countries; consequently, the pharmaceutical industries are not interested in investing in new antichagasic drugs and their excessive costs for research in developing countries [122].

NFX was the first drug developed for this purpose and has been used since 1965 and BZN since 1971. BZN was first produced by the pharmaceutical company Roche (Rochagan[®] and Radanil[®]), now it is manufactured by the Pharmaceutical Laboratory of the State of Pernambuco (Lafepe), Brazil, and by the private laboratory in Elea (Abarax[®]), Argentina. NFX is free of charge through a WHO-Bayer agreement [17, 123, 124].

Studies of the mechanisms of both drugs are not fully understood yet. NFX and BZN are prodrugs and require activation by enzymatic activity. Wilkinson and coworkers [125] proposed that nitroreductase is the main enzyme involved in the activation of nitro-heterocyclic drugs in *T. cruzi*. In general, nitroreductases reduce the nitro group present in both the nitro-heterocyclic compounds, generating

Table 1 The main contraindications and indications for Benznidazole and Nifurtimox

Drug	Contraindications	Indications
 <p>(1) Nifurtimox (NFX)</p>	The first trimester of pregnancy; Woman who are breastfeeding; Patients with a history of mental problems or seizures; Patients with a history of psychiatric or neurological disorders [122]	Congenitally infected newborns Acute phase
 <p>(2) Benznidazole (BZN)</p>	Liver failure, kidney failure, pregnancy [17]	Acute phase Congenitally infected newborns Acute phase Children under long-term therapy

metabolites with different reactivities for each drug, such as molecules considered free radicals (e.g., R–NO⁻) and electrophilic metabolites (e.g., R–NHOH) [3, 126].

However, NFX and BZN require distinct enzymes for biological activation. For NFX, the proposed mechanism is through the one-electron route (type II nitroreductase). A re-oxidation leads to reactive oxygen species formation in cells (oxidative stress); the production of highly toxic oxygen metabolites and oxidative stress to the parasite by generating the nitro radical, redox cycling, and production of O₂⁻ and H₂O₂ [3, 122, 127, 128]. In addition, some studies have proposed that the reduction of NFX by nitroreductase can open the furan ring producing an unsaturated open-chain nitrile that is as cytotoxic as the parent compound. This suggests that NFX trypanocidal activity does not necessarily involve oxidative stress [129, 130].

For the benznidazole the two-electron route (type I nitroreductase) in the mechanism forming reactive nitroso and hydroxylamine intermediates (chemical stress). The hydroxylamine derivative (R–NHOH) converts to glyoxal, a highly cytotoxic and mutagenic compound [129]. Some studies indicate that the reactive species generated in BZN metabolites can improve phagocytosis, increase trypanosome death through IFN- γ induction, and inhibit *T. cruzi* NADH fumarate reductase (thus) inducing mitochondrial DNA damage. The *T. cruzi* cytotoxic activity of these metabolites may involve covalent modification of macromolecules such as DNA, proteins, and lipids [131, 132].

The chemical species generated by these drugs are highly reactive and can affect other molecules, especially in the vertebrate host. Consequently, this low specificity of action on the parasite contributes to the cytotoxic effects observed in treatments with patients. *T. cruzi* is probably deficient in the metabolic detoxification mechanisms for oxygen and that makes it very susceptible to partial reduction products of oxygen, consequently, it is more sensitive to oxidation than the vertebrate cells [3, 127, 128].

NFX and BZN are almost 100% effective in curing CD if given soon after infection, at the onset of the acute phase, in cases of congenital transmission and for those in whom the infection has been reactivated due to immunosuppression. However, the efficacy of both reduces as time passes [17]. BZN is usually the first-line treatment in most countries because it has a better safety record and efficacy profile than Nifurtimox, mainly for adult patients that require prolonged administration [129]. In the acute and undetermined chronic phases, treatment with BZN has proven to be highly effective and about 80% of patients have no sign of parasites in the blood after 12 months of treatment. The other benefits of treatment with BZN are the high cure rates in infants with congenital infection and in children with chronic infection [122].

In 2011, a partnership between DNDi and Lafepe laboratory (Brazil) allowed the development of the first pediatric formulation of benznidazole for the treatment of children with CD. Following partnerships with Chemo Research, Exeltis USA, Mundo Sano Foundation and Laboratory ELEA PHOENIX were set up aiming to enable a broader registration in endemic countries, with a commitment to make the pediatric formulation more widely available. In 2017, the FDA granted approval for

the use of benznidazole in children with CD aged between 2 and 12 years old [17, 123, 133].

The treatment of the chronic phases of CD in adults with BZN on the growth rates of negative parasitemia increases with treatment time but there are no significant modifications with increased drug dosages or modifications in the drug formulation. Studies have demonstrated that negative parasitemia in patients with chronic CD has been observed after treatment from 55.97% after 2 months to 62.59% after 8–16 months and 72.81% after 9–11 years [122, 123, 133, 134]. Although BZN is able to reduce serum parasite detection in chronic CD, it does not significantly reduce cardiac clinical deterioration [135, 136].

NFX treatment studies of 114 adults with chronic Chagas were conducted. These studies demonstrated positive serology after an average of 6.6 years for all of them; and *T. cruzi* was not detected in 93.9% of the cases after treatment. However, in these cases, according to the current cure criterion, it is not possible to interpret the obtained results as a parasitological cure [137]. In treatment with both drugs, BZN and NFX, therapeutic failures are common for reasons that include influences of the parasite and host genetics, and the effects of toxicity on adherence to the treatment. The long-term therapy increases the chances of NFX presenting more intense adverse effects than BZN. The adverse reactions are more frequently reported with older aged patients with both NFX and BZN. In general, NFX has shown higher toxicity and adverse effects than BZN, including increased oxidative stress in rat pancreas and heart [135].

Problems related to suffering from adverse drug reactions (ADRs) are reported in more than 30% of the patients treated with BZN, especially those in the chronic phase. These effects involve dermatitis, disorders in vision, myelosuppression, and peripheral polyneuropathy, as well as gastrointestinal system disorders [16, 122, 138, 139].

The main contraindications to treatment with BZN are liver or kidney failure and pregnancy. NFX is contraindicated in patients with a history of psychiatric or neurological disorders (Table 1) [140, 141].

Additionally, significant problems of resistance have emerged with both drugs [17, 123]. The mechanism of BZN and NFX resistance has been associated with the deletion of copies of genes encoding two different nitroreductases, namely old yellow enzyme (TcOYE) or prostaglandin synthase, and trypanosomal type I nitroreductase [122].

4 New Drugs and Clinical Trials

4.1 Drugs in Clinical Trials

Since the introduction of pharmacotherapy for CD with BZN and NFX, only allopurinol and some azoles, such as: itraconazole, fluconazole, ketoconazole,

posaconazole (POSA), E1224/ravuconazole (RAV) and fexinidazole have been tested for the treatment of CD [3].

4.1.1 Fexinidazole: First Global Approval

Fexinidazole (FXN) is a promising broad-spectrum antiparasitic agent with clinical trials already taking place. FXN was first discovered by Sanofi (former Hoechst AG) and was identified by the DNDi in 2005 as having activity against *Trypanosoma brucei gambiense*, *T. b. rhodesiense*, and *T. cruzi* [142]. This compound has a nitro group in its composition that is metabolized by the parasite nitroreductases, forming reactive species that inhibits DNA synthesis [124].

Clinical studies with FXN have been performed with both CD and visceral leishmaniasis patients [143]. Dose-finding studies over a maximum treatment time of 8 weeks were conducted with chronic indeterminate Chagas disease patients. A Phase II Proof of Concept (PoC) study of fexinidazole was initiated in 2014 in Cochabamba and Tarija, Bolivia. However, the study was interrupted due to safety and tolerability problems. Further analysis of the results showed high efficacy findings were at the lowest dose tested for all treatment durations. The higher doses of the drug were considered unsafe after only 14 days. However, the clinical study follow-up was extended to 12 months [140]. FXN has demonstrated its therapeutic potential against CD, since it has been approved by the European Medicines Agency (EMA) for the treatment of African trypanosomiasis, in both adults and children [124, 142].

4.1.2 E1224/Ravuconazole

E1224 (fosravuconazole or fosravuconazole L-lysine ethanolate) is a prodrug of ravuconazole (RVZ), which is a potent inhibitor of ergosterol biosynthesis with activity against a wide range of fungal species [144–146]. RVZ is a potent CYP51 inhibitor, but studies have shown that it has a short terminal half-life, where in studies with murine models it presented limited in vivo activity due to unfavorable pharmacokinetic properties [147, 148]. In studies with canine models, the results were suppressive but not curative due to the short terminal half-life [147]. In a clinical study with humans led by DNDi, the drug E1224 presented a rapid, but transient, parasite clearance effect – suggesting that this drug has a static rather than a parasitocidal mechanism of action [145, 146]. It was also observed that the combination of RVZ with BZN did not significantly affect efficacy [144]. According to Spósito et al. [148] the authors suggest that therapeutic failure may be related to inadequate azole levels in tissues and suboptimal drug exposure [148].

4.1.3 New Drugs Discovery

The knowledge of parasite biology has, over the last decades, discovered small molecules that could be potential chemotherapeutic targets in the parasite for CD treatment, such as: enzymes that participate in the synthesis of ergosterol in *T. cruzi*; enzymes of the trypanothione metabolism such as trypanothione reductase; Cruzipain; Carbonic anhydrases; among others [84].

The potent activity of azole antifungal drugs against *T. cruzi* were reported more than 30 years ago. Since then, several azole antifungal drugs such as ketoconazole, fluconazole, itraconazole, posaconazole, D0870 and others, have been reported as sterol 14 α -demethylase inhibitors (CYP51) [149]. Triazole compounds such as posaconazole and D0870 have been shown to be effective at curing mice with chronic CD. On the other hand, clinical studies with ketoconazole or itraconazole in humans with chronic CD have not presented any significant cure of the disease. However, studies have been published demonstrating the synergistic activity of azole drugs with various other compounds, indicating that a combination of chemotherapies may be an effective strategy as this field moves ahead [149]. VNI is the first nonantifungal compound CYP51 inhibitor to target the 14 α -demethylase activity of *T. cruzi*. This compound demonstrated a cure for both the acute and chronic phases of infection with the Tulahuen *T. cruzi* strain in mice but failed to cure mice infected with the Y and Colombian *T. cruzi* strains in both phases of the infection [150].

Several compounds have been shown to inhibit *T. cruzi* trypanothione reductase and affect parasite growth in vitro or in vivo, such as nitrofurans and naphthoquinones, phenothiazines and crystal violet, diphenyl sulfide derivatives, polyamine derivatives, dibenzazepines, bisbenzylisoquinoline alkaloids, ajoene, acridines, terpyridine platinum complexes, and Mannich bases as well as some natural products [84, 151]. Buthionine sulfoximine (BSO) has been shown to be a potential drug candidate against *T. cruzi* alone, or jointly with free radical-producing drugs such as nitrofurazone and benzimidazole [84]. K777, a vinyl sulfone derivative, is a potentially irreversible inhibitor of cruzipain. Nevertheless, preclinical studies demonstrated poor tolerability even at low doses in primates and dogs. Novel scaffolds for the inhibition of cruzipain were identified from high-throughput screening (HTS) of GlaxoSmithKline HAT (Human African Trypanosomes) and Chagas chemical boxes [84].

Several aromatic/heterocyclic sulfonamides have been studied as carbonic anhydrases of *T. cruzi* (TcCA) inhibitors. The studies were performed against mammalian enzymes (hCA I and hCA II) and the inhibitors showed a greater inhibition of the *T. cruzi* TcCA indicating a positive selectivity [58]. Additionally, a series of the 4,5-dihydroisoxazoles incorporating hydroxamate moieties showed that they could also act as inhibitors of the *T. cruzi* α -CA and of peptidases from this pathogen, such as the cysteine and metallo-peptidase. These series were assayed in vitro against the tree forms of *T. cruzi* and in vivo. These studies showed that a leading compound had potential values for the growth inhibition of all three developmental forms of the Y strain of *T. cruzi* at relatively low concentrations [61].

5 Omics Platforms in Chagas Disease

Even after more than 100 years of the discovery of Chagas disease, and despite an enormous scientific research effort, this disease is still a major threat in several Latin American countries and an emerging global health problem [16, 152]. In work carried out by Alonso-Padilla et al. on strategies to diagnose and treat patients with CD, the authors cite the lack of public policies and funding as factors that make facing the problem even more challenging [10]. The authors attest that when there is no treatment ~30% of those chronically infected will develop cardiac and/or digestive disorders. According to the authors, a deeper understanding of the parasite's biology and its interactions with the host is of fundamental importance for the discovery of safer drugs or vaccines. Therefore, studies on the genomics of the parasite and the host combined with other omics are essential to determine the factors that lead to the development of CD [10]. Research into the biology of parasites requires a sophisticated and integrated computational platform that allows the analysis of large volumes of data [153].

Platforms with large amounts of omic data (proteomics, genomics, transcriptomics, metabolomics, etc.) have already been created and continue to be developed at an ever faster rate, but the databases have heterogeneous formats that are often difficult to integrate with experimental data [153]. According to Wooden et al., transcriptomic, genomic, and omic data (proteome, metabolome, kinome, methylome, acetylome, lipidome, microbiome, phenome, exposome, meta-genome, and interactome) have been increasingly deposited for public use, however, this excess of information has made the analysis of these omic data sets a challenging task [152].

In relation to CD, other types of genetic studies, such as transcriptomics and epigenetics, are needed to expand and integrate the genomic data already studied, in addition to the need to understand how environmental factors imply susceptibility to the disease [154]. According to Talavera-López and Andersson [155], integrative omic approaches will be able to treat, on a large scale, biological information from the most diverse areas such as drug resistance, epidemiology, genetic exchange, immune evasion mechanisms, genetic function, etc. However, according to these authors, additional work is also needed for a complete characterization of individual targets and families of genes, and yet other aspects of parasitic biology are still relatively unexplored. Therefore, they emphasize that a lot of basic research is needed before these issues can be effectively addressed [155]. In summary, Talavera-López and Andersson highlighted some points that make parasitic research problematic: (a) Sampling that is difficult to perform. When they are carried out, they are often not collected in a standardized manner, limited metadata and often there is no sampling strategy guided by a specific biological issue. (b) Requirement for field studies with visits to remote locations that implies complex and costly logistics. (c) Some parasites such as *Leishmania*, *T. cruzi*, *T. rangeli*, etc. can only grow in vitro using culture media and even cloning by limiting dilution. In this way, a level of uncertainty is introduced in the experiment, considering that the parasites can

change according to the culture medium in which they grow. And often the clones that dominate the culture may not be the same ones that occur in the host [155].

According to Sánchez-Ovejero et al., the integration of omic data is essential to understand the host–parasite relationship [156]. However, as demonstrated by Vermelho et al. [16], the technological advances to date have not resulted in the discovery of new drugs since the 1960s, nor in the cure of CD. However, with the advancement of metagenomics and metabolomics technologies, discoveries pertinent to various human conditions have been made, and for this reason, new light has begun to be shed on neglected tropical diseases [157]. Following this direction, in a review carried out by Kules et al. [158], the authors claim that high-throughput technologies, such as whole-genome sequencing, omics (metagenomics and meta-proteomics), and mass spectrometry (MS), open new opportunities for the diagnosis of Vector-Borne Diseases. According to Kratz [159], even with all the recent advances associated with the discovery of drugs for CD, the likelihood of a new ideal treatment emerging in the coming years is still uncertain. The author claims that success in this discovery does not depend only on new tools and technologies, but also on the availability of financing and collaborative R&D models, in addition to a deepening of the understanding of the pathophysiology of the disease [159]. Financial support is needed for the initial stages of research and development of medication for CD [160]. In a work carried out by Calogeropoulou et al., the authors attest that many research programs are successful and have initial leads, however, they fall prematurely into the “valley of death” of candidate compounds. Therefore, they suggest that drug discovery for CD should become collaborative and include academic research, national and international organizations, government initiatives and private research centers as well as pharmaceutical companies [160].

Currently, modern drug design and discovery is based on computational methods that predict and evaluate binding of ligands to receptors related to various pathologies [161]. Therefore, the methods that are capable of promoting correct data acquisition, mining, and analysis are crucial in order to be able to obtain reliable results [162]. Traditional drug discovery and development is known to be time consuming and cost-intensive to both biotechnology and pharmaceutical companies [163]. According to Shaker et al. computer-aided drug design (CADD) offers methods to discover and optimize potent drugs *in silico*, aiming to screen millions of substances in order to identify chemical compounds that can geometrically and chemically bind to a specific cavity on a target protein [164].

In a study for drug repositioning, Sayé et al. used computational methods to discover substances with a structure similar to crystal violet (CV) [165]. CV was chosen because it has been used in blood banks for several years to eliminate the parasite *Trypanosoma cruzi*. The authors claim that the mechanism of action of CV is the inhibition of proline uptake by the parasite. Thus, the *in-silico* drug repurposing strategy through a similarity-based virtual screening protocol was able to identify compounds structurally related to CV (loratadine, cyproheptadine, olanzapine, and clofazimine). As a result, they observed that loratadine, cyproheptadine, and clofazimine inhibit the proline transporter TcAAAP069 and also they had a trypanocidal effect against all stages of *T. cruzi* [165].

2D QSAR is a tool that seeks to explain the relationships between chemical structures and experimental observations so that predictions of new compounds with certain desired properties can be made [166]. In a recent review, Halder and Dias Soeiro Cordeiro [168] analyzed different *in silico* approaches that were successfully applied in the discovery of anti-leishmaniasis (anti-LM) and anti-trypanosomiasis (anti-TP) drugs. After reviewing these works, the authors [168] identified two *in silico* approaches that have been little explored in recent years, but that had a high potential in the development of anti-LM and anti-TP agents, which are QSARs with.

In the search for molecules with action against *Trypanosoma cruzi*, [169] used the quantitative structure-activity relationship (QSAR) approach to investigate how molecular physical-chemical characteristics affect biological activity. These authors assumed that there are compounds that at some point show activity against *T. cruzi* but occasionally fail when tested in “whole-cell phenotypic assays.” However, according to the authors, this result may be due to several factors such as inadequate physical-chemical and pharmacokinetic properties, resulting in molecules with little capacity to cross cell membranes. In this work, the authors applied artificial neural networks (ANNs) and kernel-based partial least squares regression (KPLS) to anti-*T. cruzi* activity data (Fig. 3). Through the analysis of atomic contribution maps, the authors found that fluorine and, in general, the heterocyclic aromatic rings and piperazine rings contributed positively to anti-T activity. Therefore, the integration of the ANN and KPLS analyses enabled the generation of a collection of key fragments strongly correlated with anti-*T. cruzi* compounds, providing valuable information to guide the design of new antichagasic agents with enhanced properties [169].

In 2012, Vincent et al. demonstrated the use of the metabolomic platform to elucidate the mode of action of an anti-trypanosomal drug [170]. According to Trochine et al., the first option for the treatment of CD is the administration of BZN, although its mode of action is not yet fully understood [171]. In order to analyze the metabolic response of *T. cruzi* to BZN, the authors [171] used a non-targeted MS-based metabolomics approach. Thus, the global changes in the metabolites that occur when BZN enters the parasite could be monitored. As a result, the authors concluded that treatment with BZN mainly affects molecules containing thiol in *T. cruzi*, therefore, this interference in thiol metabolism contributes to the action of the drug [171]. Later, the same group monitored, using the same metabolomic strategy, changes of low-mass metabolites in the epimastigote forms of *T. cruzi* treated with Bestatin [172]. Bestatin is a natural product with a broad-spectrum of inhibitory action on metalloaminopeptidases. Their results showed that Bestatin did not have a toxic effect directly on the parasite, but it had a substantial effect on the dipeptide pool, demonstrating its action as an inhibitor compound of dipeptidase enzymes [172]. In conclusion, the authors attested that the metabolomic platform has great potential for *in situ* analysis of enzymatic inhibition by pharmacological agents, and is an alternative for the evaluation of metabolic changes that occur after the exposure to a compound [172].

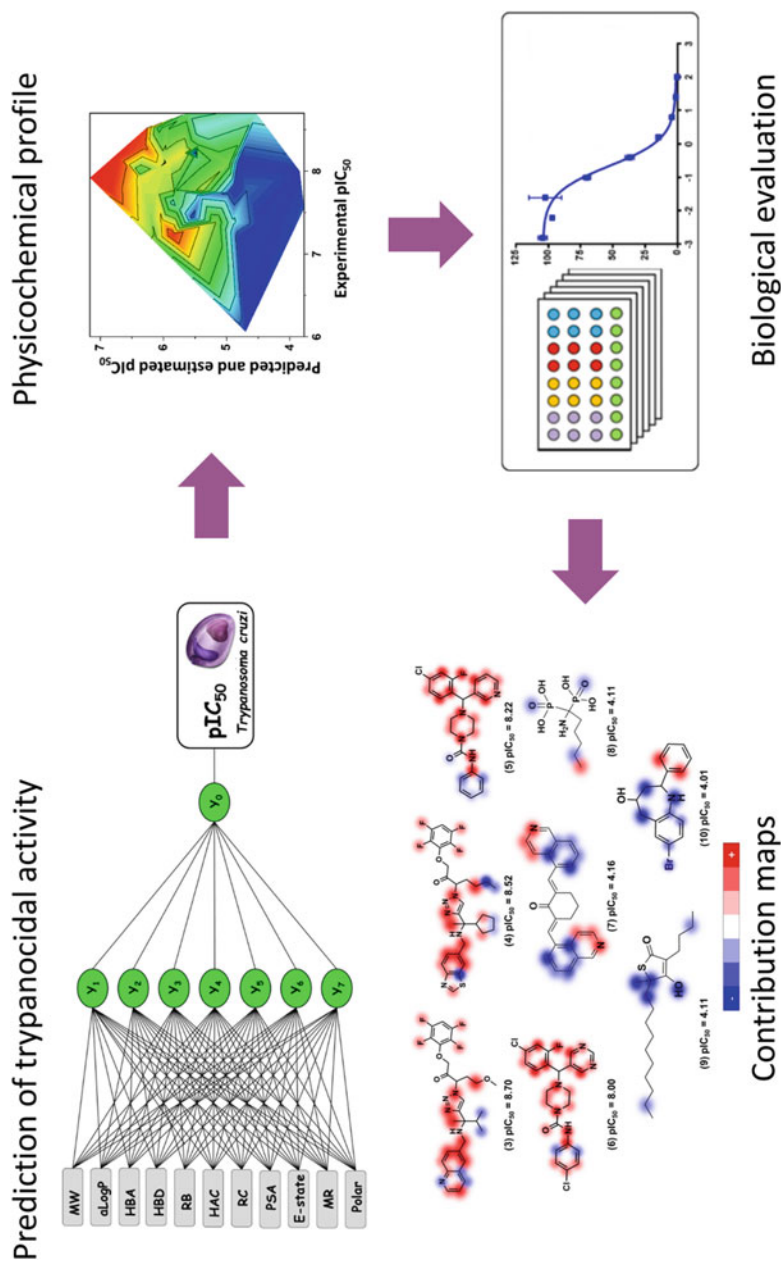


Fig. 3 Proposed workflow for the design of new anti-*Trypanosoma cruzi* compounds based on the generation of contribution maps, prediction of trypanocidal activity using artificial neural networks and analysis of physical-chemical properties [169]

In a study on the metabolic changes that occur during the phases of exponential and stationary growth in *T. cruzi* epimastigotes, Barisón et al. studied the 47 metabolic intermediates of the most important pathways for energy metabolism and oxidative imbalance [173]. As a result, the authors showed for the first time that *T. cruzi* epimastigotes exhibit an adaptive metabolic mechanism that allows alternating the consumption of glucose to amino acids, in the transition from the exponential to the stationary phase. Mosquillo et al. carried out the first work that combined massive data from transcriptome and translome, aiming to unravel the mechanism of action of two organometallic compounds (Pd-dppf-mpo and Pt-dppf-mpo) with trypanocidal activity [174]. As a result, the authors identified modified and/or metabolic enzymes present in the parasite, but absent in the mammalian host, which may become targets for rational drug planning. In a study conducted by Zrein and Chatelain on monitoring the status of CD in infected patients, the authors state that despite the great effort in basic research that seeks to help in the management of CD, to date there has been little translation into available products [175]. The authors pointed out that disease control becomes even more challenging due to the lack of methods that allow the effectiveness of treatments to be evaluated safely. Currently, patients infected with *T. cruzi* are diagnosed using antibody detection methods in serological assays [175]. However, the authors claim that it is essential to change the paradigm of serological methods in order to be able to monitor the elimination of the parasites correctly. Thus, they argued that analyzing the diversity of antibodies is more informative of the clinical status than the conventional serological tests that are designed for global detection of antibodies [175].

Therefore, to date, there are relatively few validated drug targets for CD. As seen in this section, the combination of different types of omics data (proteomics, genomics, transcriptomics, metabolomics, etc.) is necessary for a correct understanding of the functioning of the disease. As well as the resolution of how environmental factors and culture media can change the characteristics of the parasite under study. Thus, understanding the host–parasite relationship is fundamental for extracting useful information for the development of an effective drug against CD.

6 Perspectives: Challenges in New Drugs Discovery

Several recent reviews in the literature discuss this important point about the challenges present in new drugs discovery [16, 30, 56, 176–180].

According to the roadmap of Echeverría et al., prevention, diagnosis, and treatment are the three major levels of intervention for which it is necessary to seek solutions [1]. Progress has been made in the purpose of new drug discovery for treatment of CD. Parameters that need to be improved have been defined and joint actions bringing companies, support entities, and academies together have been established [181]. A successful drug discovery campaign typically takes 10–15 years [30, 56].

However challenges are still present such as a drug effective against the acute and chronic phase, difficulties in the translation process, genetic diversity of *T. cruzi*, development of standard tests to monitor the course of treatment with drugs to certify the cure of CD among other factors [16, 182]. New potential and promising targets, new methodologies including animal models of tropical disease infections that represent human disease are needed. Evolution in the standardization, as well as innovative strategies, is currently being developed to improve all process related to drug discovery [183]. Proteomic studies provide information on the pathogenic mechanisms of CD and other neglected tropical diseases, identifying molecular targets for drug discovery and development promising biomarkers to detect both stages of the disease and for potential use in diagnosis [184].

We can consider that despite all the difficulties, the panorama of discovering new drugs has improved in these years. A better understanding of the problems and challenges and greater integration between the pharmaceutical industries, academics, governmental and non-governmental intuitions are accelerating all the processes involved in developing new drugs to find the best treatment for Chagas disease and other neglected diseases.

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Compliance with Ethical Standards **Conflict of Interest:** The authors declare that they have no conflict of interest.

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