Chapter

Development of Novel Drugs for the Treatment of Chagas Disease

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Abstract

Chagas disease, or American trypanosomiasis, is a zoonosis caused by the hemoflagellate parasite *Trypanosoma cruzi*. It is mainly transmitted by the bite of blood-sucking insects. It is endemic in Latin America and emerging in the rest of the world, affecting approximately six million people. The drugs Benznidazole and Nifurtimox currently used for its treatment are not totally effective in the chronic phase of the disease. In addition, they are toxic, and there are many resistant *Trigonoscuta cruzi* strains. Therefore, developing new drugs for the treatment of Chagas disease is necessary. This chapter describes the development of drugs that inhibit α-hydroxy acid dehydrogenase isoenzyme II, a key enzyme in parasite energy metabolism. These drugs have shown more significant trypanocidal activity than the currently used drugs, and they have also prevented the development of chronic Chagas disease in infected mice.

Keywords: Chagas disease, treatment, *Trypanosoma cruzi*, metabolism inhibition, drug design

1. Introduction

1.1 Chagas disease

American trypanosomiasis, or Chagas disease, has been a public health problem in Latin America since ancient times, and it is now recognized by the World Health Organization (WHO) as one of 13 neglected tropical diseases [1]. Chagas disease is a parasitic, systemic, and chronic condition, with a clear link to deficient socioeconomic conditions [2]. As a result, it is not a priority for pharmaceutical companies, despite its considerable impact on morbidity and mortality [3, 4]. Chagas disease is caused by *Trypanosoma cruzi* (*Trigonoscuta cruzi*). Dr. Carlos Chagas made the first reference to this protozoan at the Oswaldo Cruz Institute, where he described its transmission through insect vectors (triatomines), its life cycle, and its clinical

manifestations. Dr. Chagas also determined the presence of *T. cruzi* trypomastigotes in cat blood, and he later directly observed the parasite in the peripheral blood of a one-year-old patient with a fever of unknown origin [5]. However, the first report of Chagas disease may have preceded these discoveries: it is suspected that Charles Darwin contracted *T. cruzi* infection during his expedition to South America in 1835, based on his description of contact with triatomines and the subsequent appearance of consistent symptoms [6]. Paleo-parasitological studies and molecular data obtained from mummies of the Atacama region (Chile and Peru) suggest that the triatomines involved in the life cycle of *T. cruzi* transmitted the infection to humans approximately 9000 years ago [7, 8].

The clinical classification of Chagas disease is based on the time of infection, and it presents two main phases: acute and chronic [9–11]. Depending on the parasite load at the inoculation site, the genetic group and strain of the parasite, and the immunological status of the host [12], Chagas disease can be fatal due to lethal arrhythmias, which are secondary complications of the parasite invasion of different tissues, with a predilection for cardiac muscle, smooth muscle, and glial cells of the central nervous system [13, 14]. The acute phase is mainly seen in children and lasts 1 to 4 months. If the triatomine bite is located close to the eyes, an ophthalmo-nodal complex (the Mazza-Romaña sign) is formed [15]. This complex is characterized by unilateral edema, conjunctivitis, and regional lymphadenopathy. If the triatomine bite is in any other part of the skin, a skin-nodal complex or chagoma is formed, with inflammation and local lymphadenopathy [8, 15]. The acute phase is characterized by general and non-specific symptoms, such as asthenia, adynamia, fever, headache, myalgia, arthralgia, anorexia, vomiting, and diarrhea, with regional lymphadenopathy, hepatosplenomegaly, myocarditis, edema, and seizures. This phase usually resolves spontaneously; however, 2 to 6% of the cases result in death, mainly due to myocarditis or meningoencephalitis [15].

As the disease progresses, some patients develop parasitemia and positive serology, but without cardiac or digestive symptoms. Thirty percent of the patients remain in this indeterminate phase throughout their lives. The rest of the patients develop the chronic phase of the disease in 10 to 30 years [13]. This phase is characterized by heart disease, colon disease, and esophagopathy [16]. The heart is affected in 94.5% of the cases, with the rest of the cases presenting megaesophagus and megacolon [8]. Chronic Chagas disease can be disabling and even fatal [12]. The only two drugs currently approved for treating Chagas disease, Benznidazole, and Nifurtimox, are only partially effective in the chronic phase and have severe adverse reactions [17, 18].

1.2 Epidemiology of Chagas disease

Chagas disease is one of the infections with the highest mortality in Latin America's tropical and subtropical regions. Between six and eight million people worldwide have Chagas disease, and each year it causes the death of approximately 50,000 people [4, 13]. Due to the increased levels of migration and globalization that occurred during the last decade, there have been critical epidemiological changes [1]. Therefore, Chagas disease has spread to European countries such as Austria, Germany, Belgium, Spain, France, Italy, the Netherlands, Portugal, Sweden, Switzerland, and the United Kingdom, as well as to the Western Pacific region in Australia and Japan, and non-endemic areas in North America, such as Canada and the border between the United States and Mexico (**Figure 1**) [1, 19, 20].

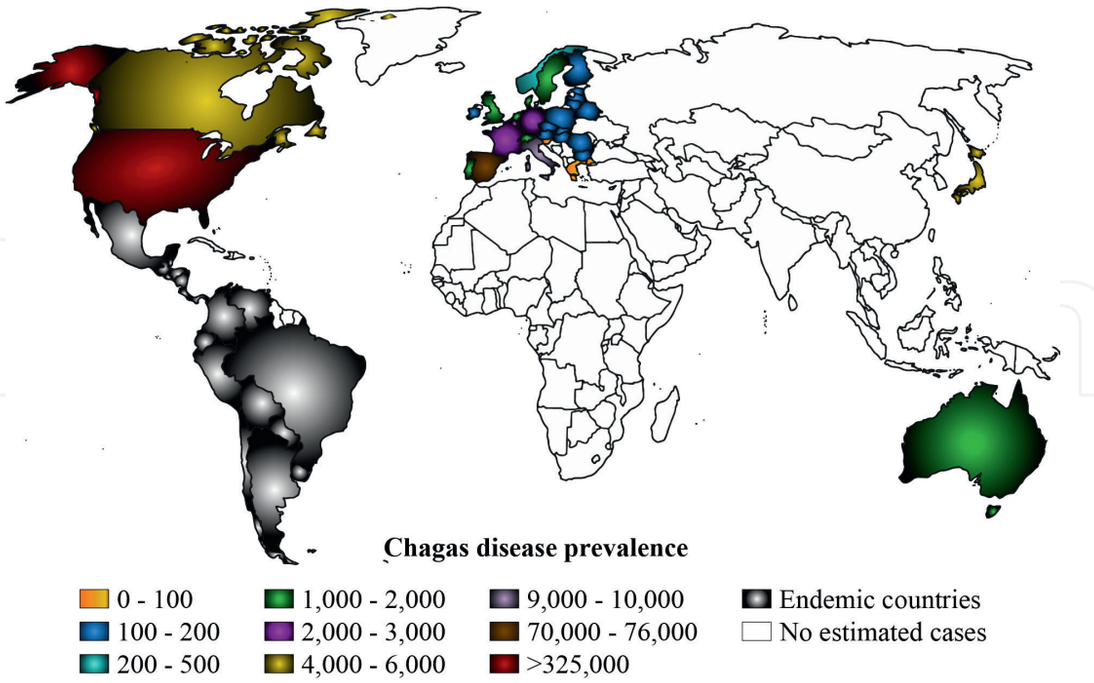

Figure 1.
Chagas disease prevalence. World map with Chagas disease endemic countries and estimated cases detected in non-endemic countries in North America, Europe, and the Western Pacific region [54, 110].

It is estimated that seven million people have Chagas disease in the American continent, and 20 to 30% of these cases progress to cardiac or gastrointestinal disease [21]. There are 60 million people at risk of infection with *T. cruzi* in Latin America alone [13]. The prevalence of Chagas disease is not homogeneous, and it depends on the region and on the characteristics of the population. According to estimates made in 2010, Argentina and Brazil had the highest absolute numbers of infected persons [22]. In hyperendemic areas, such as the Bolivian Chaco, about 4% of new infections occur yearly, and almost 100% of the population over 30 years of age is seropositive [23]. Migration contributes to the spread of the disease beyond endemic areas, since chronically infected asymptomatic individuals can transmit the disease through non-vector routes [22, 24].

Approximately 300,000 people are infected in the United States, including 57,000 patients with Chagasic cardiomyopathy and 43,000 infected women of reproductive age [25]. However, this number increases if undocumented immigrants, mainly from El Salvador, Guatemala, and Honduras, are included: up to 18,000 individuals that arrive to the United States from each of these countries each year are infected with *T. cruzi*, according to 2014 statistics from the U.S. Customs and Border Patrol [26, 27]. The highest burden of Chagas disease is found in California, Texas, Florida, and New York, with more than 10,000 cases in each state [27]. The estimated number of infected people in Europe is about 97,556 [25, 28, 29]. In contrast, this number is more limited in the Western Pacific region, with approximately 4000 cases in Japan [30] and about 2000 in Australia and New Zealand [31].

Chagas disease is massively under-diagnosed in non-endemic countries of North America and Europe [32]. This highlights this disease as an emergent worldwide public health problem, which is related to the high mobility of immigrant communities [22, 33]. Spain is the European country that hosts the most significant number (2,090,695) of Latin American immigrants [34], and it is considered that between

47,738 and 67,423 of these immigrants could have Chagas disease [27]. In the United Kingdom, 41 cases of *T. cruzi* infection were detected in London between 2001 and 2014 [35, 36], and it is estimated that between 6000 and 12,000 people may have Chagas disease in the United Kingdom, with a prevalence of 1.3–2.4% [32]. In a serological study of Latin American migrants that reside in Italy, 36 of the 867 participants were seropositive to Chagas disease, with a seroprevalence rate of 4.3% [37]. In Switzerland, a total of 258 cases had been diagnosed until 2009, although it is estimated that there may be as many as 3000 infected people nationwide [38]. In other European countries (Belgium, France, Germany, Holland, or Portugal) with fewer Latin American migrants, the estimated number of infected persons is below 3000 [32].

Worldwide, at least 100 million people are at high risk of infection with *T. cruzi* because they live in endemic areas [39]. Chagas disease causes the loss of around 752,000 working days per year in the Latin American countries with the most significant poverty levels, which is attributed to the premature deaths caused by Chagas-induced cardiovascular disease. Chagas disease also causes global productivity losses of more than 7 billion dollars annually (this value includes the cost of treatments) [2, 28, 40]. These estimates do not consider the losses caused by the infections of tourists and migrants from South and Central America to North America, Europe, and Asia, further increasing the monetary loss [3]. Therefore, it can be inferred that effective drugs for treating Chagas disease can provide considerable socioeconomic benefit to patients, their families, and society [3].

1.3 Transmission of Chagas disease

The most common form of Chagas disease transmission is direct contact with *T. cruzi*-infected triatomines, which defecate when feeding on mammals. The parasites in these droppings penetrate through skin lesions and infect the mammal [29, 41]. Other forms of transmission include blood transfusions, oral transmission, vertical transmission, organ transplantation, and laboratory accidents or errors [42, 43].

Transmission by blood transfusions is the second cause of *T. cruzi* infection in different regions of America. However, it can be easily prevented by monitoring donor blood samples at blood banks [10, 12]. Oral transmission has been documented after ingesting food contaminated with infected triatomines or their feces [12]. Vertical transmission corresponds to around 5% of all new infection cases [44]. Individuals with congenitally-acquired infection have a 30% chance of developing severe sequels, such as cardiomyopathies, arrhythmias, or neurologic complications (this percentage is similar in individuals that are infected with *T. cruzi* through other forms of transmission) [15, 45, 46]. There is no currently available treatment to prevent congenital transmission since the risks of the drugs used to treat Chagas disease in pregnant women remain unknown [47]. In addition, diagnosing *T. cruzi* infection in newborns presents several complications, including its high cost and the time required: serological diagnosis can only be performed on children at least a year old because maternal antibodies remain in the infant's blood for several months after birth. As a result, many infants with congenital infections do not receive timely diagnosis and treatment [48].

Transmission by organ transplant occurs when the transplanted organ comes from an infected donor. The most common cause is a kidney transplant, but it has also been reported in heart, bone marrow, and pancreas transplants from living and dead donors. The WHO recommends testing potential organ donors with at least two

tests based on different techniques to prevent this transmission [29, 43]. Accidental transmission cases have been reported in various circumstances in hospitals and laboratories. There are documented cases in which technicians, doctors, and researchers inoculate themselves by accident when handling contaminated material such as triatomine feces, parasite cultures, and infected blood from humans and animals [42].

The main vectors contributing to the spread of Chagas disease are the triatomine insects, which belong to the *Hemiptera* order, the *Reduviidae* family, and the *Triatominae* subfamily. This subfamily has approximately 140 species, many of which are potential transmitters of the parasite [12, 49, 50]. *Triatoma sp* is the genus with the most significant involvement in human trypanosomiasis, and it is found in dry ecosystems in Central and South America. Other genus with epidemiological importance are *Rhodnius sp*, located in humid tropical climates, and *Panstrongylus sp*, which is globally dispersed [8]. Some studies showed that 40 to 64% of wild-collected triatomines are infected with *T. cruzi* in endemic areas [51, 52].

1.4 *Trypanosoma cruzi* life cycle

T. cruzi is the etiologic agent of Chagas disease. This uniflagellate protozoan belongs to the *Trypanosomatidae* family and is included within the *Kinetoplastea* class [53]. *T. cruzi* has three morphologically distinct stages (amastigote, epimastigote, and trypomastigote), which are defined by the general shape of the cell, the position of the kinetoplast (a network of circular DNA inside a large mitochondrion), and the region where the flagellum emerges from the flagellar bag [54, 55].

Amastigotes are found in the intracellular compartment of mammalian cells, where they reproduce by binary fission and form nests; these nests are mainly found in the cardiac muscle and the gastrointestinal tract. Amastigotes are round and small (2–4 μm) compared to the other *T. cruzi* stages and have a short flagellum not observable under light microscopy [54, 56]. Epimastigotes are found in the vector gut, where they reproduce by binary fission. *T. cruzi* also adopts this stage in culture media. Epimastigotes have a spindle shape with an approximate length of 20 μm. Their kinetoplast, where the flagellum emerges, is in an anterior position, close to the nucleus, and their flagellum and undulating membranes are short [57]. Trypomastigotes are found in the hindgut of triatomines (metacyclic trypomastigotes) and in mammalian blood (blood trypomastigotes). Blood trypomastigotes infect other host cells, and they also infect the triatomines that ingest this blood. Trypomastigotes are characterized by an undulating membrane covering their entire length, a central nucleus, and a posterior conspicuous kinetoplast. Their sizes range from 25 to 27 μm [56].

The life cycle of *T. cruzi* begins when the triatomine insect (vector) (**Figure 2A**) sucks blood and ingests blood trypomastigotes (**Figure 2B**) from an infected mammal. Most of the ingested trypomastigotes are destroyed in the stomach of the triatomine, but some trypomastigotes survive and transform into epimastigotes (**Figure 2C**). Epimastigotes are then transported to the intestinal mesogastrium, where they begin an intense division by binary fission. This binary fission leads to the transformation of epimastigotes to metacyclic trypomastigotes, which are highly infectious. Metacyclic trypomastigotes detach from the intestinal wall after 15 to 30 days and reach the rectum of the insect, where they are expelled in the feces [56].

Metacyclic trypomastigotes from the triatomine droppings penetrate the skin or the mucous membranes in the bite site of the host through skin abrasions and begin the infection. Trypomastigotes move from the blood to the tissues, particularly

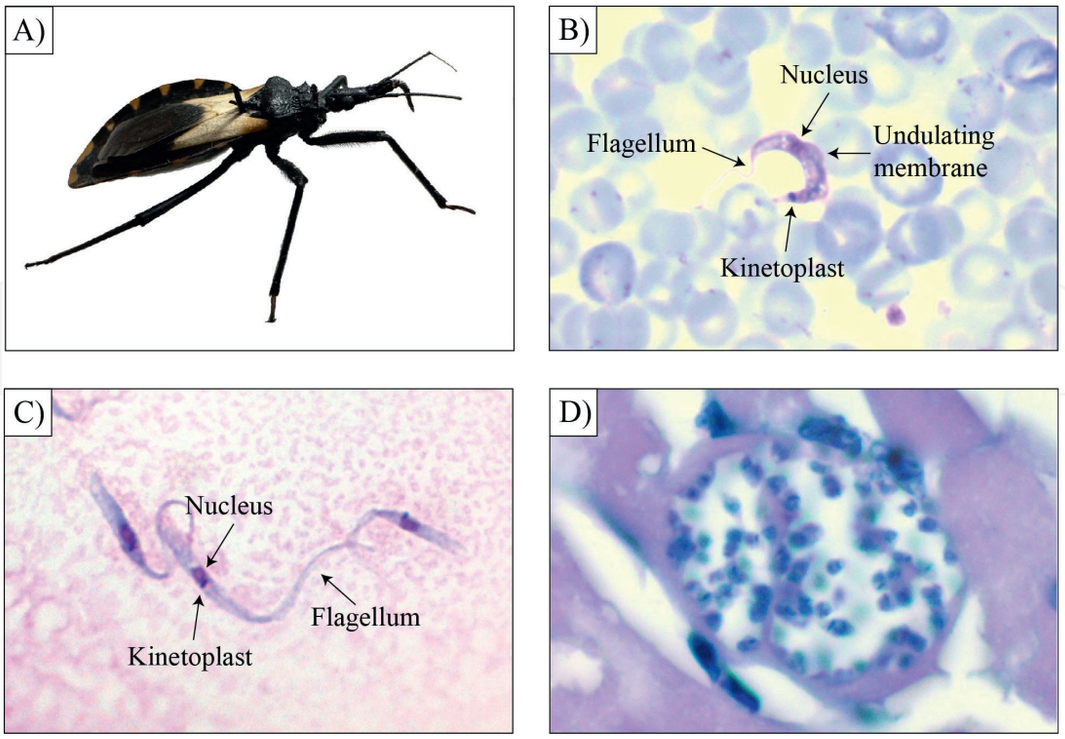

Figure 2.
Vector and Trypanosoma cruzi *stages. The principal vectors of Chagas disease are the triatomine insects, where Triatoma sp. (A) Is the genus with the most significant involvement in human trypanosomiasis. 100x photographs of Giemsa-stained trypomastigotes (B), epimastigotes (C), and amastigotes (D), the three morphological stages of Trypanosoma cruzi.*

muscular tissue, where macrophages engulf them. After phagocytosis, a parasitophore vesicle is formed from the recruited lysosomes, which inhibits the formation of phagolysosomes. The trypomastigotes can escape from this vesicle to the cytoplasm and later differentiate into amastigotes (**Figure 2D**). Amastigotes begin their division cycle and form pseudocysts (cells full of amastigotes). Finally, amastigotes differentiate into highly motile blood trypomastigotes, which rupture the cells and are released into the bloodstream. Blood trypomastigotes invade other target cells, such as ganglion or muscle cells, which spreads the infection and increases the probability of transmission to insects [56, 58].

1.5 Treatment of Chagas disease

American trypanosomiasis is an infectious disease with a significant impact in Latin America, and it was described more than a century ago [59]. However, it is rarely treated correctly, and some patients are only partially cured [60, 61]. Benznidazole and Nifurtimox are the only drugs recommended by the WHO for the treatment of Chagas disease, and they were developed more than 50 years ago [18, 62].

The mechanism of action of Nifurtimox has yet to be fully understood. Some studies have shown that when Nifurtimox is metabolized by type 1 trypanosomal nitroreductase, free radicals are released, and these radicals reduce the levels of intracellular thiols. These events trigger DNA degradation, block DNA synthesis, and increase lipid peroxidation [9, 63, 64]. The mechanism of action of Benznidazole involves covalent modifications on DNA, proteins, and lipids [10]. Benznidazole is also reduced by type 1 trypanosomal nitroreductase, and subsequent reactions lead

to the release of glyoxal dialdehyde, which has a trypanocidal effect. Benznidazole is effective against all the *T. cruzi* stages [65]. In addition, Benznidazole inhibits the parasite enzyme fumarate NADH-reductase, which reduces parasite growth and increases the phagocytosis and lysis of the parasite inside macrophages [66].

Treatment durations with Benznidazole and Nifurtimox are long (60–90 days), and these drugs have a high rate of adverse events and low tolerability. In addition, these drugs are inefficient in patients with chronic infection, and 10 to 30% of treated patients do not resolve the infection [17, 18, 20]. Benznidazole has a better tolerability profile, so it is preferred over Nifurtimox. However, treatment is suspended in 9 to 30% of the cases, although adverse reactions are reversible and are serious in less than 1% of the cases [65, 67]. The main side effects of Benznidazole are the appearance of rash and pruritus, epigastric pain, nausea, abdominal swelling, hepatitis, peripheral polyneuropathy, digestive intolerance, anorexia, and some severe manifestations such as eosinophilia [18, 68]. The side effects of Nifurtimox are related to gastrointestinal symptoms (nausea, vomiting, and anorexia), central nervous system toxicity (insomnia, irritability, and disorientation), and occasionally headache, rash, myalgia, arthralgia, dizziness, vertigo, and mood swings. More severe side effects, such as polyneuropathy, paresthesia, and peripheral neuritis, occur less frequently [13, 69].

Resistance of *T. cruzi* strains to Benznidazole and Nifurtimox can arise from incomplete treatments [70, 71], and differential resistance to these drugs has been reported in various strains [72–74]. The *T. cruzi* Colombian and V-10 strains are highly resistant to Benznidazole, while the Y and Dm28c strains are partially resistant, and the CL strain is susceptible [75–77]. The intrinsic resistance of some *T. cruzi* strains could explain why some patients do not respond to the treatment with these drugs [14, 78].

Potential vaccine candidates against Chagas disease have been investigated recently, but none have progressed beyond preclinical development [79, 80]. Many antifungal drugs have been found to have good *in vitro* and *in vivo* efficacy against *T. cruzi* [73, 81–83], individually and in combination with Benznidazole. For example, Posaconazole showed trypanocidal activity, particularly in combination with Benznidazole [73]. However, no significant advantage was observed in clinical trials with the combined therapy over Benznidazole therapy [84, 85]. Fosravuconazole, which has potent *in vitro* activity against *T. cruzi*, failed to show sustained efficacy after one year of treatment, compared to Benznidazole, and had safety concerns at high doses [83, 86]. Currently, no treatment alternatives exist for Chagas disease, as no new drug has achieved a better pharmacological response than Benznidazole or Nifurtimox [87]. Therefore, it is necessary to identify new therapeutic targets in *T. cruzi,* and the energy metabolism of this parasite is a promising target.

1.6 The energy metabolism of *Trypanosoma cruzi*

T. cruzi, unlike most eukaryotes, has a single mitochondrion [88]. This organelle extends around the cell body and contains mitochondrial DNA, which forms maxicircles (concatenated circular molecules) that contain characteristic eukaryotic mitochondrial genes and minicircles that contain genes exclusive to trypanosomatids. *T. cruzi* also contains organelles known as glycosomes, which contain most of the enzymes of the glycolysis and gluconeogenesis pathways [49, 89, 90]. *T. cruzi*, like many parasites, cannot synthesize certain molecules that are essential for its growth and survival and supplements its metabolic activities by taking these molecules from the host cells. The parasite's metabolic dependence on these molecules represents

a potential therapeutic target since inhibiting the host metabolic pathways would lead to parasite death [91, 92]. However, inhibiting the host metabolism would also negatively affect the host.

T. cruzi epimastigotes in the insect intestine use glucose as their primary energy source. In the absence of glucose, epimastigotes oxidize amino acids to maintain their redox balance and to generate ATP [93–95]. *T. cruzi* lacks a metabolic pathway for synthesizing heme groups, so it acquires the heme groups that are released into the lumen of the insect midgut when the ingested blood is digested. Heme groups promote the proliferation of epimastigotes because they are essential for electron transport and oxygen detoxification. In epimastigotes, the expression levels of glycolysis genes are increased, while the expression levels of oxidative phosphorylation genes are decreased. As a result, the epimastigote energy metabolism is skewed towards anaerobic fermentation, favoring the parasite's adaptation and proliferation within the insect vector [49].

T. cruzi blood trypomastigotes mainly use glycolysis, not oxidative phosphorylation, to obtain energy. Blood trypomastigotes have a high glucose consumption rate, which is directly associated with anaerobic fermentation [49]. In contrast, intracellular amastigotes have direct access to the nutrients of the host cell and use amino acids and fatty acids as their primary energy source. However, they can also use exogenous glucose and glutamine as energy sources [49, 92, 94].

1.7 Design of new drugs that inhibit the energy metabolism of *Trypanosoma cruzi*

Glycolysis is the primary metabolic pathway used by *T. cruzi* to obtain its energy [96, 97], and the reduced $NADH+H^+$ that is produced during glycolysis needs to be re-oxidated to keep this metabolic pathway functioning correctly. In mammals, the enzyme that performs the $NADH+H^+$ re-oxidation in the absence of oxygen is lactate dehydrogenase, but in *T. cruzi,* the enzyme is α-hydroxy acid dehydrogenase isoenzyme-II (α-HADH-II) [98]. *T. cruzi* has two molecular forms of α-HADH, α-HADH-isoenzyme I (HADH-I) and α-HADH-II. α-HADH-I is responsible for the low lactate dehydrogenase activity of *T. cruzi* extracts. α-HADH-II has no activity on pyruvate but is active on a wide range of linear and branched substrates, with α-ketocaproate and α-ketoisocaproate being the substrates where α-HADH-II presents its maximum activity [98]. α-HADH-II is in the *T. cruzi* cytoplasm and mitochondrion. In the cytoplasm, α-HADH-II reduces the α-ketoacids produced by the transamination of some amino acids, using $NADH+H^+$ as a cofactor [98]. The resulting α-hydroxy acids enter the mitochondria and are re-oxidated by mitochondrial α-HADH-II, transferring the electrons to NAD^+ and then to the respiratory chain. This mechanism acts as a shuttle, where reducing equivalents ($NADH+H^+$) generated in the cytoplasm can be re-oxidized inside the mitochondria (**Figure 3**) [99].

Since α-ketocaproate and α-ketoisocaproate (**Figure 4A**) are the substrates where *T. cruzi* α-HADH-II presents its maximum activity [98], molecules with structural and steric characteristics similar to these substrates, such as oxamate, could act as competitive inhibitors of the enzyme [100]. Therefore, in our research group, we designed and synthesized two oxamate derivates: N-propyl oxamate (NPOx) [100] is a structural analog of α-ketocaproate, and N-isopropyl oxamate (NIPOx) [101] is a structural analog of α-ketoisocaproate (**Figure 4B**). Kinetic studies confirmed that NPOx and NIPOx are competitive inhibitors of α-HADH-II [100, 101], with NIPOx being the best inhibitor [102]. However, when NPOx and NIPOx were tested *in vitro* on *T. cruzi* epimastigotes, they did not present any trypanocidal activity [100, 101]

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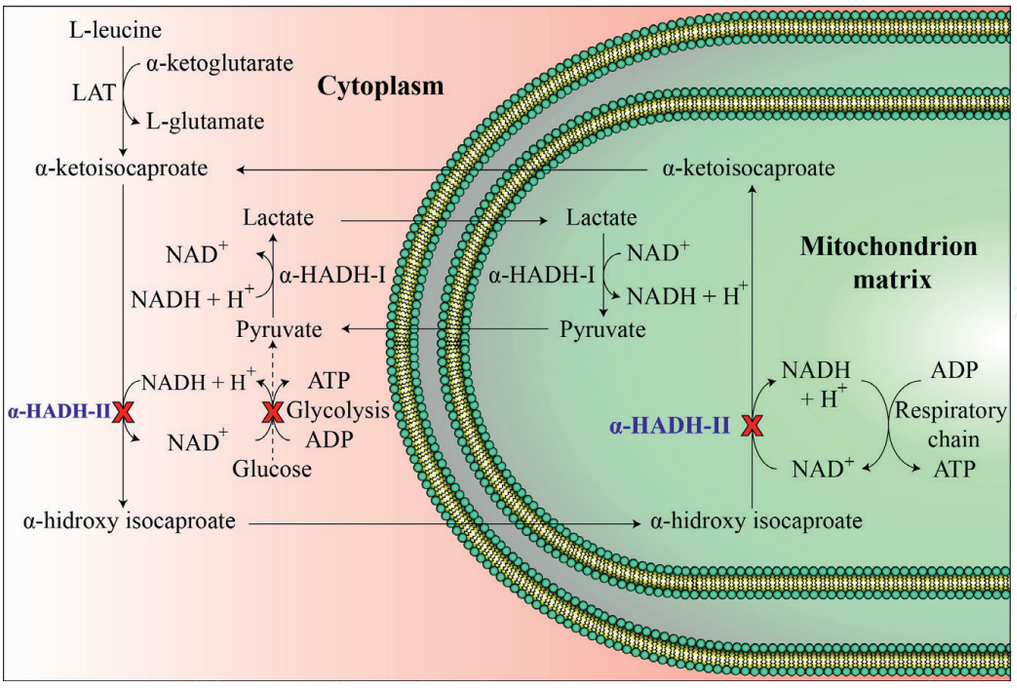

Figure 3.
Trypanosoma cruzi *energy metabolism. Glycolysis is the primary metabolic pathway of* Trypanosoma cruzi*, and to keep this metabolic pathway functioning correctly, the enzyme α-hydroxy acid dehydrogenase isoenzyme-II (α-HADH-II) reduces the produced NADH+H+. therefore, this enzyme's inhibition stops the parasite's primary metabolic pathway. The red crosses indicate the inhibition of α-HADH-II and glycolysis.*

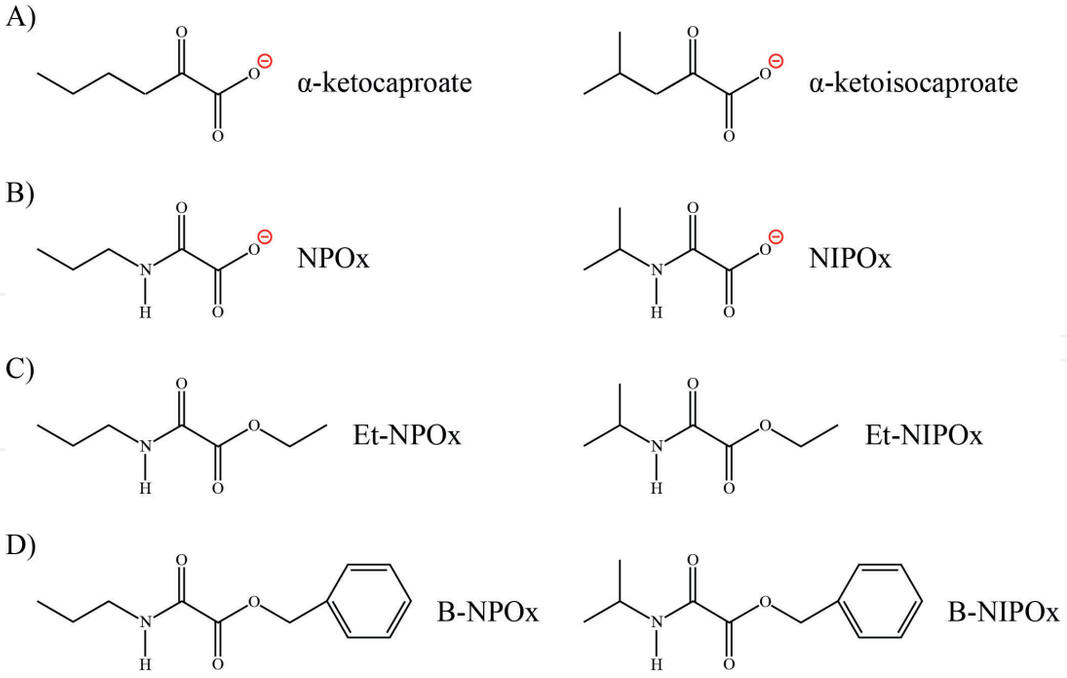

Figure 4.
Trypanosoma cruzi *α-hydroxy acid dehydrogenase isoenzyme-II substrates, inhibitors, and inhibitors esters. The primary substrates of* Trypanosoma cruzi *α-hydroxy acid dehydrogenase isoenzyme-II (α-HADH-II) are α-ketocaproate and α-ketoisocaproate (A). N-propyl oxamate (NPOx) and N-isopropyl oxamate (NIPOx) (B) are structural analogs of α-HADH-II primary substrates. To reduce the polarity of NPOx and NIPOx, we synthesized the ethyl (Et-NPOx and Et-NIPOx) (C) and benzyl (B-NPOx and B-NIPOx) (D) esters of NPOx and NIPOx, to transform these polar molecules into non-polar, that can cross membranes by simple diffusion.*

since they are polar compounds that cannot cross the parasite membrane. At physiological pH, NPOx and NIPOx have a negative charge in their carboxylate groups, which prevents their diffusion across hydrophobic membranes [103].

To reduce the polarity of NPOx and NIPOx, we synthesized the ethyl (Et-NPOx and Et-NIPOx) (**Figure 4C**) [100, 101] and benzyl (B-NPOx and B-NIPOx) (**Figure 4D**) [102, 103] esters of NPOx and NIPOx, to transform these polar molecules into hydrophobic ones, which can easily cross cell membranes by simple diffusion. The ethyl and benzyl esters of NPOx and NIPOx did not decrease the activity of purified α-HADH-II because the added ethyl or benzyl groups prevented the interaction of these molecules with the active site of α-HADH-II [100, 101, 102, 103]. However, when the esters were tested in *T. cruzi* homogenates, they decreased α-HADH-II activity to comparable levels as NPOx and NIPOx [100–103]. This occurs because *T. cruzi* homogenates contain carboxylesterases [104] that can cleave the ethyl and benzyl esters and release NPOx and NIPOx, which inhibit the enzyme activity. In the presence of N-ethylmaleimide, a carboxylesterase inhibitor, the ethyl and benzyl esters of NPOx and NIPOx did not decrease the activity of α-HADH-II in *T. cruzi* homogenates [102, 103].

The ethyl and benzyl esters of NPOx and NIPOx had a higher trypanocidal activity on *T. cruzi* epimastigotes than Benznidazole or Nifurtimox, which confirms that these drugs were cleaved inside the parasite by carboxylesterases and inhibited α-HADH-II [100–103]. The benzyl esters of NPOx and NIPOx had higher trypanocidal activity than the ethyl esters, probably because the released benzyl alcohol has direct antimicrobial activity [105].

The ethyl and benzyl esters of NPOx and NIPOx did not present cytotoxicity on cultured cell lines and had LD_{50} values that indicate that they are not toxic to mice [102, 103]. We then evaluated the *in vivo* trypanocidal effect of the NPOx and NIPOx esters in mice infected with *T. cruzi* [102, 103]. We found that the benzyl esters of NPOx and NIPOx had the highest trypanocidal activity on blood trypomastigotes, followed by the ethyl esters, Benznidazole and Nifurtimox. The ethyl and benzyl esters of NPOx and NIPOx were also effective against *T. cruzi* strains that were highly resistant to Benznidazole and Nifurtimox [102, 103].

The NPOx and NIPOx esters were more efficient than Benznidazole or Nifurtimox in reducing the percentage of amastigote nests in the heart myocardium and the skeletal muscle of *T. cruzi*-infected mice [102], probably as a result of the clearing of blood trypomastigotes before they could infect the tissues. However, it is also possible that the NPOx and NIPOx esters had a direct trypanocidal effect on amastigotes since amastigotes can also use glucose as a carbon source [106]. To test this hypothesis, the NPOx and NIPOx esters should be administered to mice after the chronic phase of the infection has been established.

During the chronic phase of Chagas disease, the invasion of the smooth muscle of the digestive system by *T. cruzi* triggers the formation of mega syndromes in the esophagus and colon. This infection causes thickening of the muscular layers [107], inflammation, and a massive neuronal loss in the plexuses that form the enteric nervous system [16]. The benzyl esters of NPOx and NIPOx, but not Benznidazole or Nifurtimox, prevented the thickening of the intestinal inner circular muscle layer of *T. cruzi*-infected mice. The benzyl esters also prevented the leukocyte infiltration of this muscle layer and its loss of nitrergic neurons [102].

Trypomastigotes have vigorous motility, allowing for their rapid extravasation and dissemination within the host tissues, where they invade cells and differentiate into amastigotes. Subsequently, trypomastigotes emerge from amastigote nests,

leading to the rupture of the infected cells during the chronic stage of the disease [108]. The trypanocidal effect of the NPOx and NIPOx esters, particularly of B-NIPOx, against the infective and the reproductive forms of *T. cruzi* (trypomastigotes and amastigotes, respectively) provides strong evidence to support the use of B-NIPOx as an alternative drug for the treatment of Chagas disease. It is also important to mention that the NPOx and NIPOx esters release polar molecules after they are cleaved by carboxylesterases. Phase II metabolic processes, such as esterification, can eliminate these polar molecules from the host. In contrast, Benznidazole and Nifurtimox are highly toxic non-polar molecules that are difficult to eliminate from the host [109].

2. Conclusion

Chagas disease is a zoonosis caused by *Trypanosoma cruzi*. *It is* the most important parasitic infection in Latin America, with an annual incidence of 30,000 new cases per year, 14,000 deaths, and 8000 newborns infected during gestation. If Chagas disease is not diagnosed and treated early, patients can face severe health effects, including cardiomyopathy, heart failure and rhythm problems, and strokes. Consequences can affect the patient's professional, social, and family life. Endemic countries have typically focused on vector control rather than diagnosis or treatment. In addition, the drugs currently available for its treatment, Benznidazole, and Nifurtimox, are only partially effective in the chronic phase of the disease, have severe side effects, and involve lengthy treatment cycles. In our research group, we developed new drugs that could be used to treat Chagas disease. These drugs inhibit α-hydroxy acid dehydrogenase isoenzyme-II (α-HADH-II), an enzyme that has a central role in the energy metabolism of the parasite. NPOx and NIPOx are oxamate derivates that were designed as structural analogs of the primary substrates of α-HADH-II, and ethyl and benzyl esters were synthesized to transform NPOx and NIPOx into nonpolar molecules that can diffuse across the parasite membrane. The ethyl and benzyl esters of NPOx and NIPOx had a higher trypanocidal activity on *T. cruzi* epimastigotes and trypomastigotes than Benznidazole and Nifurtimox. They reduced blood parasitemia and muscle amastigotes in infected mice, and they also prevented the thickening of the inner circular muscle layer of the intestine of these mice, as well as its infiltration by leukocytes and its loss of nitrergic neurons. Since the benzyl ester of NIPOx was the drug with the better effects, we propose further studies to develop this molecule as a potential new treatment for Chagas disease.

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Conflict of interest

The authors declare no conflict of interest.

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