European Review for Medical and Pharmacological Sciences 2019; 23: 2576-2586

Developments on treatment of Chagas disease – from discovery to current times

T.O. CUSTODIO LEITE

Universidade Federal Fluminense, Instituto de Química, Departamento de Química Orgânica, Programa de Pós-Graduação em Química, Rio de Janeiro, Brazil.

Abstract. – OBJECTIVE: This work aims to collect publications of available drugs for reposition and new substance development against the Chagas disease, since they represent the beginning of a path for new discoveries of viable alternatives to improve the prognosis of millions of patients around the world.

PATIENTS AND METHODS: An extended research on English and Portuguese-language literature in the Scientific Electronic Library Online – Scielo, SciFinder and PubMed – database was made. The bibliography was screened using the keywords "Chagas Disease" and "Treatment".

RESULTS: Despite the low resources available for research and development of drugs against Chagas disease, the knowledge produced in this area is large but not directly proportional to the therapeutic advances. Two categories were analyzed, such as drug repositioning, and new substances were researched.

CONCLUSIONS: Even if great findings were reported, more efforts are necessary to find new therapies against *Trypanosoma cruzi (T. cruzi)*.

Key Words:
Alternatives, Antichagasic, Chagas, Development, Drug reposition. nifurtimox and benznidazole

Introduction

The Chagas disease, also known as American Trypanosomiasis, was discovered by the Brazilian epidemiological physician Carlos Justiniano Ribeiro Chagas in 1909 in Lassance, a municipality located in the state of Minas Gerais, Brazil[1]. This pathology is caused by the flagellated protozoan *Trypanosoma cruzi (T. cruzi)* transmitted by triatomine bugs, has infected 8 million people and is the cause of thousands of premature deaths each year across the Americas, especially in Latin America, where it is endemic[2,3]. It is important to mention that less than 1% of infected people have access to diagnosis and/or treatment[4].

The vector combat is the main prophylactic strategy to control the disease, such as chemical treatment (pesticides) and the improvement of housing conditions[5], since the bug has the habit of living inside the houses, a characteristic of its adaptation of life in the domestic environment. A better screening of blood bags used in blood donation is also involved[6].

It is also relevant to emphasize that, although Chagas disease predominates in countries with low socioeconomic development, increased tourism and migrations have raised the importance of greater investments directed to research and development of more effective treatments besides vaccines, to avoid its dissemination around the world[7].

Trypanosoma Cruzi: Transmission and Life Cycle

The parasite which causes Chagas disease can infect more than 100 mammal species and can be transmitted by over 150 species of *hematophagous* arthropod from the *Triatominae* family, especially *Triatoma, Panstrogylus,* and *Rhodnius*[8,9]. Their nocturnal habits make this period more propitious for disease transmission, since the infected insect, while sucking human's blood, simultaneously defecates and eliminates the protozoa. Because of the abrasion on the skin, caused by individual itching, the protozoa is inoculated to human tissue, and quickly reaches the bloodstream[10,11]. The penetration can also be successful through the intact mucosae, such as conjunctiva[12].

There are other forms of Chagas transmission with major epidemiological relevance also reported on literature, such as blood transfusion, breastfeeding, congenital infection, organ donation or contaminated food – such as sugar cane juice and Brazilian fruit açaí[3,1315]. The exacerbation of oral contamination with food ingestion was also related to treatment with acetylsalicylic acid, because

Corresponding Author: Talita Odriane, Custodio Leite, MD; e-mail: talitacustodio@id.uff.br

of its gastric mucosal damage which facilitates *T. cruzi* infection[16].

The life cycle of this parasite is very complex and involves two different types of hosts: (i) invertebrate host, represented by the *triatomine* bug and (ii) vertebrate host, represented especially by the human. Different developmental stages are related to this parasite as it migrates from one host to another, and four steps of development are needed to complete the parasite stages: (i) replicative epimastigote; (ii) replicative amastigote; (iii) non-replicative trypomastigote, and (iv) blood trypomastigotes[17].

In general, as shown in Figure 1, a triatomine vector insect infected with the parasite in its metacyclic trypomastigote form bites the host to feed on blood and eliminates the trypomastigote forms of the parasite through its faeces, near the site of the bite. Thus, trypomastigotes enter the host organism through the wound of the bite or mucous membranes. Inside the host, the trypomastigote forms invade nucleated cells close to the inoculation site and differentiate into intracellular form the amastigote, which multiplies in the cellular cytoplasm. Such amastigote forms differentiate again into trypomastigotes and thus are released into the bloodstream, infecting cells from various tissues, and again transformed into intracellular amastigotes into new sites of infection. Clinical manifestations may arise from this continuous infective cycle. The triatomine insect is infected by the blood ingestion of host's blood containing a circulating parasite on trypomastigote form, which after achieving the vector's medium intestine, differentiates into epimastigote form. In this stage, after reaching the posterior intestine, they are differentiated into the infectious form, the metacyclic trypomastigote[17].

Phases of the Disease and Diagnosis

From the analysis of clinical and laboratory aspects of patients with this disease, three stages of infection were determined[18-20]: (i) acute, occurring in the first four months of the protozoan in the human body, with presence of an inflammatory lesion on the skin (inoculation chagoma or Romaña's sign, if parasitic inoculation occurred in the ocular region), followed by degenerative and inflammatory tissue alterations, fever, malaise, subcutaneous oedema, lymph adenomegaly, splenomegaly and hepatomegaly, which may last for decades; (ii) an indeterminate phase, usually asymptomatic for a period of 10 to 20 years; and (iii) chronic, with the gradual disappearance of clinical manifestations, low parasitaemia (not detectable in direct parasitological clinical tests) and elevated immunoglobulin G (IgG) antibodies presented under three clinical aspects, such as undetermined, cardiac (more severe, with progressive myocardial damage) and digestive (megaoesophagus and megacolon, with destruction of the nervous cells of the enteric sys-

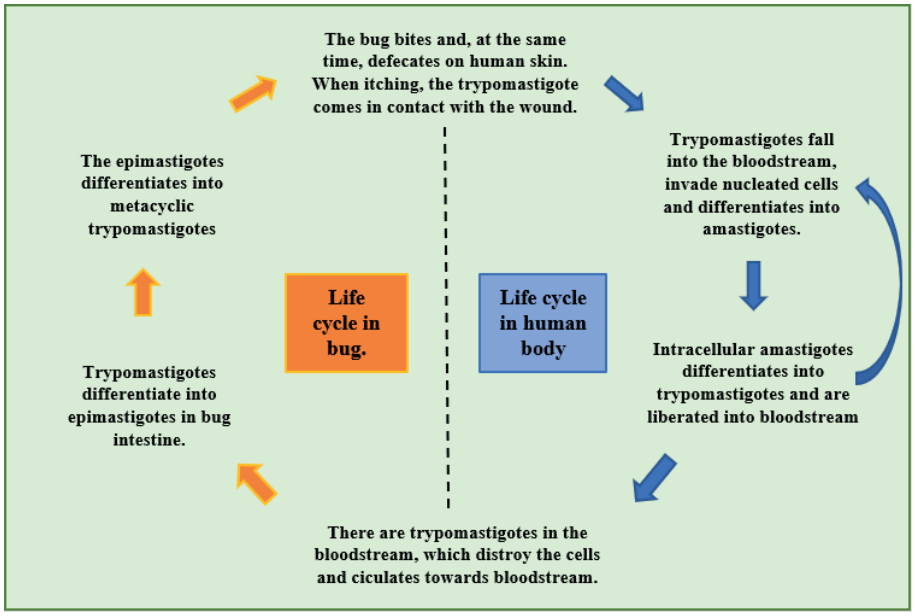

Figure 1. Life cycle of T. *cruzi*.

Table I. Clinical and laboratory aspects of Chagas disease on acute and chronic phases.

Disease Phases	Clinical Aspects	Laboratory Aspects
Acute	Depends on inoculation region. Skin chancre or orbital unilateral oedema (Romaña's sign), with local lymph adenomegaly and fever for weeks. Multiple lymph adenopathy, hepatosplenomegaly and myocarditis (chest pain, cardiac failure), meningoencephalopathy (not usual – seizure, paralysis).	Parasite detection on blood and lymph nodes. Positive serological test for antibodies against *Trypanosoma cruzi.*
	Asymptomatic patients.	Examination in the faeces of uninfected triatomine fed with the patient's blood.
Chronic	Long and latent period, with cardiac lesions (arrhythmia, cardiomyopathy, thromboembolism). Mostly asymptomatic.	Positive serological test for antibodies against *Trypanosoma cruzi.*

tem), as the parasite reaches and directly harms vital organs, such as heart, oesophagus and colon, respectively (Table I).

Infected patients usually exhibit normal life for decades as the infection remains latent. The specific treatment can interfere with the disease evolution, preventing or obstructing its continuity[21]. Thus, one of the major questionings in this field is related to the therapeutic involving distinct phases of the disease.

The diagnosis of the disease is carried out directly or indirectly. The direct form occurs in the acute phase, which consists in the search of the trypomastigote form of the parasite in the blood. This investigation is held through a blood drop between glass blade and coverslip or taking thick drop colored with Giemsa or Leishman pigment. Therefore, an approach is very difficult due to an irregular or low parasitemia. This is the reason why an indirect method is in most of the cases required, such as xenodiagnoses, blood culture and the use of molecular and serological strategies, such as enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence, or indirect hemagglutination[22,23].

Guidelines for Chagas Disease Treatment

Chagas disease is a well-known illness with many published articles and permanent investigation of the life cycle and physiology of the parasite. After its discovery, some compounds, such as arsenic derivative, fuchsine, emetic tartar, and mercury chloride ($HgCl_2$) were used, but no good clinical result was achieved[24,25].

Until now, only nifurtimox and benznidazole are available to treat patients. The excessive cost for research and development of new drugs associated with its major presence in emergent countries makes the pharmaceutical industries still not interested in investing in new antichagasic drugs[25].

Table II. National Guideline for the Chagas' disease treatment in acute and chronic phase.

Chagas's Disease Phases	Available Drugs	
	Nifurtimox	Benznidazole
Acute	Patients under 40 kg: 10 to 12 mg/kg/day in 2 to 3 doses for 30 to 60 days. Patients over 40 kg: 8 mg/kg/day in 2 to 3 doses for 30 to 60 days. Do not use in children under 12 years of age.	Patients under 40 kg: 7.5 mg/kg/day in 2 to 3 doses for 30 to 60 days. Patients over 40 kg: 5 mg/kg/day in 2 to 3 doses for 30 to 60 days. Children under 12 years-old: weight under 40 kg 7.5 mg/kg/day in 2 to 3 doses for 30 to 60 days; weight over 40 kg 5 mg/kg/day in 2 to 3 doses for 30 to 60 days.
Chronic	Children over 12 years-old and adults: 8 to 10 mg/kg/day in 2 to 3 doses for 30 to 60 days.	Children over 12 years-old and adults: 5 mg/kg/day in 2 to 3 doses for 30 to 60 days.

Nifurtimox was the first drug developed for this purpose and has been used since 1965. However, studies indicate that long-term therapy increases the chances of intense adverse effects. In addition, women in the first trimester of pregnancy or the ones who are breastfeeding are forbidden to use it, as well as patients with a history of mental problems or seizures because of its collateral effects. The mechanism of action of nifurtimox involves: (i) parasite oxidative stress induction; (ii) reduction activation by type I eukaryotic Nitroreductase; (iii) inhibition of parasite dehydrogenases activity; and (iv) mitochondrial membrane potentia[26,27].

Therefore, the use of benznidazole is recommended for a long-period treatment[26]. Benznidazole is an antiparasitic drug with significant activity on the acute phase with positive prognosis in around 80% of the patients. It became an alternative for nifurtimox since 1971. Nevertheless, problems related to toxicity are intensely reported, involving dermatitis, myelosuppression, and peripheral polyneuropathy, especially on chronic phase[14,28-30]. Drug resistance has also been observed with some parasite strains[31].

The Benznidazole Evaluation for Interrupting Trypanosomiasis "BENEFIT" initiative, a randomized, double-blind, placebo-controlled study, was made to conquer new methods using this drug. But no clinical improvements for patients were demonstrated[32]. The same pursuit was made on the TRAENA initiative, a randomized placebo-controlled evaluation of benznidazole treatment on long-term disease progression in adults with chronic Chagas disease, which showed that rates of negative parasitemia increased with time after treatment from 55.97% after two months to 62.59% after 8-16 months and 72.81% after 9-11 years[33]. Even modifications on drug formulation or drug dose were proposed, but no significant modifications were found[34-36].

The mechanism of action of benznidazole involves: (i) free radical generation; (ii) formation of metabolites capable of reacting with nucleophiles; (iii) mitochondrial DNA damage induction[29]. There is also a description of natural resistance development towards this drug by the parasite through the mechanism of aldo-ceto reductase and alcohol dehydrogenase of *T. cruzi*, which makes this drug not capable of reaching high rates of remission, especially in the chronic phase of the disease[37].

Since both drugs were discovered, despite advances in knowledge of the disease, no other drug was introduced in this field of treatment. The low investment in research in this area is the main cause of this problem. Thus, Chagas' disease can be considered (an) extremely neglected (disease)[38]. It is important to highlight the pioneer teams involved in the treatment of Chagas' disease, such as Rassi[39], Miranda et al[40], Viotti et al[41], Fragata Filho et al[42], Coura et al[24] as well as Cançado[43]. Their work was crucial to develop a treatment for chronically infected patients and vastly contributed to clinical knowledge nowadays.

The National Guideline for the treatment of the disease (Table II) determined from the findings based on the research available in Brazilian or international territory clearly demonstrates the limited presence of available antichagasic therapy. Treatment with these drugs will depend on the age, weight, and phase of each patient's illness[44].

The research on Chagas' disease can be explored through two approaches: (i) development of a preventive vaccine; (ii) discovery of new effective drugs. But unfortunately, none of these events are tangible. First, a vaccine has not been reached nor is close to be created, and as described above, no other antichagasic therapy is available and the median results with nifurtimox and benznidazole must be overtaken[45]. Therefore, this work aims to investigate drugs and substances with promising activity against Chagas disease. This is an attempt to instigate an evolution in the fight against this injury.

Patients and Methods

For this overview analysis, articles on Chagas Disease and Treatment published on Scielo, Sci Finder and Pubmed databases have thoroughly been studied, revised and updated. The results were selected in terms of authors of scientific articles, focused on relevance, especially – not limited to – date publication between 2012 and 2018 with no limitation of language and divided into two categories: (i) drugs already available in the market; and (ii) development of new substances. For this study, the information was collected in literature on "Chagas Disease" or/and "Treatment" already published in databases, hence the ethical approval was not required.

Results

The evolution of the research related to this disease is not very extensive. Even after one century

from its discovery, only two drugs are indicated for treatment, nifurtimox and benznidazole. After that, most of the research is focused on drugs already available in the market for other diseases and not in the development of new drugs. The manual elimination of articles describing the epidemiology and clinical evaluation was performed in order to find the most adjusted results for this paper.

The research on pharmacological targets is also very well documented and is crucial to determine new development directions. It was possible to define that these targets were involved in enzymes or metabolic pathways, which are exclusive and present in the parasite organism, such as the trypanothione reductase, ergosterol inhibition, polyphosphate metabolism, purine and protein synthesis, and cysteine proteinase (cruzipain). Lysophospholipid analogues and natural products were also included in the available data, with remarkable results[46,47]. All data is shown below, divided into two categories, drugs reposition and new developments.

Discussion

Drugs Available in the Market – Drug Reposition

The repositioning of drugs is a strategy used in the search for treatments for several diseases, especially those neglected. Such tactic is beneficial due to the long duration and excessive costs involved in the development of new drugs. This method is also advantageous since these drugs already have a toxicological and pharmacokinetic profile investigated when inserted in its original therapeutic indication. Thus, compounds of various pharmacological classes were tested against the *T. cruzi* parasite[48].

The usage of drugs related to the ergosterol biosynthesis of the parasite as antichagasic was considered due to the similarity with the biosynthesis of this sterol in fungi. Sterols are essential for eukaryotes, so the inhibition of its synthesis causes membrane damage in addition to toxins accumulation. Therefore, azole derivatives were, in theory, promising for these tests. They are antifungal drugs which act by blocking ergosterol biosynthesis in fungi via inhibition of the cytochrome (CYP) P450 enzyme.

Lepesheva[49] tested five azoles approved for clinical systemic use: ketoconazole, itraconazole, posaconazole, fluconazole, and voriconazole. As a result, fluconazole (6) showed a relatively weak inhibitory potency as the *T. cruzi* CYP51 inhibitor in vivo. Its close-derivative Voriconazole (7), however, has been recently reported to reveal some suppressive effect in a mouse model (75% of survival rate at the 40 mg/kg/day dosage administered for 30 days). Another work[50] demonstrated that Itraconazole managed to block the evolution of the disease in the chronic phase.

Other researches were guided for posaconazole and have demonstrated significant *in vitro* and *in vivo* activity against *T. cruzi* (the half maximal inhibitory concentration "IC_{50}" between 1 and 11 nM depending on the strain or clone). Its performance involves: (i) ergosterol blockade, with a higher inhibition rate of the parasite when acting on the proliferation of the epimastigote form; (ii) alteration in the phospholipid profile of the parasite. Even so, clinical studies have demonstrated that its activity is closely related to the exposure to the substance, and depends on the strain tested, which made this research not very successful. However, the experimental models associated with the favorable safety profile make it potential in the combined use of other therapies[51,52].

The activity of posaconazole was compared in another clinical trial named CHAGASAZOL (NCT01162967), and posaconazole showed significantly more patients who had treatment failure during the follow-up as compared to benznidazole patients. The association of both substances was also evaluated in a randomized, double-blind, placebo-controlled phase II study, called Stop Chagas trial (NCT01377480), demonstrated that posaconazole does not improve therapeutic response compared to placebo in participants with a diagnosis of asymptomatic chronic Chagas disease[53].

The use of Ravuconazole, the first drug developed for Chagas' disease after decades, was performed, and its potency and specificity related to *T. cruzi in vitro* was found interesting. However, initially, in *in vivo* studies with mice it was limited (probably because of unfavorable pharmacokinetics in this kind of animal)[54]. Because of that, other investigations have been posteriorly developed, and a prodrug of this substance demonstrated efficacy and safety in its use as monotherapy or even in combination with benznidazole as demonstrated on the E1224 trial (NCT01489228)[55].

Another drug under test, which will have its efficacy published in 2019, is Fexinidazole. Its activity in this field was investigated *in vivo* in mice infected with resistant strains and characteristics of partial resistance to benznidazole and nifurtimox. The study demonstrated efficacy in

suppressing parasitemia and healing around 80% of the animals, in addition to the myocarditis reduction[56]. As a continuation, the Phase II study began in 2014 in Bolivia and demonstrated high efficacy at low doses[57].

Allopurinol is a purine synthesis inhibitor which acts directly on the synthesis of the genetic code of the trypanosome. Its structure is equivalent to hypoxanthine and acts mimicking it as a substrate for hypoxanthine-guanine phosphoribosyl transferase and, when it becomes a nucleotide analogue, it interferes in the synthesis of RNA. Its antichagasic activity has been briefly demonstrated and should be considered as an alternative for subsequent research[58,59]. Also, the combination of Allopurinol and Itraconazole produced beneficial results in patients with chronic disease[60].

When it comes to cardiac complication caused by Chagas' disease, Amiodarone is an antiarrhythmic drug of significant importance. The reputation of this drug is also related to the direct action against *T. cruzi*, with considerable clinical improvements in the individuals tested. In this study[61,62], the synergism of Amiodarone with Posaconazole was also analyzed and identified.

Another drug tested was the Risedronate which also presented activity against the *T. cruzi*. Although it is a drug used to treat and prevent osteoporosis in men and women[63], *in vitro* and *in vivo* tests indicated enzyme inhibition in the protozoan organism, in addition to decreasing parasitemia and increasing human infected survival. The tests were performed in rats and there is still a need for further development in research with this drug[64].

Natural drugs were also tested such as Aphidicolin, which is isolated from the fungus *Nigrospora sphaeric* and is an inhibitor of human αDNA polymerase, described as a potent antiparasitic. Thus, Santos et al[65] performed structural modifications in the nucleus of this drug and verified that, after certain changes, new bioactive substances were found, achieving IC_{50} of 1.7 μM in the *T. cruzi* cells. The results demonstrated that their potency and safety against the amastigote form of trypanosome made them excellent candidates to be included in the small group available for the treatment of this disease.

With the development of genome projects, several pharmacological targets have been identified in the organism of the parasite. Cruzipain, an essential protease for the Trypanosoma, was one of them and had its inhibition evaluated by drugs already used in certain treatments. Benidipine, regularly used in the treatment of cardiovascular diseases as interfering in calcium channels, and antibiotic Clofazimine, used in the treatment of leprosy, are promising because they can reduce the parasitic load in blood and skeletal tissues of mice[66].

Even with the already reported use of benznidazole, studies are still conducted with this drug to find adjuvant therapies which may improve its beneficial effects, as is the case of its use together with Clomipramine, a drug with an antidepressant activity. García et al[67] demonstrated a synergistic effect in the combination of both substances with activity against the trypomastigote form of the parasite, effectively suppressing parasitemia. Its use in the chronic phase of the disease still indicates less damage to the heart tissue. Therefore, when both drugs are used concomitantly, they are also suggestive of potential alternatives for the fight against *T. cruzi*.

New Developments on Chagas Treatment

The development of new substances involves many steps and demands a great amount of investment. That is the reason for several studies being focused on already available drugs. However, in the last decade, many articles related to new substances which presented antichagasic activity were published. Their structures are illustrated in Figure 2.

The compound GNF6702 (1) showed significant activity as an antiprotozoal drug, with IC_{50} = 35 nM. Its broad spectrum includes the activity against leishmaniasis, Chagas disease, and sleeping sickness. It is responsible for the non-competitive inhibition of parasite essential enzyme called proteasome[68].

Quinone represents a broad family of compounds with natural distribution and vast bioactivity, including trypanosomicide, as described for lapachol (2), α-lapachone (3), β-lapachone (4), and juglone (5). With the exception of 22 (IC_{50} > 50 μM), the other quinones achieve IC_{50} smaller than 5 μM when compared to benznidazole (IC_{50} = 2.13 μM). The mechanism of action of these substances is related to oxidative stress which induces the formation of reactive oxygen species (ROS), such as superoxide anion radical (O_2•−) and hydroxyl radical (•OH) which can alter redox balance and promote parasite death[69,70].

Gallic acid derivatives linked to a triphenylphosphonium group also demonstrated activity against *T. cruzi* Y strain trypomastigotes. The substances TPP^+-C_{10} (**6**) and TPP^+-C_{12} (**7**) were the most potent in both models, with half maximal

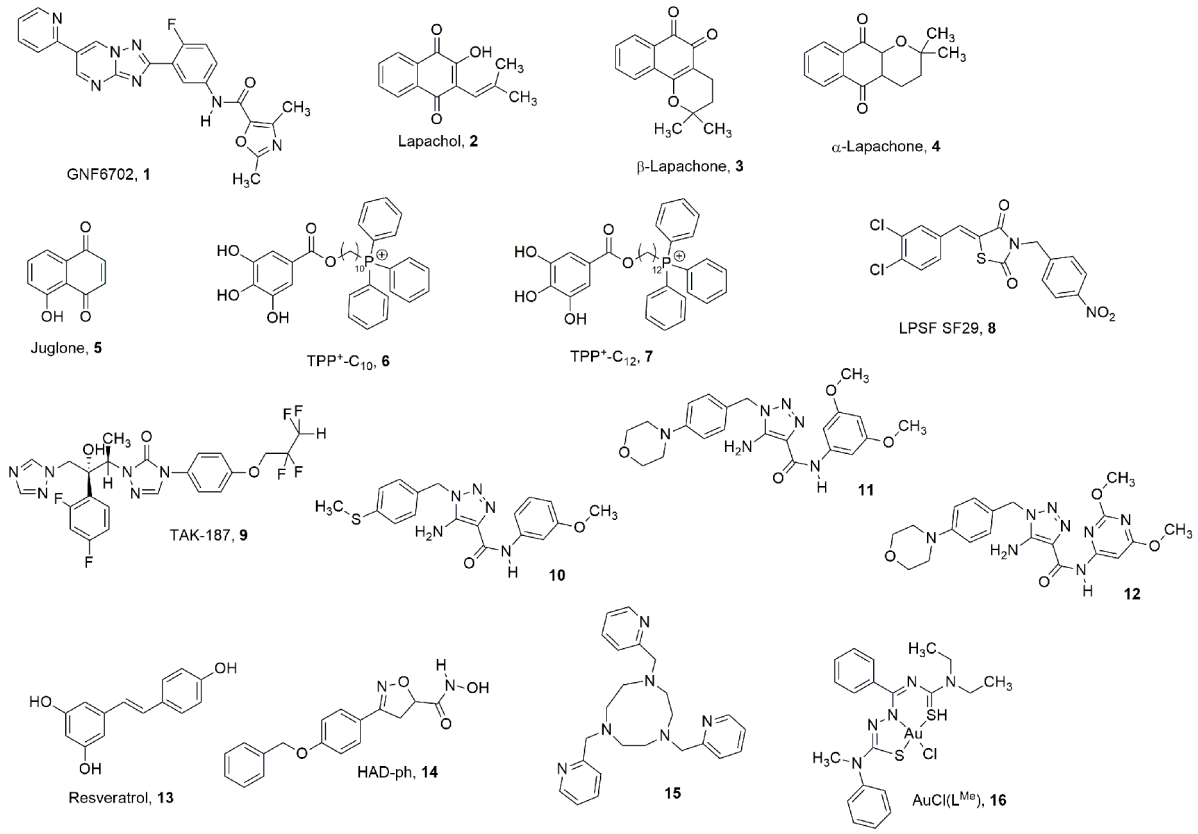

Figure 2. Chemical structure of tested substances against *T. cruzi* collected in this article.

effective concentration "EC_{50}" values (in isolated parasites) of 1.0 ± 0.6 and 1.0 ± 0.7 μM, respectively, and were significantly more potent than nifurtimox (EC_{50} = 4.1 ± 0.6 μM). The mechanism of action is related to mitochondrial toxicity[71].

The thiazolidine LPSF SF29 (8) was also tested against the *T. cruzi* epimastigote. The results demonstrated a dose- and time-dependent effect on parasite growth, with an IC_{50} of 8.7 ± 0.83 M, a lower value than the IC_{50} of the reference drug benznidazole (10.4 M). Its mechanism of action involves the interference on the synthesis of Trypanothione reductase, unique in the parasite and responsible for diminishing reactive oxygen species (ROS) on its organism, and mitochondrial alterations[72].

The derivative TAK-187 (9), an ergosterol biosynthesis inhibitor, showed activity against *T. cruzi*, also preventing cardiac damage in a murine model of Chagas disease as compared to benznidazole[73,74]. This path is very determinant in many publications for the last decade. Other substances, such as 10, 11, and 12 (Figure 2) were identified by Brand et al[75], and demonstrated promising results, achieving EC_{50} of 6.6, 7.1, and 7.4, respectively, the protozoan parasite *Trypanosoma cruzi*.

Another interesting advance was performed by Resveratrol (13), known for its antioxidant properties, even without a direct activity against T. cruzi, it can improve heart function of infected mice when treatment was started late after infection, while benznidazole failed. In this way, the risk factor related to cardiac failure is reduced, activating the AMPK enzyme pathway, reducing parasite burden and heart oxidative stress[76].

Two hydroxamic acid derivatives HAD-ph (14) were also synthetized and showed a significant reduction in the number of intracellular parasites and consequently demonstrated relevant activity against parasite multiplication. The potent activity (greater than benznidazole) against all evolutive forms of *T. cruzi*, reached an IC_{50} value from 7 to 51 μM for different strains of the parasite[77].

Tetraamine-based compounds were prepared to determine the trypanocide effects against *Trypanosoma cruzi* and its cytotoxicity. In this study, the compound **15** (Figure 2) induced a remarkable decrease in the reactivation of parasitaemia after

immunosuppression and curative rates of 33%, with IC_{50} of 7.3 ± 0.8 μM against epimastigote forms 7.0 ± 0.9 μM against amastigote forms and 5.7 ± 0.8 μM against trypomastigotes forms when compared to benznidazole (15.8 ± 1.1 μM, 23.3 ± 4.6 μM and 22.4 ± 1.9 μM respectively)[78].

An interesting approach was obtained with gold (III) complexes with ONS-Tridentate thiosemicarbazones as described in literature and compound (**16**) showed great results, with IC_{50} of 2.48 μM against amastigote forms when compared to benznidazole (13.67 μM)[79].

Conclusions

Chagas' disease is one of the neglected diseases with a profound impact worldwide. Several publications related to the *T. cruzi* are available and diverse investigations of the life cycle and physiology of the parasite are constantly published. However, the knowledge produced to date was still not transformed into therapeutic advances, since until now only two drugs are being used for the treatment of patients with this illness: nifurtimox and benznidazole. However, their adverse effects and the low percentage of positive prognosis with their use keeps the dissemination of the disease at a very worrying level. To make the situation even less favorable, a solution to obtain a vaccine to prevent it is far from being found and only a few substances in clinical studies have been described in literature in the last decades.

Moreover, there are many drugs with anticha-gasic activity at various stages of the protozoan. This indicates that alternatives can already be considered to improve the prognosis of millions of patients around the world. This research was able to denigrate some of the paths already taken (by researchers), which should be analyzed with great attention.

In addition, there should be more efforts to ensure that the knowledge and results obtained related to *T. cruzi* are effectively returned to society, especially to those living in areas affected by Chagas' disease. As seen in this work and in several others found in scientific literature, various research groups around the world are willing to collaborate with this turnover.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Chagas CJR. Nova tripanozomiaze humana: estudos sobre a morfolojia e o ciclo evolutivo do schizotrypanum cruzi n. gen., n. sp., ajente etiolojico de nova entidade morbida do homem. Mem Inst Oswaldo Cruz 1909; 1: 159-162.
2) Diniz Lde F, Urbina JA, de Andrade IM, Mazzeti AL, Martins TA, Caldas IS, Talvani A, Ribeiro I, Bahia MT. Benznidazole and posaconazole in experimental Chagas disease: positive interaction in concomitant and sequential treatments. PLoS Negl Trop Dis 2013; 7: 1-8.
3) De Urioste-Stone SM, Pennington PM, Pellecer E, Aguilar TM, Samayoa G, Perdomo HD, Enríquez H, Juárez JG. Development of a community-based intervention for the control of Chagas disease based on peridomestic animal management: an eco-bio-social perspective. Trans R Soc Trop Med Hyg 2015; 109: 159-167.
4) DNDi. Fact Sheet on Chagas disease. Available at: https://www.dndi.org/wp-content/uploads/2017/08/Factsheet_2016_Chagas.pdf cited date Sep 04, 2018.
5) Dias JVL, Queiroz DRM, Diotaiuti L, Pires HHR. Conhecimentos sobre triatomíneos e sobre a doença de Chagas em localidades com diferentes níveis de infestação vetorial. Ciênc Saúde Col 2016; 21: 2293-2304.
6) Coura JR, Viñas PA. Chagas disease: a new worldwide challenge. Nature 2010; 465: S6-7.
7) Valle-Reyes JS, Melnikov V, Dobrovinskaya O, Rodriguez-Hernández A, Wookee-Zea C, Pimientel-Rodrigez V, Rueda-Valdovinos G, Delgado-Enciso I, López-Lemus UA, Espinoza-Gómez F. Antiprotozoal drug nitazoxanide enhances parasitemia, tissue lesions and mortality caused by trypanosoma cruzi in murine model. Exp Parasitol 2017; 172: 44-50.
8) Sherlock IA. Vetores. In: trypanosoma cruzi e doença de Chagas. 2nd ed. Guanabara Koogan Publishing, 2000.
9) WHO. Chagas. Available at: http://www.who.int/chagas/en/ cited date Sep 22, 2018.
10) Dias JCP. Epidemiologia. In: trypanosoma cruzi e doença de Chagas. 2nd ed. Guanabara Koogan Publishing, 2000.
11) Pinheiro E, Brum-Soares L, Reis R, Cubides JC. Chagas disease: review of needs, neglect, and obstacles to treatment access in Latin America. Rev Soc Bras Med Trop 2017; 50: 296-300.
12) Kashiwabara YB, Paiva RMRA, Nakaoka VY, Silva E, Kashiwabara TGB. Doença de Chagas - Revisão de Literatura. Braz J Surg Clin Research 2010; 4: 49-52.
13) Shikanai-Yasuda MA, Marcondes CBP, Guedes LA, Siqueira GS, Barone AA, Dias JCP, Amato Neto V, Tolezano JE, Peres BA, Arruda Júnior ER, Lopes MH, Shiroma M, Chapadeiro E. Possible oral transmission of acute Chagas' disease in Brazil. Rev Inst Med Trop 1991; 33: 23-28.
14) De Andrade AL, Zicker F, de Oliveira RM, Almeida Silva S, Luquetti A, Travassos LR, Almeida IC, de Andrade SS, de Andrade JG, Martelli CM. Ran-

domised trial of efficacy of benznidazole in treatment of early trypanosoma cruzi infection. Lancet 1996; 348: 1407-1413.

15) Barbosa-Ferreira JM, Guerra JAO, Santana Filho FS, Magalhães BML, Coelho LIARC, Barbosa MGV. Acometimento cardíaco em Casos de Doença de Chagas Aguda da Amazônia. Arq Bras Cardiol 2010; 94: 95-98.

16) Cossentini LA, Da Silva RV, Yamada-Ogatta SF, Yamauchi LM, De Almeida Araújo EJ, Pinge-Filho P. Aspirin treatment exacerbates oral infections by trypanosoma cruzi. Exp Parasitol 2016; 164: 64-70.

17) Calderano SG, Moretti NS, Araújo C, da Silva MS, de Jesus TCL, Lima LPO, Lopes MC, Augusto LS, Cunha JRC, Elias MC, Schenkman S. The cellular organisation of trypanossomatids during life cycle. In: frontiers in parasitology – molecular and cellular biology of pathogenic trypanosomatids. Bentham Books, 2017.

18) Chagas CJR. Processos patojenicos da tripanozomiase americana. Mem Inst Oswaldo Cruz 1916; 8: 5-36.

19) Moncayo A, Silveira AC. Current epidemiological trends of Chagas disease in Latin America and future challenges: epidemiology, surveillance, and health policies. Mem Inst Oswaldo Cruz 2009; 104: 17-30.

20) Zingales B. Trypanosoma cruzi genetic diversity: Something new for something known about Chagas disease manifestations, serodiagnosis and drug sensitivity. Acta Tropica 2017; S0001-706X: 1-67.

21) Coura JR. Tripanossomose, Doença de Chagas. Cienc Cult 2003; 55: 30-33.

22) Rassi A, Marin-Neto JA. Chagas disease. Lancet 2010; 375: 1388-1402.

23) Mattos ER, Berto BP. Doença de Chagas: Uma breve revisão das recentes ocorrências, vias de transmissão e métodos diagnósticos. Saúde & Ambiente em Revista 2012; 6: 40-45.

24) Coura JR, de Castro SL. A critical review on Chagas disease chemotherapy. Mem Inst Oswaldo Cruz 2002; 97: 3-24.

25) Dias LC, Dessoy MA, Silva JN, Thiemann HO, Oliva G, Andricopulo AD. Quimioterapia da doença de Chagas: estado da arte e perspectivas no desenvolvimento de novos fármacos. Quím Nova 2009; 32: 2444-2457.

26) Forsyth CJ, Hernandez S, Olmedo W, Abuhamidah A, Traina MI, Sanchez DR, Soverow J, Meymandi SK. Safety profile of nifurtimox for treatment of Chagas disease in the United States. Clin Infect Dis 2016; 63: 1056-1062.

27) Boiani M, Piacenza L, Hernández P, Boiani L, Cerecetto H, González M, Denicola A. Mode of action of nifurtimox and N-oxide-containing heterocycles against Trypanosoma cruzi: is oxidative stress involved? Biochem Pharmacol 2010; 79: 1736-1737.

28) Sosa Estani S, Segura EL, Ruiz AM, Velazquez E, Porcel BM, Yampotis C. Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas' disease. Am J Trop Med Hyg 1998; 59: 526-529.

29) Rajão MA, Furtado C, Alves CL, Passos-Silva DG, de Moura MB, Schamber-Reis BL, Kunrath-Lima M, Zuma AA, Vieira-da-Rocha JP, Garcia JB, Mendes IC, Pena SD, Macedo AM, Franco GR, de Souza-Pinto NC, de Medeiros MH, Cruz AK, Motta MC, Teixeira SM, Machado CR. Unveiling benznidazole's mechanism of action through overexpression of DNA repair proteins in Trypanosoma cruzi. Environ Mol Mutagen 2014; 55: 309-321.

30) Trochine A, Creek DJ, Faral-Tello P, Barret MP, Robello C. Benznidazole biotransformation and multiple targets in trypanosoma cruzi revealed by metabolomics. PLoS Negl Trop Dis 2014; 8: 1-15.

31) Mejía-Jaramillo AM, Fernández G, Montilla M, Nicholls RS, Triana-Chávez O. Trypanosoma cruzi strains resistant to benznidazole occurring in Colombia. Biomedica 2012; 32: 196-205.

32) Morillo CA, Marin-Neto JA, Avezum A, Sosa-Estani S, Rassi A Jr, Rosas F, Villena E, Quiroz R, Bonilla R, Britto C, Guhl F, Velazquez E, Bonilla L, Meeks B, Rao-Melacini P, Pogue J, Mattos A, Lazdins J, Rassi A, Connolly SJ, Yusuf S. Randomized trial of benznidazole for chronic Chagas' cardiomyopathy. N Engl J Med 2015; 373: 1295-1306.

33) DNDi. TRAENA study: Evaluation of potential biomarkers of therapeutic efficacy. Available at: http:// www.dndi.org/wp-content/uploads/2010/03/NewsletterChagas.Eng.2.pdf cited date Ago 31, 2018.

34) Alvarez MG, Vigliano C, Lococo B, Petti M, Bertocchi G, Viotti R. Seronegative conversion after incomplete benznidazole treatment in chronic Chagas disease. Trans R Soc Trop Med Hyg 2012; 106: 636-638.

35) Tarragona S, Salomón C, Jiménez-Kairuz A, Lamas M, Orlandi S, Leonardi D, Maggia N, Paredes A, Romañuk C, García M. Design and preparation of pharmaceutical solid dosage forms of benznidazole for the treatment of chagas disease. Rev Argent Salud Pública 2013; 4: 6-13.

36) Manarin R, Lamas MC, Bottasso E, Serra E, Revelli S, Salomón CJ. Efficacy of novel benznidazole solutions during the experimental infection with Trypanosoma cruzi. Parasitol Int 2013; 62: 79-81.

37) González L, GarcíaÐHuertas P, TrianaÐChávez O, García GA, Murta SMF, Mejía Jaramillo AM. Aldo-keto reductase and alcohol dehydrogenase contribute to benznidazole natural resistance in Trypanosoma cruzi. Mol Microbiol 2017; 106: 704-718.

38) FIOCRUZ. Doenças Negligenciadas. Available at: https://agencia.fiocruz.br/doenças-negligenciadas cited date Oct 4, 2018.

39) Rassi A. Etiological treatment of Chagas' disease. Arq Bras Cardiol 1982; 38: 277-281.

40) Miranda L, Campos G, Marins N, Lobo V. História natural da forma crônica da doença de Chagas x tratamento específico. Rev Centro-Oeste Cardiol 1994; 1: 25-29.

41) Viotti R, Vigliano C, Armenti H, Segura E. Treatment of chronic Chagas' disease with benznidazole: clinical and serologic evolution of patients with

long-term follow-up. Am Heart J 1994; 127: 151-162.

42) Fragata Filho AA, da Silva MA, Boainain E. Ethiologic treatment of acute and chronic Chagas' disease. Sao Paulo Med J 1995; 113: 867-872.

43) Cançado JR. Long term evaluation of etiological treatment of Chagas disease with benznidazole. Rev Inst Med Trop Sao Paulo 2002; 44: 29-37.

44) Protocolo Clínico e Diretrizes Terapêuticas: Doença de Chagas. Available at: http://conitec.gov.br/images/Relatorios/2016/PropostaEscopo_PCDT_DoencaChagas.pdf cited date Sep 18, 2018.

45) Sales Junior PA, Molina I, Fonseca Murta SM, Sánchez-Montalvá A, Salvador F, Corrêa-Oliveira E, Carneiro CM. Experimental and clinical treatment of Chagas disease: a review. Am J Trop Med Hyg 2017; 97: 1289-1303.

46) Porrás AI, Yadon ZE, Altcheh J, Britto C, Chaves GC, Flevaud L, Martins-Filho OA, Ribeiro I, Schijman AG, Shikanai-Yasuda MA, Sosa-Estani S, Stobbaerts E, Zicker F. Target product profile (TPP) for Chagas disease point-of-care diagnosis and assessment of response to treatment. PLoS Negl Trop Dis 2015; 9: 1-8.

47) Bermudez J, Davies C, Simonazzi A, Real JP, Palma S. Current drug therapy and pharmaceutical challenges for Chagas disease. Acta Tropica 2016; 156: 1-16.

48) Alberca LN, Sbaraglini ML, Balcazar D, Fraccaroli L, Carrillo C, Medeiros A, Benitez D, Comini M, Talevi A. Discovery of novel polyamine analogs with anti-protozoal activity by computer guided drug repositioning. J Comput Aided Mol Des 2016; 30: 305-321.

49) Lepesheva GI. Design or screening of drugs for the treatment of Chagas disease: what shows the most promise? Expert Opin Drug Discov 2013; 8: 1-17.

50) Apt W, Arribada A, Zulantay I, Rodríguez J, Saavedra M, Muñoz A. Treatment of Chagas' disease with itraconazole: electrocardiographic and parasitological conditions after 20 years of follow-up. Journal of Antimicrob Chemother 2013; 68: 2164-2169.

51) Molina I, Salvador F, Sánchez-Montalvá A. The use of posaconazole against Chagas disease. Curr Opin Infect Dis 2015; 28: 397-407.

52) Morillo CA, Waskin H, Sosa-Estani S, Del Carmen Bangher M, Cuneo C, Milesi R, Mallagray M, Apt W, Beloscar J, Gascon J, Molina I, Echeverria LE, Colombo H, Perez-Molina JA, Wyss F, Meeks B, Bonilla LR, Gao P, Wei B, McCarthy M, Yusuf S; STOP-CHAGAS Investigators. Benznidazole and posaconazole in eliminating parasites in asymptomatic T. Cruzi carriers: the STOP-CHAGAS trial. J Am Coll Cardiol 2017; 69: 939-947.

53) Molina I, Gómez I, Prat J, Salvador F, Treviño B, Sulleiro E, Serre N, Pou D, Roure S, Cabezos J, Valerio L, Blanco-Grau A, Sánchez-Montalvá A, Vidal X, Pahissa A. Randomized trial of posaconazole and benznidazole for chronic Chagas' disease. N Engl J Med 2014; 15: 1899-1908.

54) Urbina JA, Payares G, Sanoja C, Lira R, Romanha AJ. *In vitro* and *in vivo* activities of ravuconazole on Trypanosoma cruzi, the causative agent of Chagas disease. Int J Antimicrob Agents 2013; 21: 27-38.

55) Torrico F, Gascon J, Ortiz L, Alonso-Vega C, Pinazo MJ, Schijman A, Almeida IC, Alves F, Strub-Wourgaft N, Ribeiro I; E1224 Study Group. Treatment of adult chronic indeterminate Chagas disease with benznidazole and three E1224 dosing regimens: a proof-of-concept, randomised, placebo-controlled trial. Lancet Infect Dis 2018; 18: 419-430.

56) Bahia MT, de Andrade IM, Martins TA, do Nascimento ÁF, Diniz Lde F, Caldas IS, Talvani A, Trunz BB, Torreele E, Ribeiro I. Fexinidazole: a potential new drug candidate for Chagas disease. PLoS Negl Trop Dis 2012; 6: 1-9.

57) DNDi. Fexinidazole (Chagas). Available at: https://www.dndi.org/diseases-projects/portfolio/fexinidazole-chagas/ cited date Sep 18, 2018.

58) Gallerano RH, Marr JJ, Sosa RR. Therapeutic efficacy of allopurinol in patients with chronic Chagas' disease. Am J Trop Med Hyg 1990; 43: 159-166.

59) Rassi A, Luquetti AO, Rassi A Jr, Rassi GG, Rassi SG, DA Silva IG, Rassi AG. Specific treatment for Trypanossoma cruzi: lack of efficacy of allopurinol in the human chronic phase of Chagas disease. Am J Trop Med Hyg 2007; 76: 58-61.

60) Apt W, Arribada A, Zulantay I, Sanchez G, Vargas SL, Rodriguez J. Itraconazole or allopurinol in the treatment of chronic American trypanosomiasis: the regression and prevention of electrocardiographic abnormalities during 9 years of follow-up. Ann Trop Med Parasitol 2003; 97: 23-29.

61) Bellotti G, de Moraes AV, Silva LA, Galiano N, Rati M, Esteves Filho A, Ramires JA, Pileggi F. Effects of amiodarone on the elastic properties of the left ventricle in chronic Chagas' cardiomyopathy. Arq Bras Cardiol 1983; 41: 71-75.

62) De Barros MA, Maciel RM. Prospective study of the effects of amiodarone on thyroid function in chagasic patients in an area of iodine deficiency. Rev Soc Bras Med Trop 1994; 27: 149-155.

63) Välimäki MJ, Farrerons-Minguella J, Halse J, Kröger H, Maroni M, Mulder H, Muñoz-Torres M, Sääf M, Snorre Øfjord E. Effects of risedronate 5 mg/d on bone mineral density and bone turnover markers in late-postmenopausal women with osteopenia: a multinational, 24-month, randomized, double-blind, placebo-controlled, parallel-group, phase III trial. Clin Ther 2007; 29: 1937-1949.

64) Bouzahzah B, Jelicks LA, Morris SA, Weiss LM, Tanowitz HB. Risedronate in the treatment of murine Chagas' disease. Parasitol Res 2005; 96: 184-187.

65) Santos GB, Krogh R, Magalhaes LG, Andricopulo AD, Pupo MT, Emery FS. Semisynthesis of new aphidicolin derivatives with high activity against Trypanosoma cruzi. Bioorg Med Chem Left 2016; 26: 1205-1208.

66) Sbaraglini ML, Bellera CL, Fraccaroli L, Larocca L, Carrillo C, Talevi A, Alba Soto CD. Novel cruzipain inhibitors for the chemotherapy of chronic Chagas disease. Int J Antimicrob Agents 2016; 48: 91-95.

67) García MC, Ponce NE, Sanmarco LM, Manzo RH, Jimenez-Kairuz AF, Aoki MP. Clomipramine and benznidazole act synergistically and ameliorate the outcome of experimental chagas disease. Antimicrob Agents Chemother 2016; 60: 3700-3708.

68) Khare S, Nagle AS, Biggart A, Lai YH, Liang F, Davis LC, Barnes SW, Mathison CJ, Myburgh E, Gao MY, Gillespie JR, Liu X, Tan JL, Stinson M, Rivera IC, Ballard J, Yeh V, Groessl T, Federe G, Koh HX, Venable JD, Bursulaya B, Shapiro M, Mishra PK, Spraggon G, Brock A1, Mottram JC, Buckner FS, Rao SP, Wen BG, Walker JR, Tuntland T, Molteni V, Glynne RJ, Supek F. Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness. Nature 2016; 537: 229-233.

69) Da Silva MN, Ferreira VF, De Souza MC. Um panorama atual da química e da farmacologia de naftoquinonas, com ênfase na β-lapachona e derivados. Quim Nova 2003; 26: 407-416.

70) Da Silva MN, Ferreira SB, da Rocha DR, Ferreira VF. Chagas disease: challenges in developing new trypanocidal lead compounds. Rev Virtual Quim 2012; 4: 46-72.

71) Cortes LA, Castro L, Pesce B, Maya JD, Ferreira J, Castro-Castillo V, Parra E, Jara JA, López-Muñoz R. Novel gallate triphenylphosphonium derivatives with potent antichagasic activity. PLoS One 2015; 28: 1-17.

72) De B. Moreira TL, Barbosa AF, Veiga-Santos P, Henriques C, Henriques-Pons A, Galdino SL, de Lima Mdo C, Pitta Ida R, de Souza W, de Carvalho TM. Effect of thiazolidine LPSF SF29 on the growth and morphology of Trypanosoma cruzi. Int J Antimicrob Agents 2013; 41: 183-187.

73) Corrales M, Cardozo R, Segura MA, Urbina JA, Basombrío MA. Comparative efficacies of TAK-187, a long-lasting ergosterol biosynthesis inhibitor, and benznidazole in preventing cardiac damage in a murine model of Chagas' disease. Antimicrob Agents Chemother 2005; 49: 1556-1560.

74) Creek DJ, Vincent IM, Barrett MP. Pharmacological metabolomics in trypanossomes. In: trypanosomatid diseases: molecular routes to drug discovery. Wiley-VCH, 2013.

75) Brand S, Ko EJ, Viayna E, Thompson S, Spinks D, Thomas M, Sandberg L, Francisco AF, Jayawardhana S, Smith VC, Jansen C, De Rycker M, Thomas J, MacLean L, Osuna-Cabello M, Riley J, Scullion P, Stojanovski L, Simeons FRC, Epemolu O, Shishikura Y, Crouch SD, Bakshi TS, Nixon CJ, Reid IH, Hill AP, Underwood TZ, Hindley SJ, Robinson SA, Kelly JM, Fiandor JM, Wyatt PG, Marco M, Miles TJ, Read KD, Gilbert IH. Discovery and optimization of 5-Amino-1, 2, 3-triazole-4-carboxamide series against Trypanosoma cruzi. J Med Chem 2017; 60: 7284-7299.

76) Vilar-Pereira G, Carneiro VC, Mata-Santos H, Vicentino AR, Ramos IP, Giarola NL, Feijó DF, Meyer-Fernandes JR, Paula-Neto HA, Medei E, Bozza MT, Lannes-Vieira J, Paiva CN. Resveratrol reverses functional Chagas heart disease in mice. PLoS Pathog 2016; 12: 1-19.

77) Rodrigues GC, Feijó DF, Bozza MT, Pan P, Vullo D, Parkkila S, Supuran CT, Capasso C, Aguiar AP, Vermelho AB. Design, synthesis, and evaluation of hydroxamic acid derivatives as promising agents for the management of Chagas disease. J Med Chem 2014; 57: 298-308.

78) Olmo F, Costas M, Marín C, Rosales MJ, Martín-Escolano R, Cussó O, Gutierrez-Sánchez R, Ribas X, Sánchez-Moreno M. Tetradentate polyamines as efficient metallodrugs for Chagas disease treatment in murine model. J Chemother 2016; 29: 83-93.

79) Rettondin AR, Carneiro ZA, Gonçalves AC, Ferreira VF, Oliveira CG, Lima AN, Oliveira RJ, de Albuquerque S, Deflon VM, Maia PI. Gold (III) complexes with ONS-tridentate thiosemicarbazones: toward selective trypanocidal drugs. Eur J Med Chem 2016; 120: 217-226.