**Cancer Research Journal**
2014; 2(6-1): 11-29
Published online December 26, 2014 (http://www.sciencepublishinggroup.com/j/crj)
doi: 10.11648/j.crj.s.2014020601.12
ISSN: 2330-8192 (Print); ISSN: 2330-8214 (Online)

# New treatments for Chagas disease and the relationship between chagasic patients and cancers

**Viviane M. de Oliveira[1], Luana T. Mendes[1], Durinézio J. Almeida[2], Lucas V. B. Hoelz[3], Pedro H. M. Torres[3], Pedro Geraldo Pascutti[3], Guilherme Barroso L. de Freitas[1, 4, *]**

[1]Mid-West State University (UNICENTRO), Department of Pharmacy, Guarapuava, Brazil
[2]Maringá State University (UEM), Programme of Comparative Biology, Maringá, Brazil
[3]Federal University of Rio de Janeiro (UFRJ), Department of Biophysics, Rio de Janeiro, Brazil
[4]Post-Degree Program in Internal Medicine and Health Sciences at UFPR, Department of Clinical Patology, Federal University of Parana, Curitiba, Parana, Brazil

## Email address:

vyvymatoso@hotmail.com (V. M. de Oliveira), lucashoelz@yahoo.com.br (L. V. B. Hoelz), prof.gbarroso@yahoo.com.br (G. B. L. de Freitas)

## To cite this article:

Viviane M. de Oliveira, Luana T. Mendes, Durinézio J. Almeida, Lucas V. B. Hoelz, Pedro H. M. Torres, Pedro Geraldo Pascutti, Guilherme Barroso L. de Freitas. New Treatments for Chagas Disease and the Relationship between Chagasic Patients and Cancers. *Cancer Research Journal.* Special Issue: Lifestyle and Cancer Risk. Vol. 2, No. 6-1, 2014, pp. 11-29. doi: 10.11648/j.crj.s.2014020601.12

**Abstract:** Chagas disease is an infectious illness with a broad distribution throughout the South American and African continents, importantly influencing human morbidity and mortality and a controversial relationship with the onset of cancers, especially of the gastrointestinal tract system. In addition, it is listed by the World Health Organization (WHO) as one ofthe most neglected tropical diseases. Although Chagas disease (CD) was discovered more than 100 years ago, the existing therapies show low efficacy and serious side effects and developing safer and more effective drugs remains a hard challenge. Thus, this review highlights the main, novel and promising treatments against Trypanosoma cruzi, including biomacromolecules, natural products, vaccines, and metabolic pathway targets and highlights a worsening of esophageal cancer prognosis in chagasic patients. Moreover, we also discuss the perspectives of obtaining original optimized drugs that take advantage of organic and inorganic medicinal chemistry advances, as well as molecular modeling and biotechnology.

**Keywords:** Chagas, Cancer, Drug, Neglected Disease, *Trypanosoma cruzi*

## 1. Introduction

American trypanosomiasis (Chagas disease) is a zoonosis or an anthropozoonosis caused by the flagellated protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). Despite the alarming rates of infections, this disease is recognized worldwide as only one of the 13 most neglected tropical diseases [1]. It is estimated that there are about 13 million people infected only in Latin America, and more than 60 million people may be at risk of transmission in about 18 endemic countries [2]. On top of that, amongst the infected group, about 25% of cases will develop chronic myocardial disease in the next years or decades [3].

Although the incidence of disease transmission in the last 15 years has been decreasing in southern region of South America (a decline of approximately 73%), due to a reduction of transmission through of the vector and by blood transfusion in countries like Uruguay and Brazil, combating the disease in the Andean and Central America countries is complicated dueto the large number of vectors. Additionally, in Colombia, almost 5% of the population is infected and about 20% are in imminent danger of acquiring the disease [4]. In Brazil, three factors can be pointed as responsible for the vast spread of the disease: the immense forest area, the sharing habitat of populations of low profits with *T. cruzi* transmitters and the poor housing conditions. Due to these factors, it is estimated that there are about 5 million chagasic patients and thousands of others with serious risk, mainly in the northern region of Brazil [2]. While tropical countries have the highest rate of incidence, the increasing globalization and the easiness of movement raise the risk of cross-contamination between healthy people and chagasic patients [5].

The infection can be very serious, leading to high mortality in children in the acute phase. During the chronic phase it is

common for adults to develop cardiac and/or digestive problems, leading to 400 thousands deaths per year in Latin America [6]. Furthermore, endemic countries suffer with high medical costs and social loses due to the existing comorbidities and inabilities generated by the disease. In 1991, the social costs of CD can reach $ 1 billion per year only in Brazil, however, actualized values are not available [7].

The intermediate stage of the disease, also known as "indeterminate phase" (a term currently under revisionbecause of the several pathophysiological mechanisms thatcan occur during this phase), has a period of two to three decades. In this stage, the only detectable manifestation of the disease is the immune response and some degrees of autonomic dysfunction. During the chronic phase that follows, most patients remain asymptomatic (20 to 50% of the cases). However, it is possible to observe some people with severe symptoms like cardiac, digestive or neurological disturbances [8].

In 1964, Chapadeiro *et al.* related for the first time a possible relationship between Chaga`s disease and the occurence of malignant neoplasms [9]. Since this point, several others papers were published, however, at the moment there is not conclusion if *T. cruzi* induced or decreases cancer development. Therefore, is evident the importance of understand the cellular alterations caused by this parasite.

Actually, in spite of the illness severity, there are no really effective drugs that agree with the rules of the WHO. Following the requirements of this organization, an ideal drug against CD should fulfill the following requirements: (i) parasitological cure of acute and chronic cases; (ii) effective insingle or few doses; (iii) accessible to the patients, i.e., low cost; (iv) no collateral or teratogenic effects; (v) no need of hospitalization for treatment; (vi) no induction of resistance [9]. Logically, all of these precepts dictated by the WHO are very difficult to achieve for the majority of available drugs in the market.

In 2005, genome sequencing of *T. cruzi* was completed, providing new insights into the drug design for the treatment of CD, i.e., molecules with therapeutic profile closer to an “ideal” drug [10]. Despite advances, many issues must beanswered, especially regarding to treatment methods. Although efforts towards the development of a definitive cure exists, the advancement of therapeutic understanding related to CD is much slower when compared to other diseases like cancer, AIDS, inflammation, and, even, erectile dysfunction or hair loss [11].

Therefore, the main objective of this review is to report the treatments for CD, new therapeutic targets, the relationship with cancer and prospects in the short to medium time using an effective treatment. To carry out an updated study, a systematic review was realized, looking for some recent impressive results for therapeutic strategies against CD.

## 2. Life Cycle of T. Cruzi

*T. cruzi* life cycle scheme starts in an animal reservoir. These reservoirs are usually mammals, wild or domestic, and include humans. The reduviid bug, a triatomine, serves as the vector while feeding on an infected host blood, it ingests *T. cruzi*. In the reduviid bug, they go into the epimastigote form, the parasite`s reproductive morphological stage. After reproducing through mitosis, the epimastigotes move onto the rectal cell wall, where they become infectious. Infectious *T. cruzi* are called trypomastigotes. Then, while the infected triatomine are feeding from a human, they simultaneously expel the trypomastigotes in the feces. The trypomastigotes enter the human host through the bite wound or by crossing mucous membranes. When they enter a human cell, they become amastigotes (another reproductive stage). After reproducing through mitosis until a large amount of amastigotes are in a cell, pseudocysts are formed in infected cells. The amastigotes then turn back into trypomastigotes, and the cell bursts. The trypomastigotes swim along to either infect other cells are ingested by other reduviid bugs [12].

The parasite causes both acute and chronic inflammation during the initial and more advanced infection. Because of this inflammation, some problems became frequently and are responsible for morbility and mortality related to Chagas disease.

## 3. The Relationship between Chagas Disease and Cancer

When we seek scientific studies with the descriptors "Chagas disease" and "cancer" can be found more than 4130 results, which more than half were published in the last 10 years. Nevertheless, few articles discuss the clinically CD and the relation with different types of cancer.

Most of the patients that have CD get infected in childhood and develop cell destruction and proliferation due to the inflammatory process caused by parasite. This chronic cell inflammation and proliferation are thought to facilitate the occurrence of neoplasias in such individuals [13]. The inflammatory process can generate reactive oxygen species that induce DNA damage at the same time that can hamper cells' DNA strand repair process. These two mechanisms together lead to the occurrence of mutation, evasion of the defense mechanisms of the human host, cell invasion process metastasis [14] and activation of angiogenic pathways [15].

Chagas disease mainly affects the nervous system, gastrointestinal tract (GIT) and heart. A frequently problem in GIT is the denervation, which occurs to variable degrees, is irregular and noncontinuous, and probably depends on both parasitic and host factors [16]. It can leads to loss of motor coordination and achalasia of the sphincters, preventing these segments from emptying semisolid material, thereby causing dilatation. Approximately one third of patients can develop gastrointestinal manifestation including the dilatation of organs (megasyndromes), e.g. megacolon, megaesophagus, mega stomach, mega duodenum, mega jejunum, mega gallbladder and mega choledochus. The megasyndromes develops in about 10% to 15% of chronically infected patients [17,18]. Patients with megaesophagus also have an increased prevalence of cancer of the esophagus. One of the most

important points to explain this relationship is the increase of gastroesophageal reflux in megaesophagus. However, an increased frequency of colorectal cancer has not been reported in patients with megacolon.

A review of 4,690 necropsies and 24,209 surgical specimens showed that the prevalence of malignant tumors of the large bowel was not higher in chagasic megacolon [19]. In order to evaluate if chagasic patients may have an increased risk of developing other type of cancer when compared to non-chagasic, few studies have been conducted.

Dominical *et al.*, studied the epidemiological profile of patients with CD and gynecologic neoplasia (GN) and concluded that CD was not a risk factor or protective against the development of GN [14].

Interacting cells have been reported to exchange membranes and associated proteins, highlighting trogocytosis by its relation with *T. cruzi* [20, 21]. Trogocytosis occurs when cells, not only cells of the immune system, interact physically enabling transfer of cellular material (membranes and proteins) between these cells [22].

Recently, was reported trogocytosis between unrelated eukaryotic organisms (*Entamoeba histolytica* and host cells) [23]. This fact was also observed between trypomastigotes and amastigotes of *T. cruzi* and the mammalian cells it infects [22].

In the study about the functionality of trogocytosis process to *E. histolytica*, it was concluded that this mechanism contributes to killing of host cells and tissue invasion and destruction [23]. However, in the case of T. cruzi, we do not know the precise reason for the transfer of surface molecules, but it is evident a change of parasite cell phenotype and probably an increase of survival advantage for the parasite [22]. Although, apparently trogocytosis to be a survival process for *T. cruzi*, more studies are required to assess if this mechanism affect carcinogenesis by increasing free radical or modification of apoptosis factors.

Although antitumor effects have been reported for a variety of parasites [24], *T. cruzi* molecules and possible mechanism responsible for these effects, have been poorly defined.

While the prevalence of tumor aggressions in *T. cruzi* infected humans has not been assessed, more than 100 different kinds of tumors are frequently diagnosed in other infected host mammal species. Calreticulin (CRT) as a multi-functional endoplasmic reticulum protein is involved in a important cellular processes which ranges from chaperoning [25], integrin binding [26], attachment to steroid hormone receptors [27], $Ca^{2+}$ binding and finally malignant formation and progression. Interestingly, Ramirez *et al.*. observed that *T. cruzi* calreticulin has antiangiogenic activity which allows a turnaround in relation to carcinogenesis. Nonetheless, further studies are necessary to confirm this property [28].

While there is no conclusion on the possible carcinogenic or antitumor effects, it becomes necessary to develop effective drugs to combat the disease in both acute and chronic phases with low side effect

# 4. Treatment against Chagas Disease

### *4.1. The Emergence of the First Drugs*

Accompanied by the finding of CD and confirmation of the health problems caused by this disease, there was an urgent needing of drugs to treat the affected patients. Several potential trypanocidal compounds have been tested before nifurtimox and benznidazole (the first two drugs used against *T. cruzi*) breakthrough. Experiments were conducted with drugs like atoxil, fuchsin and mercury, which however, showed no efficacy [29]. In 1933, Stein tested bismuth derivatives in animal models and after the World War II other drugs were tested, but none succeeded. In 1972, the prodrug nifurtimox was released, showing reduction of parasitemia and lethality of the treated patients, but it was actually withdrawn from the market because of its high toxicity [30]. Nifurtimox has been used for more 40 years to treat CD and its part of a recently approved combinational therapy that targets West African trypanosomiasis. The current specific chemotherapy, is based on two drugs introduced for clinical use in late 1960 and early 70s, nifurtimox (NFX, Lampit®) and benznidazole (BZN, Rochagan®) [31].

Both drugs are nitroheterocyclic compounds, i.e. they contain a nitro group linked to a furan or imidazole ring, respectively. At the moment, its mechanism of action is poorly understood. It is believed that the formation of reactive oxygen and nitrogen intermediates from nifurtimox induces oxidative stress in the target cell. The precise mode of action of nitroheterocyclic drugs in trypanosomes was originally unclear, with two hypotheses proposed.

The first emerged from observations that activation of either drug can lead to the formation of reactive oxygen species (ROS) [32, 33], a process that involves a one-electron reduction of the drug by trypanosomal type II nitroreductase (type II NTR) activity. In the presence of oxygen, this promotes superoxide anion production and drug regeneration, a process known as futile cycling (Figure 1). To date, several trypanosomal flavin adenine dinucleotide (FAD)-containing enzymes, including trypanothione reductase (TR), lipoamide dehydrogenase and cytochrome P450 reductase have been implicated in nifurtimox reduction [34-36]. This mode of action was considered attractive because trypanosomes were thought to lack many of the “classical” eukaryotic enzymes responsible for ROS detoxification. However, far from being deficient in such activities, trypanosomatids actually possess a series of novel oxidative defense pathways. The only direct link between drug-induced ROS formation and trypanocidal activity comes from gene deletion experiments on *T. brucei* SODB1, which encodes a superoxide dismutase. Parasites lacking SODB1 are hypersensitive to nifurtimox and benznidazole. Functional analysis of other oxidative defense pathways has failed to find a link with the trypanocidal activity of nitroheterocyclic drugs [37].

The second hypothetical mechanism was based on the demonstrated antimicrobial activity of nitrofurans [38]. Here, flavin mononucleotide (FMN)-containing, oxygen-insentive type I NTRs mediate a series of two-electron reductions of the

conserved nitrogroup, through nitroso, to a hydroxylamine derivative, using reduced nicotinamide adenine dinucleotide (phosphate) [NAD(P)H] as the source of reducing equivalents. The hydroxylamine can react to generate nitrenium cations (Figure 1) which promote DNA breakdown [39]. In addition to this, the highly electrophilic intermediaries may affect other molecules in the cell. The decrease in free thiol content observed in nifurtimox- and benznidazole-treated *T. cruzi* could be due to conjugation of the thiol with the nitroso from of the drugs, thus affecting the redox status of the cell. Two trypanosomal enzymes with this type I activity have been reported. The first is a prostaglandin F2alpha synthase (PGFS) that can only mediate two-electron reduction of nifurtimox under anaerobic conditions [40]. The second, for wich there is now strong experimental evidence is a type I NTR [41]. This class of enzyme had been regarded as specific to bacteria, and absent in eukaryotes; trypanosomes are now a major exception.

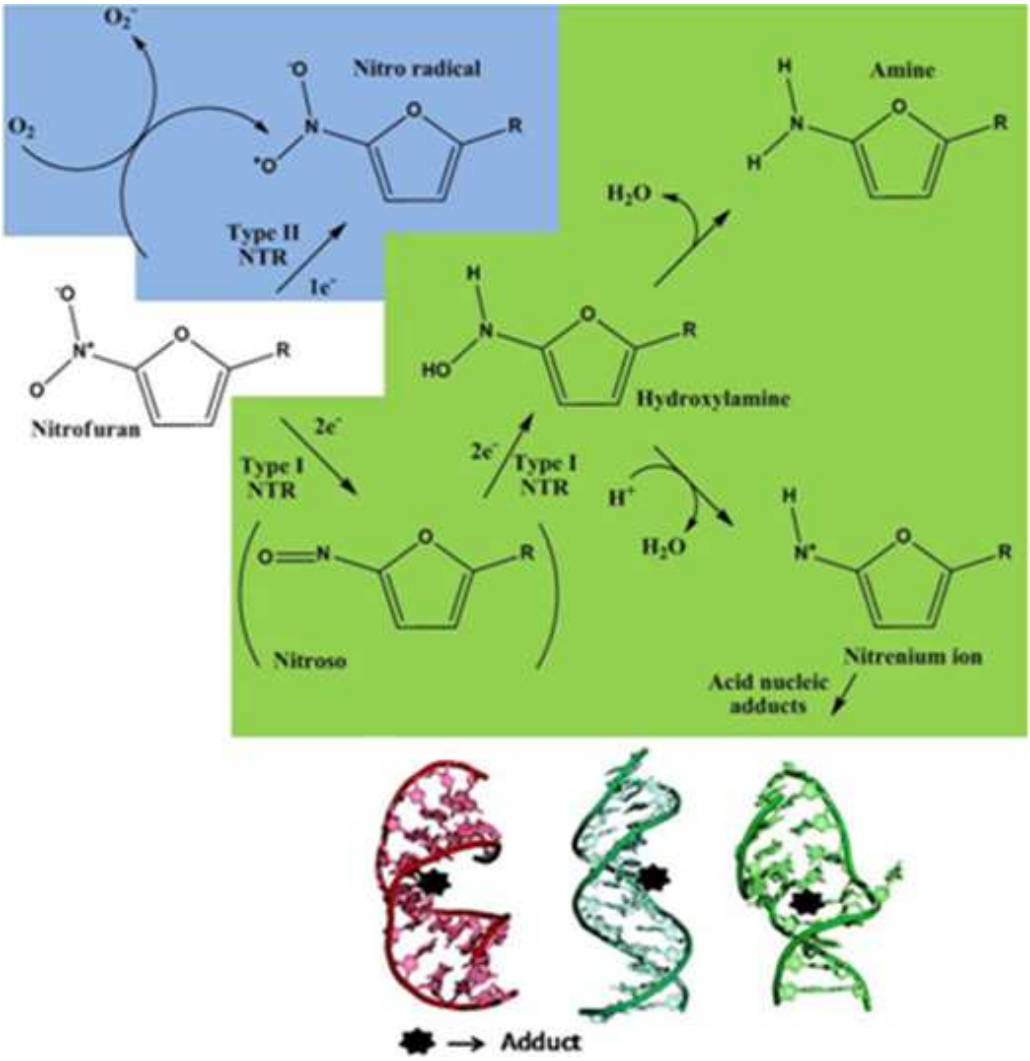


***Figure 1.*** *Reduction of nitrofuran ring by nitroreductases from two ways, Type I (highlighted in green) and Type II (highlighted in blue). The nucleic acid adducts can change the conformation of DNA, often causing mutations or cell death.*

Nowadays, the only commercially available trypanocidal drug is the benznidazole, which generates various adverse drug reactions (ADRs) such as dermatitis, bone marrow depression, thrombocytopenia and agranulocytosis. In the acute phase, the effectiveness of this medication is 70 to 100%. However, some studies have reported treatment failure in some chagasic patients in acute phase, likely due to infections by strains naturally resistant to the drug [4]. The difference in effectiveness varies according to geographical area, probably due to distinctions in susceptibility among unlike strains of *T. cruzi* [31]. The clinical limitations of this compound regarding the antiparasitic activity arise during the chronic disease, where over 80% of patients are not cured parasitologically when the disease reaches this phase. The reasons behind the large difference in the antiparasitic efficacy of heterocyclic compounds on acute and chronic stages are still unclear [42], but they could arise due to pharmacokinetic properties, such as short half-life of the drug and limited tissue penetration. These properties limit the action in the chronic stage when the parasites are mostly confined to the deep tissues and exhibit slow replication [43].

Conversely to what was observed in the treatment of adults, in infants and children, treatment with benznidazole seems associated with a lower incidence and decreased severity of

ADRs, but these effects have not been clearly characterized [44]. It is possibly related to pharmacokinetic effects, as the larger clearance of benznidazole in children when compared to adult patients. This physiologic difference between metabolism and consequent clearance causes an increase on drug concentration in adults, which might lead to the toxicity observed in older people.

### *4.2. New Drugs and Therapeutic Targets*

Nowadays, the researchers are looking for novel drugs in order to eliminate the parasite and minimize the ADRs caused by currently available therapy. The development of new antiparasitic therapy is given mainly in three ways: using the active metabolites of plants commonly used in folk medicine, testing drugs previously approved for the treatment of other diseases (through high-throughput screenings - HTS) or determining specific targets present in metabolic pathways of the parasite that are fundamental for its life [45]. Recent efforts to develop new drugs are occurring almost exclusively as preclinical research, although phase II studies for the antifungal posaconazole and ravuconazole (prodrug) are being planned [46]. The fact that since the discovery of CD, only a few compounds have advanced into clinical trials, suggests, at least in part, the lack of a consensus on appropriate protocols for tests in vivo and in vitro, e.g. standardizing the pharmacological steps. In order to fulfill that need, an algorithm for in vivo and in vitro tests was elaborated to serve as a protocol to evaluate drugs for potential treatment against *T. cruzi* [47], however, not every study follow this protocol, which impairs the pharmacological results qualitative analysis.

#### *4.2.1. Inhibitors of the Ergosterol Synthesis*

Research during the last two decades has consistently demonstrated that the protozoan *T. cruzi*, like most fungi and yeasts, requires specific sterols to maintain cell viability and proliferation in all stages of its life cycle. In particular, the ergosterol biosynthesis pathway has been chemically validated in vitro at many different steps [31, 49]. The ergosterol is the main sterol required for *T. cruzi*, fungi and yeasts. It is crucial for their proliferation and cell viability, because the microorganisms are not able to use cholesterol from the vertebrate host. Therefore obviously inhibiting the biosynthesis of ergosterol became an important strategy for selective drug development against CD. Figure 2 outlines some therapeutic classes which possess the mechanism of action related directly to the biosynthesis of ergosterol [48]. Notice that knowing the enzymes involved in this biosynthetic pathway becomes crucial to understanding and predicting the planning of new drugs.

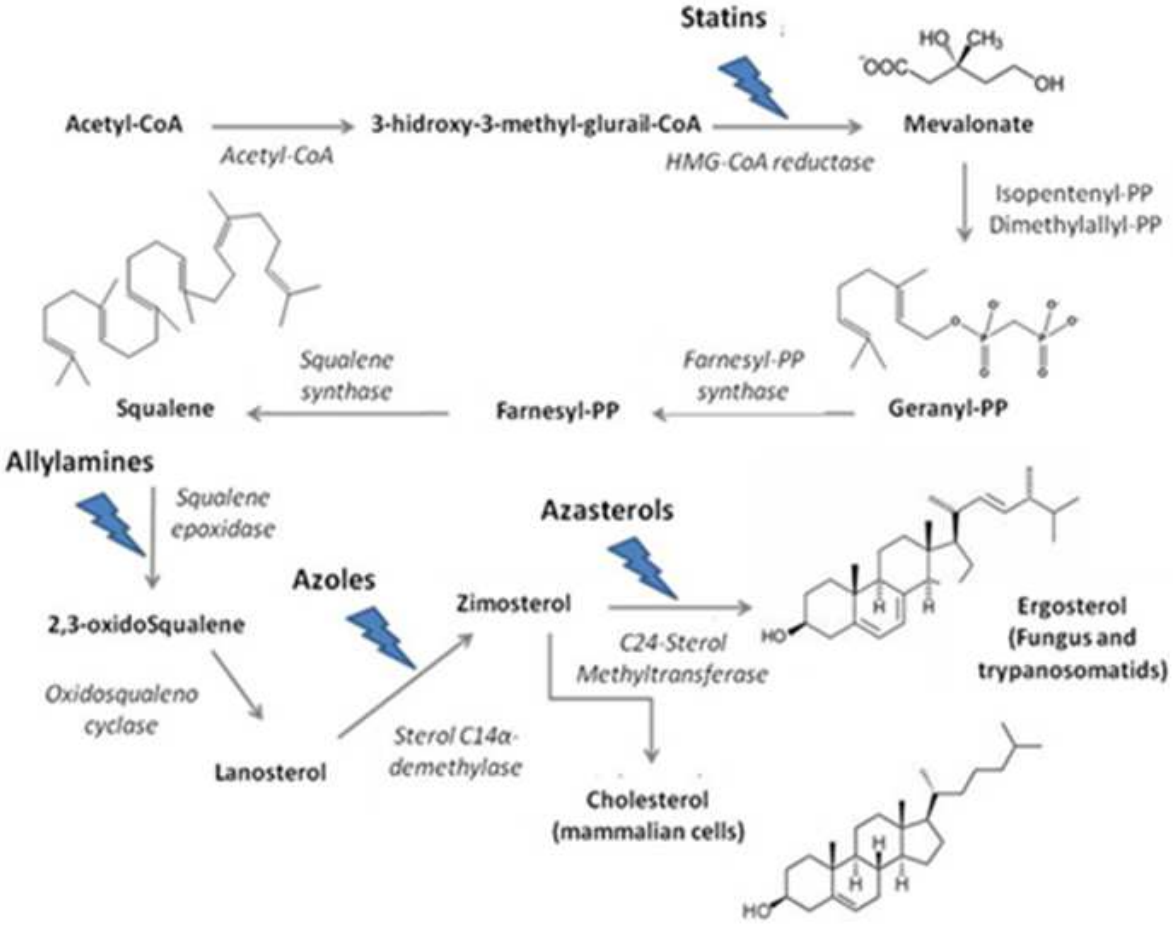


***Figure 2.*** *The biosynthesis of ergosterol and cholesterol, showing the main steps, the enzymes involved, and the known inhibitors.*

#### *4.2.2. Hydroxymethylglutaryl-Coenzyme a Reductase (HMG-COA Reductase or HMGR)*

HMGR catalyses the important reduction of hydroxymethyl glutaryl-coenzyme A to mevalonate. HMGR Inhibitors, more commonly referred as statins, are effective and safe drugs widely prescribed in cholesterol-lowering therapy. Statins act blocking this enzymatic mechanism inhibiting, ultimately, the synthesis of cholesterol and ergosterol, as can be seen in Figure 2 [49-51].

One of the most popular drugs of this therapeutic class is the lovastatin, behaving as a competitive inhibitor of HMG-CoA of *T. cruzi* epimastigotes. Nevertheless, when combined with ketoconazole or terbinafine, statin drugs optimizes the antiproliferative effects both in vitro and in vivo [52, 53].

Experimental and clinical studies have indicated that statins, present cardiovascular protective properties that complement their lipid-lowering effects [54, 55]. Chagas cardiomyopathy (CC) is a progressive inflammatory disease caused by the *T. cruzi* that has been considered the most common form of non-ischemic cardiomyopathy worldwide [56]. CC is initially marked by the presence of an inflammatory infiltration leading, in some years, to conductive and functional heart alterations. The pro-inflammatory cytokines: tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) play a key role controlling tissue parasitism during *T. cruzi* infection, whereas regulatory cytokine interleukin-10 (IL-10) seems to moderate this response, suggesting a protective state to the infected host [57].Statins have also shown the ability to reduce systemic inflammation, improve endothelial function, stimulate angiogenesis and mobilize bone marrow derived stem cells. Evidence from several large clinical trials highlighted a reduction of morbidity and mortality in patients with distinct cardiovascular diseases, contributing to the improvement of left ventricular function and the prevention or attenuation of progressive left ventricular remodeling in heart failure [55, 58, 59]. Mello *et al.* (2011) reinforced the benefits of statin therapy using dog models, mainly after 6 months of treatment, when it became possible to identify the difference in the quantification of collagen (fibrosis) [57]. According to them, another important factor is that, besides interfering in the blood parasite replication during acute phase, the chronic therapy of simvastatin was able to modulate the inflammatory response without inducing a global immunosuppressive state on dogs and consequently, did not alter parasite replication in the chronic phase. In a nutshell, statin drugs have two therapeutic actions against CD, one presenting direct effect on biosynthesis of ergosterol and another that minimizes the cardiac effect, through anti-inflammatory effects.

#### *4.2.3. Farnesyl Pyrophosphate Synthase (FPPS)*

Another tripanossomal enzyme emerging as a potential target for new drugs is FPPS that catalyzes the biosynthesis of ubiquinones, heme and sterols of the parasite [60]. Sigman and colleagues (2006) studied the structure of the *T. cruzi* farnesyl pyrophosphate synthase (TcFPPS) in order to obtain a structural model allowing the design of new inhibitors that may be useful for the treatment. The knowledge of amino acid distribution at the active site can be exploited to rationally design TcFPPS inhibitors [61].

Scientific evidences have shown that a variety of bisphosphonate compounds, such as alendronate, pamidronate and risedronate are potent inhibitors of TcFFPS [61-65]. In a study conducted with animals, risedronate showed a reduction of over 90% of the number of parasites in the blood, a significant increase on animal survival as well as on the decrease of mortality, suggesting a trypanocidal activity of the compound [66]. Esteva and colleagues (2005) synthesized farnesyl transferase inhibitors analog to benzophenone and observed that derivatives of R-phenylalanine and N-propylpiperazin achieved excellent in vitro results. The tests carried out in vivo obtained, after 115 days of infection, a survival rate ranging between 60-80% which confirms the potential of the tested prototypes [67].

There was a significant reduction in mortality, but irrelevant changes regarding myocardial disease when risedronate was administered subcutaneously thrice a week in mice infected with a Brazilian strain of *Trypanosoma cruzi* [68]. Some complexes including risedronate and metallic ions such as Cu, Co, Mn and Ni were synthesized and tested in vitro against epimastigotes and intracellular amastigotes of *T. cruzi*. The results showed that the complexes have a better antiproliferative effect against *T. cruzi* than the free ligand. Furthermore, some pharmacokinetic studies evaluated the interaction with proteins and confirmed that the complexes have a strong interaction with albumin, facilitating their transport to the tissues in vivo [69]. Potential binding to plasma proteins (e.g. albumin) prolong the plasma half-life time which may be useful to optimize the therapeutic diets as well as the adherence of the patient to the treatment.

#### *4.2.4. Squalene Synthase (SQS)*

SQS (or Farnesyl-diphosphate farnesyltransferase - FDFT1) catalyzes the biosynthesis of squalene, a key ergosterol precursor, through a reductive dimerization of two farnesyl diphosphate (FPP) molecules. The reaction is unique when compared with those of other FPP-utilizing enzymes and proceeds in two distinct steps, both of which involve the formation of carbocationic reaction intermediates. Because FPP is located at the final branch point in the isoprenoid biosynthesis pathway, its conversion to squalene through the action of SQS represents the first committed step in the formation of ergosterol, making it an attractive target for therapeutic intervention [70].

The first reports of active oral inhibitors of this enzyme in *T. cruzi* were associated to the compounds E5700 and ER119884. The compounds were classified as potent noncompetitive inhibitors, and E5700 was able to reduce mortality and parasitemia in vivo experiments [71]. The characteristic of noncompetitive inhibition is interesting to the treatment because it does not depend on the concentration of endogenous substrate.

In addition, it was demonstrated that aryloxyethyl

thiocyanate derivatives are potent inhibitors of *T. cruzi* proliferation [72]. It has been found that growth inhibition of *T. cruzi* epimastigotes induced by 4-Phenoxyphenoxyethyl thiocyanate also known as WC-9 was associated with a reduction in the content of the parasite's endogenous sterols due to a specific blockade of their de novo synthesis at the level of squalene synthase [73].

Previous studies on WC-9 have indicated that structural variation at the B ring had no influence on biological action [74, 74], it was thought that the introduction of a fluorine atom at the B ring would benefit biological activity. The estimated log P values for this modification increased from 4.51 (WC-9) to 4.71 (fluorine-containing analog). As the drugs must penetrate two cellular bilayers to reach TcSQS, a better biological action could be anticipated. Then, fluorine-containing drugs were designed, synthesized, and biologically evaluated against the intracellular and the epimastigote forms of *T. cruzi*, showing good effects. These promissing compounds were potent growth inhibitors of the intracellular form of *T. cruzi*. In fact, these drugs exhibited IC50 values of 4.3 μM (meta-substituted) and 3.7 μM (para-substituted), respectively being 4-fold more potent than the well-known antiparasitic agent WC-9 (lead compound), used as a positive control, under the same assay conditions. The same behavior was observed against the epimastigote form of *T. cruzi* where the analogs were more potent than WC-9 but in a lesser extent than against amastigotes [72]. After analyzing the structure-activity relationship of WC-9 and its fluorine-containing analogues has become possible to observe that the increase of Log P values optimizes the efficiency of the lead compound. Therefore, it is expected that the next planned analogs should contain lipophilic substituents on *meta* and *para* positions. There are important scientific studies that can support a rational design of new SQS inhibitors using molecular modeling [76, 77] and structure activity relationship [71, 78, 79].

#### *4.2.5. Oxidosqualene Cyclase (OSC)*

Ergosterol or similar 24-alkyl sterols are one of the final products of the sterol synthetic pathway of some pathogenic protozoa including Trypanosoma species. In 2001, Buckner and colleagues reported the development of oxidosqualene cyclase enzyme inhibitors [80]. The activity of these inhibitors can be explained as a consequence of depletion of the ergosterol or of accumulation of toxic intermediates and side products [81].

The activity of a potential compound, N-(4E,8E)-5,9,13-trimethyl-4,8,12-tetradecatrien-1-ylpyridin ium, causes accumulation of 2,3-oxidoesqualeno but, on the other hand it induces a reduction of lanosterol and ergosterol synthesis in *T. cruzi*, This effect causes a mechanism for inhibiting the growth of the plasmid. Besides this compound, other 27 derived from it were tested and, among them, 12 showed activity against trypomastigotes [80].

In the last years, many studies to indentify new OSC inhibitors were carried out. Among them, a set of compounds, previously described as effective inhibitors of human oxidosqualene cyclase [82], were tested for their ability to inhibit oxidosqualene lanosterol cyclases from *T. cruzi* and *P. carinii* expressed in an OSC-defective *Saccharomyces cerevisiae* strain. Balliano and collaborates (2009), made a preliminary screening of 10 compounds from different chemical classes, carried out in cell free homogenates, identifying a potent inhibitor of OSC from *T. cruzi* (IC50 0.07 μM) [83].

Balliano and colleagues (2009) tested a series of 25 compounds, some of which, previously described as inhibitors of human liver microsomal OSC. The screening detected three derivatives particularly promising for the development of novel anti-Trypanosoma agents which showed an activity ≤ 0.3 Mm [83].

Although many studies are looking for potential ligands, at the moment there isn't a tridimensional model of *T. cruzi* OSC`s active site, which would be useful to quantitative and qualitative in silico studies. On the other hand, the homology model for human OSC was obtained and could be useful to correlate the structure and activity of compounds to the differences in the active site of target enzymes [84].

#### *4.2.6. Demethylase C14α-Sterol (CYP51)*

Numerous studies have shown that commercially available ergosterol biosynthesis inhibitors (EBI), which are highly successful for the treatment of fungal diseases, e.g. ketoconazole, itraconazole or terbinafine, have suppressive but not curative effects against *T. cruzi* infections in humans or experimental animals and are unable stop the progression of the disease [85].

Ketoconazole was the first imidazole to display activity against the acute phase of *T. cruzi*; nonetheless, it was proved to be ineffective in chronic phase [86]. In 2005, Santa-Rita and collaborates investigated the synergistic effects of two analogues, edelfosine and miltefosine, when associated with ketoconazole and found an improvement in results. Edelfosine and ketoconazole when administrated non-associated induce morphological changes in the plasma membrane of the parasite, while the combined administration led, in addition, to severe mitochondrial damage, formation of autophagic structures and multinucleation [87].

Fluconazole, itraconazole and other azoles were also subjects of several studies, despite of the induction of resistance to these drugs. This process of increasing resistance was observed through in vitro experiments with fluconazole, ketoconazole, miconazole and itraconazole [88]. Nonetheless, second (R66905, BAY R-3783, SCH39304 and D0870) and third-generation (voriconazole, posaconazole, ravuconazole and TAK-187) triazoles which are potent and selective inhibitors of fungal and protozoan cytochrome P-450-dependent CYP51, have been found to induce radical parasitological cure in vitro [89] and murine models of acute and chronic CD [43].

The antifungal posaconazole (PCZ) is currently the most advanced candidate for the treatment of CD. Its activity results in potent antiparasitic effects both in vitro and in animal models of CD. Molina *et al.*. (2000) demonstrated the potent

trypanocidal effect of posaconazole. Recent studies showed that posaconazole is able to selectively eliminate intracellular amastigotes of *T. cruzi* present in culture of cardiomyocytes [90]. Furthermore, this compound allows complete reorganization of the host cell cytoskeleton and contractile apparatus during in vitro tests [91]. When it was compared to benzinidazol, the anti-Trypanosoma activity of posaconazole is less dependent on interferon gamma and B lymphocytes [92]. A more recent study evaluated the activity of demethylase C14α-sterol enzyme inhibitors, which possess simpler molecular structure than posaconazole, a fact that would allow a reduction in costs in the production of the compound. The research confirmed the activity of two compounds in reducing the parasitemia of infected animal models in the acute phase, showing comparable efficacy to posaconazole [93]. In 2010, the pharmaceutical Merck announced plans to phase II clinical studies evaluating the use of posaconazole for the treatment of chronic CD, to be conducted with 160 adult patients for 360 days. The proposed study is placebo controlled and uses an oral suspension of posaconazole (400 mg twice daily) for 60 days, tested as monotherapy and in combination with benzidazole [94]. The association between posaconazole and amiodarone results in antiproliferative synergism of the drugs (fractional inhibitory concentration < 0.5). This combination of drugs resulted in autophagic death of the intracellular amastigote [95].

The ravuconazole has potent antifungal activity in vitro and in vivo with both broad spectrum and long half-life in humans. Tests carried out on mice with acute experimental infections caused by different nitrofurans and nitroimidazoles resistant *T. cruzi* strains, showed that the ravuconazole was able to induce parasitological cure in all animals infected with CL strain (resistant strain) and in 58% of animals infected with Y strain (partially sensitive to nitrofuran and nitroimidazole). Nevertheless, in animals already infected with the Colombiana strain there was no cure [96].

Diniz and collaborates (2010) tested the ravuconazole in vivo activity on infected dogs [97]. There were no significant side effects and parasitemia was permanently reduced to undetectable levels from the first day of treatment, regardless of analyzed strain. One month after treatment, the results of PCR tests for *T. cruzi* were negative for three of five animals infected with the Y strain and two of five animals infected with Berenice-78. All dogs treated with ravuconazole had negative serological results during up to 30 days after treatment, regardless of the regimen used. The ravuconazole was unable to induce parasitological cure on chronic phase, but has suppressive activity in dogs with the disease is in acute phase. Since the half-life of ravuconazole in humans (4-8 days) is extremely higher than in dogs (8.8 hours) the drug is said to be promising for treatment in humans.

In 2000, infected mice were treated with D0870, which obtained as a result parasitological cure at the acute phase (70 to 100%) in 7 of 9 strains tested, and a lower incidence of death was highlighted [90]. In another study, Molina *et al.* (2001) treated mice infected with CL and Y strains during 30 consecutive days using bis-triazole DO870 incorporated into nanospheres, intravenously and obtained a cure rate of 60 to 90% with both strains at the dose 3 mg / kg / day [98]. However, ketoconazole and itraconazole embedded in nanoparticles did not led to parasitological cure, a fact that still is under study [98].

Another triazole derivative, UR-9825, was also active against epimastigotes and amastigotes of the parasite [99]. In 2003, an experimental triazole called TAK-187 was tested in mice infected with *T. cruzi* in acute and chronic periods of the illness and was considered in vivo as a potent anti-*T. cruzi* agent, even against strains resistant to nitrofuran and nitroimidazole [43].

#### *4.2.7. Cruzipain Enzyme (GP57/51)*

Cruzipain, a member of the papain superfamily, is a cysteine peptidase of *T. cruzi* that is an important virulence factor of this parasite, which is involved in several crucial steps in the interaction with mammalian cells, such as in the host cell invasion (in trypomastigote form), and parasite survival, differentiation and multiplication within host cell [100]. Its role in host cell invasion is mediated through at least two distinct mechanisms [101, 102]. One pathway involves the triggering of the B2 type of bradykinin receptor (B2R), whereas the other pathway is independent of the kinin receptors [101], and is involved in the mobilization of endothelin receptors during the invasion of smooth muscle [103]. Moreover, cruzipain may act negatively on all subclasses of human IgG, which consists one of the escape mechanisms from the adaptive immune response [104]. Thus, cruzipain is considered a validated target for the development of new chemotherapies which can be decisive for the intracellular replication and consequentely the differentiation of trypomastigotes to amastigotes.

Engel *et al.* showed that cruzipain inhibitors are responsible for an accumulation of this enzyme in vesicular compartments, causing abnormalities in the Golgi apparatus and endoplasmic reticulum, which leads to disruption of cellular protein transportation systems and induces parasitic cell death. It has been hypothesized that the trypanocidal effect of cysteine protease inhibitors results from the direct inhibition of the active cruzain contained in the lysosomes [105]. There are several potential inhibitors of the enzyme as peptide and non-peptide derived (triazoles, pyrimidines, chalcones and thiosemicarbazones), complexes of rhenium and gold (oxorhenium and cyclometalated) and nitric oxide donor compounds (nitrosothiols and complexes of iron and ruthenium).

The peptide derivatives are the most important class of inhibitors of cruzipain. In experiments made on immunocompromised mice infected by *T. cruzi*, it was observed that the treatment with the N-methyl-Pip-F-homoF-vinyl sulfonyl phenyl (N-methyl-Pip-F-hF-VS phi) dipeptidic inhibitor prevented the death of these animals, and increased the survival of the most animals treated. It was confirmed by negative PCR results and normal histopathology exam [106]. However, a possible limitation in the use of these inhibitors would be the

development of resistance to these drugs, as observed in investigations conducted by Yong *et al.* [107]. Other *T. cruzi* proteases have also been subject of studies such as cysteine proteases, serine proteases, threonine proteases and metalloproteinases.

Reis and collaborators (2007) reported potent and selective inhibition of cruzipain by the recombinant full-length prodomain of cruzipain. The propeptide did not inhibit human cathepsins S, K or B or papain at the tested concentrations, and moderately inhibited human cathepsin V [108]. Human cathepsin F was very efficiently inhibited (*K*i of 32 pm), an interesting finding indicating that cruzipain propeptide is able to discriminate cathepsin F from other cathepsin L-like enzymes. Studies of comparative structural modeling and analysis recognized the interaction between the *beta*1p-*alpha*3p loop of the propeptide and the propeptide-binding loop of mature enzymes as a probable cause of the observed inhibitory selectivity [108].

Chen *et al.* synthesized an analogue of K11777, known as WRR-483, which showed cruzain-inhibiting effect and blocked the proliferation of *T. cruzi* in cell culture [109]. This compound demonstrated great potency against cruzain eliminating the parasitic infection in mouse models of CD in the acute phase, suggesting then that WRR-483 has a potential to be a new therapeutic tool. The analysis of the several existing protein-ligand complexes (PDB: 2AIM, 3LXS, 3KKU, 3IUT, 3IO6, 3HDE, among others) may help to elucidate how the interaction between cruzipain and these inhibitors occurs.

Figure 3 represents the pharmacophoric residues in cruzipain active site (PDB: 3LXS, resolution of 1.50 Å), and might explain the differences between the pharmacodynamic properties of the inhibitors WRR-483 and K11777. Cruzipain is a dual-specificity protease that binds to substrates containing phenylalanine or arginine in the P2 site, with a better afinity for phenylalanine [109].

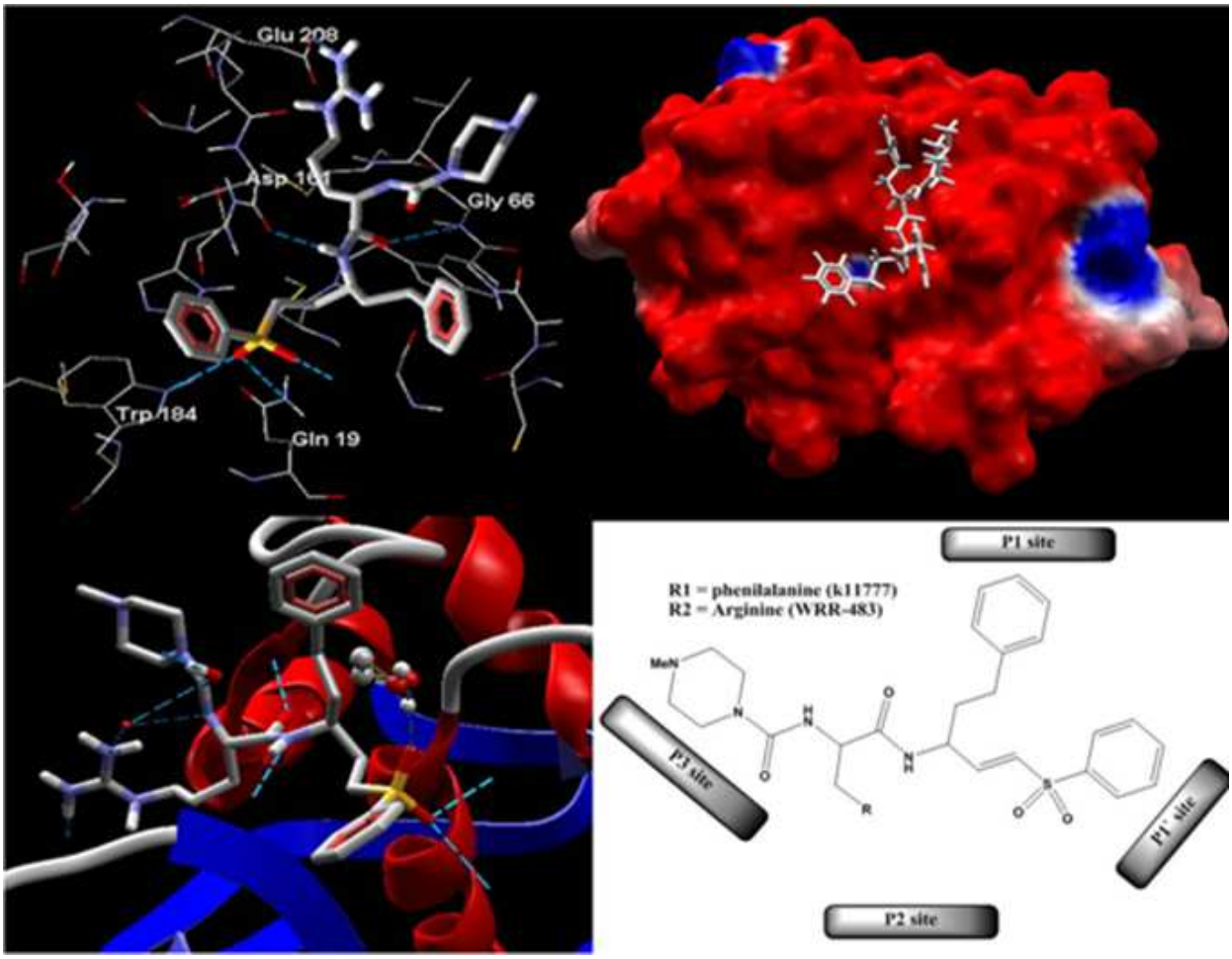


***Figure 3.*** *View of WRR-483 ligand doched in the active site of cruzain, highlighted the electrostatic potential of receptor, secondary structury and residues involved in binding. Structure of vinyl sulfone inhibitors, K11777 (R1) and WRR-483 (R2) are also represented together with the site specific of binding. This figure was built by using the program Molegro Molecular Viewer®, Version 5.5.0.*

### *4.2.8. Trypanothione Reductase (TR or [T(SH)2])*

Discovered in 1985, TR is found in many parasites such as *T. cruzi*, *T. brucei, T. congolense* and *L. donovani*. These parasites have a unique trypanothione-based thiol metabolism, in which the glutathione/glutathione reductase, present in most living organisms, is replaced by trypanothione/trypanothione reductase. TR is a NADPH-dependent flavoprotein that maintains trypanothione in its reduced form. Accordingly, T(SH)2 was soon implicated

in the defense against oxidants, xenobiotics, heavy metals, and in regulatory processes making TR a valid and attractive target for new trypanocidal drugs [110]. The designand testing of specific inhibitors is currently underwayand several families of compounds have been identified as specific TR inhibitors and trypanocidal agents in vitro.

Some alkaloids compounds such as clomipramine and thioridazine were also effective in experimental animal models showing improve in the cardiac function and survival. Clomipramine inhibits trypanothione reductase enzyme from *T. cruzi* leading it to death, and prevents the cardiac damage in mice on acute infection. Current studies confirmed that clomipramine when used in chronic asymptomatic stage of infection with *T. cruzi* modifies the natural evolution of Chagas' heart disease and increases survival of animals [111].

Thioridazine, a known inhibitor of TR in vitro is able to reduce the parasitemia, increase survival and prevent cardiac damage in murine models of acute CD, but no parasitological cures were obtained. Both thioridazine and clomipramine can be used in the chronic phase, the critic step for the patient [112]. They produce a decrease in electrocardiographic alterations, fewer modifications in the affinity and density of cardiac beta-receptors, and fewer isolated areas of fibrosis in the heart. Survival in treated mice was 100% for benznidazole and 88% for thioridazine, independent of the parasite strain; survival for untreated mice was 30% and 40% for Tulahuen strain and SGO-Z12 isolate, respectively. Based on the promising results observed in the chronic phase, new analogues from thioridazine and clomipramine should be planned to avoid the central nervous system and hence side effects [112].

Another class of hit compounds largely studied are the derivatives containing two 1,4-naphtoquinone moieties linked by a polyamine spacer they have exhibited significant inhibition of TR in the low micromolar range (IC50 < 5 μM), particularly when the length of the alkyl linker reached a maximum of 4 or 5 methylene groups [113].

#### *4.2.9. Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT)*

Unlike mammals, protozoan parasites do not possess the enzymes necessary for the *de novo* synthesis of purine nucleotides (auxotrophic for purines) and must rely on salvage pathways to obtain them. In *T. cruzi*, the hypoxanthine phosphoribosyl transferase (HPRT) is responsible for the salvage of the purine nucleotides hypoxanthine (Hx) and guanine (G) [114]. HGPRT catalyzes the magnesium-dependent reversible transfer of a phosphoribosyl group from 5-phospho-α-d-ribosyl-1-pyrophosphate (PRPP) to a purine base (G) to form a purine ribonucleotide, guanosine monophosphate (GMP) [115]. Moreover, it can also initiate the metabolism of certain cytotoxic purine base analogs that have little effect on the mammalian host. This implies that either inhibitors or substrates of HGPRT might serve as efficacious and selective agents for the treatment of diseases for which trypanosomatids are the etiologic agent [116].

In silico techniques through molecular docking studies characterized the HPRT active site and gave a view of essential interactions. The closed conformation of the *T. cruzi* HPRT encompasses an active site that spans approximately 11 X 11 X 7 Å and encloses a volume of approximately 610 Å3. In the crystal structure, this space is entirely filled by well-ordered ligand atoms; these include the PRPP substrate, a purine substrate analog, two magnesium ions and a total of 14 water molecules, five of which are in the first coordination spheres of the two magnesium ions [117]. Five segments of the protein backbone enclose the region: residues 50-53 (loop I), 79-85 (loop II), 111-120 (loop III), 163-165 and 169-173 (loop IV), determining the exact size to optimize the efficacy of new drug candidates [118]. After knowing the residues properties many hits were synthesized as potent and selective inhibitors, such as 6-(2,2-dichloroacetamido) chrysene, which has nanomolar efficacy on HGPRT inhibition.

Other tests have showed that allopurinol induces suppression of *T. cruzi* in mice, primarily in epimastigotes. Allopurinol acts as an alternative substrate for the HGPRT system; it is incorporated into the RNA and leads to the formation of a non-physiological nucleotide that blocks *de novo* synthesis of purines. Nevertheless studies conducted by Lauria-Pires *et al.* showed that allopurinol was ineffective during the acute phase of CD [119].

The combined drug "cocktail" effect has been tested. Actually, some studies have investigated the effect of the clomipramine (trypanothione reductase inhibitor) and allopurinol combination on the treatment of experimental CD in the acute phase. The results show that this relationship produces a greater increase in the rate survival of animals tested compared with the single use of clomipramine. Despite the clinical importance of HGPRT, at the moment, few studies are investigating the protein-ligands interaction, the strucutural basis of the catalysis [120] or rational drug design.

#### *4.2.10. DNA Topoisomerase I and II*

DNA topoisomerases are enzymes that regulate the DNA topological state by introducing or removing supercoiling, knots or catenations in DNA molecules. The dynamic nature of DNA is required for its essential biological functions such as replication, transcription, recombination, repair and DNA segregation [121]. In the last 30 years, they have attracted the attention of the scientific community, because is essential to DNA replication of *T. cruzi* [122]. Currently DNA topoisomerases have been recognized as potential chemotherapeutic targets for antitumor and antiparasitic agents. DNA topoisomerases are ubiquitous enzymes that control many vital cellular processes by making reversible DNA breaks, enabling a specific tyrosyl residue in the enzyme to covalently link to the phosphoryl group at the DNA break via a phosphodiester bond. They have been classified into two types. The type I enzymes make a transient single stranded nick in absence of any high energy cofactor, whereas the type II enzymes make double-stranded breaks in the presence of ATP, which allows supercoils to be removed from the circular DNAs. Both types of enzymes have been characterized in kinetoplastid hemoflagellated protozoan parasites [123].

Most topo I-targeted drugs act by stabilizing the covalent topoisomerase–DNA complex, thus preventing re-ligation. As a consequence, topo I inhibitors exhibit cell cycle arrest with activation of DNA damage signals that induce DNA repair mechanisms or apoptosis [124]. Topo II inhibitors such as nalidixic acid, etoposide and mitoxantrone enhance irreversible topo II-mediated DNA cleavages, thus transforming this essential enzyme into a potent cellular toxin. Conversely, topo II inhibitors, which affect enzyme activity, do not have the ability to stimulate DNA cleavage like novobiocin, aclarubicin, merbarone and staurosporine [125].

In trypanosomatids, a few reports have proposed that such groove-binder drugs interact preferentially with AT-rich mitochondrial DNA rather than with nuclear DNA. This is in agreement with the proposal that topo II is unable to interact with DNA sites where the minor groove is occupied by ligand drugs [122].

Some effects related with these targets were observed when ofloxacin, a nalidixic acid derivative, was tested. This drug inhibits topoisomerase II causing blockage in the differentiation of amastigotes in cell culture without however, presenting parasitological cure in trials with mice. Later, it was shown that even some drugs like pentamidine, ISM (isometamidium chloride hydrochloride) and diminazene inhibited the mitochondrial topoisomerase II, but not the nuclear topoisomerase. In 1995, Bodley and Shapiro described the camptothecin as topoisomerase I inhibitor, which induced the cleavage of nuclear and mitochondrial DNA in *T. cruzi* [126]. There are some commercially available antineoplasic drugs that act as inhibitors of topoisomerase I (irinotecan [Camptosar®] and topotecan [Hycamtin®]) and as inhibitors of topoisomerase II (etoposide [Vepesid®] and teniposide [Vumon®]). Anthracyclines, camptothecin, acridines and fluoroquinolone form well-known inhibitors classes, which show good results against blood trypomastigotes of *T. cruzi*.

Recently, a study about the effects of various topoisomerase inhibitors (camptothecin, rebeccamycin, merbarone, mitoxantrone, norfloxacin and enoxacin) and DNA-binding (berenil and distamycin) drugs tested the ultrastructure and cellular proliferation of *T. cruzi* epimastigote form. *Blastocrithidia culicis* was used as a comparative model, which has a more relaxed kinetoplast DNA (kDNA) organization [122]. The results showed that the eukaryotic topoisomerase I inhibitors camptothecin and rebeccamycin were the most effective compounds in the arrest of *T. cruzi* proliferation, i.e. unpacking of heterochromatin and mitochondrial swelling. The eukaryotic topoisomerase II inhibitors, mitoxantrone, but not merbarone, was effective against cell proliferation. The prokaryotic topoisomerase II inhibitors norfloxacin and enoxacin targeted the kinetoplast specifically, thus promoting ultrastructural kDNA rearrangement in *B. culicis*. Of the DNA-binding drugs, berenil caused remarkable kDNA disorganization. It is noteworthy that although in this study kinetoplast ultrastructural alteration in the *T. cruzi* epimastigote form treated with distamycin was not observed, another research reported intense kDNA network disruption in the trypomastigote form after treatment with several aromatic diamidines [127]. Unfortunately, although agents that affect DNA topoisomerases have proven to be useful to treat cancer, there have been very few studies on trypanosomatids effects.

#### *4.2.11. Glycolysis*

The infective trypomastigote forms of *T. cruzi* depend on glycolysis in order to produce ATP because it does not possess the tricarboxylic acid cycle. Thus, this pathway becomes an attractive target for the development of new drugs to treat CD. *Trypanosoma cruzi*, the etiologic agent of CD, has an unusual ATP-dependent hexokinase (TcHK) that is not affected by D-glucose 6-phosphate, but is non-competitively inhibited by inorganic pyrophosphate (PPi), suggesting a heterotropic modulator effect [128].

Recently, a different class of bisphosphonates (aromatic amino-methylene derivatives) has been identified as potent and selective inhibitors of *T. cruzi* hexokinase [129]. It was observed that these compounds blocked glycolysis and growth of both extracellular epimastigotes and intracellular amastigotes, with a selectivity ratio of > 3 orders of magnitude. Such compounds are the first known selective inhibitors of glycolysis in Trypanosomatid parasites and constitute a promising new class of anti *T. cruzi* agents [129]. Obviously, we should not be little other studies in this field that described the kinetic and metabolic [128] and QSAR properties [130, 131].

Other enzymatic targets are aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, glycerol-3-phosphate dehydrogenase and phosphofructo kinase, whose inhibition could lead to a reduction in parasital glycolysis [132, 133]. Among the compounds described as inhibitors of the glycolytic pathway nucleosides, nitric oxide, nitroxyl and peroxinirito stand out, as well as some secondary metabolites like coumarins and flavonoids [134, 135].

#### *4.2.12. Other Promising Targets*

Trans-Sialidase, an enzyme from *Trypanosoma cruzi* (TcTS) that is involved in glycosylation of mucins for recognition and adhesion of the parasite in the host cell, has also been studied. Benzoic acid and pyridine derivatives have shown to be weak inhibitors of the enzyme. Nevertheless, the 4-acetylamino-3-hydroxymethylbenzoic and 5-acetylamino-6-aminopyridine-2-carboxylic acids are considered lead compounds to the development of new inhibitors [136]. The compound 3-benzothiazole-2-yl-4-phenyl-but-3-enoic acid, has proved to be an effective prototype through in silico studies, but need more safe results through in vitro and vivo studies [4].

A library prepared from the Evotec database of commercially available compounds was screened using the molecular docking program GOLD, following the application of drug-likeness filters. After that, top-scoring ligands were purchased and assayed using a fluorimetric assay, where 3-benzothiazole-2-yl-4-phenyl-but-3-enoic acid stood out [4]. Using the available TcTs crystal structure it became possible

to identify pharmacophoric regions of the active site. Specifically, hydrogen bonding interactions with Tyr119, Arg314, Arg245, Arg53, Asp96, Asp59 and hydrophobic interaction with Trp312. Docking studies identified two pharmacophoric residues, i.e. Tyr119 and Trp312, which should be explored by techniques of medicinal chemistry.

Authors have investigated the trypanocidal activity of N-allyl (NAOx) and N-propyl (NPOX) oxamats, as well as N-allyl (Et-NAOx) and N-propyl (Et-NPOX) oxamatethyl esters in vitro and in vivo, using murine model and five different strains. The ethyl esters (Et-NAOx and Et-NPOX) exhibited trypanocidal activity on five tested strains of *T. cruzi* [137]. In subsequent studies using ethyl esters of N-propyl (Et-NPOX) and N-isopropyl (Et-NIPOX) oxamats, there was a marked reduction of parasitemia in infected mice with effects especially on amastigotes [138]. Kinetic studies showed that NAOx and NPOX were competitive inhibitors of α-hydroxyacid dehydrogenase isozyme II (II-Hadham isoenzyme) that participates in energy metabolism of *T. cruzi* [139].

Unsaturated fatty acids (UFAs) are components of biological membranes that are essential for determining their structure and functions. They maintain the correct viscosity of the hydrophobic core of the bilayer, and are, therefore, responsible for the mobility and function of embedded proteins and for providing flexibility and selective permeability to cellular membranes. In addition, they serve as precursors for a number of biologically active molecules such as prostaglandins, leukotrienes and tromboxanes. Parasitic protists, including trypanosomatids like *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* spp., have a high proportion of UFAs. This fact could indicate that the biosynthetic pathways of these fatty acids (FAs) are essential in these flagellates. This would not be unexpected, considering the complexity of the life cycles of these parasites, during which they have to adapt to dramatic differences of temperature and change their morphology. Highly fluid membranes may be advantageous to handle these changes [140]. Synthesis of UFAs involves the activity of desaturases, which are enzymes that introduce double bonds in the aliphatic chain of FAs, using molecular oxygen and reducing equivalents. The structural differences and lack of activity of oleate desaturase in mammalian cells, also makes this enzyme a promising therapeutic target. Four isomers of tiastearato and isoxil (thiocarlide) on epimastigotes were tested and proven potential inhibitors of oleate desaturase [141].

Another important drug target receiving considerable attention is the *T. cruzi* is the flavoenzyme dihydroorotate dehydrogenase (TcDHODH; EC1.3.99.11), which catalyzes a coupled redox reaction in which dihydroorotate is oxidized to orotate and fumarate is reduced tosuccinate. In DHODH knockout studies, *T. cruzi* did not express the enzyme and could not survive even in the presence of pyrimidine nucleosides, confirming its dependence on *de novo* biosynthesis [142]. The study of Cheleski and collaborates showed that the physical-chemical characteristics of DHODH are an important tool to design new inhibitors against *T. cruzi*. Surveys that assessed a series of methanolic extracts of green, brown and red algae have showed that they present biological activity as dihydroorotate dehydrogenase (DHOD) inhibitor [142]. The extracts of *Fucus Evanescense* and *Babingtonii Pelvetia algae*, provided a reduction of up to 59% in activity of DHOD [143].

Another therapeutic strategy is to block the biosynthesis of mRNA expression of the parasite. Many drugs were evaluated and the most promising compounds were hydroxymethylnitrofurazone (NFOH) and nitrofurazone, which significantly reduced the RNA expression, leading to a decrease in the number of parasites [144]. Hydroxymethylnitrofurazone (NFOH) is a prodrug that is active against *Trypanosoma cruzi*, however, it presents low solubility and high toxicity. In order to solve this issue the drug-delivery system formed by hydroxypropyl-β-cyclodextrin (HP-β-CD) has been used, because it improves the physical-chemical properties of NFOH [144].

The dUTpase is an additional enzymatic target involved in metabolism and replication of nucleotides. The native conformation of dUTpase from *T. cruzi* was determined and it revealed stumpy structural similarities with human dUTpase. This suggests certain specificity, making this enzyme another potential therapeutic target until today; however, no drug had demonstrated potential inhibition [145].

The enzyme dihydrofolate reductase (DHFR) has also been studied [146, 147]. The role of DHFR is to catalyse the NAPDHdependent reduction of dihydrofolate to give tetrahydrofolate, a central component in the single carbonmetabolic pathway. The tetrahydrofolate is methylated to methylene tetrahydrofolate, which is directly involved in thymidine synthesis (assisting the methylation of deoxyuridine monophosphate to give thymidine monophosphate) and indirectly implicated in the metabolism of amino acids and purine nucleotide. Inhibition of DHFR thus prevents biosynthesis of DNA leading to cell death [147]. During the trials, methotrexate and some synthetic derivatives showed selectivity for tripanosomal DHFR, in addition, two of these compounds showed activity in vivo [148, 149]. Inhibitory activity was also found in a series of compounds synthesized from 2,4-diaminoquinazolines [150, 151]. Another study involving a series of 2-exo-aryl-1,4-epoxy-2,3,4,5-tetrahydronaphtho [1,2-b] azepines and cis-2-aryl-4-hydroxy tetrahidronaphtho-2,3,4,5-[1,2-b] azepines analogs, have been active against the free and intracellular forms of *T. cruzi*, with low toxicity in mammalian cells [152]. Thiocyanate derivatives have also proved effective against the proliferation of *T. cruzi*, although their mechanism of action has not yet been determined [72].

Glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) is a glycolytic enzyme that catalyzes the oxidativephosphorylation of glyceraldehyde-3-phosphate (GAP) to 1,3-bisphosphoglycerate (BGP). The three dimensionalstructure of *T. cruzi* gGAPDH shows differences, when compared to the homologous human enzyme that could

be exploited for selective inhibition [153]. In 2010 a survey experimented nitric oxide donor compounds such as ruthenium which showed potent trypanocidal activity in vitro and in vivo. The study has also revealed that the mechanism of action of these compounds was related with GAPDH [154]. Moreover, three new flavonoids extracted from *Neoraptua magnifica* var. have showed inhibitory potentials [155]. Furthermore, several synthetic analogs of adenosine compounds had their inhibitory effect against GAPDH tested, showing promising results [156]. Studies of structure-activity relationship (SAR) of certain inhibitors, including a series of coumarins, flavonoids, nucleosides, as well as tests of inhibitory activity of the enzyme GAPDH by anacardic acid and semi-synthetic products were also carried out in order to allow better development of new ligands [134, 157-159].

In addition to the aforementioned synthetic compounds, a series of products which include thiadiazine, oxadiazole, pyridine, acridine and phenothiazine derivatives, metal chelators and metal complexes derived from propylene-1-amine have been studied [160]. Trossini has also revealed in a study of synthesis of Mannich bases of hydroxyfurazone, some compounds with anti-chagasic potential [161]. Many natural products such as quinones, flavonoids, terpenes, alkaloids, neolignans, benzofurandion, xanthones, taxoids, propolis, gangliosides, and inhibitors of juvenile hormone have been studied [160, 162]. Leite and collaborates, reported a novel family of antimicrobial and anti-protozoan peptide extracted from the skin secretion of two Brazilian Phyllomedusa species (*Phyllomedusa oreades* and *Phyllomedusa hypochondrialis*) named phylloseptins [163]. These new peptides (PS-1, -2, -3, -4, -5, and -6) have molecular mass ranging from 1.7 to 2.1 kDa, amongst which PS-4 and -5 stood out showing anti-*Trypanosoma cruzi* activity.

Recently, studies have demonstrated that the poison from *Apis mellifera*, popularly known as western honey bee or European honey bee, can affect the growth, viability and ultrastructure of all *Trypanosoma cruzi* developmental forms, including intracellular amastigotes. That was possible at concentrations 15- to 100-fold lower than those required to cause toxic effects in mammalian cells [164]. Further details about other anti-chagasic metabolites to treat can be found in a substantial study by Saúde-Guimarães and Faria [162].

#### *4.2.13. Immunization*

Basombrio and Besuschio used *T. cruzi* culture as vaccine to prevent chronic Chagas' disease in mice [165]. The immunized groups have obtained lower mortality rates at 2 months postchallenge (9% versus 23%; P = 0.059), lower early peaks of parasitemia, lower percentages of positive xenodiagnoses at 5.5 months (40 versus 80%; P = 0.061), and lower incidences of tissue lesions in the skeletal muscle at 2, 6, and 10 months post challenge [165].

Cardiac lesions caused by *T. cruzi* infeccion are mediated by autoimmune antiheart reactions. Is possible that the mechanisms by which these reactions occur may involve cross-reacting antigens shared by *T. cruzi* and heart muscle [166]. The antigen called SRA is present on the surface of cardiac myofibers and is able to produce autoimmune cell-mediated antiheart reactions and myocardial lesions identical to those found in individuals with long-term *T. cruzi* infections. Unfortunately there are few studies about this antigen, therefore, it can still be considered just cleared.

According to Planeles *et al.*, immunization with murine *Trypanosoma cruzi* KMP11-HSP70 fused genes but not the KMP11 gene alone elicited both an immunoglobulin G2a long-lasting humoral immune response against KMP11 protein and activation of CD8+ cytotoxic T lymphocytes specific for two KMP11 peptides containing A2 motifs [167].

Garg *et al.*. tested the effectiveness of the *T. cruzi* trans-sialidase family (ts) genes ASP-1, ASP-2, and TSA-1 as genetic vaccines [168]. The results showed that 90% of control mice injected with empty plasmid DNA died during the acute phase of infection while the pool of three ts genes provided no greater protection than the most effective single gene (ASP-2) either with or without coadministration of cytokine plasmids. Other studies associated with production of interferon- γ [169] and immunization using recombinant adenovirus, are also found in literature [170] with promising outcomes.

## 5. Conclusions

Although CD is a serious medical and social problem that afflicts millions of people around the world, its prevention, control and treatment are still a great challenge. at first, it was believed that all cancers of the gastrointestinal tract could be directly related to T. cruzi due to chronic inflammatory process identified in patients and due to possible cell and tissue damage. However, of all cancers evaluated only the esophageal was related to the disease. It was explained by the increase of gastroesophageal reflux observed in chagasic patients. Despite great advances in the medical field, the success of therapy has bogged down in issues such as the prolonged treatment regimen, genetic variability of the parasite strains, natural resistance to drugs and adverse reactions. Therefore, there is still a long way to go before science makes available new safe and effective drugs. This review showed the most important therapeutic molecular targets of CD known to date and tried demonstrate the difficulties of developing effective treatments in spite of all searchs and strategies, such as molecular modeling (in silico studies), biotechnology (vaccines), structure–activity relationship and even high-throughput screening (HTS) realized by pharmaceutical industry. Among the strategies to develop such a treatment and possibly the cure for CD, the most promising is clearly through understanding the interactions between the lead compounds and the active sites of the various targets involved with the *T. cruzi* fisiology. Thereafter, the design of new drugs will be closer to reality and these new breakthroughs will greatly benefit chagas patients. To find efficient and safe therapies may reduce recurrent episodes of oesophageal cancer in chagasic patients.

# References


[1] Hotez P J, et al. (2007) Control of neglected tropical diseases. N Engl J Med 357, 1018 - 27.

[2] WHO (World Health Organization). (2002) Expert Committee on the Control of Chagas Disease. Control of Chagas disease: second report of the WHO expert committee. Geneva. 1 - 109.

[3] Muratore C A, Baranchuk A. (2010) Current and emerging therapeutic options for the treatment of chronic chagasic cardiomyopathy. Vasc Health Risk Manag 6, 593 – 601.

[4] Neres J, et al. (2009) Discovery of novel inhibitors of Trypanosoma cruzi trans-sialidase from in silico screening. Bioorg Med Chem Letters 19 (3), 589 - 96.

[5] Schmunis G A, Yadon Z E (2010) Chagas disease: A Latin American health problem becoming a world health problem. Acta Trop 115 (1-2), 14 – 21.

[6] Pozas R, Carballo J, Castro C, Rubio J. (2005) Synthesis and in vitro antitrypanosomal activity of novel nifurtimox analogues. Bioorg Med Chem Letters 15 (5), 1417 - 21.

[7] Schofield C J, Dias J C P. (1991) A cost-benefit analysis of Chagas disease control. Mem Inst Oswaldo Cruz 86 (3), 285 – 95.

[8] Rosenbaum M B (1964) Chagasic myocardiopathy. Prog Cardiovasc Dis 7, 199 – 225.

[9] Chapadeiro E, Lopes E R, Mesquita P M, Pereira F E L. (1964) Incidências de mega associadas à cardiopatia chagásica. Rev Inst Med Trop 6, 287 - 91.

[10] Alviano D S, et al. (2007) Conventional therapy and promising plant-derived compounds against trypanosomatid parasites. Front Microbiol 3, 283.

[11] El-Sayed N M, et al. (2005) The genome sequence of Trypanosoma cruzi, etiologic agent of Chagas disease. Science 309 (5733), 409 – 15.

[12] Silva A L M, et al. (2007) Desenvolvimento de método analítico por CLAE em comprimidos de benznidazol para a doença de chagas. Quim Nova 30 (5), 1163 - 6.

[13] Coura J R, Borges-Pereira J. (2010) Chagas disease: 100 years after its discovery. A systemic review. Acta Trop 115 (1-2), 5 – 13.

[14] Lustig E S, et al. (1980) Association of Chagas disease and cancer. Medicina (B Aires) 40, 43 - 6.

[15] Dominical V M, et al. (2010) Chagas disease and gynecologic neoplasias. Ann Diagn Pathol 14 (5), 337 – 40.

[16] Granger D N, Senchenkova E S R Inflammation and the Microcirculation. Angiogenesis. Morgan & Claypool Life Sciences; 2010, Chapter 6

[17] Jr A R, Rassi A, Rezende J M (2012) American Trypanosomiasis (Chagas Disease). Infect Dis Clin N Am 26 (2), 275 – 91.

[18] Jr A R, Rassi A, Marin-Neto J A. (2010) Chagas disease. Lancet 375 (9723), 1388 – 402.

[19] Rassi A, et al. Clinical phases and forms of Chagas disease. In: Telleria J, Tibayrenc M, editors. American trypanosomiasis (Chagas disease). One hundred years of research. 1st edition. Burlington (MA): Elsevier Inc; 2010, p. 709–41.

[20] Meneses A C, et al. (1989) Megas and cancer. Cancer of the large intestine in chagasic patients with megacolon. Arq Gastroenterol. 26 (1-2), 13 - 6.

[21] Joly E, Hudrisier D. (2003) What is trogocytosis and what is its purpose? Nat Immunol 4 (9), 815.

[22] Vanherberghen B, et al. (2004) Human and murine inhibitory natural killer cell receptors transfer from natural killer cells to target cells. Proc Natl Acad Sci USA 101(48), 16873 - 8.

[23] Mukherjee S, et al., (2014) Trypanosoma cruzi invasion is associated with trogocytosis. Microb Infect 1-19.

[24] Ralston K S, Solga M D, Mackey-Lawrence N M, Bhattacharya S, Petri Jr WA. (2014) Trogocytosis by Entamoeba histolytica contributes to cell killing and tissue invasion. Nature 508, 526 - 30.

[25] Kim J O, et al. (2007) Inhibition of Lewis lung carcinoma growth by Toxoplasma gondii through induction of Th1 immune responses and inhibition of angiogenesis. J Korean Med Sci 22, S38 – S46.

[26] Michalak M, Robert Parker J M, Opas M (2002) Ca2+ signaling and calcium binding chaperones of the endoplasmic reticulum. Cell Calcium 32 (5–6), 269 – 78.

[27] Rojiani M V, Finlay B B, Gray V, Dedhar S. (1991) In vitro interaction of a polypeptide homologous to human Ro/SS-A antigen (calreticulin) with a highly conserved amino acid sequence in the cytoplasmic domain of integrin alpha subunits. Biochem 30 (41), 9859 – 66.

[28] Burns K, et al. (1994) Modulation of gene expression by calreticulin binding to the glucocorticoid receptor. Nature 367 (6462), 476 – 80.

[29] Ramírez G, et al. (2012) Roles of Trypanosoma cruzi calreticulin in parasite–host interactions and in tumor growth. Mol Immunol. 52 (3-4), 133 - 40.

[30] Mayer M, Rocha H L. (1912) Zur Entwicklung von Schizotrypanum cruzi in Saengatieren. Archiv fur schisffs- und Tropen-Hygiene 16, 90 - 94.

[31] Bock M, Haberkorn A, Herlinger H, Mayer K H, Petersen S. (1972) The struture activity relationship of 4-5'-nitrofufurylidene-amino-tetrahydro-4H-1,4-thiazine-1,1-dioxides active against Trypanosoma cruzi. Arzneim-Forsh. 22, 1564 - 9.

[32] Urbina J A. (2009) Ergosterol biosynthesis and drug development for chagas disease. Mem Inst Oswaldo Cruz 104 (1), 311 - 318.

[33] Docampo R, Mason R P, Mottley C, Muniz R P. (1981). Generation of free radicals induced by nifurtimox in mammalian tissues. J Biol Chem 256 (21), 10930 - 3.

[34] Docampo R. (1990) Sensitivity of parasites to free radical damage by antiparasitic drugs. Chem Biol Interact 73, 1-27.

[35] Viodé C, et al. J. (1999) Enzymatic reduction studies of nitroheterocycles. Biochem Pharmacol 57 (5), 549 - 57.

[36] Hall B S, Bot C, Wilkinson S R. (2011) Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. J Biol Chem 286 (15), 13088 - 95.

[37] Blumenstiel K, Schöneck R, Yardley V, Croft S L, Krauth-Siegel R L. (1999) Nitrofuran drugs as common subversive substrates of Trypanosoma cruzi lipoamide dehydrogenase and trypanothione reductase. Biochem Pharmacol 58 (11), 1791 - 9.

[38] Wilkinson S R, Kelly J M. (2009) Trypanocidal drugs: mechanisms, resistance and new targets. Expert Rev Mol Med 11, e31.

[39] Roldán M D, Pérez-Reinado E, Castillo F, Moreno-Vivián C. (2008) Reduction of polynitroaromatic compounds: the bacterial nitroreductases. FEMS Microbiol Rev 32 (3), 474 - 500.

[40] Streeter A J, Hoener B A. (1988) Evidence for the involvement of a nitrenium ion in the covalent binding of nitrofurazone to DNA. Pharm Res 5 (7), 434 - 6.

[41] Kubata B K., et al. (2002) A key role for old yellow enzyme in the metabolism of drugs by Trypanosoma cruzi. J Exp Med 196 (9), 1241 - 51.

[42] Wilkinson S R, Taylor M C, Horn D, Kelly J M, Cheeseman I. (2008). A mechanism for cross-resistance to nifurtimox and benznidazole in trypanosomes. Proc Natl Acad Sci USA 105 (13), 5022 - 7.

[43] Cançado J R. (2002) Long term evaluation of etiological treatment of chagas disease with benznidazole. Rev Inst Med Trop 44 (1), 29 - 37.

[44] Urbina J A, Docampo R. (2003) Specific chemotherapy of chagas disease: controversies and advances. Trends Parasitol 19 (11), 495 - 501.

[45] Altcheh J, Moscatelli G, Moroni S, Garcia-Bournissen F, Freilij H. (2011) Adverse events after the use of benznidazole in infants and children with Chagas disease. Pediatrics.127 (1), e212 - 8.

[46] Soeiro M N C, De Castro S L. (2009) Trypanosoma cruzi targets for new chemotherapeutic approaches. Exp Opin Ther Targets 13 (1), 105 - 21.

[47] Buckner F S, Navabi N. (2010) Advances in Chagas disease drug development: 2009-2010. Curr Opin Infect Dis 23 (6), 609 - 16.

[48] Romanha, A.J., et al. (2010). In vitro and in vivo experimental models for drug screening and development for Chagas disease. Mem Inst Oswaldo Cruz 105 (2), 233 - 8.

[49] Dias L C, et al. (2009) Quimioterapia da doença de Chagas: estado da arte e perspectivas no desenvolvimento de novos fármacos. Quim Nova 32 (9), 2444 - 57.

[50] Corsini A, Bellosta S, Baetta R. (1999) New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol Ther 84 (3), 413 - 428.

[51] Itsvan E S, Deisenhofer J. (2001) Structural Mechanism for Statin Inhibition of HMG-CoA Reductase. Science 292 (5519), 1160 - 4.

[52] Yasmin S, et al. (2012) Mevalonate governs interdependency of ergosterol and siderophore biosyntheses in the fungal pathogen Aspergillus fumigatus. Proc Natl Acad Sci USA 109 (8), E497 - 504.

[53] Hankins E G, Gillespie J R, Aikenhead K, Buckner F S. (2005) Upregulation of sterol C14-demethylase expression in Trypanosoma cruzi treated with sterol biosynthesis inhibitors. Mol Biochem Parasitol 144 (1), 68 - 75.

[54] Urbina J A, et al. (1993) Mevinolin (lovastatin) potentiates the antiproliferative effects of ketoconazole and terbinafine against Trypanosoma (Schizotrypanum) cruzi: in vitro and in vivo studies. Antimicrob Agents Chemother 37 (3), 580 – 91.

[55] Shanes J G, Minadeo K N, Moret A, Groner M, Tabaic S A. (2007) Statin therapy in heart failure: prognostic effects and potential mechanisms. Am Heart J 154 (4), 617 – 23.

[56] Weis M, Heeschen C, Glassford A J, Cooke J P. (2002) Statins have biphasic effects on angiogenesis. Circulation. 105 (6), 739 – 45.

[57] Feldman A M, McNemara D. (2000). Medical Progress: Myocarditis. N Engl J Med 343, 1388 – 98.

[58] Melo L, et al. (2011) Low doses of simvastatin therapy ameliorate cardiac inflammatory remodeling in Trypanosoma cruzi-infected dogs. Am J Trop Med Hyg 84 (2), 325 - 31.

[59] Cheng X, et al. (2005) Effects of Atorvastatin on Th polarization in patients with acute myocardial infarction. Eur J Heart Fail 7 (7), 1099 – 104.

[60] Robinson J G. (2008) Models for describing relations among the various statin drugs, low-density lipoprotein cholesterol lowering, pleiotropic effects and cardiovascular risk. Am J Cardiol 101 (7), 1009 – 15.

[61] Montalvetti A, et al. (2003) Farnesyl pyrophosphate synthase is an essential enzyme in Trypanosoma brucei. J Biol Chem 278 (19), 17075 – 83.

[62] Sigman L, Sánchez V M, Turjanski A G. (2006) Characterization of the farnesyl pyrophosphate synthase of Trypanosoma cruzi by homology modeling and molecular dynamics. J Mol Graphics Mod 25 (3), 245 – 52.

[63] Montalvetti A, et al. (2001) Bisphosphonates are potent inhibitors of Trypanosoma cruzi farnesyl pyrophosphate synthase. J Biol Chem 276 (36), 33930-7.

[64] Szajnman S H, Montalvetti A, Wang Y, Docampo R, Rodriguez J B. (2003) Bisphosphonates derived from fatty acids are potent inhibitors of Trypanosoma cruzi farnesyl pyrophosphate synthase. Bioorg Med Chem Lett 13 (19), 3231 - 5.

[65] Szajnman S H, Ravaschino E L, Docampo R, Rodriguez J B. (2005) Synthesis and biological evaluation of 1-amino-1,1-bisphosphonates derived from fatty acids against Trypanosoma cruzi targeting farnesyl pyrophosphate synthase. Bioorg Med Chem Lett 15 (21), 4685 - 90.

[66] Martin B M, et al. (2001) Bisphosphonates Inhibit the Growth of Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondii, and Plasmodium falciparum: A Potential Route to Chemotherapy. J Biol Chem 44 (6), 909 – 16.

[67] Garzoni L R, et al. (2004) Antiparasitic activity of risedronate in a murine model of acute Chagas' disease. Int J Antimicrob Agents 23 (3), 286 - 90.

[68] Esteva M I, et al. (2005) Benzophenone-based farnesyltransferase inhibitors with high activity against Trypanosoma cruzi. J Med Chem 48 (23), 7186 - 91.

[69] Bouzahzah B, Jelicks L A, Morris S A, Weiss L M, Tanowitz H B. (2005) Risedronate in the treatment of Murine Chagas' disease. Parasitol Res 96 (3), 184 - 7.

[70] Demoro B, et al. (2010) Risedronate metal complexes potentially active against Chagas disease. J Inorg Biochem 104 (12), 1252 – 1254.

[71] Pandit J, et al. (2000) Crystal structure of human squalene synthase. A key enzyme in cholesterol biosynthesis. J Biol Chem 275 (39), 30610 - 7.

[72] Urbina J A, et al. (2004) In vitro and in vivo activities of E5700 and ER-119884, two novel orally active squalene synthase inhibitors, against Trypanosoma cruzi. Agents Chemother 48 (7), 2379 - 87.

[73] Linares G G., et al. (2007) Fluorine-containing aryloxyethyl thiocyanate derivatives are potent inhibitors of Trypanosoma cruzi and Toxoplasma gondii proliferation. Bioorg. Med Chem Letters 17 (18), 5068 - 71.

[74] Urbina J A, et al. (2003) Parasitological cure of acute and chronic experimental Chagas disease using the long- acting experimental triazole TAK-187.Activity against drug-resistant Trypanosoma cruzi strains. Int J Antimicrob Agents 21 (1), 39 - 48.

[75] Szajnman S H, et al. (2000) Design and synthesis of aryloxyethyl thiocyanate derivatives as potent inhibitors of Trypanosoma cruzi proliferation. J Med Chem 43 (9), 1826 – 40.

[76] Elhalem E, et al. (2002) Design, synthesis and biological evaluation of aryloxyethyl thiocyanate derivatives against Trypanosome cruzi. J Med Chem 45, 3984 – 99.

[77] Pandit J, et al. (2000) Crystal structure of human squalene synthase. A key enzyme in cholesterol biosynthesis. J Biol Chem 275 (39), 30610 - 7.

[78] Oldfield E. (2010) Targeting isoprenoid biosynthesis for drug discovery: bench to bedside. Acc Chem Res 43 (9), 1216 - 26.

[79] Sealey-Cardona M, et al. (2007) Kinetic characterization of squalene synthase from Trypanosoma cruzi: selective inhibition by quinuclidine derivatives. Antimicrob Agents Chemother 51 (6), 2123 - 9.

[80] Orenes S L, et al. (2005) Biphenylquinuclidines as inhibitors of squalene synthase and growth of parasitic protozoa. Bioorg Med Chem 13 (10), 3519 - 29.

[81] Buckner F S, Griffin J H, Wilson A J, Van Voorhis W C. (2001) Potent anti-Trypanosoma cruzi activities of oxidosqualene cyclase inhibitors. Antimicrob. Agents Chemother. 45 (4), 1210 - 5.

[82] Oliaro-Bosso S, et al. (2005) Analogs of squalene and oxidosqualene inhibit oxidosqualene cyclase of Trypanosoma cruzi expressed in Saccharomyces cerevisiae. Lipids 40 (12), 1257 - 62.

[83] Dehmlow H, et al. (2003) Synthesis and structure-activity studies of novel orally active non-terpenoic 2,3-oxidosqualene cyclase inhibitors. J Med Chem 46 (15), 3354 - 70.

[84] Balliano G., et al. (2009) Oxidosqualene cyclase from Saccharomyces cerevisiae, Trypanosoma cruzi, Pneumocystis carinii and Arabidopsis thaliana expressed in yeast: A model for the development of novel antiparasitic agents. Bioorg Med Chem Lett 19 (3), 718 – 23.

[85] Thoma R, et al. (2004) Insight into steroid scaffold formation from the structure of human oxidosqualene cyclase. Nature 432 (7013), 118 - 22.

[86] Urbina J A, Payares G, Sanoja C, Lira R, Romanha A J. (2003) In vitro and vivo activities of ravuconazole on Trypanosoma cruzi, the causative agent of Chagas diseases. Int J Antimicrob Agents 21 (1), 27 - 38.

[87] De Castro S L. (1993) The challenge of Chagas disease chemotherapy: an update of drugs assayed against Trypanosoma cruzi. Acta Trop 53 (2), 83 - 98.

[88] Santa-Rita M R, Lira R, Barbosa H S, Urbina J A, De Castro S L. (2005) Anti-proliferative synergy of lysophospholipid analogues and ketoconazole against Trypanosoma cruzi (Kinetoplastida: Trypanosomatidae): cellular and ultrastructural analysis. J Antim Chemother 55 (5), 780 - 4.

[89] Buckner F S, Wilson A J, White T C, Van Voorhis W C. (1998) Induction of resistance to azole drugs in Trypanosoma cruzi. Antimicrob. Agents Chemother. 42 (12), 3245 - 50.

[90] Chen C K, et al. (2009) Trypanosoma cruzi CYP51 Inhibitor Derived from a Mycobacterium tuberculosis Screen Hit. PLoS Negl Trop Dis 3 (2), e372.

[91] Molina J, Brener Z, Romanha A J, Urbina J A. (2000) In vivo activity of the bis-triazole D0870 against drug-susceptible and drug-resistant strains of the protozoan parasite Trypanosoma cruzi. J Antim Chemother 46 (1), 137 - 40.

[92] Silva D T, De Nazareth S L M, Meirelles M, Almeida D, Urbina J A, Pereira M C. (2006) Cytoskeleton reassembly in cardiomyocytes infected by Trypanosoma cruzi is triggered by treatment with ergosterol biosynthesis inhibitors. Int J Antimicrob Agents 27 (6), 530 - 7.

[93] Ferraz M L, Gazzinelli R T, Alves R O, Urbina J A, Romanha A J. (2007) The Anti-Trypanosoma cruzi activity of posaconazole in a murine model of acute Chagas' disease is less dependent on gamma interferon than that of benznidazole. Antimicrob Agents Chemother 51(4), 1359-64.

[94] Suryadevara K P, et al. (2009) Structurally simple inhibitors of lanosterol 14α-demethylase are efficacious in a rodent model of acute Chagas disease. J Med Chem 52 (12), 3703 – 15.

[95] Olivieri B P, et al. (2010) A comparative study of posaconazole and benznidazole in the prevention of heart damage and promotion of trypanocidal immune response in a murine model of Chagas disease. Int J Antimicrob Agents 36 (1), 79 - 83.

[96] Veiga-Santos, P, et al. (2012) Effects of amiodarone and posaconazole on the growth and ultrastructure of Trypanosoma cruzi. Int J Antimicrob Agents 40 (1), 61 - 71

[97] Urbina J A, Concepcion L J, Montalvetti A, Rodriguez B J, Docampo R. (2003) Mechanism of action of 4-phenoxyphenoxyethyl thiocyanate (WC-9) against Trypanosoma cruzi, the causative agent of Chagas' disease. Antimicrob Agents Chemother 47 (6), 2047 – 50.

[98] Diniz L F, et al. (2010) Effects of Ravuconazole Treatment on Parasite Load and Immune Response in Dogs Experimentally Infected with Trypanosoma cruzi. Antimicrob Agents Chemother 54 (7), 2979 - 86.

[99] Molina J, et al. (2001) Cure of experimental Chagas' disease by the bis-triazole DO870 incorporated into 'stealth' polyethyleneglycol-polylactide nanospheres. J Antim Chemother 47 (1), 101 - 4.

[100] Urbina J A, Lira R, Visbal G, Bartroli J. (2000) In vitro antiproliferative effects and mechanism of action of the newtriazole derivative UR-9825 against the protozoan parasite Trypanosoma (Schizotrypanum) cruzi. Antimicrob Agents Chemother 44 (9), 2498 - 2502.

[101] Uehara L A, et al. (2012) Cruzipain promotes Trypanosoma cruzi adhesion to Rhodnius prolixus Midgut. PLoS Negl Trop Dis 6 (12), e1958.

[102] Scharfstein J, et al. (2000) Host cell invasion by Trypanosoma cruzi is potentiated by activation of bradykinin B(2) receptors. J Exp Med 192 (9), 1289 - 1300.

[103] Aparicio I M, Scharfstein J, Lima A P. (2004). A new cruzipain-mediated pathway of human cell invasion by Trypanosoma cruzi requires trypomastigote membranes. Infect Immun 72 (10), 5892 - 902.

[104] Andrade D, et al. (2012) Trypanosoma cruzi Trypanosoma cruzi invades host cells through the activation of endothelin and bradykinin receptors: a converging pathway leading to chagasic vasculopathy. Br J Pharmacol 165 (5), 1333 - 47.

[105] Berasain P, Carmona C, Frangione B, Cazzulo J J, Goñi F. (2003) Specific cleavage sites on human IgG subclasses by cruzipain, the major cysteine proteinase from Trypanosoma cruzi. Mol Biochem Parasitol 130 (1), 23 - 9.

[106] Engel J C, Doyle P S, Palmer J, Bainton D F, McKerrow J H. (1998) Cysteine protease inhibitors after Golgi complex ultra structure and function in Trypanosoma cruzi. J Cell Science 111 (5), 597 - 606.

[107] Doyle S P, Zhou M Y, Engel C J, McKerrow H J. (2007) A cysteine protease inhibitor cures Chagas' disease in an immunodeficient-mouse model of infection.Antimicrob. Agents Chemother 51 (11), 3932 – 9.

[108] Yong V, et al. (2000) Altered expression of cruzipain and a cathepsin B-like target in a Trypanosoma cruzi cell line displaying resistance to synthetic inhibitors of cysteine-proteinases. Mol Biochem Parasitol 109 (1), 47 - 59.

[109] Reis F C, et al. (2007) The propeptide of cruzipain--a potent selective inhibitor of the trypanosomal enzymes cruzipain and brucipain, and of the human enzyme cathepsin. F FEBS J 274 (5), 1224 - 34.

[110] Chen Y T, et al. (2010) In vitro and in vivo studies of the trypanocidal properties of WRR-483 against Trypanosoma cruzi. PLoS Negl Trop Dis 4 (9), e825.

[111] Flohé L. (2012) The trypanothione system and its implications in the therapy of trypanosomatid diseases. Int J Med Microbiol 302 (4-5), 216 - 20.

[112] Graebin C S, et al. (2009) Antiprotozoal agents: an overview. Anti-Infective agents Med Chem 8, 345-366.

[113] Lo Presti M S, et al. (2004) Thioridazine treatment prevents cardiopathy in Trypanosoma cruzi infected mice. Int J Antimicrob Agents 23 (6), 634 - 6.

[114] Salmon-Chemin L, et al. (2001) 2- and 3-substituted 1,4-naphtoquinone derivatives as subversive substrates of trypanothione reductase and lipoamide dehydrogenase from Trypanosoma cruzi: synthesis and correlation between redox cycling activities and in vitro cytotoxicity. J Med Chem 44 (4), 548 - 65.

[115] Wenck M A, Medrano F J, Eakin A E, Craig S P. (2004) Steady-state kinetics of the hypoxanthine phosphoribosyltransferase from Trypanosoma cruzi. Biochem Biophys Acta 1700 (1), 11 - 8.

[116] Canyuk B, Medrano F J, Wenck M A, Focia P J, Eakin A E, Craig S P 3rd. (2004) Interactions at the dimer interface influence the relative efficiencies for purine nucleotide synthesis and pyrophosphorolysis in a phosphoribosyltransferase. J Mol Biol 335 (4), 905 - 21.

[117] Ullman B, Carter D. (1997) Molecular and biochemical studies on the hypoxanthine-guanine phosphoribosyltransferases of the pathogenic haemoflagellates. Int J Parasitol 27 (2), 203 - 13.

[118] Focia P J, Craig III S P, Eakin A E. (1998) Approaching the transition state in the crystal structure of a phosphoribosyltransferase. Biochem 37 (49), 17120 - 7.

[119] Freymann D M, et al. (2000) Efficient identification of inhibitors targeting the closed active site conformation of HPRT from Trypanosoma cruzi. Chem Biol 7 (12), 957 - 68.

[120] Lauria-Pires L, Castro C N, Emanuel A, Prata A. (1998) Ineficácia do allopurinol em pacientes na fase aguda da doença de Chagas. Rev Soc Med Trop 21 (2), 79 - 80.

[121] Héroux A, White E L, Ross L J, Borhani D W. (1999) Crystal structures of the Toxoplasma gondii hypoxanthine-guanine phosphoribosyltransferase-GMP and -IMP complexes: comparison of purine binding interactions with the XMP complex. Biochemistry 38 (44), 14485 - 94.

[122] Cortés F, Pastor N, Mateos S, Domínguez I. (2003) Roles of DNA topoisomerases in chromosome segregation and mitosis. Mutat Res 543 (1), 59 - 66.

[123] Zuma A A, Cavalcanti D P, Maia M C P, De Souza W, Mottaa M C M. (2011) Effect of topoisomerase inhibitors and DNA-binding drugs on the cell proliferation and ultrastructure of Trypanosoma cruzi. Int J Antimicrob Agents 37 (5), 449 – 56.

[124] Ray S, Hazra B, Mittra B, Das A, Majumder H K. (1998) Diospyrin, a bisnaphthoquinone: a novel inhibitor of type I DNA topoisomerase of Leishmania donovani. Mol Pharmacol 54 (6), 994 – 9.

[125] Teicher B A. (2008) Next generation topoisomerase I inhibitors: rationale and biomarker strategies. Biochem.Pharmacol. 75 (6), 1262 – 71.

[126] Palchaudhuri R, Hergenrother P J. (2007) DNA as a target for anticancer compounds: methods to determine the mode of binding and the mechanism of action. Curr Opin Biotechnol 18 (6), 497 – 503.

[127] Bodley A L, Shapiro T A. (1995) Molecular and cytotoxic effects of camptothecin, a topoisomerase I inhibitor, on trypanosomes and Leishmania. Proc Natl Acad Sci USA 92 (9), 3726 - 30.

[128] Batista D G., et al. (2010) Biological, ultrastructural effect and subcellular localization of aromatic diamidines in Trypanosoma cruzi. Parasitol 137 (2), 251 - 9.

[129] Sanz-Rodríguez C E, Concepción J L, Pekerar S, Oldfield E, Urbina J A. (2007) Bisphosphonates as inhibitors of Trypanosoma cruzi hexokinase: kinetic and metabolic studies. J Biol Chem 282 (17), 12377 - 87.

[130] Urbina J A. (2010) Specific chemotherapy of Chagas disease: relevance, current limitations and new approaches. Acta Trop 115 (1-2), 55 - 68.

[131] Hudock P M, et al. (2006) Inhibition of Trypanosoma cruzi hexokinase by bisphosphonates. J Med Chem 49 (1), 215 – 23.

[132] Klebe G., Abraham U, Mietzner T. (1994) Molecular similarity indices in a comparative analysis (CoMSIA) of drug molecules to correlate and predict their biological activity. J Med Chem 37 (24), 4130 - 46.

[133] Bakker M B, Westerhoff V H, Opperdoes R F, Michels A M P. (2000) Metabolic control analysis of glycolysis in trypanosomes as an approach to improve selectivity and effectiveness of drugs. Molec Biochem Parasitol 106 (1), 1 - 10.

[134] Urbina J A, Crespo A. (1984) Regulation of energy metabolism in Trypanosoma (schizotrypanum) cruzi epimastigotes, I. Hexokinase and phosphofructokinase. Mol Biochem Parasitol 11, 225–239.

[135] Leitão A, et al. (2004) Structure-activity relationships of novel inhibitors of glyceraldehyde-3-phosphate dehydrogenase. Bioorg Med Chem Letters 14 (9), 2199 - 204.

[136] Silva J J N, Osakabe A L, Pavanelli W R, Silva J S, Franco D. (2007) In vitro and in vivo antiproliferative and trypanocidal activities of ruthenium NO donors. Br J Pharmacol 152 (1), 112 – 21.

[137] Neres J, et al. (2007) Benzoic acid and pyridine derivatives as inhibitors of Trypanosoma cruzi trans-sialidase. Bioorg Med Chem 15 (5), 2106 - 19.

[138] Alvarado A C, Martínez Z F, Páez R L, Nogueda B, Baeza I, Wong C. (2007). In vitro and in vivo trypanocidal activity of the ethyl esters of N-allyl and N-propyl oxamates using different Trypanosoma cruzi strains. J Enzym Inhib Med Chem 22 (2), 227 - 33.

[139] Alvarado A C, et al. (2010) Trypanocidal activity of the ethyl esters of N-propyl and N-isopropyl oxamates on intracellular amastigotes of Trypanosoma cruzi acute infected mice. J Enzym Inhib Med Chem 25 (1), 111 - 5.

[140] Chena M A, Elizondo-Jiménez S, Rodríguez-Páez L, Nogueda-Torres B, Baeza-Ramírez I, Wong-Ramírez C. (2004) Trypanosoma cruzi: inhibition of alpha-hydroxyacid dehydrogenase isozyme II by N-allyl and N-propyl oxamates and their effects on intact epimastigotes. Mem Inst Oswaldo Cruz 99 (8), 831 - 7.

[141] Alloatti A, Uttaro A D. (2010) Highly specific methyl-end fatty-acid desaturases of trypanosomatids. Mol Biochem Parasitol 175 (2), 126 - 32.

[142] Alloatti A, Testero S A, Uttaro A D. (2009) Chemical evaluation of fatty acid desaturases as drug targets in Trypanosoma cruzi. Int J Parasitol 39 (9), 985 - 93.

[143] Cheleski J, et al. (2010) Novel insights for dihydroorotate dehydrogenase class 1A inhibitors discovery. Eur J Med Chem 45 (12), 5899 - 909.

[144] Rocha O P, et al. (2011) Chemical Profile and Biological Potential of Non-Polar Fractions from Centroceras clavulatum (C. Agardh) Montagne (Ceramiales, Rhodophyta). Molecules. 16 (8), 7105 – 14.

[145] Barbosa C F, Okuda E S, Chung M C, Ferreira E I, Cicarelli R M B. (2007) Rapid test for the evaluation of the activity of the prodrug hydroxymethylnitrofurazone in the processing of Trypanosoma cruzi messenger RNAs. Braz J Med Biol Res 40 (1), 33 - 9.

[146] Harkiolaki M, Dodson E J, Bernier-Villamor V, Turkenburg J P, González-Pacanowska D, Wilson K S. (2004) The crystal structure of Trypanosoma cruzi dUTPase reveals a novel dUTP/ dUDP binding fold. Structure 12 (1), 41 - 53.

[147] Gilbert I H. (2002) Inhibitors of dihydrofolate reductase in leishmania and trypanosomes. Biochem. Biophys. Acta 1587 (2-3), 249 - 57.

[148] Zuccotto F, et al. (2001) Novel inhibitors of Trypanosoma cruzi dihydrofolate reductase. Eur J Med Chem 36 (5), 395 - 405.

[149] Zuccotto F, Brun R, Gonzalez-Pacanowska D, Ruiz-Perez L M, Gilbert I H. (1999) The structure-based design and synthesis of selective inhibitors of Trypanosoma cruzi dihydrofolate reductase. Bioorg Med Chem Lett 9 (10), 1463 - 8.

[150] Chowdhury S F, et al. (1999) Design, Synthesis, and Evaluation of Inhibitors of Trypanosomal and Leishmanial Dihydrofolate Reductase. J Med Chem 42 (21), 4300 - 12.

[151] Khabnadideh S, et al. (2005) Design, synthesis and evaluation of 2,4-diaminoquinazolines as inhibitors of trypanosomal and leishmanial dihydrofolate reductase. Bioorg Med Chem 13 (7), 2637 - 49.

[152] Schormann N, et al. (2010) Synthesis and characterization of potent inhibitors of Trypanosoma cruzi dihydrofolate reductase. Bioorg Med Chem 18 (11), 4056 - 66.

[153] Palma A, Yepes A F, Leal S M, Coronado C A, Escobar P. (2009) Synthesis and in vitro activity of new tetrahydronaphtho[1,2-b]azepine derivatives against Trypanosoma cruzi and Leishmania chagasi parasites. Bioorg Med Chem Letters 19 (8), 2360 - 3.

[154] Souza D H, et al. (1998) Trypanosoma cruzi glycosomal glyceraldehyde-3-phosphate dehydrogenase: structure, catalytic mechanism and targeted inhibitor design. FEBS Lett 424 (3), 131 - 5.

[155] Silva J J N, et al. (2010) Novel ruthenium complexes as potential drugs for Chagas's disease: enzyme inhibition and in vitro/in vivo trypanocidal activity. British J Pharmacol 160 (2), 260 – 9.

[156] Tomazela D M, et al. (2000) Pyrano chalcones and flavone from Neoraputia magnífica and their Trypanosoma cruzi glycosomal glyceraldehyde-3-phosphate dehydrogenase-inhibitory activities. Phytochem 55 (6), 643 - 51.

[157] Bressi C J, et al. (2001) Adenosine Analogues as Selective Inhibitors of Glyceraldehyde-3-phosphate Dehydrogenase of Trypanosomatidae via Structure-Based Drug Design Med Chem 44 (13), 2080 – 93.

[158] Ladame S, et al. (2003) Crystal structure of Trypanosoma cruzi glyceraldehyde-3-phosphate dehydrogenase complexed with an analogue of 1,3-bisphospho-D-glyceric acid. Eur J Biochem 270 (22), 4574 – 86.

[159] De Marchi A A, et al. (2004) New 3-piperonylcoumarins as inhibitors of glycosomal glyceraldehyde-3- phosphate dehydrogenase (gGAPDH) from Trypanosoma cruzi. Bioorg Med Chem 12 (18), 4823 - 33.

[160] Pereira J M, et al. (2008) Anacardic acid derivatives as inhibitors of glyceraldehyde-3-phosphate dehydrogenase from Trypanosoma cruzi. Bioorg Med Chem 16 (19), 8889 - 95.

[161] Coura R J, De Castro L S. (2002) A Critical Review on Chagas Disease Chemotherapy. Mem Inst Oswaldo Cruz 97 (1), 3 - 24.

[162] Trossini G H G. (2004) Antichagásicos potenciais: síntese de bases de Mannich do hidroximetilnitrofural. Dissertation. University of São Paulo, Pharmaceutical Science Program.

[163] Guimarães S A D, Faria R A. (2007) Substâncias da natureza com atividade anti-Trypanosoma cruzi. Braz J Pharmacogn 17 (3), 455 - 65.

[164] Leite J R, et al. (2005) Phylloseptins: a novel class of anti-bacterial and anti-protozoan peptides from the Phyllomedusa genus. Peptides 26 (4), 565 - 73.

[165] Adade C M, Chagas G S F, Souto-Padrón T. (2012) Apis mellifera venom induces different cell death pathways in Trypanosoma cruzi. Parasitol 139 (11), 1444 - 61.

[166] Basombrío M A, Besuschio S. (1982) Trypanosoma cruzi culture used as vaccine to prevent chronic Chagas' disease in mice. Infect Immun 36 (1), 351 – 6.

[167] Acosta A M, Santos-Buch C A. (1985) Autoimmune myocarditis induced by Trypanosoma cruzi. Circulation. 71 (6), 1255 - 61.

[168] Planelles L, Thomas M C, Alonso C, López M C. (2001) DNA Immunization with Trypanosoma cruzi HSP70 Fused to the KMP11 Protein Elicits a Cytotoxic and Humoral Immune Response against the Antigen and Leads to Protection. Infect Immun 69 (10), 6558 – 63.

[169] Garg N, Tarleton R L. (2002) Genetic immunization elicits antigen-specific protective immune responses and decreases disease severity in Trypanosoma cruzi infection. Infect Immun 70 (10), 5547 - 55.

[170] Laucella S A, et al. (2004) Frequency of interferon-γ-producing T cells specific for Trypanosoma cruzi inversely correlates with disease severity in chronic human chagas disease. J Infect Dis 189 (5), 909 - 18.

[171] Machado A V, Cardoso J E, Claser C, Rodrigues M M, Gazzinelli R T, Bruna-Romero O. (2006) Long-term protective immunity induced against Trypanosoma cruzi infection after vaccination with recombinant adenoviruses encoding amastigote surface protein-2 and trans-sialidase. Hum Gene Ther 17 (9), 898 - 908.