UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

TÍTULO: Advances in the Chemotherapy of Chagas Disease

Trabajo de integración curricular presentado como requisito para la obtención del título de Ingeniero Biomédico

Autor:

André Joshua Saigua Encalada

Tutor:

Reneé Lira, PhD

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A mi mamá,

A mi papá desde el cielo,

A mi seres amados.

André Joshua Saigua Encalada

AGRADECIMIENTO

A mis padres, Sonia y Luis, por haberme apoyado cada uno a su manera y confiado en mí. A mis amigos que son como mi familia. A mis profesores, en especial a mi tutora Renée Lira por haberme enseñado con calidad y paciencia. A mi familia y amigos.

André Joshua Saigua Encalada

RESUMEN

La enfermedad de Chagas, también llamada tripanosomiasis americana, es una de las enfermedades parasitarias más desatendidas del mundo. Su agente infeccioso es el parásito protozoario *Trypanosoma cruzi* con síntomas que progresan desde una inflamación leve hasta insuficiencia cardíaca. El descubrimiento de los derivados nitroheterocíclicos como fármacos antiparasitarios: Nifurtimox y Benznidazol para el tratamiento de la enfermedad de Chagas, debido a su eficacia en la fase aguda, sin embargo. una reducida eficacia en la fase crónica. Las principales limitaciones de estos fármacos son la administración a largo plazo y los efectos secundarios graves.

En el contexto de esta revisión, se describe el efecto de los inhibidores de la biosíntesis de esteroles en *T. cruzi* como blancos quimioterapéuticos de los fármacos anti-tripanosomátidos. La vía de biosíntesis de esteroles ahora está bien establecida como una vía metabólica importante en los hongos y en los miembros de la familia Trypanosomatidae, estas vías producen ergosterol y otros 24-metil esteroles, que son necesarios para el crecimiento y la viabilidad de los parásitos, pero están ausentes en los mamíferos células huésped. Además, se muestran los avances en la quimioterapia de Chagas mediante el uso Alquilfosfolípidos (ALP). Debido a la actividad citotóxica de las ALP, esta clase de moléculas ha demostrado ser eficaz contra muchas enfermedades desatendidas como el Chagas, la leishmaniasis y la filariasis, así denominadas por la iniciativa de Medicamentos para Enfermedades Desatendidas (DNDi).

PALABRAS CLAVES

Alquilfosfolipidos, ruta de biosíntesis de esteroles, *Trypanosoma cruzi*.

ABSTRACT

Chagas disease, also called American trypanosomiasis, is one of the most neglected parasitic diseases in the world. Its infectious agent is the protozoan parasite *Trypanosoma cruzi* with symptoms progressing from mild swelling to heart failure. The discovery of the nitroheterocyclic derivatives as antiparasitic drugs Nifurtimox and Benznidazole (1970) brought new perspectives for the treatment of Chagas disease due to their efficacy in the acute phase, however a reduced effectiveness in the chronic phase. The main limitations of these drugs are the long-term administration and the severe side effects.

In the context of this review, we describe the effect of sterol biosynthesis inhibitors in *Trypanosoma cruzi* as chemotherapeutic targets of anti-trypanosomatid drugs. The sterol biosynthesis pathway is now well established as an important metabolic pathway in fungi and in members of the Trypanosomatidae family, these pathways produce ergosterol and other 24-methyl sterols, which are necessary for parasite growth and viability but are absent in mammalian host cells. In addition, advances in Chagas chemotherapy through the use of Alkylphospholipids (ALP) are shown. Due to the cytotoxic activity of ALPs, this class of molecules has been shown to be effective against many neglected diseases such as Chagas, Leishmaniasis, and Filariasis so named by the Drugs for Neglected Diseases (DNDi) initiative.

KEY WORDS

Alkylphospholipids, sterol biosynthesis pathway, *Trypanosoma cruzi.*

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Chapter 1: Introduction and literature review

1.1 Introduction

Chagas disease, also called American trypanosomiasis, is a parasitic disease that has not received enough attention, which is why it has proliferated, especially in endemic areas of contagion. The disease caused by the parasite *T. cruzi*, which was detected as an infectious disease by Carlos Chagas, Brazil (Chagas, 1911), comes mainly from the tropical and endemic areas of Latin America. According to the Pan American Health Organization, it is estimated that there are approximately 8 million people infected with the parasite, 100 million at risk of contagion, 5,600 new cases a year and an average of 12,000 people dying from the disease. (PAHO, 2017).

It has been shown that there is a relationship between latitude and the site of action of the triatomine species, with a diversity towards the equator and towards the east in latitude, encompassing the Amazon (Rodriguero & Gorla, 2004). Existing geographic distribution patterns related to taxonomic groups such as the Rhodnius species associated directly with the Amazon and the periphery, extending from the west to the northern countries. (Abad-Franch & Monteiro, 2007).

Chagas disease caused by *Trypanosoma* (Schizotrypanum) *cruzi*. vector transmission by triatomines (insects) and its main route of transmission is through direct contact with the feces or infected urine of triatomines, which feed on the blood of the vertebrate host. Transmission can also be carried out by different routes, oral (food), blood (contact) congenital and organ transplants (WHO, 2020).

During this acute phase, many parasites circulate through the bloodstream, generally, there are no or minimal symptoms. In less than 50% of infected people, an initial sign is due to the presence of a skin lesion or a purple swelling of the eyelid (WHO, 2020). Acute infections can be asymptomatic and occur at any age; regarding its clinical manifestations, it can present fever, lymphadenopathy and inflammation at the site of the bite (Pérez-Molina JA, et al., 2020)

Upon reaching the next phase, which is the chronic phase, in the cellular immune response limits the proliferation of the parasite but the infection cannot be eradicated, it also leads to the development of one or more symptomatic forms of chronic disease, found in between 30 and 40% of patients, including heart failure caused by chronic Chagas cardiomyopathy, as well as digestive complications and neuropathic problems (Brener & Gazzinelli, 1997; Albareda et al., 2006; Marin-Neto et al., 2007). The main cause of deaths from this disease is chronic Chagas cardiomyopathy since it appears decades after infection and can cause cardiac arrhythmias, congestive heart failure, thromboembolism and sudden cardiac death. (Rassi et al., 2000; Marin-Neto et al., 2007; Rassi et al., 2009). Acute or initial infections, congenital diseases and reactivations in immunosuppressed patients can be diagnosed using direct visualization methods, mainly, PCR techniques (Pérez-Molina JA, et al., 2020).

Treatment of *T. cruzi* infection continues to be based on drugs such as Nifurtimox and Benznidazole, which do not guarantee efficacy, and which are mainly based on the stage of infection and the patient's age. Benznidazole has almost no benefit in the chronic phase of the infection, it also causes a large number of side effects such as nausea, abdominal swelling, rash (Oliveira et al., 2017).

The use of treatment is recommended during the acute phase of the disease, regardless of the infection mechanism since the therapy improves symptoms and elimination of the parasite. Therefore, early treatment helps prevent the development of the disease to a chronic and irremediable phase. When comparing Benznidazole and Nifurtimox, Benznidazole is commonly chosen as the main drug for treatment because it has better tissue penetration and greater efficacy (Pérez-Molina JA, et al., 2020).

The search for optimal treatment for Chagas disease has led to the combination of drugs with the aim of achieving a synergistic effect to increase the efficacy of a drug compared to monotherapy.

1.2 Statement of the problem

With the technological improvements coupled with the development of drugs directed to specific pathways for the advancement in chemotherapy of diseases, a great variety of drugs have been achieved, however, most of these compounds do not meet the expected expectations for the disease of Chagas disease, since until now there is no effective drug for the chronic phase of this disease.

The greatest challenge in the investigation of advances in chemotherapy in *T. cruzi* is to present an optimal treatment that reduces the toxic effects of drugs as well as its possible implementation.

1.3 General and Specific Objectives

General Objective

Discuss the therapeutic advances in Chagas disease in using drugs directed to different metabolic pathways as chemotherapeutic targets.

Specific Objectives

• Evaluate the chemotherapeutic strategies based on the mechanism of action of drugs currently used on ergosterol synthesis.

• Evaluate rational chemotherapy for the use of new drugs: Alkylphospholipids as Promising Chemotherapeutic Agents.

• Discuss the synergistic effects for the rational use of drugs in the treatment of Chagas disease.

Chapter 2: *Trypanosoma cruzi*: Etiology and Disease

In 1909, Carlos Chagas described the disease and its evolutionary cycle thanks to the detection of the parasite in the blood of a Brazilian boy with lymphadenopathy and fever (Chagas, 1909).

2.1 Life cycle of *Trypanosoma cruzi*

The life cycle of *Trypanosoma cruzi* is divided into two intermediate hosts, the first is an invertebrate host (triatomine insect), the second is a vertebrate host (human), and *T. cruzi* has three stages of development: trypanosome, amastigote and epimastigote (de Souza, 1984). The cycle begins with the invasion of trypanosomes into the blood of insect-infected vertebrates (Fig. 1). It is noted that most trypanosomes ingested in common media will degrade in the stomach of the insect but surviving trypanosomes will develop into the globular stage amastigote or epimastigote after a few days (Carlos et al, 2007). Once they are converted into epimastigotes, they move to the intestine where their progressive division begins and end up adhering to the perimicrovillar membranes, secreted from the midgut (Alvez et al, 2007). The aforementioned step is part of the metacyclogenesis process, which involves the transformation of epimastigotes into trypomastigotes; that is, from non-infectious to infectious, called metacyclic trypomastigotes (Ruiz et al, 1998). Invasion in vertebrates can occur through one of the three available mechanisms. In the independent mechanism of the host cell, the parasite adheres to the cell and uses the pressure of its motility to enter it. The lysosome-mediated mechanism the *T. cruzi* parasite transports lysosomes to the binding site via host microtubules, while the additional membrane allows entry into a parasitophorous vacuole. While the third cantilever mechanism of the membrane, the cell is stimulated to extend the processes in the parasite, thus facilitating entry (Yoshida, 2006).

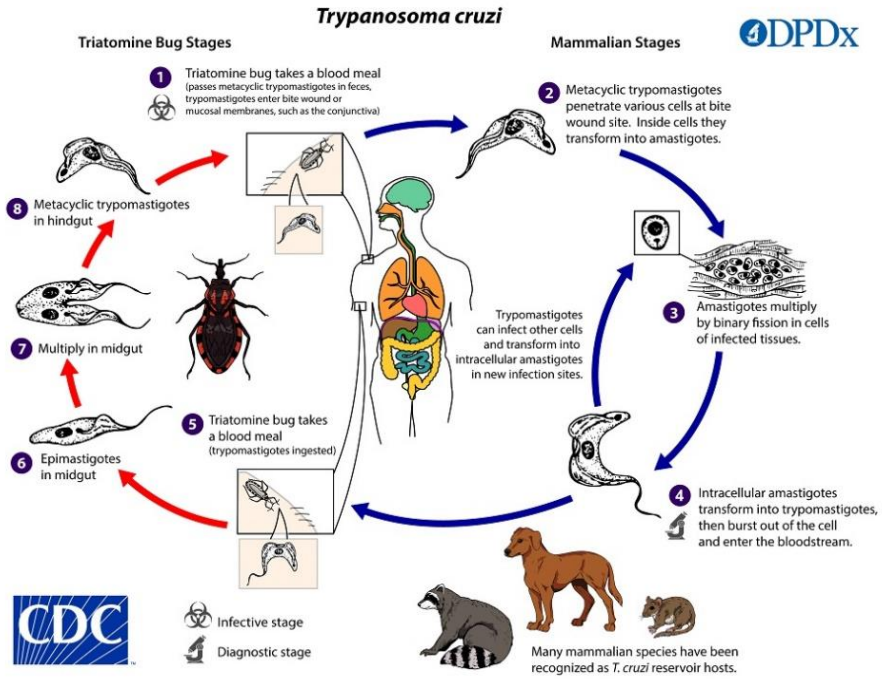


Figure 1. Life cycle of *T. cruzi* in various forms of the protozoan, in the invertebrate (triatomines) and vertebrate (mammals) hosts. From Centers of Disease Control and Prevention (2021).

2.2 Transmission of Chagas Disease

In Latin America, the parasites generated by hematophagous triatomine insects (Fig. 2) have different forms of transmission. The common factor is contact with the urine / feces of the triatomine that the infected species can deposit through a previous bite or injury.

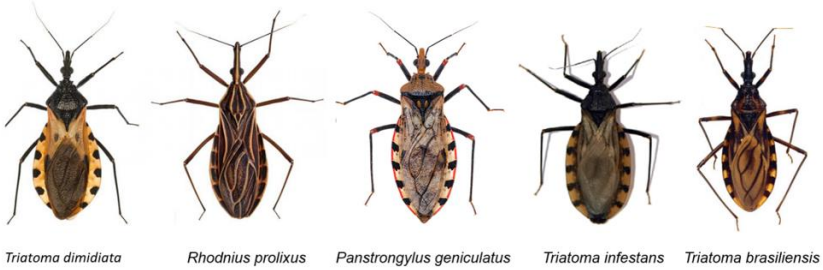


Figure 2. Vector form of the insect. Vectors found in Central and South. Centers of Disease Control and Prevention (2020).

Among the main routes of transmission of Chagas disease, we have the following:

Vector Transmission

Being the most common form of transmission, it is present in endemic areas of the infection and occurs when triatomine parasites are present in the droppings (feces / urine) of the insect penetrating the wound caused by the bite in the skin area of the animal. affected, this insertion of the parasite can be both cutaneous or due to mucus from the eyes, mouth, and nose (González et al, 2015).

Vertical Transmission

T. cruzi can be transmitted from a pregnant woman to the child since there is an increase in parasitemia in pregnancy, due to the changes produced in the immune modulation, this parasite load could occur in one or all pregnancies of the woman. This incidence can increase over time and without rapid detection (Oliveira et al, 2010)

Transfusion transmission

This is one of the main transmission routes, being in second place in the transmission method due to its frequent presence, this is due to an erroneous or lack of detection of the parasite prior to transfusion. This problem was generally from Latin America, but with the high rate of emigration, it spread to non-endemic countries of the disease (Coura & Viñas, 2010).

For this reason, a rapid detection of parasitemia is important, since years may pass without infected patients presenting symptoms of the disease and therefore not knowing their status.

Oral transmission

Oral transmission is a widespread mechanism among wild animals in the area in which the development cycle of parasitemia is located. Animals in the zones ingest the insect carrying the parasite *T. cruzi* directly or indirectly. The number of cases through transmission increases over the years, since oral transmission is also associated with the consumption of food, such as meat from infected animals in the area, homemade drinks

(with infected fruits), generating a cycle of infection of the parasite, having an indirect relationship with the insect (Coura, 2014).

2.3 Pathogenesis of Chagas Disease

Throughout several studies, it has been ensured that Chagas disease should not be overlooked since it continues to be a health problem to be prioritized, for several specific reasons, such as: maintaining constant control in the primary areas of infection as well as in residential areas, especially in South America where there is a higher prevalence of cases of infection; obtaining support from areas where the disease has been controlled/eliminated; the costs that need to be covered for the control of the disease, and depending on the phase, the change of these for others that are more efficient due to the level of severity of the parasitemia within the body (Añez N. et al, 1999). In general, epidemiological data determine that the progression of the initial heart disease caused by the parasite may be affected by the interruption of transmission by vectors (Schofield CJ, Diotaiuti L & Dujardin JP, 1999).

Acute phase and manifestations

The acute phase can appear from the first weeks or months after infection. This phase generally does not exhibit symptoms, which is why it goes unnoticed. The symptoms present are common and do not give exclusivity to Chagas disease, thus leading to an erroneous compression. Among the most common symptoms is fever, body pain, rash, headache, among others. A more in-depth physical examination includes an enlarged liver or spleen, local swelling (sting site). The acute sign of Chagas disease is Romaña, which is known as a previous inflammation of the eyelid on the face of the person close to the wound left by the insect and deposited the parasite (Fig. 3), where it may have entered the eye area. by accident by the direct contact of the patient towards the eye. These symptoms can disappear without any intervention, in a medium period of time (weeks / months), but the infection will continue if you do not receive treatment (CDC, 2017).

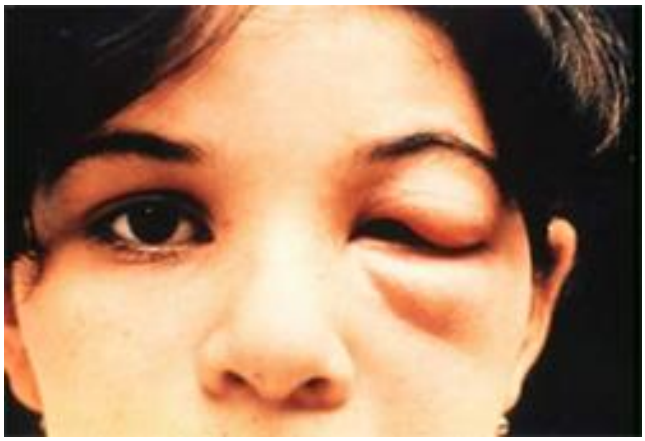


Figure 3. Acute phase of Chagas Disease. Presence of Romaña in the patient. Centers of Disease Control and Prevention (2021).

On rare occasions, this phase can lead to carrier mortality between a probability of 5 to 10% due to encephalomyelitis or severe heart disease and with a very low probability of sudden death; This varies specifically from how the immune system of the infected person presents a response to the parasite (Prata A., 2001).

Chronic phase and manifestations

After a period of 3 to 4 months, the manifestations of the acute phase disappear and therefore the parasite is hardly detected in the peripheral blood, this does not indicate that the infection process has ended, only that it has spread through the patient's body. The patient enters the chronic phase in which the infection can remain asymptomatic for years or throughout life, entering what is known as clinical latency called indeterminate form (Prata A., 2001).

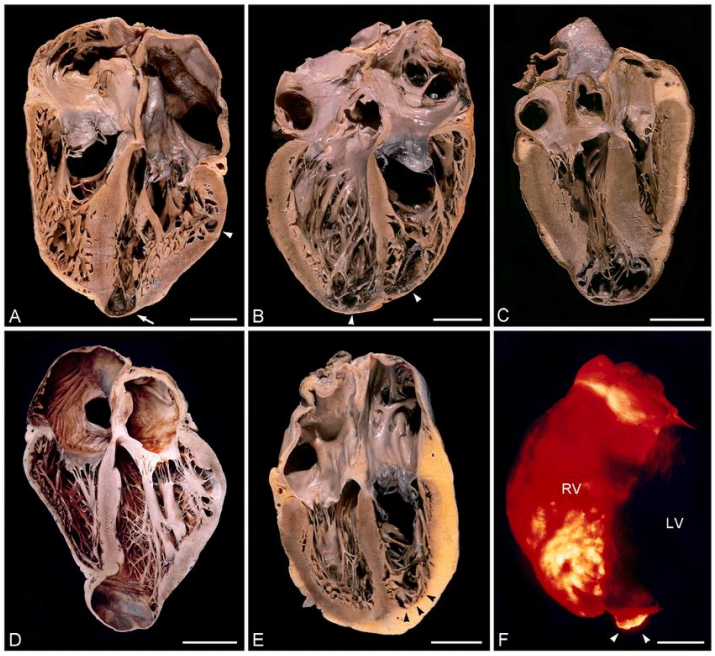


Figure 4. Chronic Phase in Chagas Disease. (A) Cardiomegaly with a aneurysm in the left apical zone (arrow). Myocardium hypertrophy. (B) Cardiomegaly. Thinning and thrombosis (arrow heads). Ventricular cardiac chamber dilation (C) Average-sized heart showing a huge aneurysm at the apex of the left ventricle (D) Slightly enlarged heart, showing four-chamber dilation. Giant left apical aneurysm. (E) Fully enlarged chronic chagasic heart with dilatation mainly affecting the right chambers. (F) Transillumination of a chagasic heart showing muscle wall thinning. From "Coronary Microvascular Disease in Chronic Chagas Cardiomyopathy Including an Overview on History, Pathology, and Other Proposed Pathogenic Mechanisms" by Rossi A. et al, 2010. *PLoS Neglected Tropical Diseases*, 4(8), e674.

A portion of the patients who completely reach the chronic phase passing through the indeterminate present different manifestations related to certain organs; as, cardiac complications in which there is an abnormal enlargement of the heart as well as heart failure, alterations in the heart rate and increased death (Fig. 4). Intestinal complications also occur, often including a disproportionate enlargement of the esophagus or colon, which presents difficulties prior to the proper functioning of each organ. The risk percentage of presenting these complications throughout the indeterminate phase is 30% (CDC, 2017).

Indeterminate Form

After several studies carried out in endemic areas of the insect and the infection, around half of the population infected by triatomines present the indeterminate form of

infection. Carriers do not present the initial symptoms, there is no immune reduction and specific tests such as the electrocardiogram, radiological examination of the heart and esophagus and colon present normally. They do not express the disease and are even unaware of the disease that PCR can identify. The delimitation made by the patients with the indeterminate form recognizes them as patients with low mortality, in which they carry out their daily activities without any impediment or complication. These patients are commonly subjected to various examinations for research purposes, such as evaluating heart or digestive diseases that could present in a period of 5 to 10 years or never present (Prata A., 2001). A variable number of patients under investigation present alterations in the tests, which are usually of low intensity and isolated. However, some of the patients have early cardiac or esophageal diseases. It is necessary to mention that the lesions are not always related to the degree of parasitemia within the patient and it can even be said that higher parasitemia will not affect the course of the disease (Zhang L & Tarleton RL, 1999).

Chapter 3: Pharmacology of Chagas disease

3.1 Drugs used to treat Chagas infection

Some of the diseases generated in tropical climates such as Chagas infection have gone unnoticed or have not been taken with due importance, for this reason some of the drugs used to combat these diseases, such as Chagas parasitic infection. All drugs must be approved and follow the guidelines established by the World Health Organization (WHO).

In experimental models, the constant antiparasitic activity of nitro-heterocyclic derivatives, motivated further work in the search for compounds of this general class with higher anti-*T. cruzi* activity and with less toxicity, this effort led to the discovery of Nifurtimox in the late 1960s and early 1970s the most active compound among the nitrofuran derivatives tested, and a 2-nitromidazole with remarkable *in vitro* and *in vivo* anti-*T. cruzi* activities. These compounds (Fig. 5 and 6) registered mainly for the treatment of acute parasitic infections by *T. cruzi*, remain until today as the only drugs available for the specific treatment of Chagas disease (Coura and de Castro, 2002; Pinto Dias, 2006).

3.1.1 Benznidazole (BZN)

Benznidazole (2-nitro-N- [phenylmethyl] -1H-imidazole-1-acetamide) (Fig. 5) is derived from nitroimidazole, this is one of the main drugs used against T. cruzi, due to its toxic effects it was lower than its competition as nitrofurazone.

Benznidazole is activated by trypanosomal nitroreductase I, it also releases molecules such as glyoxal dialdehyde, which binds the guanosine bases, causing a blockage, in this way it achieves that the parasite is exposed to oxidative damage in the stages of the life cycle of the parasite. Benznidazole is first absorbed from the gastrointestinal tract (Patterson & Wyllie, 2014)

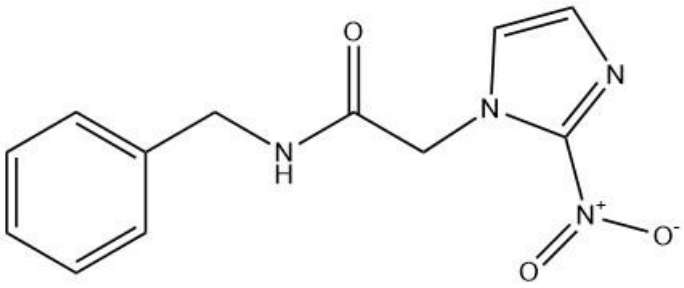


Figure 5. Benznidazol structure *Note.* Representation of Chemical Structure of Benznidazol.

BZN has a trypanocidal action, which considerably reduces parasitemia in patients in acute and chronic phases. There are cases where the parasites are more resistant and are not eliminated with the BZN, which is why in the tests (PCR) they continue to give positive carriers of the parasite. In addition, in the adult population, it is known that for the complete elimination of the parasite, it may take years or decades after treatment, which generates uncertainty in the treated population (Torrico et al, 2018).

Therefore, the efficacy of BZN in an adult population with a chronic phase will be indeterminate since it does not meet the same expected effects as with the acute phase, generating more adverse effects and failing to control cardiomyopathies and gastrointestinal pathologies in the chronic phase, even so, it can be said that in certain cases it helps the elderly to reduce mortality due to parasitic infection, for which in cases of already advanced parasitemia, the use of Benznidazole is not recommended (Chatelain, 2017).

3.1.2 Nifurtimox (NFX)

Nifurtimox (3-methyl-N - ′ [(5-nitro-2 furanyl) -methylene] -4- morpholinamine 1,1 dioxide) is derived from nitrofuran (Fig. 6), it belongs to one of the most used drugs against Chagas disease, it has demonstrated different results depending on different factors such as the age of the patient, the phase of parasitaemia, and the time of dosing. Nifurtimox generates nitro anion radicals due to the action of nitroreductases that, together with oxygen, produce free radicals that attack and damage the parasite (Docampo, Moreno & Stoppani, 1981). This joint action is carried out with Benznidazole, blocking DNA synthesis, thus accelerating its degradation. The drug is absorbed in the gastrointestinal tract and is metabolized by the liver and has the action of cytochrome P-450 (Paulos et al, 1989).

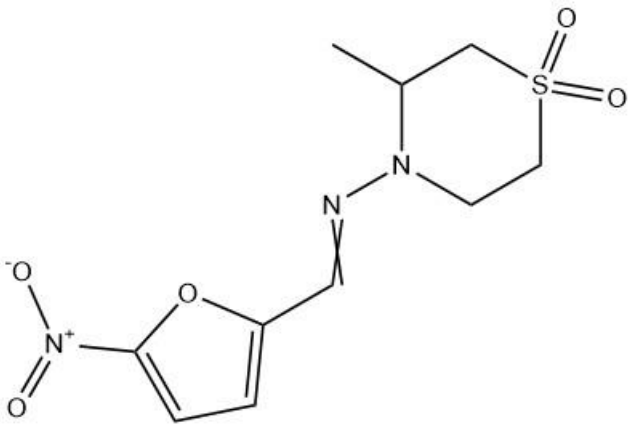


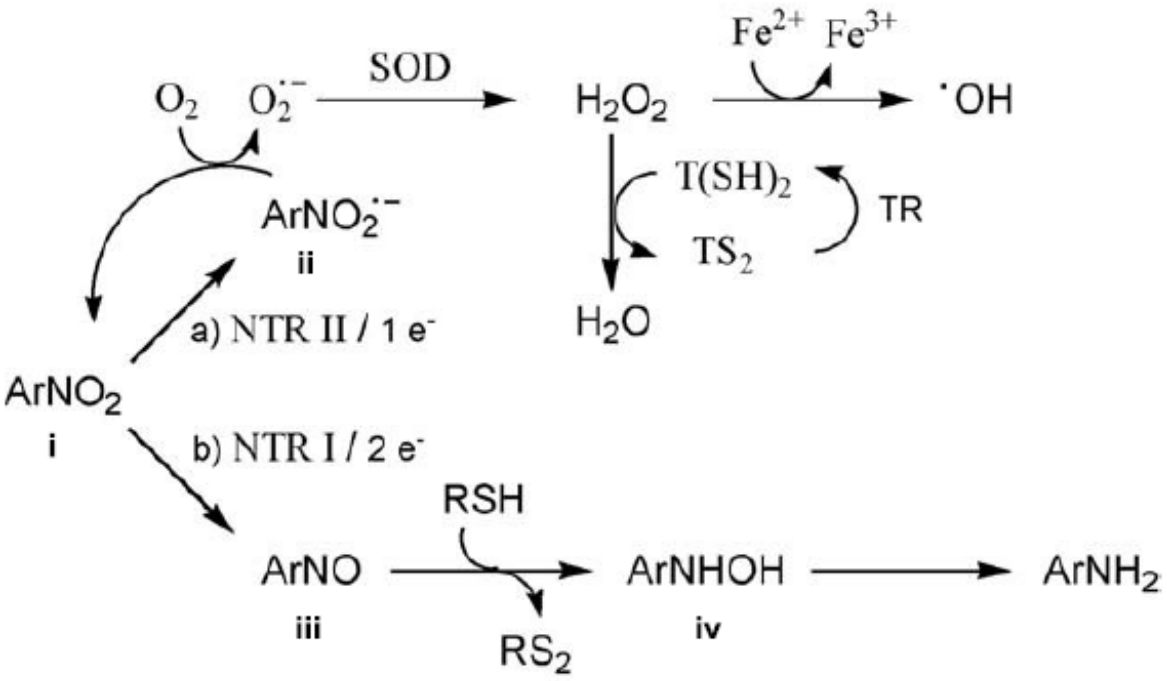
Figure 6. Nifurtimox structure *Note.* Representation of Chemical Structure of Nifurtimox.

Some adverse effects that are produced by the consumption of NFX are gastrointestinal problems such as nausea, dizziness, vomiting and abdominal pain. Other adverse effects that are generated in a smaller number of patients, such as central nervous system disorders such as seizures, confusion, and psychosis (Buekens et al 2018).

The adverse effects and toxicity of NFX led to its discontinuation of commercialization in some countries such as Brazil, Argentina, Chile and Uruguay in the early eighties. Due to the lack of alternatives in the treatments against *T. cruzi*, the drug was continued to be marketed as a second-line treatment when Benznidazole does not meet expectations, the use of Nifurtimox is reached, although there are cases in which it is preferable to use Nifurtimox before Benznidazole since it also presents toxicity in patients (Rodriguez C. & de Castro SL., 2002).

Scheme 1 shows the routes in charge of the reduction and bioactivation of nitro-heterocyclic derivatives (i). It begins with the reduction of the nitro group to the nitro-anion radical (ii) It shows the reaction catalyzed by NADPH / NADH-nitroredutase (Maya et al., 2007). Under certain specific conditions such as aerobic, the nitro-anion radical (ii) will react with oxygen, regenerating (i) and forming the superoxide anion, which will result in hydrogen peroxide and this, when reacting with superoxide dismutase (SOD) can generate the hydroxyl radical through the Haber-Weiss reaction (Haber and Weiss, 1932; Koppenol, 2001).

The accumulation of reactive oxygen species (ROS) would lead to oxidative stress in the *T. cruzi* parasite, which generates a deterioration in the detoxification mechanism (Docampo & Moreno 1984; Cadenas, 1989). Furthermore, five peroxidases were identified in *T. cruzi*: two tryparedoxin peroxidases (Wilkinson et al., 2000; Piacenza et al., 2008; Trujillo et al., 2004), two glutathione-dependent peroxidases (Wilkinson et al. 2002a; Wilkinson et al., 2002b), and an ascorbate-dependent hemoperoxidase (Wilkinson et al., 2002c) that demonstrates that the parasite has an efficient and complex system to deal with oxidative stress.



Scheme 1. Two possible routes for the metabolic reduction of the nitro group in nitro-heteroaromatic derivatives (i). The aerobic route (a) and the anaerobic route (b). NTR= nitroreductase; SOD= superoxide dismutase; TSH= trypanothione; TR= trypanothione reductase. Adapted from "Molecular Coupling Study (docking) of a series of synthetic 5-nitrofuran derivatives at the trypanotion reductase binding site of *Trypanosoma cruzi*." By Monasterios, Melina & Amesty, Ángel & Avendaño, Milagros, 2011, *Revista de la Facultad de Farmacia*. 74. 25-30.

Taking as obstacles the limitations of the drugs currently available, for the treatment of Chagas disease patients who are in the chronic phase, new studies on specific chemotherapy for Chagas disease continue to be carried out. carrying out new drugs with a short and medium term for their production, taking into account the proposed therapeutic objective.

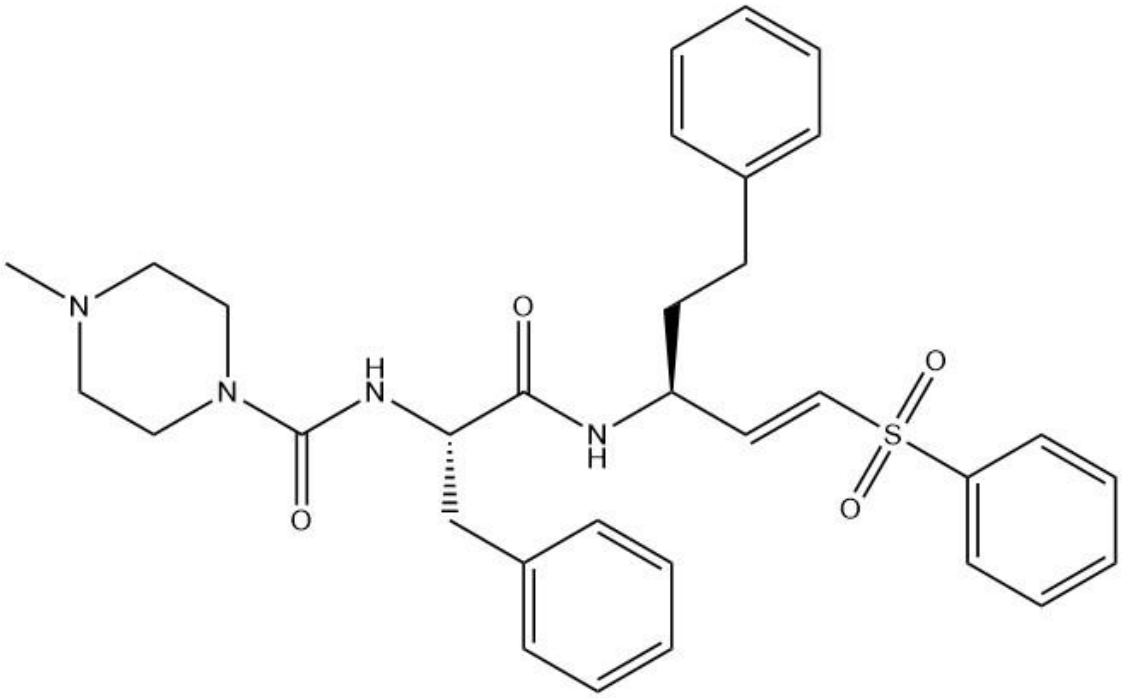
Chapter 4: Therapeutic Strategies for the development of drugs against *T. cruzi*

4.1 Protein Cruzipain as therapeutic objective

The main cysteine-protease in the *T. cruzi* parasite is cruzipain, a glycoprotein with a molecular weight of approximately 41 kDa. Cruzipain is localized in lysosomes and to a

certain extent in the plasma membrane (Robertson & Renslo, 2011). Cruzipain is considered a therapeutic target for Chagas disease chemotherapy because it plays an important role in tissue invasion and evasion of immune responses (Doyle et al, 2011). It is necessary to mention that in mouse models infected with the *T. cruzi* parasite, the compound K**777** (Fig. 7), which is a protease inhibitor, eliminates the infection in a period of 20 to 30 days, in addition to showing a synergistic effect with other drugs such as Benznidazole, giving an improvement in the survival test period of the infected muscle cells, having a survival period of 47 days, which unlike BNZ with K777 of only 27 days of survival (Doyle et al, 2007).

(A)



(B)

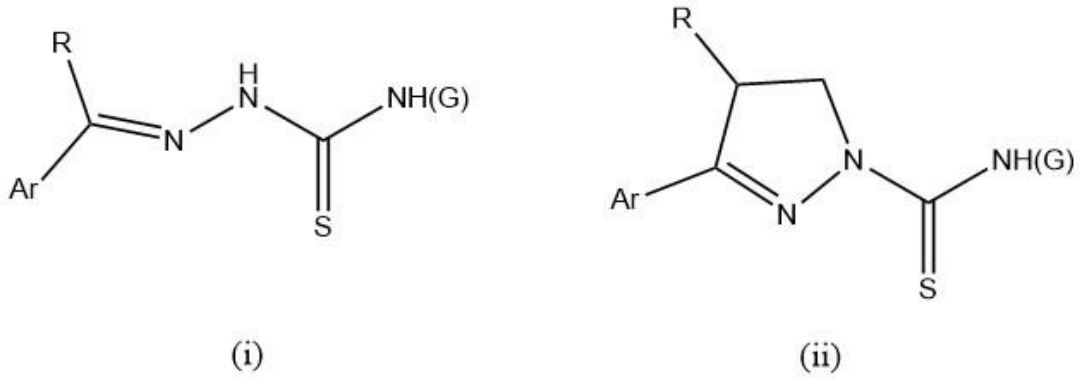


Figure 7. (A) Structures of specific as well as potent inhibitors of cruzipain, which is an essential cysteine protease of *T. cruzi*: structure of K-777, which is considered as the most advanced enzyme inhibitor, with potent and selective anti-*T. cruzi in vitro* and *in vivo*. (B) Linear (i), cyclic (ii) alkyl-thiosemicarbazone scaffolds of novel cruzipain inhibitors, with potent activity against the purified enzyme and intracellular amastigotes *in vitro*.

4.2 Cytochrome b

Different methods have been used to find therapeutic targets, which is why chemical genomics was also used to identify cytochrome b as a new potential therapeutic target for the parasitic disease of *T. cruzi*. In addition, it was discovered that the compound GNF7686 has a specific pathway to cytochrome b, which is part of the electron transport chain, crucial for the generation of adenosine triphosphate (ATP). GNF7686 (Fig. 8) proved to be a crucial inhibitor of the development of *T. cruzi*, thus giving new possibilities to the use of chemical genetics to discover new drugs that may be of great importance in the parasitemia of Chagas disease (Villalta & Rachakonda, 2019).

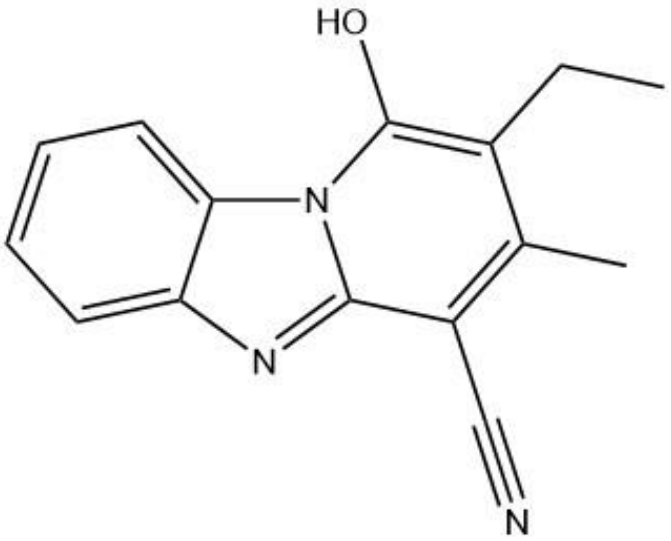


Figure 8. GNF7686 structure *Note.* Representation of Chemical Structure of GNF7686.

Chapter 5: Chemotherapeutic objectives for the development of drugs against *T. cruzi*

5.1 Ergosterol Biosynthesis Pathway and its Inhibitors

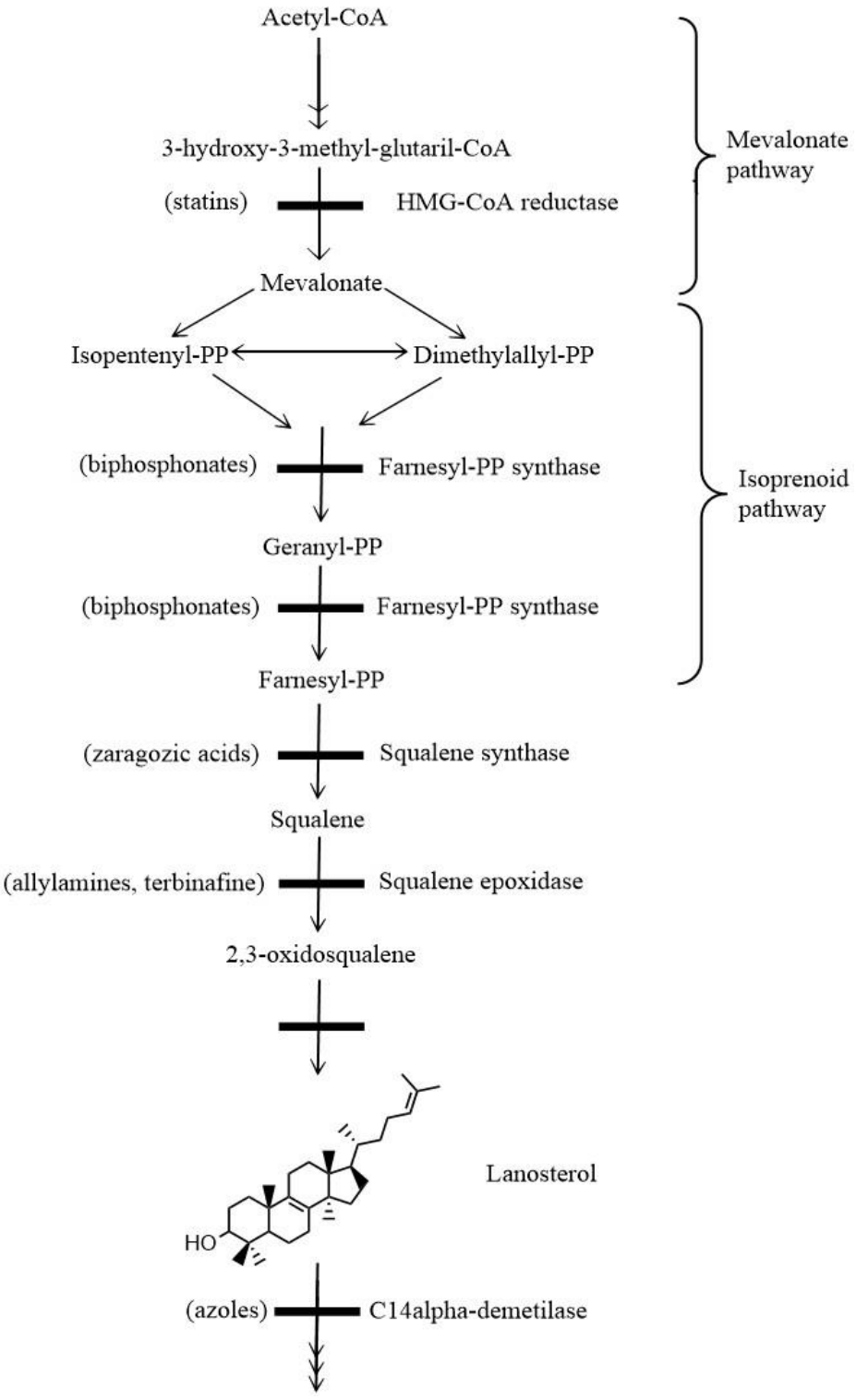
Sterols are part of cell membranes, being essential in their structure and function. In mammals, cholesterol is the main sterol present in various membranes, unlike the sterols that are predominant in microorganisms such as protozoa, trypanosomatids insert cholesterol from a culture medium or from infected animal blood, through an endocytic process that involves the development of endocytic vesicles in the cytosome and the flagellar bag with the epimastigotes of *T. cruzi* (Souza, Santanna & Cunha -e-Silva, 2009).

Therefore, in trypanosomatids, the sterol of the parasite is ergosterol, which differs from mammals (cholesterol), it is essential to provide a correct structure and function of the trypanosome membranes, it also plays a role in the reproduction of the parasite (Urbina et al, 1991).

Although sterol biosynthesis involves several steps, only two of them have become important targets of systemic clinical drugs. Statins (cholesterol-lowering agents) act before the pathway in the step of mevalonate production, these being the most frequently prescribed drugs (Superko et al., 2012). while azoles, inhibitors of CYP51, serve as the most widely used antifungals (Lass-Flörl, 2011; Denning and Bromley, 2015) and are under investigation to be repurposed for treatment of human infections with protozoan parasites (Buckner and Urbina, 2012).

In Trypanosomatidae, the fungal biosynthesis pathway will be similar to that of sterols (Fig. 1): squalene 2,3-epoxide is cyclized directly into lanosterol, and the products generated from this will be the ergosterol and its C24 alkylated analogs. The sequencing of their genomes confirmed the presence of all the necessary enzymes of the pathway (El-Sayed et al. 2005).

The main difference in sterol biosynthesis pathways (Fig. 9) between mammals and trypanosomatids is the final product presented which is ergosterol which is presented as cholesterol in mammals. Because *T. cruzi* is dependent on endogenously produced sterols for its reproduction and survival, the ergosterol biosynthetic pathway forms a target for the development of drugs against the parasite (Lepesheva et al, 2010).


Acetyl-CoA
3-hydroxy-3-methyl-glutaril-CoA
(statins)
HMG-CoA reductase
Mevalonate
Mevalonate pathway
Isopentenyl-PP
Dimethylallyl-PP
(biphosphonates)
Farnesyl-PP synthase
Geranyl-PP
(biphosphonates)
Farnesyl-PP synthase
Farnesyl-PP
Isoprenoid pathway
(zaragozic acids)
Squalene synthase
Squalene
(allylamines, terbinafine)
Squalene epoxidase
2,3-oxidosqualene
HO
Lanosterol
(azoles)
C14alpha-demetilase

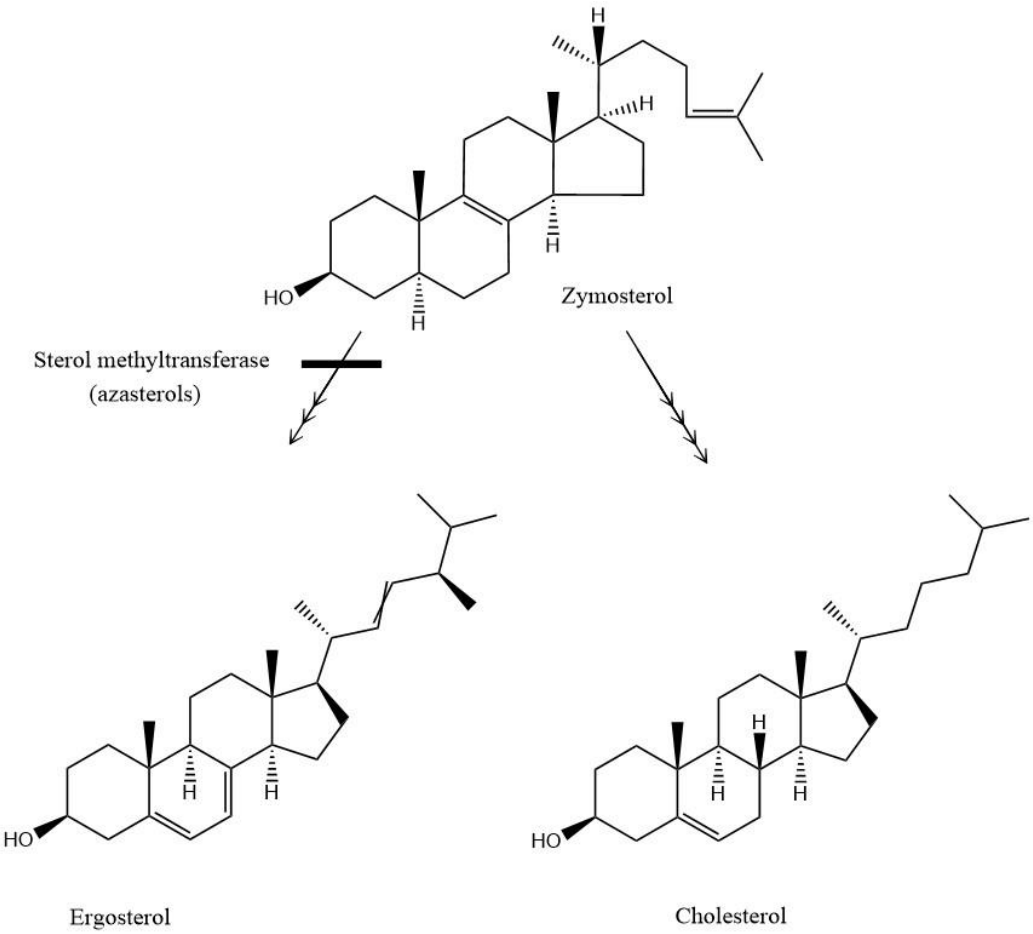


Figure 9. The biosynthesis of ergosterol and cholesterol with the main steps, the enzymes involved, and the inhibitors related.

The set of critical enzymes that play an important role in the biosynthesis of ergosterol in the parasite is also a fundamental part of the study targets since they can help understand its mechanism better and help in future research specifically directed at the critical enzymes in biosynthesis. ergosterol. Squalene synthase (SQS), squalene epoxidase (SQLE), lanosterol 14 α-demethylase (CYP51), oxidosqualene cyclase (OSC) and sterol 24-C-methyltransferase (24SMT) are part of the main enzymes dedicated to the inhibition in the sterols pathway. Oxidosqualene cyclase has shown that its inhibition directly affects ergosterol biosynthesis in the parasite (Benaim et al, 2006).

Therefore, the first step for the biosynthesis of sterols begins with the condensation of two molecules of farnesyl diphosphate to produce squalene, through a two-step reaction catalyzed by the enzyme squalene synthase (Villalta & Rachakonda, 2019).

Sterol 14-alpha demethylase (CYP51)

The sterol 14-alpha demethylase (CYP51), which is part of the ergosterol biosynthesis pathway of *T. cruzi*, is the only gene in the pathway, which has been evaluated and demonstrated, being essential for the survival of the parasite. Therefore, sterol 14-alpha demethylase is the enzyme most studied in detail as the main therapeutic target in the development of *T. cruzi* (Villalta et al, 2013).

CYP51 uses lanosterol-related compounds to produce zymosterol, which is the precursor of ergosterol. CYP51 can be inhibited in its activity using azoles (antifungal drugs). Therefore, the variety of azoles presented today have strong trypanocidal activity in vitro and in vivo (Lepesheva et al, 2015).

Human CYP51 is resistant to inhibition, raising concern that pathogenic CYP51 inhibitors would affect the human counterpart. Therefore, attempts to use human sterol 14-alpha demethylase as a pharmacological target failed, and no antifungal azole or experimental inhibitor of human pathogens CYP51 inhibits the activity of the human enzyme (Villalta & Rachakonda, 2019).

Sterol 24-C-methyltransferase (Tc24SMT)

The Tc24SMT enzyme is part of one of the most promising targets because its inhibition has not yet been studied in depth, resulting in a new option for study with beneficial studies expected. This enzyme is not found in humans. The *T. cruzi* treated with the inhibitors of 24SMT 22,26-azasterol and 24 (R, S) 25-epiminolanosterol did not show levels that indicate its presence of 24-alkyl sterols, in addition they greatly inhibited the growth of *T. cruzi.* (Urbina et al, 1996). In addition, other azasterols were synthesized, showing that they have antiproliferative effects in *T. cruzi,* however, it is necessary to deepen their research to

develop a better use in the activity and selectivity of the compounds used in the tests. On the other hand, other inhibitors of the 24SMT enzyme affect the development of epimastigote and certain ultrastructural alterations to be considered (Braga et al, 2005).

Inhibitors of the protozoan 24SMT, while under investigation, may become a potential option for the treatment parasitic and fungal diseases.

Squalene synthase (SQS)

The Squalene synthase, in its beginnings, its use as a therapeutic target in parasitemia like *T. cruzi* was discovered since its effects in vitro and in vivo of the inhibitors proved to be useful as a chemotherapeutic object. In addition, isosteric analogs were synthesized, which proved to be a good non-competitive allosteric inhibitor of the parasite (Urbina et al, 2002).

By determining the structure of the parasite and human SQS enzymes that possess four classes of inhibitors, which reduce the growth of *T. cruzi*, it also provides information on the inhibition of SQS. E5700, which is a potent quinuclidine inhibitor, binds to TcSQS and exhibited a synergistic effect together with posoconazole, in inhibiting the development of the *T. cruzi* parasite in vitro (Shang N. et al, 2014). Oral administration of E5700 provides complete survival protection from parasitic infection in mice, but curative effects have not been demonstrated in vivo. Furthermore, toxicity studies have not been conducted with TcSQS inhibitors (Villalta & Rachakonda, 2019).

Farnesyl diphosphate synthase (FPPS)

Farnesyl diphosphate synthase (FPPS) is considered a chemotherapeutic target in *T. cruzi* in addition to the mechanism of inhibition action by bisphosphonates against FPPS. Furthermore, certain approaches have been developed to design future new FPPS inhibitors for *T. cruzi* (Villalta & Rachakonda, 2019).

It is reported that there have been studies of the interaction of five 2-alkylaminoethyl-1,1-bisphosphonates, which were effective against the multiplication of *T. cruzi*, in conjunction with the structure of the farnesyl diphosphate synthase of *T. cruzi* (TcFPPS) (Aripirala et

al, 2012). The information on the structure given in the research provides the basis for the design of new compounds that are more effective for the treatment of parasitic invasion by *T. cruzi*. Thanks to the lack of pharmacokinetic studies regarding the various families of bisphosphonates that act by inhibiting TcFPPS, it is mentioned that it is important to increase the number of compounds in the process (Villalta & Rachakonda, 2019).

Oxidosqualene cyclase (OSC)

Nowadays, with the practice and investigation of the oxidosqualene cyclase (OSC), a biochemical investigation has been used for the OSC in which three inhibitors of the OSC of *T. cruzi* are evaluated, in which it was discovered that a parasitological activity was present important in vitro. It is necessary to mention that the identification and synthesis of inhibitors of *T. cruzi* of OSC has not been fully investigated, therefore there are different aspects to consider in the process of inhibition of OSC in the parasite (Villalta & Rachakonda, 2019).

Sterol 14 alpha-demethylase was the first enzyme of the ergosterol pathway of the *T. cruzi* parasite to be crystallized and through drug optimization, important inhibitors of the enzyme such as NIV and VFV were created that cure infection, which it is experimental using murine models of the disease. Recently, other enzymes in the ergosterol pathway such as SQS and FPPS have been crystallized. The structural information obtained from these enzymes may provide important future inhibitors derived from drug enhancement and optimization. It is also necessary to improve the toxicity and bioavailability problems currently generated (Villalta & Rachakonda, 2019).

5.2 Available drugs that interfere with the Sterol Biosynthesis Pathway

The development of drugs has grown nowadays, so that there are drugs that intervene in the biosynthesis of sterols, which are used mainly for the treatment of diseases of both fungal infections and high cholesterol in people.

Statin is considered one of the main inhibitors of sterol biosynthesis, since it acts on the mevalonate pathway by inhibiting the enzyme HMG-CoA reductase, which is responsible for regulating the rate of cholesterol synthesis in the liver both as in other tissues of the body (Barrett-Bee & Ryder, 1992). Statin shows a negative point in its use, since it shows an effect on the synthesis of isoprenoid compounds, which are important for cellular events. Atorvastatin (Fig. 10) of the statin class is used mainly for the treatment of hyperlipidemia.

Bisphosphonate, being an important class for the inhibition of sterol synthesis, intervenes in the isoprenoid pathway, inhibiting the step catalyzed by the enzyme farnesyl diphosphate synthase. Bisphosphonates are used mainly for the treatment of different bone resorption diseases, such as osteoporosis, among others (Rodan, 1998). Alendronate (Fig. 11) belongs to the class of bisphosphonate, which is used essentially for the treatment of bone resorption diseases such as postmenopausal osteoporosis.

The enzyme squalene synthase (SQS), which is responsible for catalyzing the first step in the biosynthesis of sterols, is inhibited both by zaragozic acids and quinuclidines. Therefore, the inhibition of the squalene synthase enzyme becomes an attractive target since it will not interfere with the production of isoprenoids, in addition to the intermediate metabolites that will be formed, they will be able to be metabolized and excreted more efficiently. (González et al, 1998). 3- (biphenyl-4-yl) -3 hydroxyquinuclidine [BPQ-OH] (Fig. 12) of the quinuclidine class, is responsible for reducing cholesterol and triglycerides by inhibiting the enzyme SQS (De Souza & Rodrigues, 2009).

On the other hand, allylamines known to be essential inhibitors of the enzyme squalene epoxidase, have different examples of drugs such as terbinafine (Fig. 13), which being of the class of allylamines inhibits squalene epoxidase, this drug has been shown to be a powerful fungicide, both orally and topically. In addition, it has a positive point, since it does not inhibit the mammalian enzyme, which is why its use in humans is more widely available (Barrett-Bee & Ryder, 1992).

Azoles are essential inhibitors of the C14 α-demethylase enzyme, they also show to be potent with fungal infection diseases, giving them an important place in the first line of fungicidal use. Posaconazole (Fig. 14) is part of the azole class, which is why it inhibits

C14 α-demethylase, being one of the first azole drugs developed, its use remained high for several years until the development of new triazoles. that proved to be more effective (De Souza & Rodrigues, 2009).

Finally, we have azasterols that are also an essential part in the inhibition of ergosterol biosynthesis. Azasterols inhibit the enzyme Δ24 (25) -sterol methyltransferase (SMT), in this part of the ergosterol biosynthesis pathway, it has a high selectivity for trypanosomatides and fungi, so this enzyme is not found in the mammalian cell, it is exclusively for mushrooms and trypanosomatids. Azasterol 22,26 (Fig. 15) is part of the azasterol class, which also has active fungicides and trypanosomicides (Urbina et al, 1996).

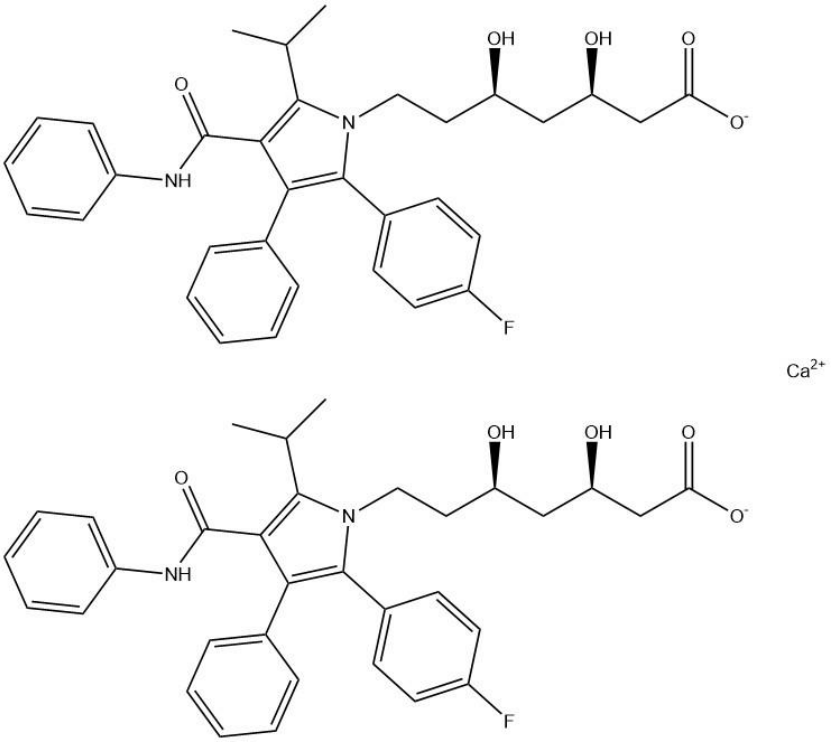


Figure 10. Atorvastatin chemical structure. Class of Statin. Inhibitor of the HMG-CoA Reductase. Used to treat hyperlipidemia in humans.

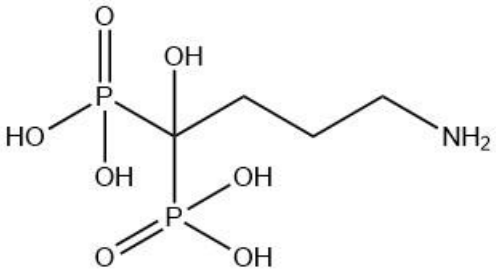


Figure 11. Alendronate chemical structure. Class of Bisphosphonate. Inhibitor of the farnesyl diphosphate synthase. Used to treat osteoporosis and different bone resorption diseases

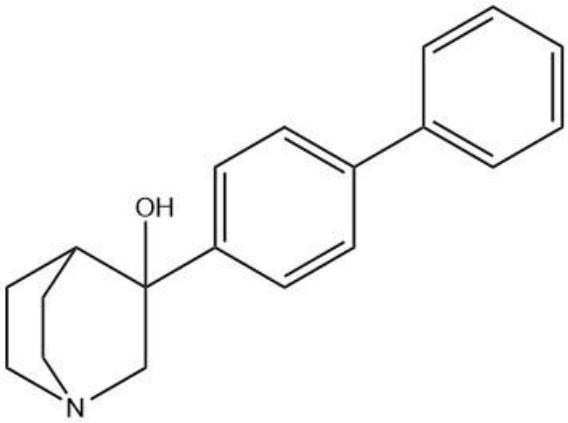


Figure 12. BPQ-OH chemical structure. Class of Quinuclidine. Inhibitor of the squalene synthase, acting in the cholesterol and ergosterol biosynthesis.

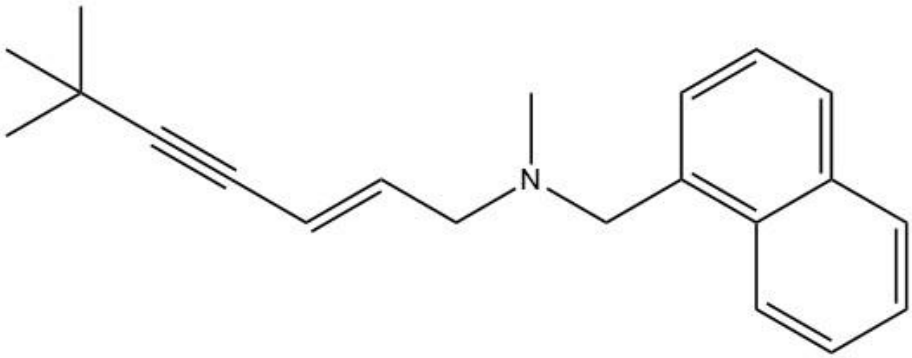


Figure 13. Terbinafine chemical structure. Class of Allylamine. Inhibitor of the squalene epoxidase. Used for a long time to treat fungal infections

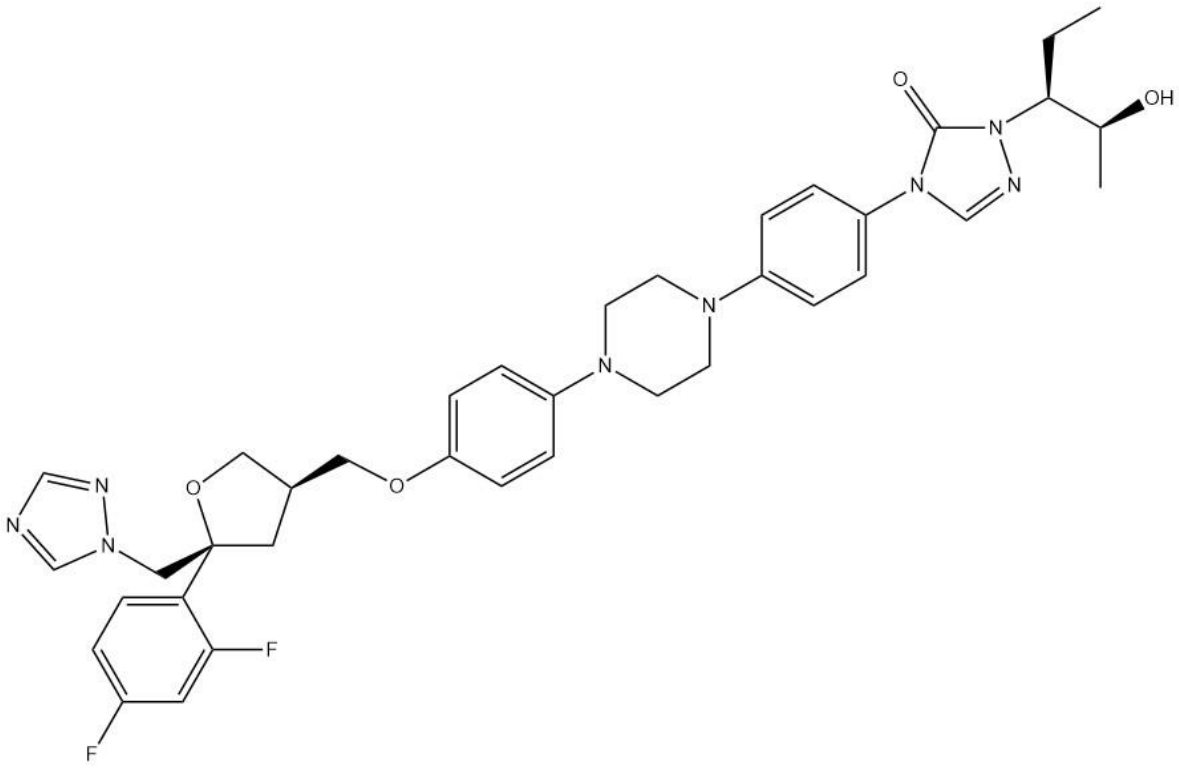


Figure 14. Posaconazole chemical structure. Class of Azole. Inhibitor of the C14α-demethylase. One of the triazole used to treat fungal infections

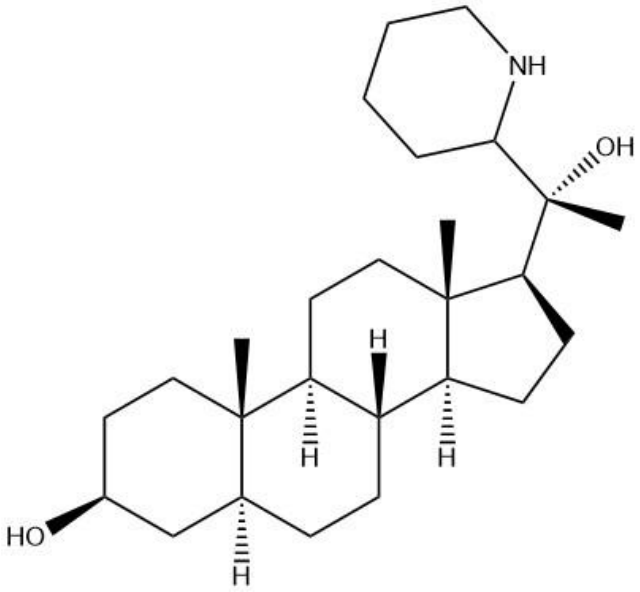


Figure 15. 22,26-azasterol chemical structure. Class of Azasterol. Inhibitor of the Δ24(25)-sterol methyltransferase, one enzyme presents exclusively in the ergosterol biosynthesis

Chapter 6: Drugs involved in the sterol biosynthetic pathway

The variety of drugs has increased on a large scale, mostly focusing on the routes of biosynthesis of sterols both eukaryotes and fungi as well as in and trypanosomatids. In fact ergosterol is the main sterol in *Trypanosoma cruzi*.

Among the main drugs used to inhibit sterol biosynthesis (Table 1) we have the statins, which act on the mevalonate pathway through the inhibition of HMG-CoA reductase, the statin is used mainly for the reduction of cholesterol in humans (Barrett- Bee & Ryder, 1992). A negative characteristic in the use of statins is the effect they produce on the synthesis of isoprenoid compounds which are necessary in various cellular events. Atorvastatin is a type of statin which is used mainly for the treatment of hyperlipidemia (Doggrell, 2006).

Another class of drug that is responsible for interfering in the isoprenoid pathway, by inhibiting the passage catalyzed by farnesyl diphosphate synthase (FPPS) are bisphosphonate. These bisphosphonates are used for a variety of bone resorption diseases such as osteoporosis, hypercalcemia caused by a malignancy, tumor metastases of the bone, and Paget's disease. Among the bisphosphonates, there is alendronate and risedronate, which are used for the treatment of bone resorption diseases (Rodan, 1998).

Among some inhibitors of the squalene synthase enzyme are zaragozic acids and quinuclidine, in which the first step of sterol biosynthesis is catalyzed, this is part of an important process because inhibition does not interfere with the production of isoprenoids and intermediate metabolites that can be formed, metabolized, and excreted in a more direct way (González-Pacanowska et al, 1988).

In the squalene epoxidase enzyme, allylamines are common inhibitors, such as terbinafine, which is a potent compound in its fungicidal use, it shows an oral efficacy as a topical. Terbinafine, by inhibiting squalene epoxidase, produces the decay of ergosterol, in

addition to an important point in its use, which is that it does not inhibit the mammalian enzyme (Barrett-Bee & Ryder, 1992).

Inhibitors of the C14 alpha-demethylase enzyme, such as azoles, because these are effective in most fungal diseases. One of the first azoles developed for antifungal use was Ketoconazole. With the appearance of new triazoles in the market for drugs such as Fluconazole, Voriconazole and Posaconazole, new alternatives were given for antifungal treatment, in addition to these triazoles showing a higher rate of effectiveness in fungicidal activity.

Azasterols, which are inhibitors of ergosterol biosynthesis, which inhibit Δ24 (25) - sterol methyltransferase (SMT). By inhibiting the ergosterol biosynthesis enzyme at this point, it was shown that it has a high selectivity in trypanosomatids, which is why this enzyme is exclusive and is not found in mammals (Ishida K., 2009).

Therefore, it is of relevant importance to explore the sterol biosynthesis pathways in trypanosomatids, especially the biosynthesis pathway the ergosterol in relation to the cholesterol route in humans.

6.1 Effects of Sterol Biosynthesis Inhibitors on the Ultrastructure of Trypanosomatids

With the use of inhibitors in the different stages of ergosterol biosynthesis, it has been shown that these induce drastic alterations in the ultrastructure of various organelles (Rodriguez et al, 2002). The alterations are carried out in the mitochondrion-kinetoplast complex, the Golgi complex, nucleus, lipid inclusions, the endoplasmic reticulum, among other structures. Therefore, the mitochondrion-kinetoplast complex is part of the target of drugs that are responsible for the inhibition of sterol biosynthesis. In addition, it is necessary to mention that *T. cruzi* has only one branched mitochondrion distributed throughout the protozoan body (De Souza, Attias & Rodrigues, 2009).

In the treatment of *L. amazonensis* with different azoles, it produced mitochondrial alterations, these alterations in the mitochondria were also evidenced in the treatment of *T. cruzi* with terbinafine, Ketoconazole Vannier-Santos et al, 1995). These alterations in the

mitochondria were evidenced by measuring the potential of the mitochondrial membrane in parasites that were permeabilized with digitone (Rodrigues et al, 2007).

Table 1. Therapeutic drugs for Chagas disease treatment

Class of Drug	Drugs	Mode of action	References
Statins	Atorvastatin Fluvastatim Lovastatin Pravastatin Rosuvastation Simvastatin	Inhibitor of the HMG-CoA reductase. Used to treat hyperlipidemia in humans.	Barrett- Bee & Ryder, 1992
Biphosphonates	Alendronato Risedronato Pamidronato	Inhibitor of the step catalyzed by the enzyme farnesyl diphosphate synthase. Biphosphonates are used for the treatment of different bone resorption diseases	Rodan, 1998
Quinuclidine	BPQ-OH	Inhibitor of the squalene synthase, acting in the cholesterol and ergosterol biosynthesis.	González et al, 1998
Allylamines	Terbinafine	Inhibitor of the squalene epoxidase. Used for a long time to treat fungal infections.	Barrett-Bee & Ryder, 1992
Azoles	Ketoconazole Fluconazole Voriconazole Posaconazole	Inhibitor of the C14α-demethylase. They also show to be potent with fungal infection diseases	De Souza & Rodrigues, 2009
Azasterols	Azasterol 22,26	Inhibitor of the Δ24(25)-sterol methyltransferase, one enzyme presents exclusively in the ergosterol biosynthesis	Urbina et al, 1996

Chapter 7: Alkylphospholipids (ALPs) as Promising Chemotherapeutic Agents

The need for new drugs at the end of the 60s, throught the use of synthetic metabolically stable analogs derivatives from lysophosphatidylcholine (LysoPC) in such a way at the end of the 60s, there was a change of a glycerol C1 ester bond in Lyso PC the C1 ester bond of glycerol in Lyso Pc in which it was transformed into an ether bond, in addition another methyl group linked to ether adhered in the C2 position. Edelfosine Et-O-

CH_3), which was the first drug molecule to be known as ALP (synthetic alkyphospholipids) (Eibl, 1967).

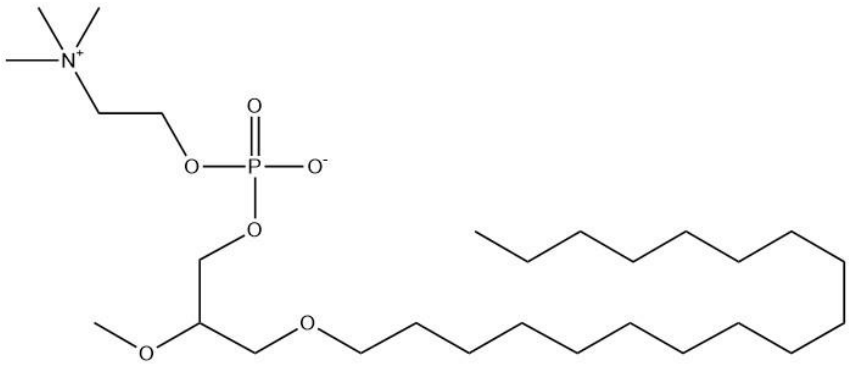


Figure 16. Edelfosine structure. Representation of Chemical Structure of Edelfosine.

In its beginnings, Edelphosine (Fig. 16) was considered a very promising molecule, since it has immunomodulatory properties as well as an inhibitory activity on the proliferation of tumor cells (Andreesen et al, 1978). Despite the first impressions given by Edelfosine, cytotoxicity tests carried out in a variety of tumors in which this phospholipid ether was used, it was shown that Edelfosine is not completely selective for tumor cells (Runge et al, 1980). Furthermore, the use of Edelfosine in clinical practice is reduced by the metabolic instability of the molecule and gastrointestinal toxicity. After the use of Edelfosine, new molecules were proposed for the creation of other drugs, in such a way that the synthesis of Ilmofosine (ILM) (Fig. 17) as a thioether analog took place (Berdel, Fink & Rastetter, 1987).

Figure 17. Ilmofosine structure. Representation of Chemical Structure of Ilmofosine.

At the end of the 80s, a new analog was discovered, which lacked glycerol, which generated that ALPs reached a clinical status to be considered. Miltefosine (MLT) (Fig. 18) was synthesized in two different groups, in which one was for the detection of analogs with anti-inflammatory purposes and the other was for the search for molecules with antitumor activity, which were synthesized in the United Kingdom and Germany, respectively (S Vink et al., 2005).

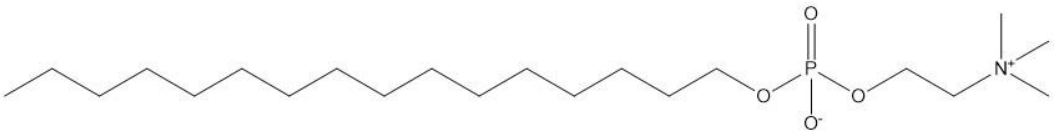


Figure 18. Miltefosine structure. Representation of Chemical Structure of Miltefosine.

Mechanisms of action of ALPs

The mechanism of action of ALPs differs from the conventional mechanisms of anticancer agents, the mechanism of these is based mainly on the interaction with the cell, on the genetic machinery (Table 2). While ALPs target the cell membrane, since it has similarities with endogenous phospholipids (Almeida et al, 2013). ALPs structurally correspond to classical surfactants and at high concentrations they can cause cell lysis. Despite the counterparts, ALPs will enter the lipid bilayer, generating biophysical alterations in cell membranes. For this reason, the activity of micelles (Fig. 19) is necessary in the cell lysis mechanism, which involves micellar dissolution, the phase transition between the lamellae and the decrease in size of the micelles (Heerklotz H, 2008).

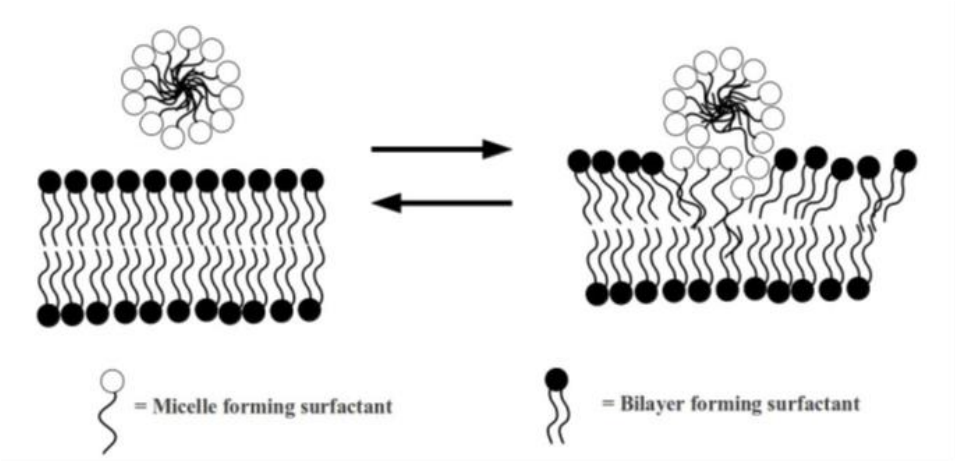


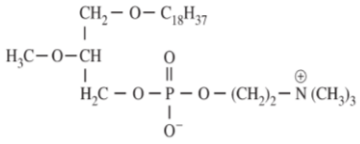
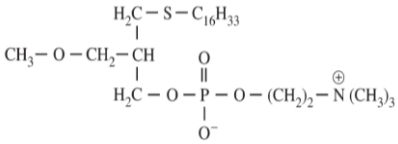
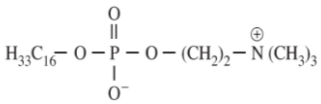
Figure 19. Process of micelle fusion and bilayer disruption. The heads of surfactant and phospholipids are represented by the black and white circles. The surfactant tails are represented by the black curved lines. From "Alkylphospholipids – A Promising Class of Chemotherapeutic Agents with a Broad Pharmacological Spectrum" by Almedia P. et al, 2013. *Journal of Pharmacy & Pharmaceutical Sciences*, 16(5), 742.

The metabolically stable ALPs are introduced into the membrane, generating the biophysical disturbance, which will intervene with the metabolism of phospholipids, their proliferation and the signaling pathways for cell survival while simultaneously activating several stress pathways. which will promote cell death (apoptosis) (Strassheim et al, 2010). It is known that ALPs will insert easily into the outer leaflet of the plasma membrane and will pass through it thanks to the ATP-dependent complex flippase. Another possible mechanism of ALPs is based on internalization in endocytosis through lipid rafts (Mollinedo F., Ruiter G.A., van Blitterswjk W.J., van der Luit A.H.). Therefore, the intervention of ALPs in the mechanism of cell membranes will trigger stress in the cell, thereby generating its subsequent death. The mechanism of the inhibition of phosphocholine (PC) biosynthesis at the beginning of cell death, further investigation is needed, therefore it is not totally clear. It is known that the lack of PC in the endoplasmic reticulum will in some way induce the stress of the pro-apoptotic transcription factor CHOP / GADD 153 (Nieto et al, 2007). ALPs have several functions both of inhibition of phosphocholine biosynthesis. They can also participate in preventing the decomposition of phosphatidic acid and in the degradation of diacylglycerol, all under certain specific signaling conditions. Phosphatidic acid and diacylglycerol, which are part of the ALP

activity, are considered second messengers, which are involved in the signaling pathways of mitogen activated protein kinase (MAPK) and Ras / Raf / MEK / ERK, which are involved in cell proliferation (Almeida et al, 2013).

The characterized target of ALP that shows optimal behavior is the AKT enzyme, which is a serine / threonine kinase, which carries out an essential process as it is a regulator of several cell survival pathways. AKT will be activated to a great extent in human cancers, it will contribute to cell growth and proliferation as well as acting on cell survival pathways, this being an important target for therapies carried out against it. cancer (Almeida et al, 2013). ALPs will prevent the activation of the AKT enzyme by intervening in the microdomains of the membrane, which are essential for growth factor signaling, or by carrying out the action of displacing the natural ligands of AKT, such as they are PIP2 (phosphatidylinositol-4,5-bisphosphate) and PIP3 (phosphatidylinositol-3,4,5-triphosphate) (Almeida et al, 2013).

Table 2. Mechanism of action of Alkylphospholipids

Alkylphospholipids	Molecular structure	Mechanism of action	References
Edelfosine (Et-18)		It acts on cellular membranes by selectively aggregating the cell death receptor Fas in membrane rafts and interference with phosphatidylcholine (PC) synthesis with subsequent induction of apoptosis.	Abramowski, P., Otto, B., & Martin, R. (2014)
Ilmofosine		Is a thioether lysophospholipid that showed early promise as an anticancer agent but is now known to have unacceptable bone marrow toxicity at reasonable exposure levels.	Girgert et al.(1995)
Miltefosine		Inhibits the synthesis of phosphatidylcholine, inhibiting the cytochrome c oxidase, it is to be expected that this potent drug also produces its effect through other targets.	Pinto-Martinez et al. (2017)

Effects of ALPs on phospholipids composition and biosynthesis in *T. cruzi* epimastigotes

Effects of ALPs on phospholipid composition and biosynthesis in *T. cruzi* epimastigotes. Based on these facts their biochemical effects on tumor cells, alteration of phospholipid synthesis, was one of the most prominent. It was demonstrated that hexadecylphosphocholine interferes with the biosynthesis of phosphatidylcholine inhibing the formation of phosphatidylcholine via CDP-choline pathway (Kennedy pathway) at the level of the rate-limiting enzyme, CTP: phosphocholine cytidyltransferase (Hasse R., 1991). A second pathway via N-methylation of phosphatidylethanolamine has also been described (Bremer, J, and Greenberg, D.M., 1961) Moreover, the lipid composition of MDCK cells was altered by treatment with 50 µM hexadecylphosphocholine for 24 h (Hasse R., 1991).

The antiproliferative synergy of LPA and ketoconazole in T. cruzi epimastigotes have shown that a secondary effect of sterol biosynthesis inhibitors is a reduction in PC content associated with an indirect inhibition of PC-PE-N- methylthransferase. The synergic effects resulting from the combined action of Et-18 and ketoconazole on the proliferation of *T. cruzi* (Lira R. et al, 2001).

Chapter 8: Conclusions

The parasitic disease generated by the protozoan *T. cruzi* needs further research. Despite the variety of alternatives given in recent years as well as the development of drugs as new perspectives for the treatment of the disease, there is still no definitive treatment for the chronic phase of Chagas disease. The traditional drugs, benznidazol and nifurtimox are effective in the acute phase although they present many undesirable side effects. Moreover, these drugs are not successful in the treatment of the chronic phase of this disease.

The research of new drugs has focused on the chronic phase. Most of the specific drugs that lead the chemotherapy of parasitic infections are heterocyclic compounds such as statin, bisphosphonate, quinuclidine, allylamine, azoles and azasteroles, these coumpounds interfere with sterol synthesis in eukaryotes, fungi and trypanosomatids. A marked

inhibition on the enzyme´s activities indicated that some of them act specifically on the pathway for sterol synthesis.

There is another group of drugs formed by Alkylphospholids (ALPs) such as Edelfosine, Miltefosine and Ilmofosine which are synthetic drugs that act in the route of biosynthesis of phospholipids and interfere with the membrane structure. The action of these drugs is mainly directed at the specific inhibition of phosphatidylcholine (PC) biosynthesis of the parasite, which begins through the Greenberg pathway (transmethylation) in the epimastigotes of *T. cruzi*, in contrast to the pathway of the CDP-choline used by the host. cells. A possible explanation for the selective anti-*T. cruzi* effects of LPAs is a specific inhibition of the parasite`s PC biosynthesis, which seems to proceed through the Greenberg`s pathways, in contrast to the CDP-choline pathway used by host cells. It is also found that this anti-proliferative activity can be potentiated by sterol biosynthesis inhibitors and provided a possible molecular explanation for these effects.

It is expected that further studies based on specific therapeutic targets can reduce the adverse effects of drugs, as well as promote the development of new optimized drugs with synergistic effects resulting from their combined use, this would be the case, for example, of the combination of azoles (i.e., Ketoconazole) and ALPs (i.e., Miltefosine).

In conclusion, research in Chagas disease should be continued in order to develop new options for its treatment and thus arrive at an ideal pharmaceutical chemotherapy.

Recommendations

- Development of drugs that are synergistic. It is contemplated that the ideal treatment for Chagas disease may involve a combination of drugs to improve both efficacy against acute or chronic infection and to minimize the risk of drug resistance.
- Minimize the unwanted side effects of available drugs.
- Any improvement in the toxicity profile of the therapy
- The most widely sourced approaches to the advancement of investigational drug candidates.
- More chances that a new treatment for this often-fatal infection.
- Since vaccines against parasitic infections are still in development and control of environmental sources of transmission is ineffective and often impractical, the need for better chemoprophylactic / chemotherapeutic approaches in controlling these pathogens is indisputable.

Bibliography

Abad-Franch, F., Monteiro, F.A., 2007. Biogeography and evolution of Amazonian triatomines (Heteroptera: Reduviidae): implications for Chagas disease surveillance in humid forest ecoregions. Mem. Inst. Oswaldo Cruz 102, 57-69.

Albareda, M.C., Laucella, S.A., et al., 2006. *Trypanosoma cruzi* modulates the profile of memory CD8+ T cells in chronic Chagas' disease patients. Int. Immunol. 18 (3), 465–471.

Abramowski, P., Otto, B., & Martin, R. (2014). The Orally Available, Synthetic Ether Lipid Edelfosine Inhibits T Cell Proliferation and Induces a Type I Interferon Response. PLoS ONE, 9(3), e91970. doi:10.1371/journal.pone.0091970

Almeida Pachioni, J. D., Magalhães, J. G., Cardoso Lima, E. J., Moura Bueno, L. D., Barbosa, J. F., Malta de Sá, M., & Rangel-Yagui, C. O. (2013). Alkylphospholipids A Promising Class of Chemotherapeutic Agents with a Broad Pharmacological Spectrum. Journal of Pharmacy & Pharmaceutical Sciences, 16(5), 742. doi:10.18433/j3cw23

Alves CR, Albuquerque-Cunha JM, Mello CB, Garcia ES, Nogueira NF, Bourguingnon SC, et al. *Trypanosoma cruzi:* Attachment to perimicrovillar membrane glycoproteins of Rhodnius prolixus. Experimental Parasitology. 2007;116(1):44-52

Andreesen R, Modolell M, Weltzien HU, Eibl H, Common HH, Löhr GW et al (1978). Selective destruction of human leukemic cells by alkyl- lysophospholipids. Cancer Res. 38: 3894-3899.

Aripirala S, Szajnman SH, Jakoncic J, et al (2012). Design, synthesis, calorimetry, and crystallographic analysis of 2-alkylaminoethyl1,1-bisphosphonates as inhibitors of *Trypanosoma cruzi* farnesyl diphosphate synthase. J Med Chem. 55(14):6445–6454

Añez N, Carrasco H, Parada H, et al. Acute Chagas' disease in Western Venezuela: a clinical seroparasitologic, and epidemiologic study. Am J Trop Med Hyg 1999, 60: 215–22.

Bargues, M.D., Klisiowicz, D.R., Panzera, F., Noireau, F., Marcilla, A., Perez, R., et al., 2006. Origin and phylogeography of the Chagas disease main vector Triatoma infestans based on nuclear rDNA sequences and genome size. Infect. Genet. Evol. 6, 46-62.

Barrett-Bee, K., & Ryder, N. (1992). Biochemical Aspects of Ergosterol Biosynthesis Inhibition. Emerging Targets in Antibacterial and Antifungal Chemotherapy, 410–436. doi:10.1007/978-1-4615-3274-3_16

Berdel WE, Fink U, Rastetter J (1987). Clinical phase I pilot study of the alkyl lysophospholipid derivative ET-18-OCH3. Lipids. 22(11): 967-969.

Benaim G, Sanders JM, Garcia-Marchán Y, et al (2006). Amiodarone has intrinsic anti-*Trypanosoma cruzi* activity and acts synergistically with Posaconazole. J Med Chem.49:892–899 PubMed

Braga MV, Magaraci F, Lorente SO, et al (2005). Effects of inhibitors of delta24(25)-sterol methyl transferase on the ultrastructure of epimastigotes of *Trypanosoma cruzi* Microsc Microanal. 11:506–515

Bremer, J., and Greenberg, D. M. (1961) Biochim. Biophys. Acta 46,. 205–216.

Brener, Z., Gazzinelli, R.T., 1997. Immunological control of *Trypanosoma cruzi* infection and pathogenesis of Chagas' disease. Int. Arch. Allergy Immunol. 114 (2), 103–110.

Buckner, F. S., & Urbina, J. A. (2012). Recent developments in sterol 14-demethylase inhibitors for Chagas disease. International Journal for Parasitology: Drugs and Drug Resistance, 2, 236–242. doi:10.1016/j.ijpddr.2011.12.002

Buekens P, Cafferata ML, Alger J, Althabe F, Belizán JM, Bustamante N, et al. (2018). Congenital transmission of trypanosoma cruzi in Argentina Honduras, and Mexico: an observational prospective study. Am J Trop Med Hyg. 98:478–85, http://dx.doi.org/10.4269/ajtmh.17-0516.

Cadenas, E. (1989). Biochemistry of oxygen toxicity. *Annual review of biochemistry*, Vol.*58*, pp.79-110.

Castro DP, Seabra SH, Garcia ES, de Souza W, Azambuja CP. Trypanosoma cruzi: Ultrastructural studies of adhesion, lysis and biofilm formation by Serratia marcescens. Experimental Parasitology. 2007;117(2):201-207

Centers for Disease Control and Prevention (2017). Detailed Fact Sheet on Chagas Disease. Retrieved from: https://www.cdc.gov/parasites/chagas/es/informativa/detallada.html

Chagas C. Nova tripanozomiase humana. Estudos sobre a morfología e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n. sp., agente etiológico de nova entidade mórbida do homem. Memórias do Instituto Oswaldo Cruz. 1909;1:159-218

Chatelain, E., 2017. Chagas disease research and development: Is there light at the end of the tunnel? Comput. Struct. Biotechnol. J. 15, 98–103.

Coura, J. R. (2014). The main sceneries of Chagas disease transmission. The vectors, blood and oral transmissions - A comprehensive review. Memórias Do Instituto Oswaldo Cruz, 110(3), 277–282. doi:10.1590/0074-0276140362

Coura J.R., Viñas PA (2010). Chagas disease: a new worldwide challenge.Nature 465 (Suppl.): S6-S7.

Coura, J. R., & Castro, S. L. de. (2002). A Critical Review on Chagas Disease Chemotherapy. Memórias Do Instituto Oswaldo Cruz, 97(1), 3–24. doi:10.1590/s0074-02762002000100001

Denning, D. W., and Bromley, M. J. (2015). How to bolster the antifungal pipeline. Science, 347(6229), 1414-1416.

De Souza W. (1984). Cell biology of *Trypanosoma cruzi.* International Review of Cytology.86:197-283

De Souza, W., & Rodrigues, J. C. F. (2009). Sterol Biosynthesis Pathway as Target for Anti-trypanosomatid Drugs. Interdisciplinary Perspectives on Infectious Diseases, 2009, 1–19. doi:10.1155/2009/642502

De Souza, W., Attias, M., & Rodrigues, J. C. F. (2009). Particularities of mitochondrial structure in parasitic protists (Apicomplexa and Kinetoplastida). The International

Journal of Biochemistry & Cell Biology, 41(10), 2069–2080. doi:10.1016/j.biocel.2009.04.007

Dias, J. C. P. (2006). The Treatment of Chagas Disease (South American Trypanosomiasis). Annals of Internal Medicine, 144(10), 772. doi:10.7326/0003-4819-144-10-200605160-00012

Docampo R, Moreno SNJ, Stoppani AOM (1981). Nitrofuran enhancement of microsomal electron transport, superoxide anion production and lipid peroxidation. Arch Biochem Biophys. 207:316–24, http://dx.doi.org/10.1016/0003-9861(81)90039-4.

Do Campo, R. & Moreno, S. N. J. (1984). Free radical metabolites in the mode of action of chemotherapeutic agents and phagocytic cells on T. cruzi. Reviews of Infectious Diseases, Vol.6, pp.223-238.

Docampo R. (1990). Sensitivity of parasites to free radical damage by antiparasitic drugs. Chemico-biological interactions, 73(1), 1–27. https://doi.org/10.1016/0009-2797(90)90106-w

Doyle, P. S., Zhou, Y. M., Engel, J. C., & McKerrow, J. H. (2007). A cysteine protease inhibitor cures Chagas' disease in an immunodeficient-mouse model of infection. Antimicrobial agents and chemotherapy, 51(11), 3932–3939. https://doi.org/10.1128/AAC.00436-07

Doyle, P.S.; Zhou, Y.M.; Hsieh, I.; Greenbaum, D.C.; McKerrow, J.H.; Engel, J.C. (2011). The *Trypanosoma cruzi* protease cruzain mediates immune evasion. PLoS Pathog. 7(9), 1-11.

Doggrell SA (2006). Is atorvastatin superior to other statins? Analysis of the clinical trials with atorvastatin having cardiovascular endpoints. Rev Recent Clin Trials. 1(2):143-53. doi: 10.2174/157488706776876508. PMID: 18473965.

D. Gonzalez-Pacanowska, B. Arison, C. M. Havel, and J. ´ A. Watson (1988). Isopentenoid synthesis in isolated embryonic Drosophila cells. Farnesol catabolism and ω-oxidation,The Journal of Biological Chemistry, vol. 263, no. 3, pp. 1301– 1306.

Eibl RH, Arnold D, Weltzien HU, Westphal O (1967). On the synthesis of alpha beta lecithins and their analogs. Justus Liebigs Ann Chem. 704: 226-230.

Girgert R, Schweizer P, Bock I, Narr R, Bruchelt G. (1995). Cytotoxicity of ether phospholipid BM 41.440 on neuroblastoma cells. J Cancer Res Clin Oncol;121(5):262-6. doi: 10.1007/BF01209591. PMID: 7768962.

González, C. R., Reyes, C., Canals, A., Parra, A., Muñoz, X., & Rodríguez, K. (2015). An entomological and seroepidemiological study of the vectorial-transmission risk of Chagas disease in the coast of northern Chile. Medical and Veterinary Entomology, 29(4), 387–392. doi:10.1111/mve.12131

Gonzalez-Pacanowska D, Arison B, Havel CM, Watson JA (1988). Isopentenoid synthesis in isolated embryonic Drosophila cells. Farnesol catabolism and omega-oxidation. J Biol Chem. 263(3):1301-6. PMID: 3335546.

Haber, F., & Weiss, J. (1932). Uber die Katalyse des Hydroperoxydes. Die Naturwissenschaften, 20(51), 948–950. doi:10.1007/bf01504715

Hasse R., Wider T., Geilen C. & Reutter W. (1991). The phospholipid analogue hexadecylphosphocholine inhibits phosphatidylcholine biosynthesis in Madin-Darby canine kidney cells. *FEBS Letters* 288, 129-32.

Heerklotz H. Interactions of surfactants with lipid membranes (2008). Q Rev Biophys. 41(3-4):205- 264

J. C. F. Rodrigues, M. Attias, C. Rodriguez, J. A. Urbina, and W. de Souza (2002). Ultrastructural and biochemical alterations induced by 22,26-azasterol, a Δ24(25)-sterol methyltransferase inhibitor, on promastigote and amastigote forms of *Leishmania amazonensis*," Antimicrobial Agents and Chemotherapy, vol. 46, no. 2, pp. 487–499.

J. C. F. Rodrigues, C. F. Bernardes, G. Visbal, J. A. Urbina, A. E. Vercesi, and W. de Souza (2007). Sterol methenyl transferase inhibitors alter the ultrastructure and function of the *Leishmania amazonensis* mitochondrion leading to potent growth inhibition,"Protist, vol. 158, no. 4, pp. 447–456.

J. C. F. Rodrigues, J. L. Concepcion, C. Rodrigues, A. Caldera, J. A. Urbina, and W. de Souza (2008). In vitro activities of ER119884 and E5700, two potent squalene synthase inhibitors, against Leishmania amazonensis: antiproliferative, biochemical, and ultrastructural effects, Antimicrobial Agents and Chemotherapy, vol. 52, no. 11, pp. 4098–4114.

Koppenol, W. H. (2001). The Haber-Weiss cycle – 70 years later. Redox Report, 6(4), 229–234. doi:10.1179/135100001101536373

Kratz, J.M., Garcia Bournissen, F., Forsyth, C.J., Sosa-Estani, S., 2018. Clinical and pharmacological profile of Benznidazole for treatment of Chagas disease. Expert Rev. Clin. Pharmacol. 11, 943–957.

K. Ishida, J. C. F. Rodrigues, M. D. Ribeiro, et al (2009). Growth inhibition and ultrastructural alterations induced by Δ24(25)- sterol methyltransferase inhibitors in Candida spp. isolates, including non-albicans organisms, BMC Microbiology, vol. 9, article 74.

Koppenol, W. H. (2002). "The Haber-Weiss cycle – 71 years later". *Redox report*, Vol.7, pp.59–60.

Lass-Flörl, C. (2011). Triazole Antifungal Agents in Invasive Fungal Infections A Comparative Review. Drugs, 71(18), 2405–2419.

Lepesheva, G. I., Park, H. W., Hargrove, T. Y., Vanhollebeke, B., Wawrzak, Z., Harp, J. M., Sundaramoorthy, M., Nes, W. D., Pays, E., Chaudhuri, M., Villalta, F., & Waterman, M. R. (2010). Crystal structures of Trypanosoma brucei sterol 14alpha-demethylase and implications for selective treatment of human infections. The Journal of biological chemistry, 285(3), 1773–1780. https://doi.org/10.1074/jbc.M109.067470

Lepesheva, G. I., Hargrove, T. Y., Rachakonda, G., Wawrzak, Z., Pomel, S., Cojean, S., Nde, P. N., Nes, W. D., Locuson, C. W., Calcutt, M. W., Waterman, M. R., Daniels, J. S., Loiseau, P. M., & Villalta, F. (2015). VFV as a New Effective CYP51 Structure-Derived Drug Candidate for Chagas Disease and Visceral Leishmaniasis.

The Journal of infectious diseases, 212(9), 1439–1448. https://doi.org/10.1093/infdis/jiv228

Lira, R., Contreras, L. M., Rita, R. M. S., & Urbina, J. A. (2001). Mechanism of action of anti-proliferative lysophospholipid analogues against the protozoan parasite Trypanosoma cruzi: potentiation of in vitro activity by the sterol biosynthesis inhibitor Ketoconazole. Journal of Antimicrobial Chemotherapy, 47(5), 537–546. doi:10.1093/jac/47.5.537

Maya, J.D.; Cassels, B.K.; Iturriaga-Vásquez, P.; Ferreira, J.; Faúndez, M.; Galanti, N.; Ferreira, A. & Morello, A. (2007). Mode of action of natural and synthetic drugs against *Trypanosoma cruzi* and their interaction with the mammalian host. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*,Vol.146, No.4, pp.601-620.

Marin-Neto, J.A., Cunha-Neto, E., et al., 2007. Pathogenesis of chronic Chagas heart disease. Circulation 115 (9), 1109–1123.

Mario Sanchez-Sanchez, Gildardo Rivera, Edgar A. Garcia and Virgilio Bocanegra-Garcia, (2016). "Therapeutic Targets for the Development of Anti-*Trypanosoma cruzi* Drugs: A Brief Review", Mini-Reviews in Organic Chemistry. 13(3) . https://doi.org/10.2174/1570193X13666160510113821

Molina I, Salvador F, Sánchez-Montalvá A, Artaza MA, Moreno R, Perin L, et al. (2017). Pharmacokinetics of Benznidazole in healthy volunteers and implications in future clinical trials. Antimicrob Agents Chemother. 61:e01912–1916, http://dx.doi.org/10.1128/AAC.01912-16.

Mollinedo F, Fernández-Luna JL, Gajate C, Martín-Martín B, Benito A, Martínez-Dalmau R *et al.* Selective induction of apoptosis in cancer cells by the ether lipid ET-18-OCH3 (Edelfosine): Molecular structure requirements,cellular uptake, and protection by Bcl-2 and Bcl-X(L). *Cancer Res*, 1997; 57(7): 1320-1328.

Monasterios, Melina & Amesty, Ángel & Avendaño, Milagros. (2011). Molecular Coupling Study (docking) of a series of synthetic 5-nitrofuran derivatives at the

trypanotion reductase binding site of *Trypanosoma cruzi*. Revista de la Facultad de Farmacia, Universidad Central de Venezuela. 74. 25-30.

M. A. Vannier-Santos, J. A. Urbina, A. Martiny, A. Neves, and W. de Souza (1995). Alterations induced by the antifungal compounds Ketoconazole and terbinafine in Leishmania, The Journal of Eukaryotic Microbiology, vol. 42, no. 4, pp. 337–346.

Nieto-Miguel T, Fonteriz RI, Vay L, Gajate C, López-Hernández S, Mollinedo F. (2007). Endoplasmic reticulum stress in the proapoptotic action of edelfosine in solid tumor cells. Cancer Res. 67(21): 10368-10378.

Olivera MJ, Cucunubá ZM, Valencia Hernández CA, Herazo R, Agreda-Rudenko D, Flórez C, et al. (2017) Risk factors for treatment interruption and severe adverse effects to benznidazole in adult patients with Chagas disease. PLoS ONE 12(9): e0185033. https://doi.org/10.1371/journal.pone.0185033

Oliveira, I., Torrico, F., Muñoz, J., & Gascon, J. (2010). Congenital transmission of Chagas disease: a clinical approach. Expert Review of Anti-Infective Therapy, 8(8), 945–956. doi:10.1586/eri.10.74

Organización Mundial de la Salud.(OMS) (2020). La enfermedad de Chagas (tripanosomiasis americana).

Organización Panamericana de la Salud.(OPS/OMS). Control, interrupción de la transmisión y eliminación de la enfermedad de Chagas como problema de salud pública. PAHO. Washington, D.C; 2019. 76 p

Patterson S, Wyllie S. (2014). Nitro drugs for the treatment of trypanosomatid diseases: past, present, and future prospects. Trends Parasitol. 30:289–98, http://dx.doi.org/10.1016/j.pt.2014.04.003.

Paulos C, Paredes J, Vasquez I, Thambo S, Arancibia A, Gonzalez-Martin G. (1989). Pharmacokinetics of a nitrofuran compound, nifurtimox, in healthy volunteers. Int J Clin Pharmacol Ther Toxicol. 27:454–7.

Pérez-Molina, J. A., Crespillo-Andújar, C., Bosch-Nicolau, P., & Molina, I. (2020). Trypanocidal treatment of Chagas disease. Enfermedades Infecciosas y Microbiología Clínica. doi:10.1016/j.eimc.2020.04.011

Piacenza, L.; Alvarez, M. N.; Kelly, J. M.; Wilkinson, S. R. & Radi, R. (2008). Peroxiredoxins play a major role in protecting Trypanosoma cruzi against macrophage- and endogenously-derived peroxynitrite. Biochemical Journal, Vol.410, pp.359–368.

Pinto-Martinez, A. K., Rodriguez-Durán, J., Serrano-Martin, X., Hernandez-Rodriguez, V., & Benaim, G. (2017). Mechanism of Action of Miltefosine on Leishmania donovani Involves the Impairment of Acidocalcisome Function and the Activation of the Sphingosine-Dependent Plasma Membrane Ca 2+ Channel . Antimicrobial Agents and Chemotherapy, 62(1). doi:10.1128/aac.01614-17

Prata, A. (2001). Clinical and epidemiological aspects of Chagas disease. The Lancet Infectious Diseases, 1(2), 92–100. doi:10.1016/s1473-3099(01)00065-2

Rassi Jr., A., Rassi, A., et al., 2000. Chagas' heart disease. Clin. Cardiol. 23 (12), 883–889.

Rassi, A., Luquetti, A.O., et al., 2007. Specific treatment for Trypanosoma cruzi: lack of efficacy of allopurinol in the human chronic phase of Chagas disease. Am. J. Trop. Med. Hyg. 76 (1), 58–61.

Rassi Jr., A., Dias, J.C., et al. (2009). Challenges and opportunities for primary, secondary, and tertiary prevention of Chagas' disease. Heart 95 (7), 524-534.

Rossi, M. A., Tanowitz, H. B., Malvestio, L. M., Celes, M. R., Campos, E. C., Blefari, V., & Prado, C. M. (2010). Coronary Microvascular Disease in Chronic Chagas Cardiomyopathy Including an Overview on History, Pathology, and Other Proposed Pathogenic Mechanisms. PLoS Neglected Tropical Diseases, 4(8), e674. doi:10.1371/journal.pntd.0000674

Ruiter GA, Zerp SF, Bartelink H, van Blitterswijk WJ, Verheij M. Alkyllysophospholipids activate the SAPK/JNK pathway and enhance radiation-induced apoptosis. Cancer Res, 1999; 59(10): 2457-2463.

Runge MH, Andreesen R, Pfleiderer A, Munder PG (1980). Destruction of human solid tumors by alkyl lysophospholipids. J Natl Cancer Inst. 64(6): 1301-1306.

Rodan, G. A. (1998). MECHANISMS OF ACTION OF BISPHOSPHONATES. Annual Review of Pharmacology and Toxicology, 38(1), 375–388. doi:10.1146/annurev.pharmtox.38.1

Rodriguero, M.S., Gorla, D.E., 2004. Latitudinal gradient in species richness of the new world Triatominae (Reduviidae). Glob. Ecol. Biogeogr. 13, 75-84

Rodriques Coura J, de Castro SL (2002). A critical review on Chagas disease chemotherapy. Memorias Inst Oswaldo Cruz. 97:3–24, http://dx.doi.org/10.1590/s0074-02762002000100001.

Robertson, S.A.; Renslo, A.R. (2011). Drug discovery for neglected tropical diseases at the sandler center. Future Med. Chem. 3(10), 1279-1288.

Ruiz RC, Favoreto S Jr, Dorta ML, Oshiro MEM, Ferreira AT, Manque PM, et al. Infectivity of Trypanosoma cruzi strains is associated with differential expression of surface glycoproteins with differential Ca2+ signaling activity. Biochemical Journal. 1998;330:505-511

Schofield CJ, Diotaiuti L, Dujardin JP. The process of domestication in triatomine. Mem Inst Oswaldo Cruz 1999; 94 (suppl I): 375–78.

Shang N, Li Q, Ko TP, et al (2014). Squalene synthase as a target for Chagas disease therapeutics. PLoS Pathog. 10(5):1004–1114. First crystallization of T cruzi squalene synthase

Strassheim D, Shafer SH, Phelps SH, Williams CL (2000). Small cell lung carcinoma exhibits greater phospholipase C-ß1 expression and edelfosine resistance compared with non-small cell lung carcinoma. Cancer Res. 60(10): 2730-2736.

Superko, H. R., Momary, K. M., & Li, Y. (2012). Statins Personalized. Medical Clinics of North America, 96(1), 123–139. doi:10.1016/j.mcna.2011.11.004

S Vink SR, Schellens JH, van Blitterswijk WJ, Verheij M. (2005). Tumor and normal tissue pharmacokinects of perifosine, an oral anti-cancer alkylphospholipid. Invest New Drugs. 23(4): 279-286.

Torrico, F., Gascon, J., Ortiz, L., Alonso-vega, C., Pinazo, M., Schijman, A., Almeida, I.C., Alves, F., 2018. Treatment of adult chronic indeterminate Chagas disease with Benznidazole and three E1224 dosing regimens: a proof-of-concept, randomized, placebo-controlled trial. Lancet Infect. Dis. 18, 419–430. https://doi.org/10.1016/S1473-3099(17)30538-8

Trujillo, M.; Budde, H.; Pineyro, M. D.; Stehr, M.; Robello, C.; Floheet, L. & Radi, R. (2004). Trypanosoma brucei and Trypanosoma cruzi tryparedoxin peroxidases catalytically detoxify peroxynitrite via oxidation of fast reacting thiols. The Journal of Biological Chemistry, Vol.279, pp.34175–43482.

Urbina, J. A., & Docampo, R. (2003). Specific chemotherapy of Chagas disease: controversies and advances. Trends in parasitology, 19(11), 495–501. https://doi.org/10.1016/j.pt.2003.09.001

Urbina, J. A. (2010). Specific chemotherapy of Chagas disease: Relevance, current limitations and new approaches. Acta Tropica, 115(1-2), 55–68. doi:10.1016/j.actatropica.2009.10.023

Urbina JA, Concepcion JL, Rangel S, et al (2002). Squalene synthase as a chemotherapeutic target in Trypanosoma cruzi and Leishmania mexicana. Mol Biochem Parasitol. 125(1–2):35–45

Urbina J. A., Lazardi K, Aguirre T, et al (1991). Antiproliferative effects and mechanism of action of ICI 195,739, a novel bis-triazole derivative, on epimastigotes and amastigotes of Trypanosoma (Schizotrypanum) cruzi. Antimicrob Agents Chemother.35 (4):730PubMed−5

Urbina JA, Vivas J, Lazardi K, et al (1996). Antiproliferative effects of delta 24 (25)sterol methyl transferase inhibitors on Trypanosoma (Schizotrypanum) cruzi: in vitro and in vivo studies. Chemotherapy. 42:294–307

van Blitterswijk WJ, Verheij M. Anticancer alkylphospholipids: mechanisms of action, cellular sensitivity and resistance, and clinical prospects. *Curr Pharm Des*, 2008; 14(21): 2061-2074

van der Luit A H, Vink S R, Klarenbeek J B, Perrissoud D, Solary E, Verheij M *et al.* A new class of anticancer alkylphospholipids uses lipid rafts as membrane gateways to induce apoptosis in lymphoma cells. *Mol Cancer Ther*, 2007; 6(8): 2337-2345

Villalta, F., & Rachakonda, G. (2019). Advances in preclinical approaches to Chagas disease drug discovery. Expert Opinion on Drug Discovery, 1–14. doi:10.1080/17460441.2019.1652593

Villalta F, Dobish MC, Nde PN, et al (2013). VNI cures the acute and chronic experimental Chagas disease. J Infect Dis.208:504–11PubMed

W. de Souza, C. Sant'anna, and N. L. Cunha-e-Silva, "Electron microscopy and cytochemistry analysis of the endocytic pathway of pathogenic protozoa," Progress in Histochemistry and Cytochemistry, vol. 44, no. 2, pp. 67–124, 2009.

Wilkinson, S. R.; Meyer, D. J.; Taylor, M. C.; Bromley, E. V.; Miles, M. A. & Kelly, J. M. (2002b). The *Trypanosoma cruzi* enzyme TcGPXI is a glycosomal peroxidase and can be linked to trypanothione reduction by glutathione or tryparedoxin. *Journal of Biological Chemistry*, Vol.277, pp.17062–17071.

Wilkinson, S. R.; Obado, S. O.; Mauricio, I. L. & Kelly, J. M. (2002c). Trypanosoma cruzi expresses a plant-like ascorbate-dependent hemoperoxidase localized to the endoplasmic reticulum. Proceedings of the National Academy of Sciences, Vol.99, pp.13453–13458, 2002c.

Wilkinson, S. R.; Taylor, M. C.; Touitha, S.; Mauricio, I. L.; Meyer, D. J. & Kelly, J. M. (2002a). TcGPXII, a glutathione-dependent Trypanosoma cruzi peroxidase with substrate specificity restricted to fatty acid and phospholipid hydroperoxides, is localized to the endoplasmic reticulum. Biochemical Journal, Vol.364, pp.787–794.

Wilkinson, S. R.; Temperton, N. J.; Mondragon, A. & Kelly, J. M. (2000). Distinct mitochondrial and cytosolic enzymes mediate trypanothione-dependent peroxide metabolism in T. cruzi. Journal of Biological Chemistry, Vol.275, pp.8220–8225.

World Health Organization, 2012. Research Priorities for Chagas Disease, Human African Trypanosomiasis and Leishmaniasis.

WHO Expert Committee on the Control of Chagas Disease (2000 : Brasilia, Brazil) & World Health Organization. (2002). Control of Chagas disease : second report of the WHO expert committee. World Health Organization.

Yoshida, N. (2006). Molecular basis of mammalian cell invasion by Trypanosoma cruzi. Anais Da Academia Brasileira de Ciências, 78(1), 87–111. doi:10.1590/s0001-37652006000100010

Zhang L, Tarleton RL. Parasite persistence correlates with disease severity and localization in chronic Chagas' disease. J Infect Dis 1999; 180: 480–86