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Artigo

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Doença de Chagas: Desafios no Desenvolvimento de Novas Substâncias Líderes Tripanomicidas

Resumo: A doença de Chagas teve seu ciclo completamente elucidado em 1909 por Carlos Chagas, quando ele relatou sua descoberta para a comunidade científica em dois artigos seminais. Hoje ainda existem inúmeros fatores que limitam o seu tratamento terapêutico. Um deles é a falta de novas drogas no mercado, pois é bem conhecido que as drogas existentes são fracamente ativas e tem baixa eficácia e consideráveis efeitos colaterais. Atualmente muitos esforços têm sido feito em química combinatória e síntese orgânica em busca de novos compostos-protótipo. A presente revisão pretende mostrar que existe uma grande variedade de estratégias em síntese orgânica que estão sendo utilizadas para a preparação de compostos bioativos contra várias cepas de *T. cruzi* e com boas perspectivas de aplicações na clínica médica.

Palavras-chave: Doença de Chagas; Substâncias tripanomicidas; Doenças negligenciadas.

Abstracts

Chagas disease cycle was fully elucidated by Carlos Chagas in 1909, when he reported his discovery to the scientific community in two seminal papers. Today remains innumerous factors that limit its therapeutic treatment. One of them is the lack of new drugs in the market since is well known that the existing drugs are poorly active with low efficacy and considerable side effects. Nowadays, many efforts have been done in combinatorial chemistry and synthesis of new compounds searching for new lead compounds. The present review intends to show that a wide variety of synthetic strategies are being used for the preparation of pharmaceutically active compounds against several strains of *T. cruzi* with a range of potential clinical applications.

Keywords: Chagas disease; Trypanocidal compounds; Neglected diseases.

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Chagas Disease: Challenges in Developing New Trypanocidal Lead Compounds

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1. Introduction

It is estimated that diseases caused by parasites affect a quarter of the world, accounting for considerable morbidity and mortality in developing countries. Among these diseases include malaria, leishmaniasis, trypanosomiasis, giardiasis and trichomoniasis (World Health Organization - WHO).[1] Daily, over 35,000 people die from infectious and neglected diseases such as leishmaniasis, schistosomiasis, Chagas disease, filariosis and sleeping sickness.[2]

Very little investment is devoted to research and develop drugs to treat diseases affecting poor populations. The lack of interest from pharmaceutical companies developing new drugs for certain diseases, is directly connected to the low purchasing power of these populations. According to a study conducted by the Non-Governmental Organization of international scope, called Doctors Without Borders, the purchasing power is the main factor in setting research priorities, which means that the health needs of poor population do not come being met. The higher purchasing power explains the high investment of the pharmaceutical products which are a highly profitable market segment in the developed countries, for example, medicines for cellulite, alopecia, stress, sleep disorders and obesity.[3]

Millions of people worldwide still die of diseases that can be prevented and treated. Inadequate treatment or non-existent, for various infectious and parasitic diseases is killing large numbers of people. The Working Group for Neglected Diseases (DNDi), formed by a multidisciplinary team of researchers, has released a document with a graphical representation that shows the imbalance in research and development of drugs, classifying diseases as global and neglected (Figure 1).[2,4]

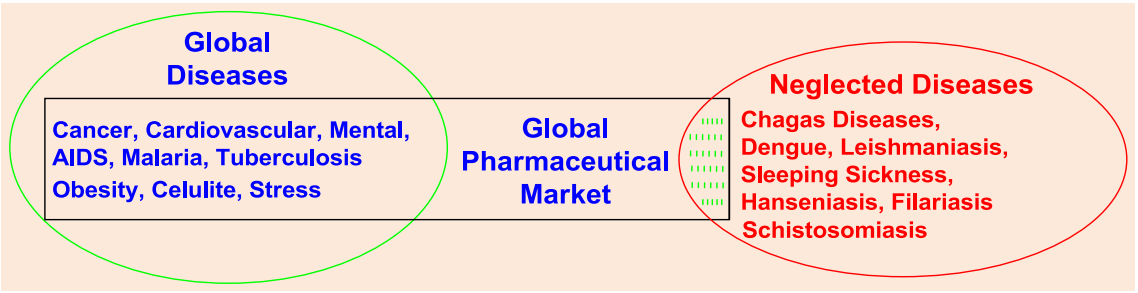


Figure 1. Representation of the disequilibrium between research and development of drugs (adapted from ref. 2)

The term "neglected diseases" is attributed to diseases that have no satisfactory treatment and, interest by the big pharmaceutical companies and insufficient investments by governments to combat such diseases.[5]

To make the picture even worse, most of the drugs used in therapies were discovered at least five decades ago, are difficult to administer, have a high toxicity, the treatments are time consuming and costly. This favors the non-compliance of the patient and that ends up generating the development of species of the causative agents resistant to drugs. Were discovered in recent years very few drugs to fight neglected diseases.[6] The urgency in finding new medicines for neglected diseases has motivated the research and development in various countries around the world.

2. Chagas Disease

Chagas disease is responsible for considerable human mortality and morbidity. Although it was first described one hundred years ago by Carlos Chagas,[7] this disease still represents an important health problem in Latin America.[8] Chagas disease, also known as American trypanosomiasis, is a parasitic disease endemic in Latin America that affects 16 million to 18 million people, with over 100 million at risk of infection.[1] Recent surveys indicate that about 200,000 new cases and 21,000 deaths are associated with this disease every year.

The etiologic agent of this disease is the flagellate protozoan parasite *Trypanosoma cruzi*, which is found in Central and South America, mainly in Brazil, but can also be found in the southern United States (Figure 2).

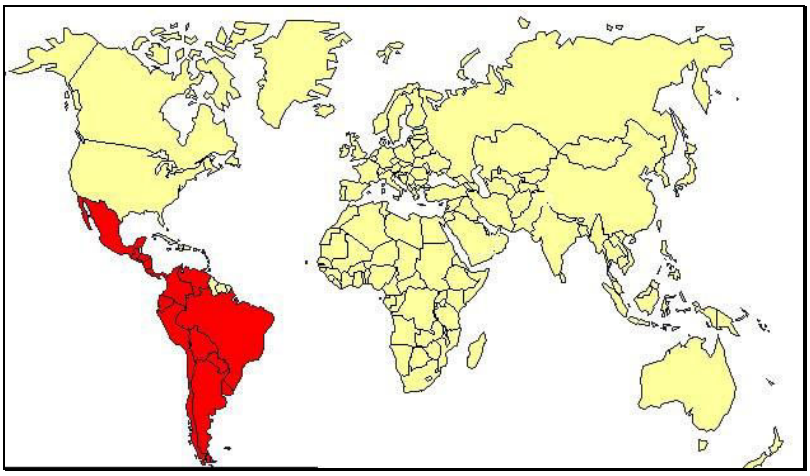


Figure 2. Geographical distribution[1]

The discovery of American trypanosomiasis by Dr. Carlos Chagas was one of the most successful and complete findings in the whole history of tropical medicine. Dr. Carlos Chagas discovered a new human disease and elucidated all the transmition cycle. He firstly discovered a new parasite and its vector, and found that cats were the domestic reservoir, thereby leading to human infection. He then discovered the wild cycle of the infection among armadillos (*Dasypus nomvemcinctus*) living in the same burrow as infected

Panstrongylus gemiculatus,[9] and subsequently the cycle among monkeys of the species *Saimiri sciureus* in the Brazilian Amazon region.[10] Soon after the discovery of Chritidias in the intestine of Conorrhinus, Chagas performed a series of experiments on laboratory animals (guinea pigs, dogs and monkeys) and studied the evolutionary cycle of *T. cruzi* in *Panstrongylus megistus,* thus covering the entire evolutionary cycle in vertebrates and invertebrates. He also studied the acute and chronic phases of the disease and its pathogenesis. [11-14]

2.1. Cycle of *Trypanosoma Cruzi*

Trypanosoma cruzi has a complex life cycle, which necessarily involves crossing a vertebrate host (mammals, including man) and invertebrate (hematophagous insects of the subfamily Triatominae) (Figure 3),[15,16] with morphological changes: the epimastigote and metacyclic trypomastigote forms found in insect, besides the amastigote and trypomastigote blood, responsible for the multiplication and spread of infection in men, respectively (Figure 4). The trypomastigote ingested by the insect differentiates into the proliferative epimastigote form that, on reaching the posterior intestine, evolves to metacyclic trypomastigotes. This latter form, following invasion of vertebrate host cells, undergoes differentiation into amastigotes, which after several reproductive cycles transform to trypomastigotes, the form responsible for the dissemination of the infection.[17]

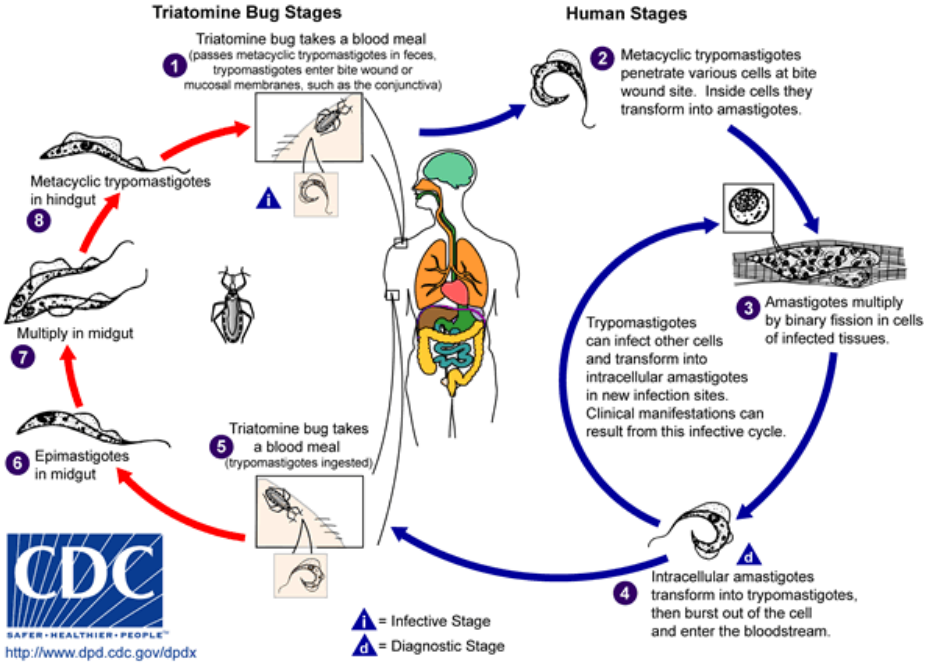


Figure 3. Life-cycle of *Trypanosoma cruzi* (Image from Centers of Disease Control and Prevention)

Figure 4. Triatomine insect[18]

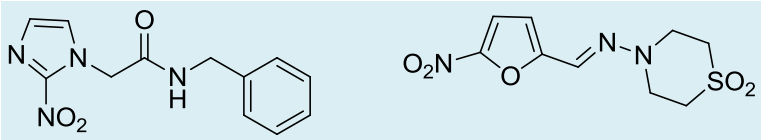
The transmission of the disease occurs mainly by the vector insects infected with *T. cruzi* via blood sucking (80 to 90%), by blood transfusion (5 to 20%) and by congenital routes (0.5 to 8%). The congenital transmission and blood are the main causes of the disease, but the congenital transmission is the most worrisome because of side effects in mothers and infants caused by medications available.[19] And there are other less common forms of infection, e.g., transmission via laboratory accident,[20] organ

transplantation[21-23] and ingestion of infected food or contaminated insects[24,25] have also been reported. Recently, Chagas disease has also been recognized as an opportunistic disease in HIV-infected individuals.[26]

Chagas disease is characterized by three clinical phases named acute, indeterminate and chronic, that differ in symptoms and morbidity. In humans, during the acute phase of Chagas disease and in the absence of specific treatment, the symptoms persist for about two months, with a mortality of 2 to 8%, especially among children. In the chronic phase, most patients remain asymptomatic, but about 20% of cases develop the symptoms characteristic of this phase, namely cardiac, digestive or neurologic disturbances. Thus, Chagas is a major cause of infectious cardiac disease in endemic areas. All available treatments have proven most effective in the acute phase of disease and in cases where this is detected in young patients.[27-29]

2.2. Chemotherapy to Chagas Disease

There are two drugs used to treat Chagas disease that was introduced in the 1960s and 1970s, benznidazole (**1**), a nitroimidazole derivative (Rochagan®, Radanil®, Roche) and nifurtimox (**2**), a nitrofuran derivative (Lampit®, Bayer) (Figure 5), the latter being no longer used therapeutically in Brazil, due to resistance problems.[30] However, neither of these therapeutics meets the precepts for a good drug in accordance with the criteria of the World Health Organization: (i) parasitological cure of acute and chronic cases of the infection; (ii) effective in a single dose or with few doses; (iii) accessible to patients, i.e., low cost and easy to obtain; (iv) no side effects or teratogenic effects; (v) no need for hospitalization for treatment and (vi) no resistance shown or induced in the etiological agent.



1, Benznidazole **2**, Nifurtimox

Figure 5. Available drugs used to treat Chagas disease

These drugs cure around 80% of acute cases and 20% of chronic cases. Patients require 60 days of treatment, with 2-3 doses per day.[31] The drugs are not accessible for patients; at least not in Brazil, where nifurtimox is unavailable and the distribution of benznidazole (**1**) is restricted to specialized clinics that require medical monitoring during the course of treatment. There are also other problems, for example, both drugs induce significant side effects, some strains of *T. cruzi* are resistant to treatment and low antiparasitic activity of these drugs in chronic disease. The data regarding their use and efficacy during the chronic phase are still controversial. This controversy is primarily due to the undesirable side effects that frequently force the abandonment of treatment, poor indices of apparent cure and a lack consensus about the available criteria for the evaluation of parasitological cure during this later phase of the disease.[16]

The significant side effects, we can highlight, anorexia, loss of weight, digestive manifestations, such as nausea or vomiting, and occasionally intestinal colic and diarrhea, peripheral polyneuropathy, psychic alterations, thrombocytopenic purpura, agranulocytosis and allergic dermopathy.[32,33]

Due to increased knowledge of physiology and biochemistry of the agent, new natural and synthetic substances, as well as also drugs in the market, have been evaluated in several biological targets of *T. cruzi*. In this regard, the search for natural compounds has been recently reviewed[34] and the focus of this review will be on synthetic compounds. Concerning the use of drugs already available for other types of diseases, an important example is the use of fungicides medicines such as itraconazole (**3**) (Sporonox®, Janssen-Cilag), ketoconazole (**4**) (Nizoral),[35] posoconazol (**5**) (SCH 56592, Schering-Plough) and ravuconazol (**6**) (BMS 207 147, Bristol-Myers Squibb, Figure 6), which were active in both chronic and acute form of the disease.[36-40] After the introduction of nifurtimox and benznidazole, despite the extensive list of classes of compounds with *in vitro* and *in vivo* activity against *T. cruzi*., with the exception of a little number of drugs, none was submitted to clinical trials.

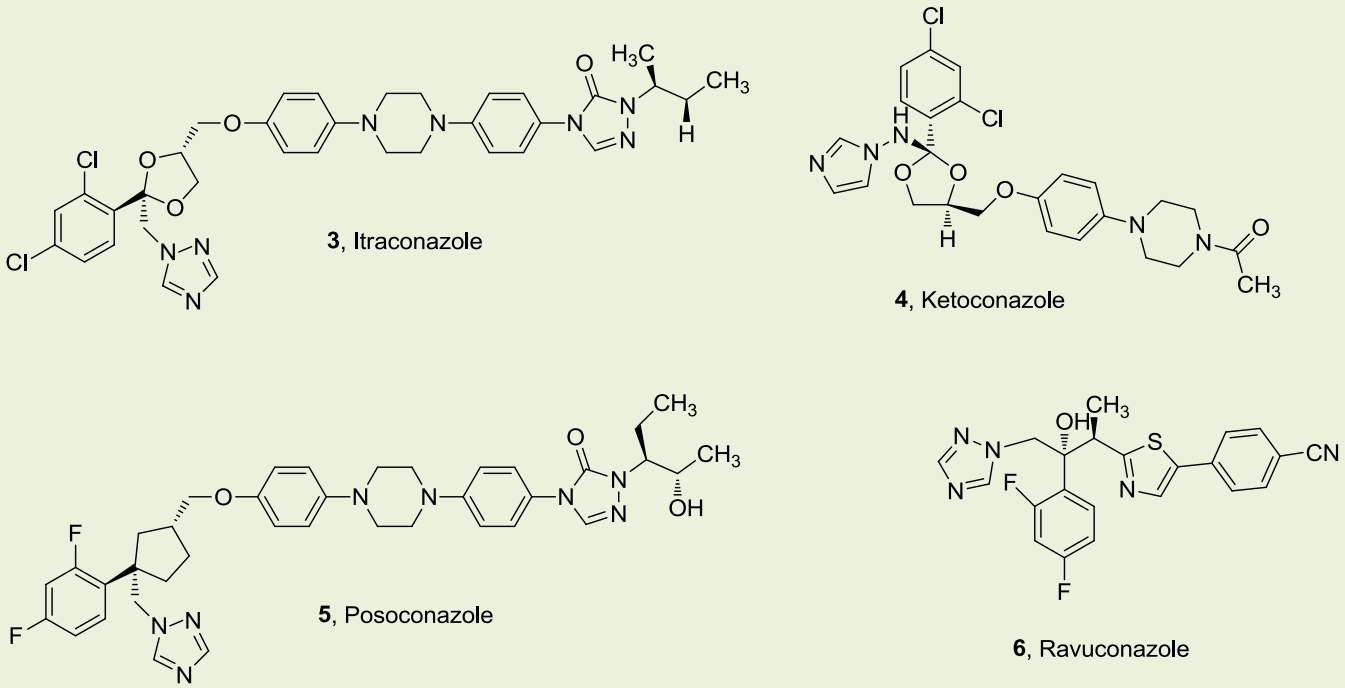


Figure 6. Other active drugs in both chronic and acute form of Chagas disease

For new drugs may be noted the TAK-187 (**7**) (Takeda Chemical Company), E1224 (**8**) (pro-drug of ravuconazole, Eisai company), K-777 (**9**) (Sandler Center for Drug Discovery), that showed trypanocidal activity both *in vitro* and *in vivo* (Figure 7). The drugs E1224 and TAK-187 had the phase I trial completed. And K-777 is in preparation for phase I safety trial.[41]

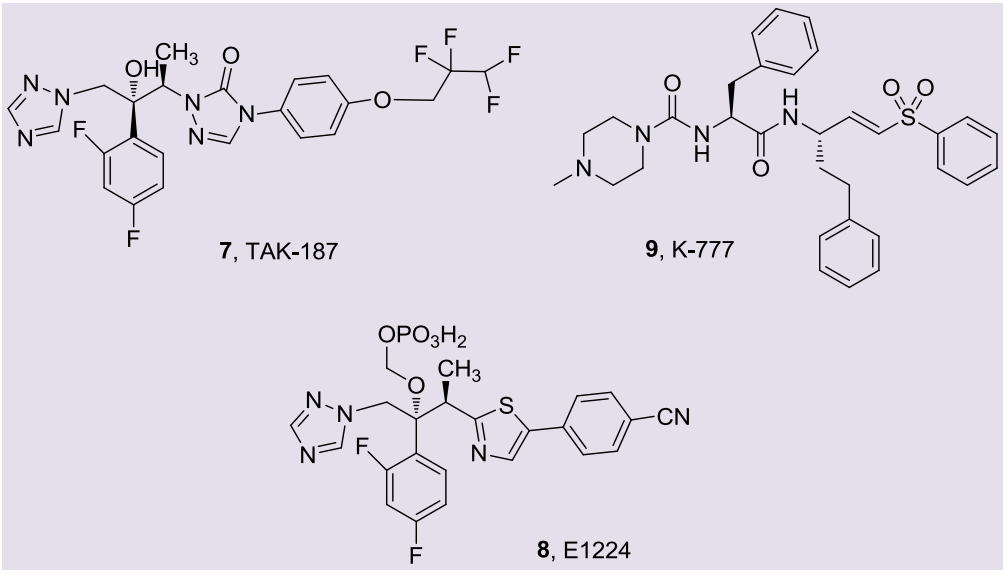


Figure 7. New hit compounds with trypanocidal activity

The knowledge of genome sequence analysis of *T. cruzi* has led to identify a large number of trypanosomatid enzymes and/or biochemical pathways with potential targets for drug development. Thus, several targets are being identified for approved drugs or new chemical structure screening with trypanocidal potential and these studies has led to structure-activity relationships for some of these molecules. Important differences were identified between these targets of the parasite and its mammalian hosts, which could be exploited as chemotherapeutic targets. The main targets that can be cited are sterol biosynthesis, purine metabolism, thiol metabolism, cysteine

proteases and polyamine biosynthesis.

3. Synthetic compounds with trypanocidal activities

The urgency for more effective chemotherapeutic agents against all strains of *T. cruzi*, and with fewer or no side effects than those currently available has prompted the synthesis of a wide number of compounds. These compounds have been assayed as trypanocidal agents and some of them have shown promising trypanocidal properties.

3.1 Quinones

Quinones are considered special structures in medicinal chemistry due to their diversity of biological activities and structural properties. They are present in various families of natural products isolated from plants and microorganism that serve as vital links in the electron transport chains in the metabolic pathway, participating in multiple biological oxidative processes.

Compounds containing the quinone moiety are found in numerous natural products and often are associated with different pharmacological activities, such as anticancer activity,[42] antibacterial,[43] antimalarial,[44] trypanocidal[45] and fungicide.[46] In recent years increased interest in these substances, not only because of its vital importance in biochemical processes, but also highlighted the increasingly are showing that in various pharmacological studies. In most cases, biological activity is related to the ability of quinones to accept one or two electrons, forming a radical anion or dianion species, this is a redox cycle.[47] Many natural compounds have been screened against *Trypanosoma cruzi* and studied as potential antichagasic drugs,[31,48-51] one of this group correspond to quinones, especially naphthoquinones. Among natural naphthoquinones, lapachol (**10**), β-lapachone (**11**),[52] α-lapachone (**12**) and juglone (**13**) have demonstrated strong trypanocidal activity (Figure 8).

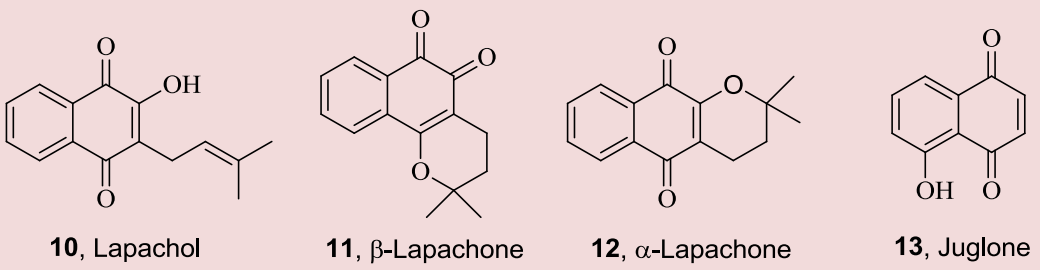


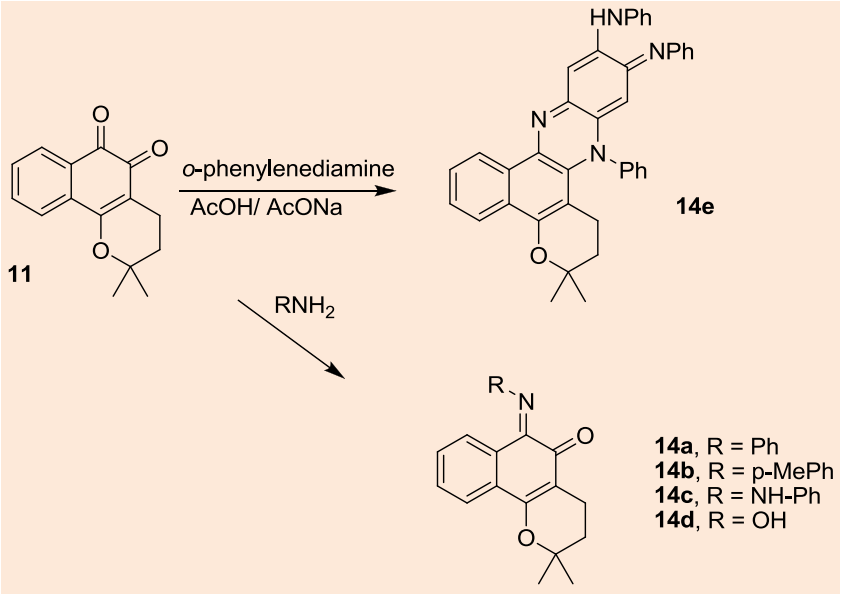
Figure 8. Natural naphthoquinones

Lapachol (**10**) is a natural naphthoquinone found in plants of the families Bignoniaceae, Leguminosae, Sapotaceae, Scrophulariaceae, and Verbenaceae Malvaceae Proteaceae.[53] However, its occurrence is higher in Bignoniaceae family, particularly in the genus Tabebuia (Tecoma). It was first isolated in 1882 from Tabebuia species avellanedae.[54] These trees are commonly known in South America as Ipês, Lapacho, Pau d'Arco, purple and lapacho Taheebo.[55] Additionally, several derivatives of lapachol (**10**) was found to have trypanocidal activities.[56-58]

β-Lapachone (**11**) is a natural *ortho*-pyran-naphthoquinone obtained as a minor component of heartwood from the Lapacho trees and it is readily obtained in high yield from lapachol (**10**) by cyclization in concentrated sulfuric acid.[59] This compound has been described as one of the most important derivatives of lapachol. This important naphthoquinone aroused the attention of the scientific community due to many different pharmacological activities. Stoppani and Cruz and Docampo were the first one to demonstrate that this compound has strong activity against the hemoflagellate protozoan *T. cruzi.*[60-63] In addition they showed that its action mechanism involves the generation of superoxide anion radicals and H_2O_2, which subsequently cause damages to several cell components and inhibit nucleic acids and protein syntheses.[64] β-lapachone never became a drug for the treatment of Chagas disease but its structure of β-lapachone (**11**) inspired the search for new derivatives with better trypanocidal activity. From this point of view, several heterocyclic derivatives have been constructed to replace the carbonyl or have been attached to the naphthoquinone nucleus, and other minor changes were introduced at the carbonyl.

Some of these new synthetic naphthoquinones were much more active and less cytotoxic than **11**.

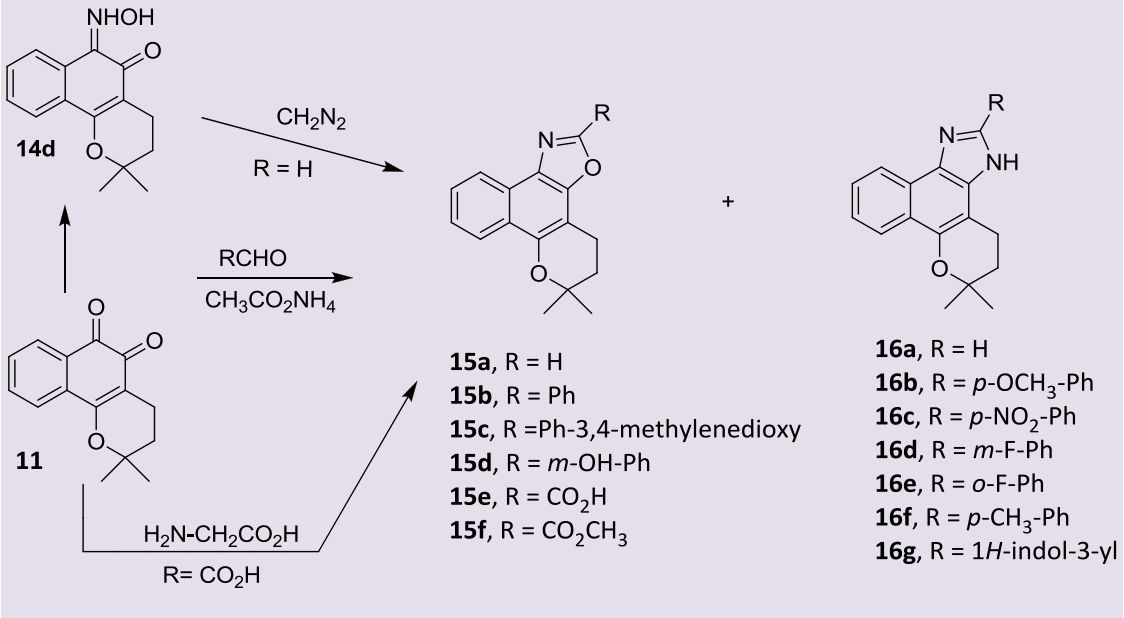
Neves et al. reported the synthesis of phenazine from β-lapachone and its potential trypanocidal activity, the compound **14e** was more active than crystal violet ($ED_{50/24h}$ = 536 ± 1 μmol.L^{-1}) against the infective trypomastigote form of *T . cruzi* (Scheme 1).[65]



Scheme 1. Synthesis of phenazine from β-lapachone (**11**) with potential trypanocidal activity

Pinto et al. have synthesized β-lapachone derivatives through the reaction of these naphthoquinones with common reagents, leading to several heterocyclic compounds. Aiming to developed a new lead compound against *T. cruzi*, the group developed new methodologies to obtain aryl-naphtho[1,2-d]oxazole and aryl-naphtho[1,2-d]imidazoles derivatives from β-lapachone (**11**), and several other 1,2-naphthoquinones (Scheme 2).[66,67] The naphtho[1,2-d]oxazole **15a** was a more active compound than **11** against *T. cruzi*. The introduction of an aromatic group to the oxazole nucleus showed a strong influence on the trypanocidal activity. Compound **15b**, with a phenyl group, and compound **15c**, with a methylenedioxy group attached to the aromatic ring, demonstrated increased activity. Also, several naphtho[1,2-d]imidazoles **16a-g** were synthesized from **11** and were generally more active than the oxazoles against trypomastigotes, epimastigotes and amastigotes forms of *T. cruzi* Among them, the derivatives **16a**, **16f** and **16g** showed the highest trypanocidal activity, **16a** being 10.6 times more active than β-lapachone (**11**).[68]

A series of *o*-naphthoquinones were investigated by Dubin et al [69,70] named CG8-935 (**17**), CG9-442 (**18**), and CG10-248 (**19**) (Figure 9). CG9-442 proved to be the most active in inducing oxidative damage in trypanosomatids. The contribution of oxygen radical production to quinone cytotoxicity was supported by the spectroscopic observation of β-lapachone, CG 8-935, CG 9-442 and CG 10-248 redox cycling, as well as by the production of the semiquinone radical, superoxide anion radical and H_2O_2 and the effect of these o-naphthoquinones on cell respiration.



Scheme 2. Several heterocyclic compounds synthesized from β-lapachone derivatives

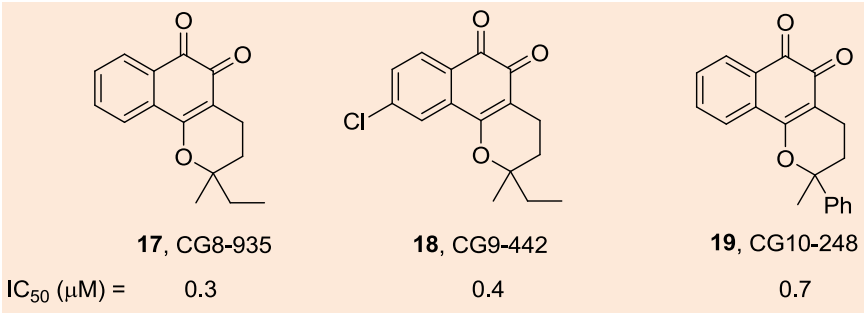
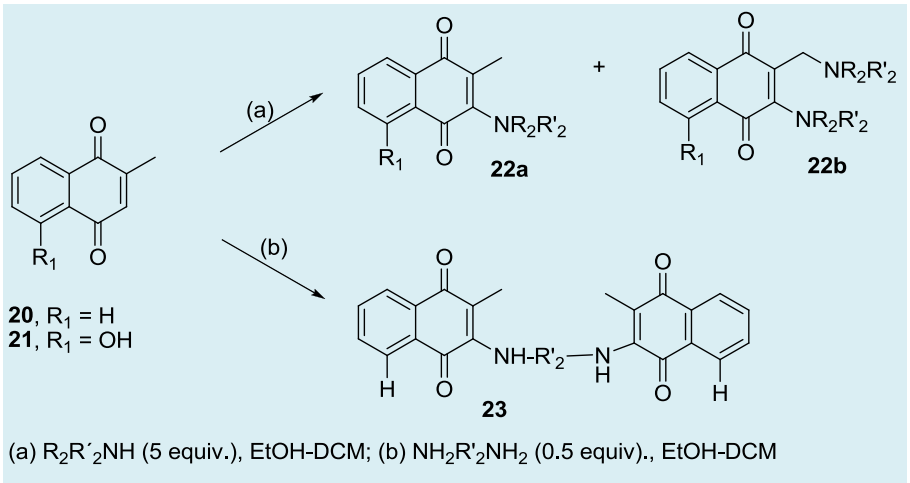


Figure 9. *o*-naphthoquinones with potential trypanocidal activity

Trypanothione reductase (TR) is both a valid and an attractive target for the design of new trypanocidal drugs.[71] Starting from menadione (**20**) and plumbagin (**21**) three distinct series of 1,4-naphthoquinones were synthesized as potential inhibitors **22a-b** and **23** of TR from *Trypanosoma cruzi* (TcTR) by Salmon-Chemin and co-workers.[72] The results obtained in this paper confirm that reduction of naphthoquinones by parasitic flavoenzymes is a promising strategy for the development of new trypanocidal drugs (Scheme 3).



Scheme 3. Synthesis of 1,4-naphthoquinones as potential inhibitors of TR

Natural naphthofurandiones such as **24 to 27**, outlined in Figure 10,[73,74] have been tested against *T. cruzi* parasites. Most of them showed an inhibitory effect on culture growth and on the parasite respiration.[75]

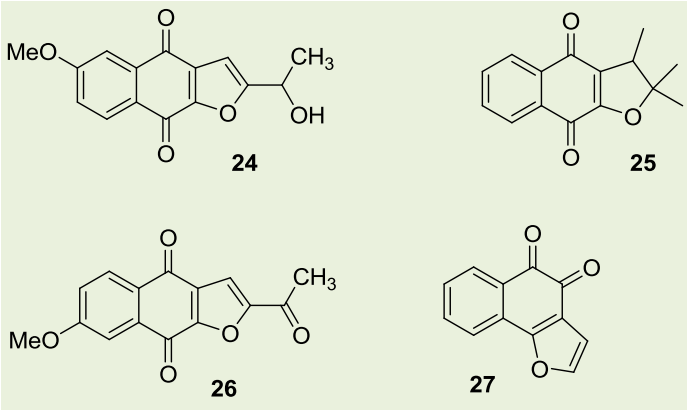
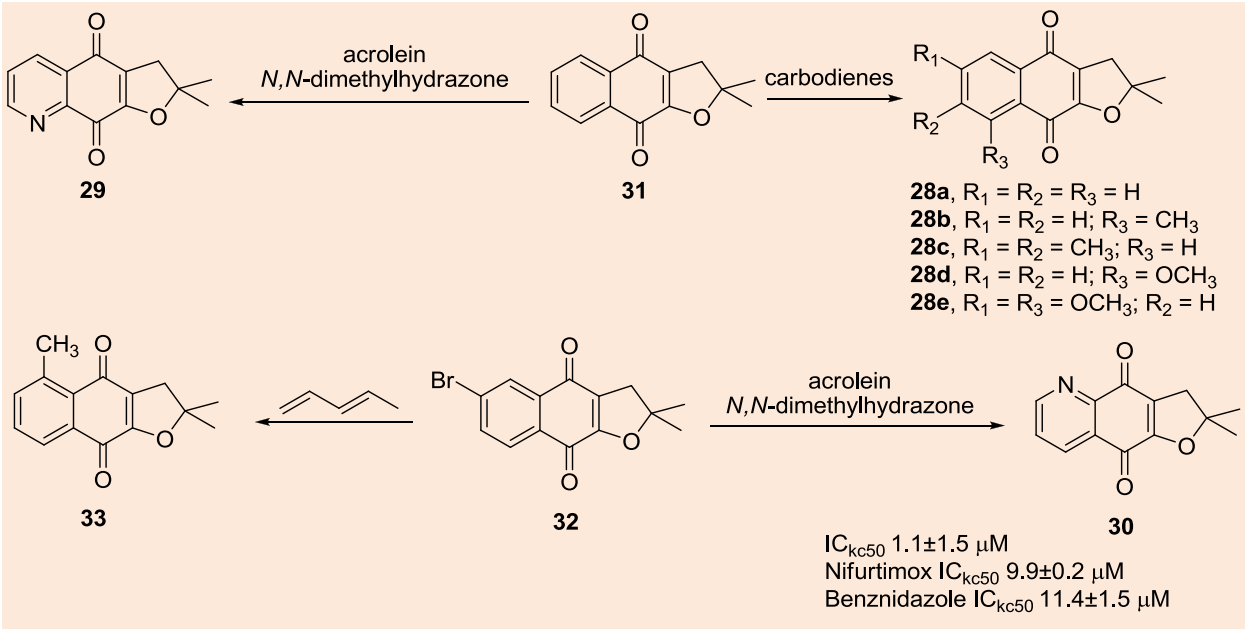


Figure 10. Natural *o*-furanquinones

In order to continue their studies with naphthofurandiones, in 2004 Tapia and co-workers prepared a series of dihydronaphthofurandiones (**28a-e**) and dihydrofuroquinolinediones (**29** and **30**) derivatives by Diels-Alder reactions of dihydrobenzofurandione (**31**) with several carbodienes or acrolein *N,N*-dimethylhydrazone. Then, the use of 5-bromobenzofurandione (**32**) toward 1,3-pentadiene and the 1-azadiene afforded quinones (**33**) and (**30**) with a total regioselectivity. All prepared quinones were tested for trypanocidal activity *in vitro* against epimastigote form of *T. cruzi* Tulahuen strain. Among the tested compounds, the furoquinolinediones **29** and **30** have shown potent trypanocidal activities but, only the 1,5-regioisomer (**30**) was found active as a redox cycling agent.[76] In the literature there isn't a correlation between the trypanocidal activity and the redox potential of naphthofurandiones has been investigated and no linear relationship was found. However, Tapia et al proposed that the angular naphthofurandiones (*o*-quinones), which are easier to reduce than linear isomers (*p*-quinones) showed higher trypanocidal activity than the latter, suggesting a contribution of the easiness of reduction on the biological activity (**Scheme 4**).



Scheme 4. Preparation of series of dihydronaphthofurandiones and dihydrofuroquinolinediones

The same group in 2008 synthesized derivatives of natural quinones with biological activities, such as lapachol, α- and β-lapachones and their trypanocidal activity evaluated in vitro in *T. cruzi* cells. All tested compounds inhibited epimastigote growth and trypomastigote viability. Several compounds showed similar or higher activity as compared with current trypanocidal drugs, nifurtimox (IC_{k50} 9.62 μM) and benznidazole (IC_{k50} 20.6 μM). The results presented by authors showed that the trypanocidal activity of the α-lapachone derivatives can be increased by the replacement of the benzene ring by a pyridine heterocyclic ring (Figure 11).[57]

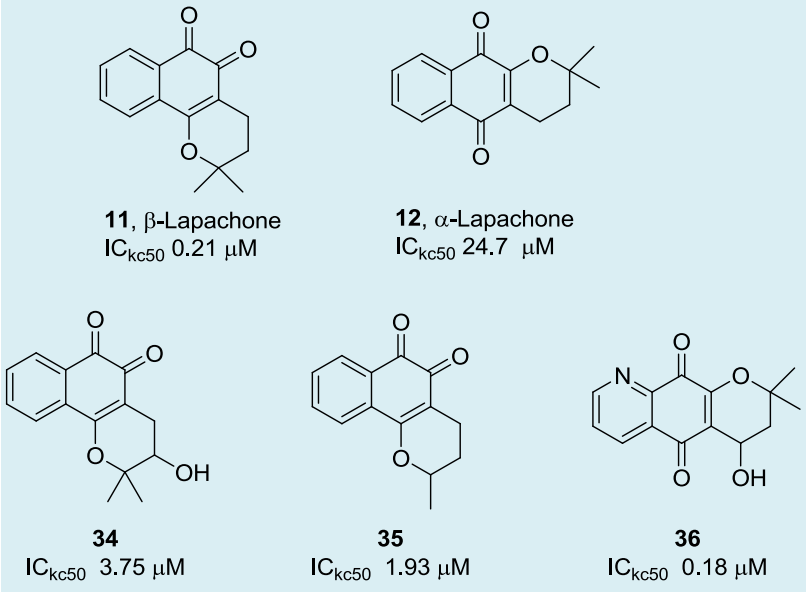
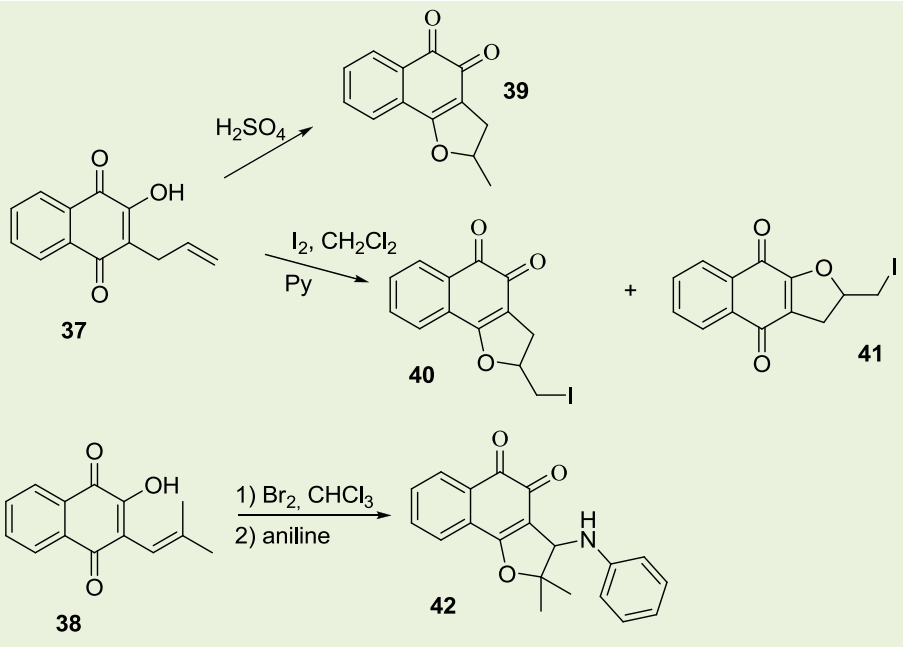


Figure 11. Natural quinones inhibited the growth of epimastigote and trypomastigote forms

In the search for new trypanocidal agents, four new naphthofuranquinones prepared by Silva et al,[77] obtained from 2-hydroxy-3-allyl-naphthoquinone (**37**) and nor-lapachol (**38**), and have their activity evaluated against *T. cruzi.* Compounds **40** and **41** were obtained by addition of iodine to **37** followed by cyclization generating a furan ring. Compound **39** was obtained through the acid-catalyzed reaction by dissolution of **37** in sulfuric acid. Compound **42** was synthesized by addition of bromine and aniline to **38**. The $IC_{50/24\ h}$ for **39-42** in assays with *T. cruzi* trypomastigotes was between 157 and 640 μM, while the value for crystal violet was 536.0 ± 3.0 μM. Compounds **39-41** also inhibited epimastigote proliferation (Scheme 5).



Scheme 5. New naphthofuranquinones obtained from 2-hydroxy-3-allyl-naphthoquinone and nor-lapachol

Ferreira and coworkers[78,79] designed some new naphthoquinone derivatives based on hybrid drugs with significant bioactivity against *T. cruzi*. In this regards, they synthesized several β-lapachone and nor-β-lapachone derivatives and assayed against bloodstream trypomastigote forms of *T. cruzi*. The best compounds for trypanocidal study were the quinones **43, 44, 45** and **46** with trypanocidal activity higher than that of benznidazole ($IC_{50/24\ h}$ 103.6 ± 0.6 μM), the standard drug (Figure 12).

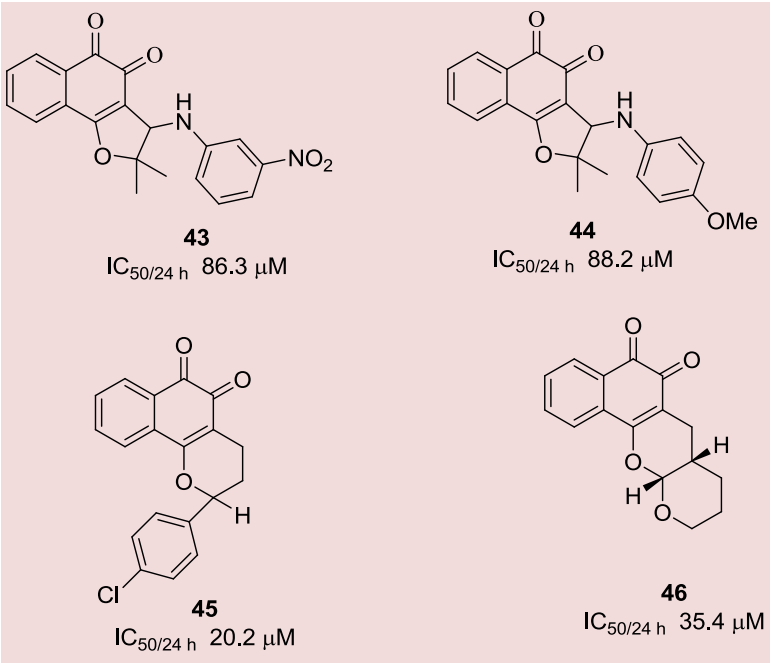


Figure 12. β-lapachone and nor-β-lapachone derivatives assayed against bloodstream trypomastigote

Although β-isomers were found more active than α-lapachone derivatives, in the last decade several α-lapachone derivatives were synthesized and their biological properties evaluated.[80-82] There are examples indicating that some structural modifications produce α-lapachone derivatives with interesting anti-trypanosoma properties.[68] This result is of great interest since usually α-lapachones have low trypanocidal activity.[83] In 2006, Ferreira and co-workers found that oxyrane derivative 4**7** (Figure 13)

showed lower cytotoxicity and high trypanocidal activity (IC_{50} 12 µM). The oxyrane (**47**) is almost strong trypanocidal agent as β-lapachone (**10**) (IC_{50} 0.9 µM).[84-86] Since the core *o*-quinone moiety was modified with the introduction of the oxyrane ring on the carbonyl C-6, it affected the formation of free radicals and reactive oxygen species. However, the oxyrane derivative of α-lapachone (**48**) showed higher trypanocidal activity (IC_{50} 1.3 µM) than α-lapachone (**11**, IC_{50}> 50 µM) without cytotoxicity to mammalian cells. Since the redox center in the *o*-quinones is the moiety responsible for the antiproliferative activity against *T. cruzi,* it seems that another mechanism of action is operating in this case. Indeed, this compound showed lethality of 97% and 84% against trypomastigotes of *T. cruzi* and to Y Colombian strains, respectively.[87] In summary, this compound is a potential candidate for chemotherapy of Chagas disease due to its trypanocidal activity with a low cytotoxicity profile to human cells.

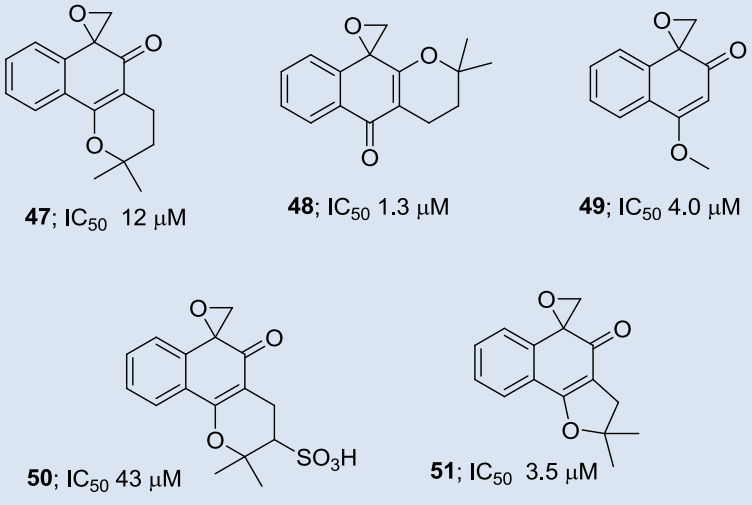


Figure 13. Series of trypanocidal oxyranes synthesized from *o*-naphthoquinones

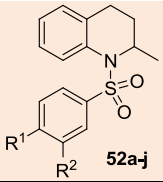
3.2. Other compounds

3.2.1. Heterocycles

Pagliero and co-workers[88] synthesized a series of 1-benzenesulfonyl-2-methyl-1,2,3,4-tetrahydroquinoline derivatives that showed moderated antiprotozoal activity including against *T. cruzi* with low cellular toxicity. All compounds with the exception of **52e** were moderately active against *T. cruzi*. Compound **52c** was the most active, with an IC_{50} of 11.44 µM, which implies only a sevenfold reduced potency when compared to that of benznidazole as the reference (IC_{50} 2.13 µM) (Table 1).

Chennamaneni and co-workers[89] developed a synthetic route to prepare isoquinoline analogs of the cancer drug clinical candidate tipifarnib and show that these compounds kill the *T. cruzi* (amastigote form) in mammalian host cells at concentrations in the low nanomolar range (Table 2).

Table 1. 1-benzenesulfonyl-2-methyl-1,2,3,4-tetrahydroquinoline derivatives and antiparasitic activity of compounds expressed as IC_{50}



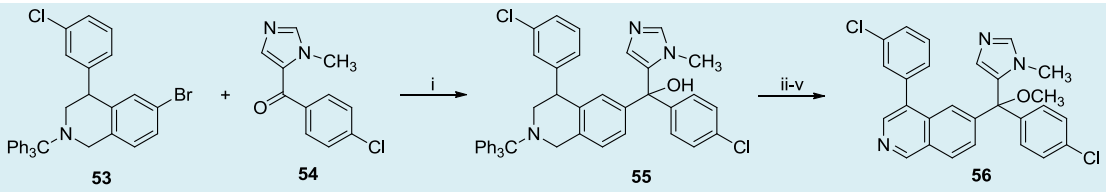
52a-j

52a, R^1 = H, R^2 = H
52b, R^1 = NHCOCH$_3$, R^2 = H
52c, R^1 = NO$_2$, R^2 = H
52d, R^1 = CH$_3$, R^2 = H
52e, R^1 = F, R^2 = H
52f, R^1 = Cl, R^2 = H
52g, R^1 = Br, R^2 = H
52h, R^1 = OCH$_3$, R^2 = H
52i, R^1 = H, R^2 = NO$_2$
52j, R^1 = NH$_2$, R^2 = H

Compounds (52a–j)	*T. cruzi* (IC_{50})	Cytotoxicity. L-6 (IC_{50})	SI *T. cruzi* (μM)[a]
Benznidazole	1.54	-	-
52a	21.26	70.03	5.28
52b	21.45	52.02	3.58
52c	11.44	248.28	30.00
52d	16.61	4.90	0.44
52e	223.74	67.57	5.58
52f	15.98	20.46	2.43
52g	13.78	7.36	1.10
52h	15.89	5.99	0.50
52i	31.94	186.97	18.08
52j	19.73	35.27	2.13

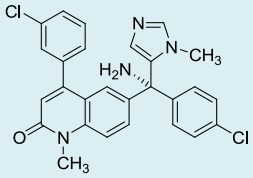
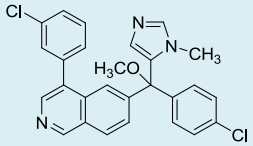
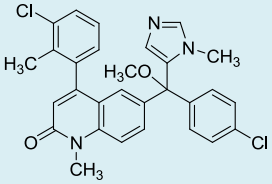
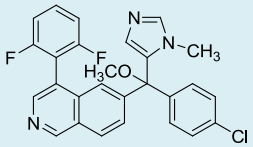
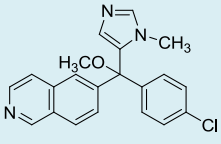
[a]Selectivity Index calculated as SI = IC_{50}L6/IC_{50} parasite.

Table 2. Synthesis of isoquinoline tipifarnib analogs and growth arrest of T. cruzi amastigotes



53 **54** **55** **56**

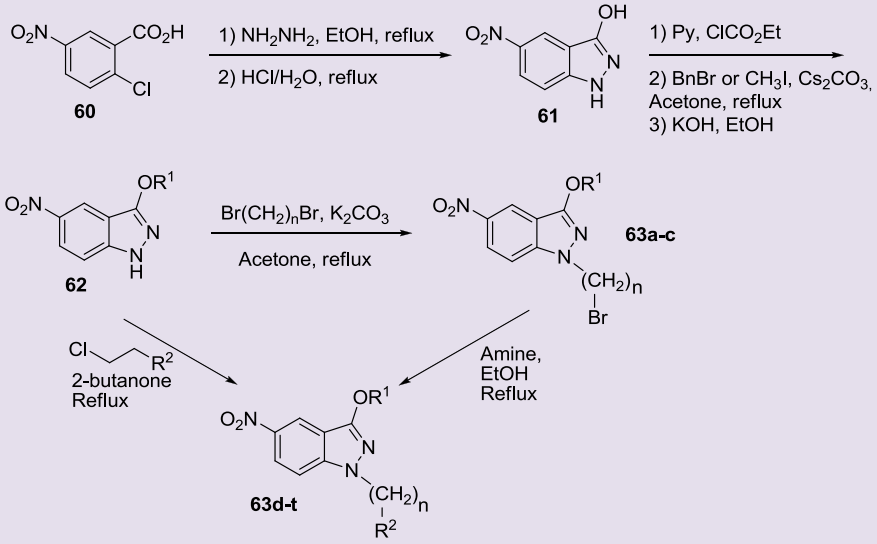
(i) n-BuLi, THF, 78 C, 56%; (ii) TFA, CH2Cl2, 85%; (iii) MnO2, dioxane, 90 C, 64%; (iv) SOCl2, rt; (v) MeOH, 90 C, 80% (two steps).

Compound	**Structure**	**EC_{50} (nM)**
Tipifarnib		4
56		0.5, 0.9, 1.1[a]
57		0.6
58		0.9, 1.3
59		120
Posaconazole		0.3

[a]The multiple numbers represent independent determination of EC_{50}.

Rodríguez et al[90,91] studied the synthesis of a series of a new 5-nitroindazole derivatives and their trypanocidal properties. Eight compounds (**63f-j**, **63l**, **63r** and **63t**) displayed remarkable in vitro activities against *T. cruzi*. Its unspecific cytotoxicity against macrophages was evaluated being not toxic at a concentration at least twice that of *T. cruzi* IC_{50}, for some derivatives (Table 3).

Table 3. Synthesis of a series of a new 5-nitroindazole derivatives and *in vitro* trypanocidal activity (epimastigote form) and unspecific cytotoxic activity (% C) against macrophages



60 61 62 63a-c 63d-t

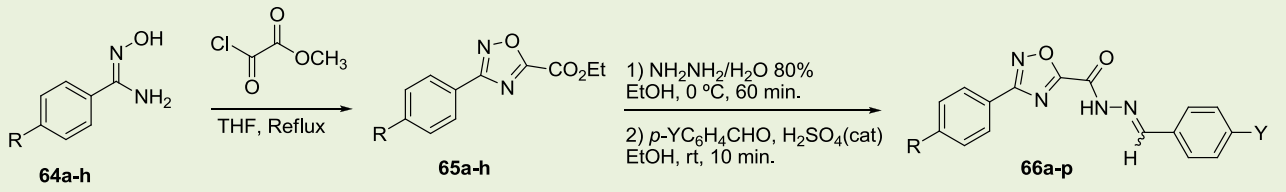
Compounds	R^1	R^2	n	IC_{50}[a]	%C[b]
63a	CH_3	Br	3	67.8	-
63b	Bn	Br	5	20.7	-
63c	Bn	Br	6	19.3	-
63d	CH_3	dimethylamino	3	>25.0	29
63e	CH_3	piperidino	3	>>25.0	0
63f	Bn	1,2,3,4-tetrahydroisoquinolyl	5	12.9	15
63g	Bn	pyrrolidino	5	7.5	25
63h	Bn	homopiperidino	5	10.5	25
63i	Bn	piperidino	6	7.4	33
63j	Bn	dimethylamino	6	9.4	40
63k	CH_3	piperidino	2	>>25.0	10
63l	Bn	piperidino	2	8.4	27
63m	CH_3	dimethylamino	2	>>25.0	15
63n	Bn	dimethylamino	2	~25.0	12
63o	CH_3	morpholino	2	>>25.0	0
63p	Bn	morpholino	2	>>25.0	0
63q	CH_3	dimethylamino	2	>>25.0	0
63r	Bn	dimethylamino	2	11.3	30
63s	CH_3	diisopropylamino	2	>>25.0	15
63t	Bn	diisopropylamino	2	9.2	0
Nifurtimox	-	-	-	3.4	40

[a]IC_{50} = concentration (μM) that inhibits 50% of *T. cruzi* growth (CL-Brener clone)
[b]% C = cytotoxicity percentages, using 25 μM as compounds concentrations.

Filho and co-workers[92] investigated the *in vitro* bioactivity of a library of sixteen 3-(4-substituted-aryl)-1,2,4-oxadiazole scaffold against epimastigote and trypomastigote forms of *T. cruzi* some then exhibited trypanocidal activity at concentrations that are not toxic to mammalian cells. The series of compounds was based on the 3-(4-substituted-aryl)-1,2,4-oxadiazole scaffold and they revealed a

remarkable effect of the substituent at the phenyl's 4-position for the inhibitory activity. The non-nitrated compounds **66a** and **66m** were found to be potent as benznidazole (Table 4).

Table 4. Synthesis of NAH **66a-p** and *in vitro* anti-Trypanosoma cruzi activity of NAH derivatives against Y strain



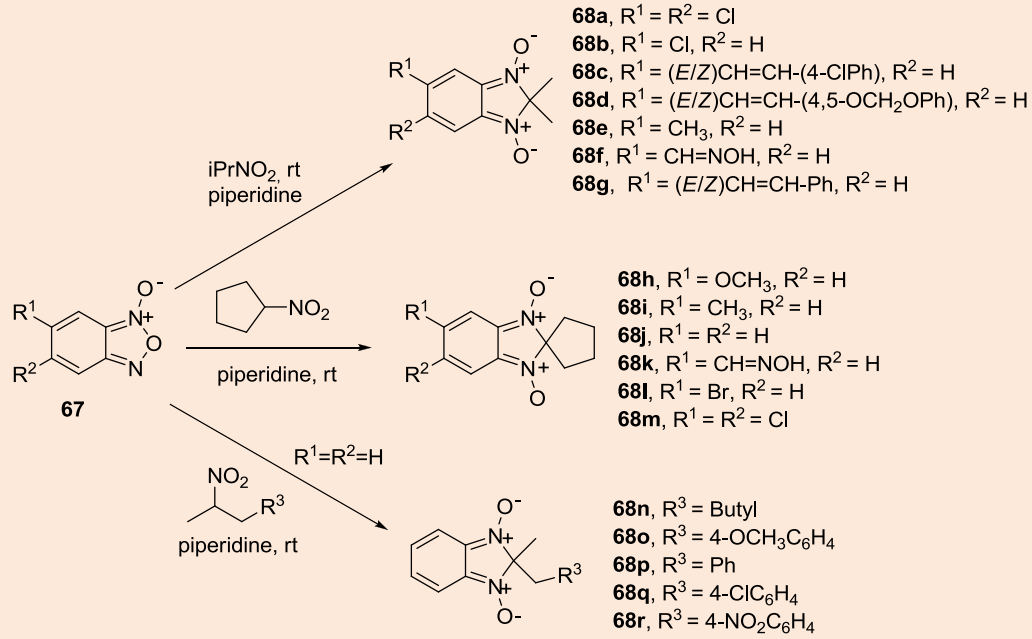
Compound	R	Y	Yield (%)	Ratio (E:Z)	Trypomastigote IC_{50} in µM	Epimastigote IC_{50} in µM	Cytotoxicity (µg/mL)[a]
66a	H	NHAc	90	100:0	3.6	14.2	33 (95)
66b	CH_3	NHAc	95	100:0	3.9	9.8	11 (30)
66c	F	NHAc	93	100:0	Nd	>150	33
66d	Cl	NHAc	98	100:0	Nd	>150	100
66e	Br	NHAc	83	100:0	Nd	>150	33
66f	NO_2	NHAc	90	100:0	Nd	>150	33
66g	OCH_3	NHAc	89	100:0	Nd	>150	100
66h	OH	NHAc	91	100:0	Nd	>150	33
66i	H	OH	98	89:11	35.7	78.3	3.3
66j	CH_3	OH	95	92:8	17.9	47.8	<1.1
66k	F	OH	85	91:9	16.7	21.0	<1.1
66l	Cl	OH	95	91:9	21.2	21.6	<1.1
66m	Br	OH	88	92:8	20.5	19.6	33 (97)
66n	NO_2	OH	85	92:8	21.3	25.2	<1.1
66o	OCH_3	OH	90	89:11	32.5	13.8	33 (85)
66p	OH	OH	85	92:8	100.3	126.6	100
Benznidazole	-	-	-	-	5.0	6.6	100
Gencian Violet	-	-	-	-	2.1	-	<1.0

[a]Expressed as the highest concentration tested non-cytotoxic for mouse splenocytes. Values in lM are shown in parentheses.

Boiani and co-workers[93,94] explored the influence of different substitution patterns of 2*H*-benzimidazole 1,3-dioxide derivatives (BzNO) we prepared fifteen new derivatives. The BzNO were tested against *T. cruzi* Tulahuen 2 strain epimastigote form and some of them presented potent trypanocidal agents. Moreover, the BzNO were able to inhibit the growth of virulent and resistant to benznidazole strains (CL Brener clone, Colombiana and Y strains). The 2*H*-benzimidazole 1,3-dioxide derivatives exhibited very high selectivity index and particularly the spiro-BzNO **68i** lowered the levels of amastigotes in Vero cells (Table 5).

Recently, new 1,2,3-triazoles[95] and pyrazoles[96] showed very good activity against *T. cruzi* strains demonstrating the importance of these moieties in the search of new molecules to Chagas disease profilaxy (Figure 14).

Table 5. Synthetic procedures used to prepare the 2H-Benzimidazole 1,3-dioxide derivatives, biological characterization against Tulahuen 2 strain and biological activity , measured as cellular viability against different T. cruzi strains



Compounds	ID_{50} (µM)	ID_{50} in µM (Percentage of cytotoxicity in %)		
		CL Brener clone	Y strain	Colombiana strain
68a	5.1	(61.4)	3.3	(35.1)
68b	12.5	>40.0 (46.6)	16.4	(21.7)
68c	3.4	8.5 (78.4)	6.0	(92.3)
68d	14.5	(88.3)	9.9	(94.4)
68e	10.1	Nd	Nd	Nd
68f	8.1	Nd	Nd	Nd
68g	3.1	Nd	Nd	Nd
68h	25.0	(62.6)	4.9	(17.3)
68i	7.9	(56.5)	15.8	(59.3)
68j	9.5	(83.7)	6.4	(94.8)
68k	19.8	(65.6)	5.7	(29.0)
68l	16.3	(91.5)	3.5	(83.8)
68m	12.0	(67.1)	5.3	(60.9)
68n	5.4	(88.2)	Nd	(77.1)
68o	>50.0	Nd	Nd	(30.8)
68p	>50.0	(47.0)	Nd	(18.6)
68q	40.0	Nd	Nd	Nd
68r	8.4	Nd	Nd	Nd
Nifurtimox	7.7	4.9 (90.0)	9.7	3.4 (87.0)
Benznidazole	7.4	Nd	Nd	Nd

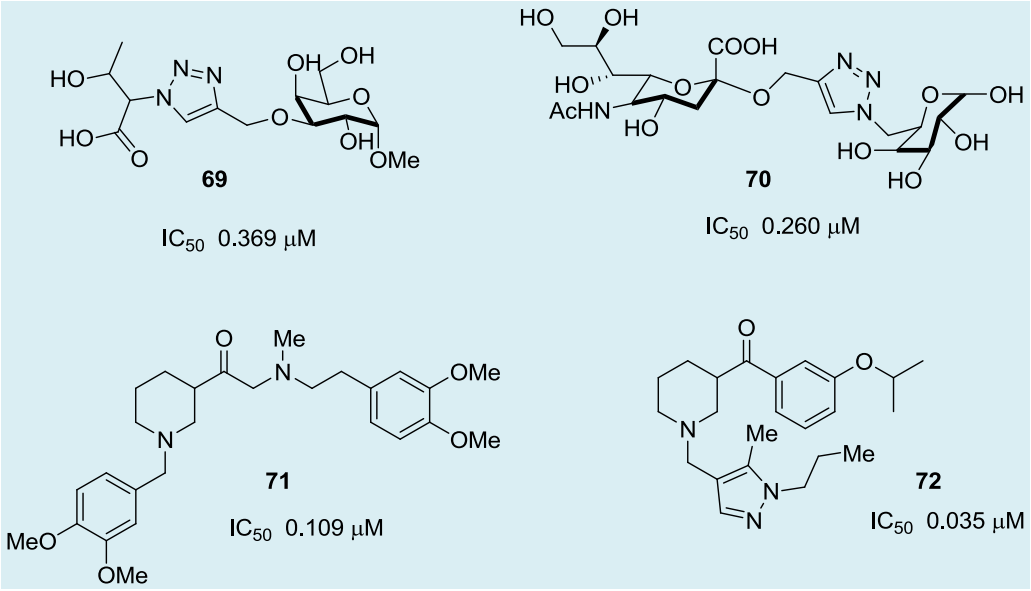
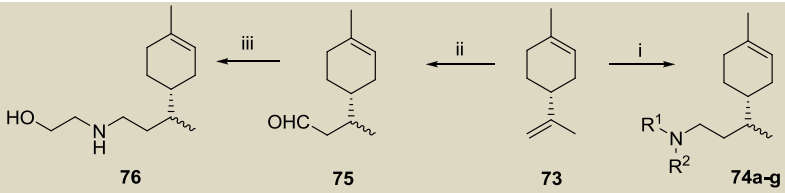


Figure 14. Heterocyclic compounds with trypanocidal activity

3.2.2 Natural Products[34]

Graebin and co-workers[97] reported the synthesis and *in vitro* activity of R(+)-Limonene derivatives against *Leishmania* and *T. cruzi* strains where two compounds showed promising new trypanocidal limonene derivatives. The results show that, compounds **74d** and **74e** are excellent hits as trypanocidal agents for further structural modifications, showing IC_{50} values in the order of the standard drug Nifurtimox (Table 6).

Table 6. Synthetic procedures used to prepare of R(+)-Limonene derivatives and *in vitro* activity against T. cruzi Tulahuen 2 strain



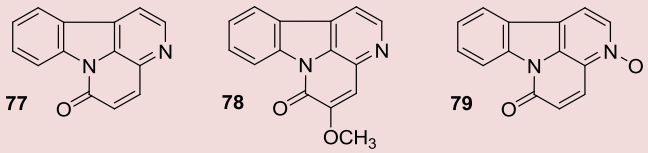
(i) $HRhCO(PPh_3)_3$ (cat.), CO (20 bar), H_2 (20 bar), amine substrate, THF, 100 °C, 5–24 h, then H_2 (40 bar), 5-48 h; (ii) $HRhCO(PPh_3)_3$ (cat.), CO (20 bar), H_2 (20 bar), THF, 100 °C, 5 h; (iii) ethanolamine (4 equiv.), AcOH (cat.), CH_2Cl_2, r.t., 5 h, then $NaCNBH_3$ (3 equiv), 24 h, r.t.

Product	R^1	R^2	Yield (%)	GI (%)[a]	IC_{50} (μM)
73 (limonene)	-	-	-	0.0	> 50.0
74a	Propyl	H	85	21.5	> 50.0
74b	Benzyl	H	44	53.5	50.0
74c	Morpholine	Morpholine	79	20.7	> 50.0
74d	Piperazine	Piperazine	89	82.4	19.2
74e	C_6H_5	H	50	70.5	29.5
74f	Furfuryl	H	75	51.4	50.0
74g	3-$CF_3C_6H_4$	CH_3	40	0.0	> 50.0
76	-	-	57	41.8	> 50.0
Nifurtimox	-	-	-	> 100	7.7

[a]Growth inhibition (%) of the given parasite at 50 μM

Ferreira and co-workers[98] studied the antiparasitic effects of canthin-6-one (**77**), 5-methoxycanthin-6-one (**78**), canthin-6-one *N*-oxide (**79**), as well as of the alkaloids isolated from *Zanthoxylum chiloperone* (*Rutaceae*) stem bark, in Balb/c mice infected either acutely or chronically with *T. cruzi*. The compounds were administered orally or subcutaneously at 5 mg/kg/day for 2 weeks, whereas the alkaloidal extract was given at 50 mg/kg/day for 2 weeks. The antiparasitic activity was compared with that of benznidazole given at 50 mg/kg/day for 2 weeks (Table 7).

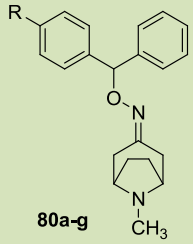
Table 7. Effect of benznidazole, canthin-6-one (**77**), 5-methoxy-canthin-6-one (**78**), canthin-6-one *N*-oxide (**79**), and crude *Zanthoxylum chiloperone* alkaloid extract in mice with acute *Trypanosoma cruzi* infection



Days post-infection	Mean parasitaemia (×104 parasites/ml blood±S. D.)								
	Control (*n* = 20)	Benznidazole (*n* = 20)	**77** (oral) (*n* = 20)	**77** (s.c.) (*n* = 20)	**78** (oral) (*n*=9)	**78** (s.c.) (*n*=8)	**79** (oral) (*n* = 9)	Crude *Zanthoxylum chiloperone* (oral) (*n*=8)	Crude *Zanthoxylum chiloperone* (s.c.) (*n*=8)
18	99.2 ± 65.1	2.6 ± 3.3	11.0 ± 7.2	15.3 ± 14.8	0.2 ± 0.3	50.9 ± 0.3	5.5 ± 4.1	20.5 ± 15.8	5.2 ± 2.3
25	323.3 ± 169.5	13.9 ± 10.7	14.9 ± 11.8	8.2 ± 8.9	9.7 ± 6.5	49.0 ± 36.0	65.4 ± 26.0	28.3 ± 18.8	81.3 ± 41.3
32	121.2 ± 91.2	12.7 ± 11.3	8.9 ± 7.5	38.5 ± 29.5	35.1 ± 28.8	15.5 ± 13.9	52.1 ± 20.7	34.3 ± 14.8	91.3 ± 50.3
39	61.6 ± 52.1	9.0 ± 8.2	5.2 ± 5.0	304.3 ± 215.2	130.9 ± 72.5	34.2 ± 14.6	30.7 ± 11.7	8.6 ± 5.3	43.6 ± 15.8
45	54.8 ± 37.7	8.2 ± 9.4	2.7 ± 2.5	8.3 ± 6.7	241.3 ± 142.8	271.3 ± 170.0	2.2 ± 1.5	3.1 ± 14.8	1.7 ± 1.1
53	43.4 ± 31.5	6.0 ± 5.8	2.1 ± 2.0	5.7 ± 4.6	206.7 ± 184.6	166.6 ± 143.4	0	3.3 ± 1.3	1.4 ± 0.8
60	115.1 ± 85.5	4.0 ± 3.7	1.1 ± 0.8	5.7 ± 4.3	13.4 ± 10.4	16.6 ± 11.9	0	1.2 ± 0.7	5.6 ± 2.4
68	71.1 ± 57.5	0.6 ± 0.5	0.1 ± 0.2	3.3 ± 2.7	49.3 ± 27.8	28.4 ± 23.7	0	0.7 ± 0.8	2.7 ± 1.7

Benzhydryl tropinone oximes have been previously identified as potently toxic to *T. cruzi*.[99] Thus, by using SAR techniques, Holloway and co-workers[100] found that part of the original compound was superfluous and all early SAR probes led to significant drops in activity. The replacement of the aryl chloride substituent with chloro homologues led to the discovery of a trifluoromethyl-containing analogue with an EC_{50} against *T. cruzi* of 30 nM and a cytotoxicity selectivity index of over 1000 relative to rat skeletal myoblast L-6 cells (Table 8).

Table 8. Series of potent trypanocides benzhydryl tropinone oximes

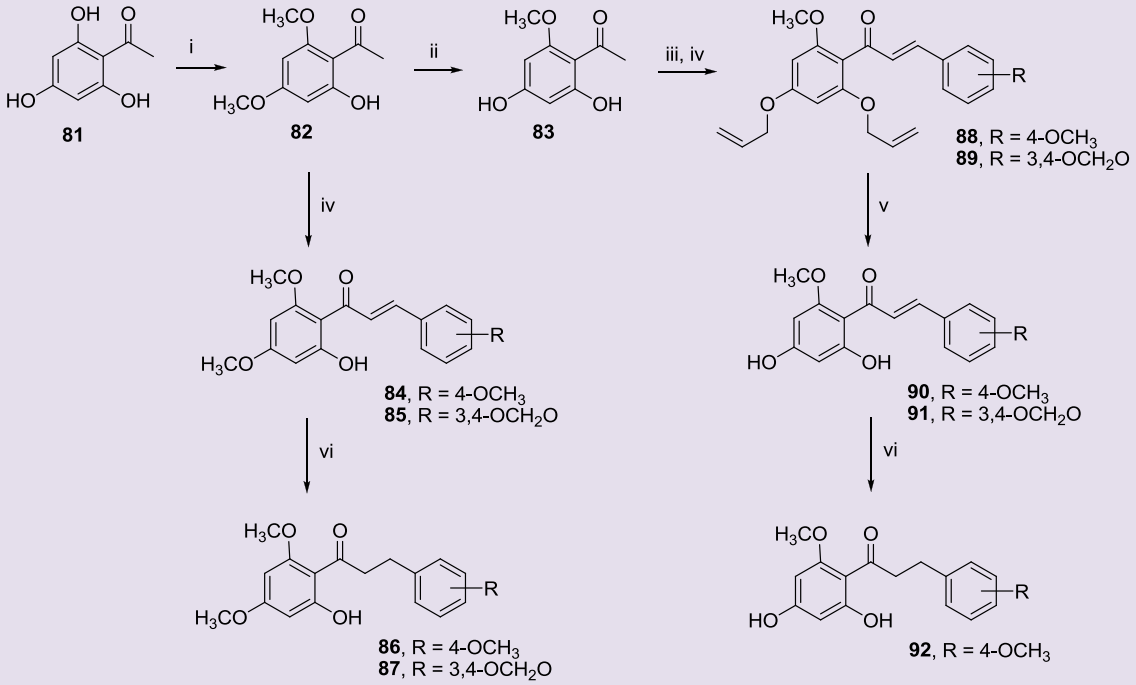


80a-g

Compound	R	EC_{50} (μM)	
		T. cruzi	Cytotoxicity
80a	Cl	0.07	15
80b	F	0.80	50
80c	Br	0.04	40
80d	I	0.04	39
80e	CH_3	0.30	51
80f	CN	0.40	53
80g	CF_3	0.03	35
Benznidazole	-	1.80	-

Aponte and co-workers[101] observed that the s cytotoxic dihydrochalcone isolated from a traditional Amazonian medicinal plant *Iryanthera juruensis* Warb (Myristicaceae) is significant trypanocidal activity. Through a comprehensive SAR analysis of synthetic saturated and unsaturated chalcone led to the identification of analogues with selective and significant *in vitro* trypanocidal activity. Further synthesis of 21 new chalcones containing two allyloxy moieties resulted in the discovery of 2',4'-diallyloxy-6'-methoxy chalcones with improved selectivity against this parasite at concentrations below 25 µM, four of which exhibited a selectivity index greater than 12 (Table 9 and Table 10).

Table 9. Strategy for the synthesis of compounds 84-92 and *in vitro* anti-*T. cruzi* activity

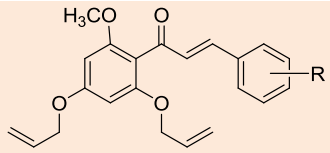


Reagents and conditions: (i) K_2CO_3, $(CH_3)_2SO_4$, $(CH_3)_2CO$, 65 °C, 6 h. (ii) $AlCl_3$, benzene, reflux, 1 h. (iii) K_2CO_3, allyl bromide, DMF, rt, overnight. (iv) Claisen-Schmidt aldol condensation of an acetophenone with an aromatic aldehyde, KOH, H_2O, CH_3OH, rt, 1-48 h. (v) K_2CO_3, catalytic $Pd(PPh_3)_4$, MeOH, 60 °C, 1 h. (vi) catalytic Pd/C 5%, H_2 gas, 250 psi, EtOAc, rt, 1.5 h.

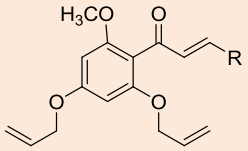
	IC_{50} (µM)		
Compound	*T. cruzi*	VERO[a]	SI[b]
84	>25	Nd	
85	>25	Nd	
86	>100	Nd	
87	>100	Nd	
88	21.4	99.9	4.7
89	13.6	73.5	5.4
90	>25	Nd	2.7
91	9.4	25.5	
92	>25	Nd	
Nifurtimox	0.52	80.1	154

[a]VERO, normal jAfrican green monkey kidney epithelial cells. [b]SI: Selectivity index = $IC_{50,VERO}/IC_{50,T.\ cruzi}$.

Table 10. *In vitro* trypanocidal Activity of Compounds **93a-n** and **94a-e**



93a-n



94a-e

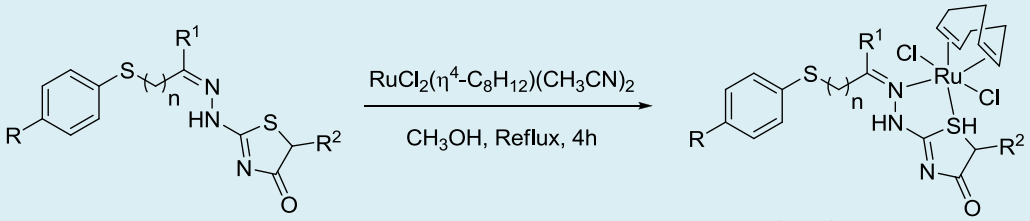
		IC_{50} (μM)		
Compound	R	*T. cruzi*	VERO[a]	SI[b]
93a	H	17.1	17.1	1.0
93b	4-CH_3	17.2	211.3	12.3
93c	3-OCH_3	14.2	141.9	9.9
93d	4-OH	20.3	76.4	3.8
93e	3-OCH_3,4-OH	>25	Nd	-
93f	2,4-OCH_3	13.1	92.6	7.1
93g	3,4-OCH_3	3.4	40.9	12.0
93h	4-CF_3	15.6	7.2	0.5
93i	4-Cl	8.6	10.4	1.2
93j	4-F	14.3	13.6	0.9
93k	2-F	6.2	16.3	2.6
93l	2-Br	13.9	13.9	1.0
93m	4-NO_2	4.1	12.6	3.1
93n	3,5-allyloxy,4-Br	6.9	96.3	13.9
94a	Styryl	>25	Nd	-
94b	pyridin-4-yl	1.5	2.8	1.9
94c	pyridin-2-yl	1.9	2.8	1.5
94d	1*H*-pyrrol-2-yl	>100	Nd	-
94e	furan-2-yl	12.2	190.9	15.6
Nifurtimox	-	0.52	80.1	154.0

[a]VERO, normal jAfrican green monkey kidney epithelial cells.
[b]SI: Selectivity index = $IC_{50,VERO}/IC_{50,T.\ cruzi}$.

3.2.3. Metal Complex

Donnici and co-workers[102] demonstrated that the complexation of bioactive ligands with ruthenium leads to a new set of trypanocidal agents with an attractive range of efficacy. The authors investigated the improvement of the ruthenium complexes on aryl-4-oxothiazolylhydrazones system against epimastigotes (proliferative form) and trypomastigotes (bloodstream form) of *T. cruzi*. In this study eight new ruthenium–ATZ complexes ($RuCl_2$ATZCOD) were prepared and evaluated *in vitro* assays against epimastigotes and trypomastigote forms of the parasite and also the cytotoxicity in mammals. Two of these complexes presented trypanocidal activity at non-cytotoxic concentrations on mammalian cells and of higher potency than its metal–free ligands, while the metallic precursor [$RuCl_2$COD$(MeCN)_2$] showed only moderate trypanocidal activity. The combined data from pharmacological tests are consistent with the conclusion that the **96h** complex constitutes an example of a potential prototype for a trypanocidal drug (Table 11).

Table 11. Synthesis of ruthenium complexes and i*n vitro* biological characterization of ATZ ligands and their ruthenium complexes



95a, R = R^1 = R^2 = H, n =1
95b, R = R^1 = H, R^2 = Et, n =1
95c, R = R^1 = R^2 = H, n =2
95d, R = H, R^1 = R^2 = CH_3, n =1
95e, R = R^2 = CH_3, R^1 = H, n =1
95f, R = CH_3, R^1 = R^2 = H, n =1
95g, R = Cl, R^1 = R^2 = H, n =1
95h, R = Br, R^1 = R^2 = H, n =1

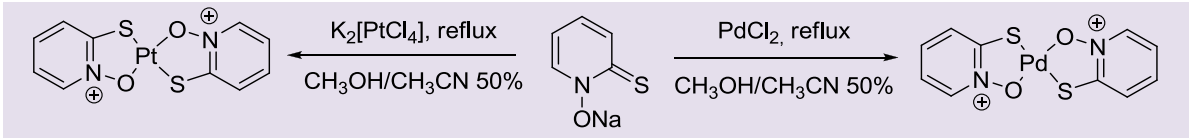
96a, R = R^1 = R^2 = H, n =1
96b, R = R^1 = H, R^2 = Et, n =1
96c, R = R^1 = R^2 = H, n =2
96d, R = H, R^1 = R^2 = CH_3, n =1
96e, R = R^2 = CH_3, R^1 = H, n =1
96f, R = CH_3, R^1 = R^2 = H, n =1
96g, R = Cl, R^1 = R^2 = H, n =1
96h, R = Br, R^1 = R^2 = H, n =1

Compounds	IC50 (µM) *T. cruzi*, Y strain		Cytotoxicity (µg/mL)[a]
	Trypomastigotes at 24 h	Epimastigotes at 11 days	
95a	7.8	35.0	>100
96a	6.2	3.8	1 (1.4)
95b	48.2	0.3	>100
96b	5.0	8.1	1.0
95c	10.0	2.9	>100
96c	6.4	10.9	1.0
95d	Nd	83.3	>100
96d	27.2	87.2	1.0
95e	84.8	63.4	>100
96e	7.0	11.4	1.0
95f	20.0	92.3	>100
96f	5.3	6.4	2 (4.5)
95g	82.4	43.9	>100
96g	3.3	2.4	1.0 (1.7)
95h	Nd	42.9	>100
96h	5.5	1.8	5.0 (8.0)
$RuCl2(\eta^4\text{-}C_8H_{12})(CH_3CN)_2$	Nd	12.7	1.0 (1.4)
Benznidazole	5.0	6.6	25
Nifurtimox	8.5	1.9	1.0 (3.4)

[a]Values in µM are showed in parentheses.

Vieites and co-workers[103] investigated the action of palladium and platinum complexes on 2-mercaptopyridine N-oxide (mpo) for new therapeutic tools against Chagas disease. Both complexes showed high in vitro growth inhibition activity (IC_{50} values in the nanomolar range) against *T. cruzi* being 39-115 times more active than the trypanocidal drug Nifurtimox (Table 12).

Table 12. *In vitro* biological activity of the free ligand and its palladium and platinum complexes and comparison of 50% inhibitory concentration (IC50) values for the parasite and for macrophages



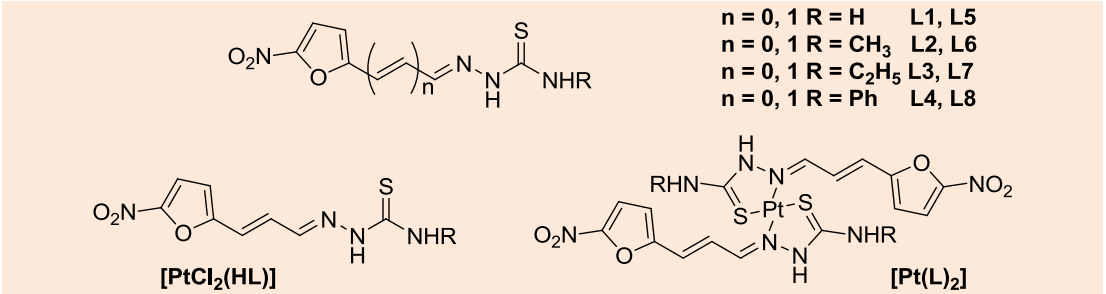
98, $Pt(mpo)_2$ **97**, Na(mpo) **99**, $Pd(mpo)_2$

Compound	IC_{50} *T. cruzi* (µM)	IC_{50} macrophages (µM)	Selectivity Index[a]
Na(mpo)	0.190 ± 0.015	0.85	4.5
$Pd(mpo)_2$	0.067 ± 0.015	0.33	4.9
$Pt(mpo)_2$	0.200 ± 0.018	>>2.0	>>10
Nifurtimox	7.700 ± 0.500	-	-

[a] IC_{50}(macrophages)/IC_{50}(*T. cruzi*)

Vieites and co-workers[104] synthesized eight new platinum(II) complexes with bioactive 5-nitrofuryl containing thiosemicarbazones (L = L1–L4) as ligands with the formula $[PtCl_2(HL)]$ and $[Pt(L)_2]$ that showed *in vitro* trypanocidal activity. Most of the Pt complexes were active against epimastigotes of *T. cruzi* Tulahuen 2 strain, showing many of them IC_{50} values of the same order than nifurtimox and the corresponding free ligands. According to the IC_{50} values, $[PtCl_2(HL1)]$ and both L2 complexes were the most active Pt complexes against this parasite strain, showing similar IC_{50} values to those of nifurtimox and benznidazol (Table 13).

Table 13. Schematic structure of 5-nitrofuryl containing thiosemicarbazones ligands and the two series of platinum(II) complexes and i*n vitro* biological activity of the Pt complexes, IC50 values of the free ligand and their Pd complexes on *T. cruzi* (Tulahuen 2 strain)



$[PtCl_2(HL)]$ **$[Pt(L)_2]$**

Compound	*T. cruzi* (Tulahuen 2)			*T. cruzi* (Dm28c)
	PGI[a]		IC_{50} (µM)	IC_{kc50} (µM)
	10 µM	25 µM		
$[PtCl_2(HL5)]$	56.1	78.6	8.6	37.04
$[Pt(L5)_2]$	37.7	50.0	25.0	6.12
$[PtCl_2(HL6)]$	50.0	74.8	10.0	26.96
$[Pt(L6)_2]$	54.3	80.5	9.1	5.89
$[PtCl_2(HL7)]$	40.2	65.1	13.7	24.40
$[Pt(L7)_2]$	6.4	21.3	>25	33.63
$[PtCl_2(HL8)]$	31.5	34.5	>25	63.23
$[Pt(L8)_2]$	4.3	8.5	>25	48.22
Benznidazol	-	-	7.4	38.00
Nifurtimox	-	-	6.1	22.79

[a]PGI: percentage of growth inhibition of *T. cruzi* epimastigote cells at the specified dose.

4. Final Remarks

Today after ten years of discovery of Chagas disease still an innumerous factors limit the therapeutic for Chagas disease. Primarily because of low efficacy, poor activity against many *T. cruzi* isolates circulating in different geographic areas and considerable side effects of existing drugs. Another factor is the cost of investments and the lack of market potential and market security in developing countries have dampened interest in developing drugs for Chagas disease. And because of that in the past few decades, few compounds have moved to clinical trials due to the minimal investments allocated to this area the lack of standardized protocols for drug screening. Fortunately, nowadays great advances are being made in many parts of world with the advent of bioinformatics, combinatorial chemistry and synthesis of new compounds, especially in the area of quinones and extensive knowledge has accumulated. These research efforts maybe bring in the future new insight toward the discovery of more selective and successful compounds.

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