Anti-*T. cruzi* Agents: Our Experience in the Evaluation of More than Five Hundred Compounds

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Abstract: Chagas' disease is the major endemic disease in South and Central America caused by a trypanosomatid parasite (*Trypanosoma cruzi*). The current treatment relies on two old and non-specific chemotherapeutic agents, Nifurtimox and Benznidazole. Despite the major advances that have been made in the identification of specific targets that afford selectivity, the drugs used today have serious side effects. Furthermore, differences in drug susceptibility among different *T. cruzi* isolates have led to varied parasitological cure rates depending on the geographical region. There is, therefore, an urgent need for the development of new antichagasic drugs. In this regard we have spent more than a decade in the search for more effective agents able to compromise the proliferation of *T. cruzi.* We began our research with our own compounds and then continued with compounds from other researcher groups. We systematically characterized representatives of a wide range of different chemical families. In this review we summarize our ongoing efforts to identify potential anti-*T. cruzi* agents using our compound-library. It is discussed and presented the structure-activity relationship observed among the different groups of chemical families.

[#]**Author Profile:** Hugo Cerecetto was born in Uruguay in 1962, studying pharmacy in Montevideo and did doctoral work on the R&D of new β3-adrenergic drugs at the University of Navarra (Spain). He came back to the University of the Republic (Montevideo, Uruguay), where he then became associate professor of organic chemistry. His research interests include the development of new chemical entities for use in the field of third world parasitic diseases, especially Chagas disease, cancer and other aging diseases.

1. INTRODUCTION

Chagas' disease (CD) or American trypanosomiasis is incited by several strains of the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*), order Kinetoplastida [1]. This disease represents a serious public health problem in the countries and zones where it is endemic (21 countries in Central and South America) because there are no effective methods of immunoprophylaxis or chemotherapy. Currently, there are an estimated 16-18 million persons infected and 100 million people at risk [2]. Cases have also been reported in the U.S. and Canada, as a result of transfusion-related infection [3-5]. *T. cruzi* can be transmitted to humans by blood-sucking reduviid bugs (*Triatoma infestans* and *Triatoma rubrovaria)* which deposit their infective faeces on the skin at the time of biting. The disease is characterized by an initial acute phase during which the flagellated parasites (trypomastigotes) multiply in the blood, followed by an indeterminate phase with very low parasitemia and no apparent pathology. In the acute form, in which 5% of infected children die, CD is manifest generally as fever, malaise, facial edema, generalized lymphadenopathy, and hepatosplenomegaly. The acute illness often spontaneously resolves in four to six weeks. Susceptible hosts then enter a chronic phase with increasing tissue damage, mostly in cardiac and skeletal muscle tissues but sometimes in the liver, spleen, colon, or esophagus, progressively leading to cardiac failure and death [6,7]. There is still considerable debate about the mechanisms involved in the pathology [8,9]. Some evidence indicates that damage is associated with the presence and replication of intracellular amastigotes in host tissues, while other studies have shown that autoimmunity induced by parasite antigens mimicking host proteins is responsible for tissue damage. Indeed, standard histopathological analysis of tissues from chronically infected hosts shows that there are abundant inflammatory cells but few or no amastigotes nests, suggesting that the pathology may develop in an almost complete absence of the parasite. A decreasing trend observed in the prevalence of house infestation by *Triatoma infestans* and in the incidence of human infection in children and youngsters is observed in the countries of the *Initiative for the Elimination of Chagas Disease in the Southern Cone Countries* (Argentina, Bolivia, Brazil, Chile, Paraguay and Uruguay) by the use of pyrethroid insecticides [10]. Two countries have already been declared free of transmission, Uruguay in 1997 and Chile in 1999. In 2001, the World Health Organization (WHO) estimated that vectorial transmission had been interrupted in the most of the endemic zones in Argentina and Brazil [11]. Currently, chemotherapeutic treatment of CD primarily involves only two drugs, Nifurtimox (**1**, **Nfx**, 4-[(5-nitrofurfurylidene) amino]-3-methylthiomorpholin-1,1-dioxide, BAY 2502, Lampit, Fig. (**1**)) and Benznidazole (**2**, **Bnz**, *N*-benzyl-2-(2-nitroimidazole)acetamide, Ro 7-1051, Radanil, Rochagan, Fig. (**1**)) [12]. Both drugs are active against the trypomastigote and amastigote forms of the parasite, but in therapy have some important limitations, including: variable efficacy - restricted to the acute phase with uncertain results during the chronic phase-, toxicity and parasite resistance, in addition to problems of supply. Gentian Violet (**3**, **GV**, [4-[bis-(*p*-dimethylaminophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-

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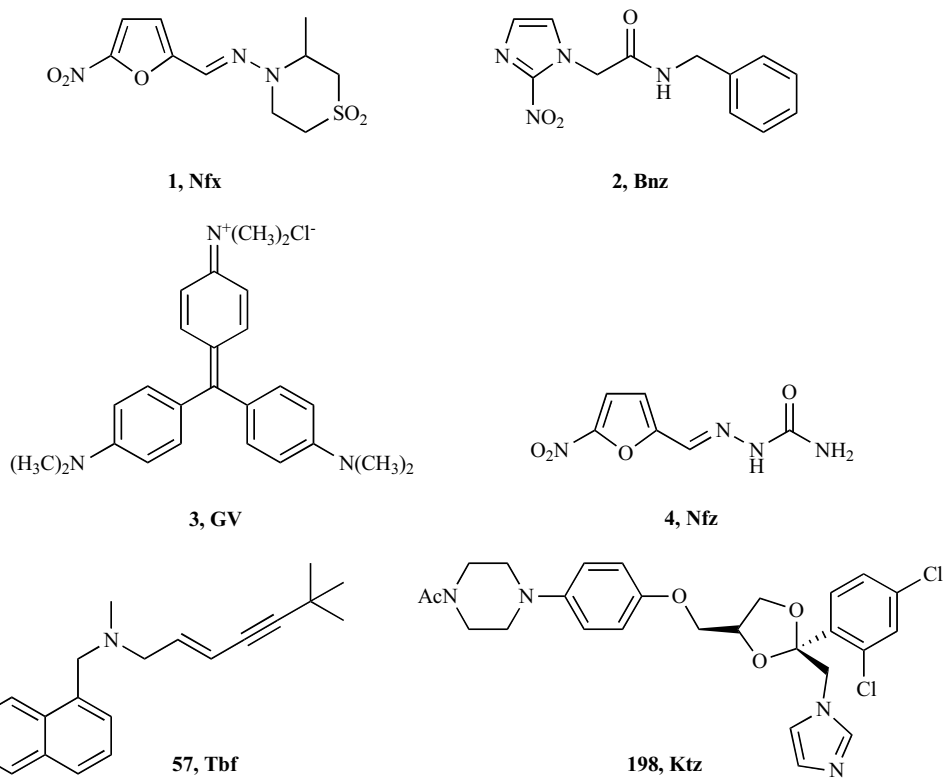


Fig. (1). Clinical and experimental drugs used as anti-*T. cruzi* agents.

N-methylmethanaminium chloride, Fig. (**1**)) is widely used in blood banks to eliminate trypomastigotes from infected human blood. The limited available treatment emphasizes the need for new and more specific therapeutic agents for more effective control. Genomic and systems-based methods continue to clarify cellular processes and thus potential specific molecular targets of *T. cruzi* and related protozoa. These efforts have helped to organize the search for novel trypanocidal agents into a far more defined process.

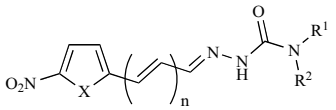
The last ten years of investigation in our laboratory are reviewed for the development of new anti-trypanosomal drugs. We describe the different families of compounds that have been studied as possible anti-*T. cruzi* agents using different *in vitro* and *in vivo* models. Proposed mode-of-action studies are also depicted and discussed. Structurally, our library of more than five-hundred compounds can be grouped as: 5-nitrofuryl- and 5-nitrothienyl-derivatives, furoxanyl- and benzofuroxanyl-derivatives, quinoxalinyl-derivatives, imidazolyl- and benzimidazolyl-derivatives, indazolyl-derivatives, metallic complexes and related derivatives.

2. 5-NITROFURYL- AND 5-NITROTHIENYL DERIVATIVES AND RELATED COMPOUNDS

The mode of action of anti-trypanosomal compounds has been studied extensively in the last forty years. Since the 1970's, the production of free radicals by a great variety of antiparasite drugs was accepted as their principal mode of action. Anti-*T. cruzi* nitroheterocycles, quinones and quinone-imine dyes, e.g., were studied from this perspective, but it became clear in some cases that the actual mechanism of parasite death was not yet fully understood [12]. We investigated a series of 5-nitrofuryl- and 5-nitrothienyl-derivatives with a potential oxidative stress (OS) mode of action, with the idea that parasite-death could be mediated by free-radicals. In this sense, we focused on six different kinds of moieties-containing 5-nitrofuryl and 5-nitrothienyl system: semicarbazone-, thiosemicarbazone-, carbazate-, ester-, amide-, and thioamide-derivatives. Chronologically, our study was initiated by developing Nitrofurazone (**4**, **Nfz**, 1-[(5-nitrofuran-2-yl)methylene]semicarbazide, Fig. (**1**)) derivatives substituting N^4-nitrogen by alkyl and aryl chains having different electronic, lipophilic and steric properties [13,14]. In this first approachs, 5-nitrothienyl analogues were produced to investigate the influence of the heterocycle on activity. Experimental and theoretical studies were done to better understand the mode of action of this series of semicarbazones [15-19]. We found that the contribution to parasite death caused by these compounds could be by OS, but the involvement of the inhibition of specific parasite enzymes was also suggested. From these studies some structural modifications were made to further test this idea, generating, e.g., 5-nitrofurylacroleine derivatives [20,21]. Table **1** summarizes the structure and properties of these studied semicarbazones, **5-30**. In an *in vivo* study, using a murine model of Chagas' disease, it was concluded that semicarbazones **5**, **7** and **19** should be tested further in additional preclinical studies.

In 2004 McKerrow *et al*. described potent small-thiosemicarbazone inhibitors of the *T. cruzi* cysteine protease cruzain [22,23]. A series of thiosemicarbazone containing 5-nitrofuranes were then developed and evaluated biologically [24] (Table **2**). Like the semicarbazone derivatives, the

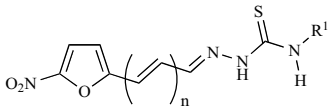
Table 1. Semicarbazones Containing 5-Nitroheterocycles Evaluated as Anti-*T. cruzi* Agents Both *In Vitro* and *In Vivo*



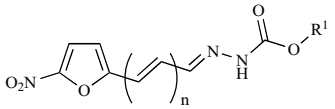
Ref.	X	n	-R^1	-R^2	PGI (%)[a,b]	*In vivo* evaluation PSA_{28}[c,d]
5	O	0	-H	$-CH_2(CH_2)_2CH_3$	51.0	100
6	O	0	-H	$-CH_2(CH_2)_4CH_3$	62.0	60
7	O	0	-H	$-CH_2CH_2OCH_3$	50.0[e]	100
8	O	0	$-NR_1R_2$= 4-morpholinyl		2.0[e]	60
9	O	0	-H	$-CH_2CH_2Ph$	16.0[e]	80
10	O	0	-H	$-CH_2CH_2$-Ph-3,4-di-OCH_3	23.0[e]	40
11	O	0	-H	$-CH_2CH_2CH_2NHPh$	28.0	ns[f]
12	O	0	-H	$-CH_2CH_2CH_2N(CH_3)_2$	1.0[e]	80
13	O	0	-H	$-CH_2CH_2CH_2N(CH_2CH_3)_2$	1.0[e]	80
14	O	0	-H	$-CH_2CH_2CH_2N(CH_2)_5$	27.0[g]	ns
15	O	0	-H	-α-naphthyl	32.0	ns
16	O	0	-H	-β-naphthyl	59.0	ns
17	O	1	-H	-H	65.0	ns
18	O	1	-H	$-CH_2(CH_2)_2CH_3$	58.0	ns
19	O	1	-H	$-CH_2(CH_2)_4CH_3$	82.0	100[h]
20	O	1	-H	$-CH_2CH_2OCH_3$	27.0	ns
21	O	1	-H	-β-naphthyl	24.0	ns
22	S	0	-H	$-CH_2(CH_2)_2CH_3$	3.0[e]	60
23	S	0	-H	$-CH_2(CH_2)_4CH_3$	4.0[e]	20
24	S	0	-H	$-CH_2CH_2OCH_3$	0.0[e]	60
25	S	0	$-NR_1R_2$= 4-morpholinyl		0.0[e]	20
26	S	0	$-NR_1R_2$= 4-methylpiperazin-1-yl		0.0[e]	ns
27	S	0	-H	$-CH_2CH_2Ph$	3.0[e]	0
28	S	0	-H	$-CH_2CH_2$-Ph-3,4-di-OCH_3	0.0[e]	40
29	S	0	-H	$-CH_2CH_2CH_2N(CH_3)_2$	1.0[e]	ns
30	S	0	-H	$-CH_2CH_2CH_2N(CH_2)_5$	33.0[g]	ns
1 (Nfx)	-	-	-	-	46.0	
4 (Nfz)	O	0	-H	-H	60.0	

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 5 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain. [c] PSA_{28}: percentage of animal survival, at day 28th, treated with studied compounds [14]. Values for **Nfx**: 100 %; **Bnz**: 95 %; control: 65 %. [d] The animals were treated orally during 10 days at 66 mg/kg body weight/day. [e] At 10 μM. [f] ns: not studied. [g] At 25 μM. [h] Data not published.

Table 2. 5-Nitrofuryl Derivatives and Trypanocidal Activities

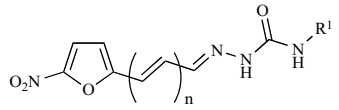


Ref.	n	-R^1	PGI (%)[a,b]
31	0	-H	69.0
32	0	$-CH_3$	51.0
33	0	$-CH_2CH_3$	51.0
34	0	-Ph	14.0
35	1	-H	74.0
36	1	$-CH_3$	60.0
37	1	$-CH_2CH_3$	74.0
38	1	-Ph	78.0



Ref.	n	-R^1	PGI (%)[a,b]
39	0	$-CH_3$	32.0
40	0	$-CH_2(CH_2)_2CH_3$	84.0
41	0	$-CH_2(CH_2)_4CH_3$	96.0
42	0	$-CH_2(CH_2)_5CH_3$	93.0
43	0	$-CH_2(CH_2)_6CH_3$	84.0
44	1	$-CH_3$	58.0
45	1	$-CH_2(CH_2)_2CH_3$	87.0
46	1	$-CH_2(CH_2)_4CH_3$	86.0
47	1	$-CH_2(CH_2)_5CH_3$	90.0
48	1	$-CH_2(CH_2)_6CH_3$	92.0
49	0	-Ph	28.0
50	1	-Ph	61.0
51	0	$-CH_2CH=CH_2$	60.0
52	0	$-CH_2$-*t*-CH=CHPh	72.0
53	1	$-CH_2CH=CH_2$	64.0
54	1	$-CH_2$-*t*-CH=CHPh	73.0

(Table 2. Contd….)



Ref.	n	-R^1	PGI (%)[a,b]
55	0	$-CH_2C(CH_3)=CH_2$	20.0
56	1	$-CH_2C(CH_3)=CH_2$	60.0
57 (Tbf)	-	-	74.0[c]

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 5 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain. [c] At 25 μM.

thiosemicarbazones derived from 5-nitrofurylacroleine were more active than those derived from 5-nitrofurfural. Moreover, derivatives **35** and **36** were less toxic than derivatives **5** and **7** (Table **1**) in an *in vivo* model of toxicity testing using healthy CD1 female mice. Studies on their mode of action have shown that thiosemicarbazones **31-34** may act on the parasite by an OS mechanism, while derivatives **35-38** do not appear to have this kind of effect [25]. Carbazate containing 5-nitrofuryl derivatives were also developed, and the following was found when assayed (Table **2**) [20,21]. In general, the carbazate derivatives were found to be better anti-*T. cruzi* agents than either the semicarbazone and thiosemicarbazone analogs *in vitro*. Interestingly, derivative **42**, unlike **Nfx**, may act by inhibiting the parasite respiratory cycle without oxygen redox cycling [26]. A series of carbazates together with two semicarbazone-containing 5-nitrofuranes have been recently developed as parasite membrane sterol biosynthesis (MSB) inhibitors (**51-56**, Table **2**) [27]. With this in mind, the compounds were designed with the incorporation of an allyl moiety much like the well known MSB inhibitor, Terbinafine (**57**, **Tbf**, (*E*)-*N*,6,6-trimethyl-*N*-(naphthalene-1-ylmethyl)hept-2-en-4-yn-1-amine, Fig. (**1**)). These compounds caused inhibition of the *de novo* sterol biosynthesis at the level of squalene epoxidase (SE). Derivatives **49** and **50** were generated as secondary products in the synthetic procedures, but without of the allyl moiety, but were still able to inhibit production of mature parasite sterols, and also caused the accumulation of squalene.

To analyze the effect of other moieties, like ester, amide, thioamide and related functional groups in the 5-nitrofuranes anti-*T. cruzi* activity, derivatives **58-75** were developed and *in vitro* evaluated (Table **3**) [20,21,28]. The best biological results were obtained with the amide derivatives and this could be interpreted as a possible adequate interaction with residues of the active site of one of the most relevant *T. cruzi* enzymes, trypanothione reductase (TR) [20,29]. Derivative **66** generates oxygen redox cycling in *T. cruzi* epimastigotes and in addition, this compound was correlated with hydroxyl radical production in the parasite much like **Nfx** as determined by electron spin resonance (ESR) [26].

Other structural approaches involved the preparation of nitroaryl derivatives by C=C formation through a condensation reaction between appropriate aldehydes and carbanion nucleophiles. These procedures were done with 5-nitrofurfural, 5-nitrothiophene-2-carbaldehyde and 4-nitrobenzaldehyde (Table **4**) [21,30]. While derivatives **76** and **77** were inactive against *T. cruzi* epimastigote, only the thienyl derivative **78** showed some activity on *T. cruzi* amastigote at one doses that does not have toxic effects in macrophages J774 [31]. The 4-nitrophenyl derivatives **88-92** had some anti-amastigote activities at the doses assayed.

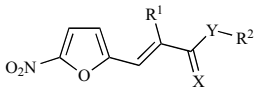
3. *N*-OXIDE CONTAINING HETEROCYCLE DERIVATIVES AND RELATED COMPOUNDS

Similar to the nitro-pharmacophore of antitrypanosomal drugs, the *N*-oxide moiety has been shown to be responsible for the biological activity of numerous drugs through the production of free radical species (including antitumoral, and antibacterial agents) [32-34]. These data suggested the possibility of designing new antitrypanosomal compounds where the *N*-oxide moiety would be the pharmacophore that generates the toxic radical species (Fig. (**2**)). Based in this idea, we designed and developed different series of *N*-oxide containing heterocycles as potential anti-*T. cruzi* agents. The next section discusses the compounds studied in chronological order.

3.1. Furoxanyl and Benzofuroxanyl Derivatives and Related Compounds

The first approach in the identification of *N*-oxide containing heterocycles with trypanocidal activity was based on the conjunction of different *N*-oxide systems and the semicarbazide moieties present in derivatives **5** and **6** (Table **1**). Previous studies [35-37] supported the idea that compounds bearing a positive charge and/or a flexible side chain "spermidine like" are able to inhibit TR, subsequently "spermidine-mimetic" residues were also included as substituents in the N^4-semicarbazide moiety. Initially we chose 1,2,5-oxadiazole *N*-oxide (furoxan) as an heteroaromatic *N*-oxide system, due to its structural similarity with the nitrofuran heterocycle (**93-100**, Table **5**) [38]. In general, the furoxan derivatives had very low activities when tested with the Tulahuen 2 epimastigote parasite at 25 μM doses *in vitro*. In a collaborative effort between our laboratory and the research group of Dr. Massimo Bertinaria, almost one hundred of furoxan derivatives and deoxygenated derivatives, e.g., furazan, from Bertinaria's chemical-library were biologically evaluated. Some relevant nitric oxide-releasing derivatives [39,40] with anti-*T. cruzi* activity (**101-106**, Table **5**) were found [41]. In compounds **101-103**, the presence and position of the *N*-oxide moiety could play a role in the observed

Table 3. Ester, Thioamide and Amide Containing 5-Nitrofuryl Derivatives with Different Anti-*T. cruzi* Activities



Ref.	-R[1]	X	-Y-	-R[2]	PGI (%)[a,b]
58	-H	O	-O-	-H	20.0
59	-H	O	-O-	-1-succinimidyl	6.0
60	-H	O	-O-	$-CH_3$	39.0
61	-H	O	-O-	$-CH_2(CH_2)_2CH_3$	42.0
62	-H	O	-O-	$-CH_2(CH_2)_4CH_3$	17.0
63	$-NH_2$	O	-O-	$-CH_2CH_3$	0.0
64[c]	$-NH_2$	O	-O-	$-CH_2CH_3$	9.0
65	-H	O	-NH-	$-CH_2(CH_2)_2CH_3$	75.0
66	-H	O	-NH-	$-CH_2(CH_2)_4CH_3$	82.0
67	-H	O	-NH-	$-CH_2CH_2OCH_3$	31.0
68	-H	O	-NH-	$-CH_2$(2-furyl)	14.0
69	-H	O	-NH-	$-CH_2CO_2CH_2CH_3$	35.0
70	-H	O	-NH-	$-CH_2CH_2Cl$	60.0
71	-H	O	-NH-	-1-adamantyl	91.0
72	[d]				47.0
73	-H	S	-NH-	$-CH_2(CH_2)_2CH_3$	52.0
74	-H	S	-NH-	$-CH_2(CH_2)_4CH_3$	11.0
75	-H	S	-NH-	$-CH_2CH_2OCH_3$	12.0

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 5 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain. [c] Thienyl derivative. [d] Chemical structure.

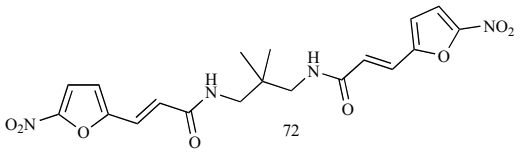


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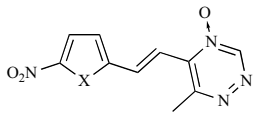
Table 4. Nitroheterocycles Condensed with Azaheterocycles Evaluated as Anti-*T. cruzi* Agents



Ref.	X
76	O
77	S

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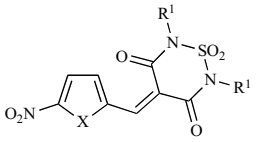


Ref.	X	-R^1	Ref.	X	-R^1	Ref.	X	-R^1
78	S	-cyclohexyl	**83**	O	-cyclohexyl	**88**	C=C	-cyclohexyl
79		$-CH_2(CH_2)_2CH_3$	**84**		$-CH_2(CH_2)_2CH_3$	**89**		$-CH_2(CH_2)_2CH_3$
80		$-CH_2(CH_2)_4CH_3$	**85**		$-CH_2(CH_2)_4CH_3$	**90**		$-CH_2(CH_2)_4CH_3$
81		-benzyl	**86**		-benzyl	**91**		-benzyl
82		$-CH_2CH_2Ph$	**87**		$-CH_2CH_2Ph$	**92**		$-CH_2CH_2Ph$

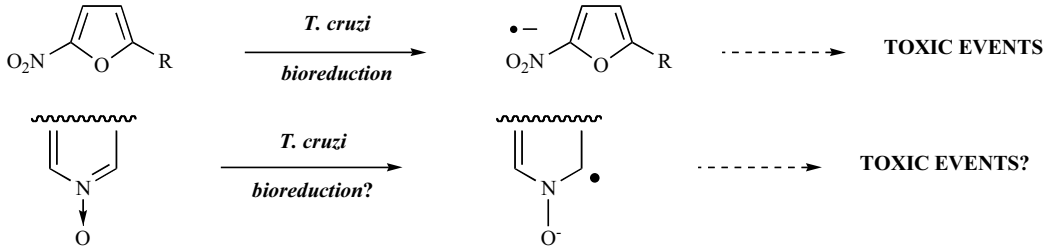
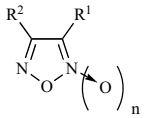


Fig. (2). Speculative mode of action of anti-*T. cruzi* activity of *N*-oxide derivatives.

Table 5. Furoxan and Furazan Derivatives Developed and Tested as Inhibitors of *T. cruzi* Proliferation



Ref.	n	-R^1	-R^2	PGI (%)[a,b]
93	1	$-CH_3$	$-CH=NNHCONHCH_2(CH_2)_2CH_3$	0.0
94	1	$-CH_3$	$-CH=NNHCONHCH_2(CH_2)_4CH_3$	3.0
95	1	$-CH_3$	-CH=NNHCONH-3-(4-morpholinyl)propyl	0.0
96	1	$-CH_3$	-CH=NNHCONH-3-(1-piperidinyl)propyl	0.0
		-R^2	-R^1	
97	1	-Ph	$-CH=NNHCONHCH_2(CH_2)_2CH_3$	17.0
98	1	-Ph	$-CH=NNHCONHCH_2(CH_2)_4CH_3$	53.0
99	1	-Ph	-CH=NNHCONH-3-(diethylamino)propyl	22.0
100	1	-Ph	-CH=NNHCONH-3-(4-morpholinyl)propyl	38.0
101	1	-Ph	-CN	100.0
102	1	-CN	-Ph	15.0
103	0	-Ph	-CN	2.0
104	1	-Ph	$-SO_2CH=CH_2$	97.0

(Table 5. Contd….)

Ref.	n	-R^1	-R^2	PGI (%)[a,b]
105	1	-$SO_2CH=CH_2$	-Ph	94.0
106	0	-Ph	-$SO_2CH=CH_2$	99.0

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain.

activities and was apparently related to nitric oxide-release capability. However, presence or position of the *N*-oxide in derivatives **104-106** did not influence the activity. Maybe a different mode of action could be involved in the anti-*T. cruzi* activities because of the cysteine proteases inhibitors properties of derivatives **104-106** [40]. A detailed study of the anti-*T. cruzi* mode of action of these derivatives is currently in progress.

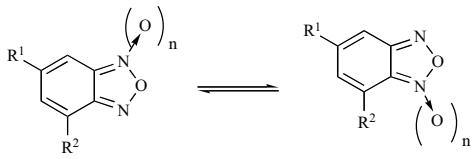
Due to the low activity observed in the first furoxan series analyzed, we extended the study to include benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxide (benzofuroxan) and quinoxaline *N,N'*-dioxide (see below). The first studied benzofuroxans were semicarbazone analogs of furoxan **93-100**, as such an amide moiety was included in this first study (**107-118**, Table **6**) [38]. Chemically, the substituted benzofuroxans were obtained as mixtures of tautomers which were inseparable at room temperature by standard purification methods (see equilibrium in Table **6**) [33]. These compounds were tested for their ability to generate free radicals in mammal microsomes, to provide support for the ability to produce an OS [42]. In order to corroborate the relevance of the *N*-oxide group in the activities of this series, some deoxygenated derivatives were developed, e.g., benzofurazan, and a complete lost of anti-*T. cruzi* activity resulted. An interesting feature in this class of compounds was that the most lipophilic derivatives had the highest activities. Therefore, in a second approach a new series of semicarbazones, amides and related compounds were developed in which the lipophilicity of the lateral amide chain was modified (derivatives **122-133**, Table **6**) [43]. One of the most lipophilic amides, **124**, was the most trypanocidal agent.

The benzofuroxans **107-118** were developed from the prototype nitrofuranes **5** and **6**, and the second-generation benzofuroxans, **122-133**, from prototypes **111** and **112**. Subsequently, we applied a synthetic plan based on the Hansch series design using cluster methodology [44] to produce a third family of derivatives. To identify the most active cluster of benzofuroxans, compounds **134-153** (Table **7**) were biologically evaluated [45,46].

Table **7** also includes previously synthesized di-substituted benzofuroxans [43] in order to show that the presence of bromine (-R^2= -Br) in the benzo-cycle, with the exception of derivative **156**, does not improve the trypanocidal activity (compare derivatives **135** and **154**, and derivatives **143** and **155**). As it had been observed previously, the absence of the *N*-oxide moiety in derivatives **161-163**, results in a total loss of activity. Gasco *et al*. [47] noted that in presence of oxyhemoglobin benzofuroxans are susceptible to being metabolized to the corresponding *o*-nitroaniline derivative. Taking into account that we incorporate hemin into the *T. cruzi* culture medium, in the *in vitro* tests, evaluation of benzofuroxan metabolically-generated *o*-nitroaniline products **164-167**, were tested to check whether the benzofuroxan biological activities were due to hemin-mediated reduction products. None of the analyzed *o*-nitroanilines significantly inhibited *T. cruzi* growth (Fig. (**3**)) showing that these species are not the active form.

These studies also involved 2D- and 3D-quantitative structure-activity relationship (QSAR) analysis, and relevant activities were identified, the best being benzofuroxan derivatives **138**, **146-149** and **156** as determined by *in vitro* trypanocidal activity. From these new prototypes we selected the best-synthetically modifiable backbone and new generations of compounds were developed from the phenylalkenes **148/149** and from the halomethyl-derivative, **146**. In this way, the bromomethyl derivative **168** (Table **7**) was prepared and its biological evaluation indicated excellent *in vitro* behavior as did the rest of the halomethyl analogues, **146** and **147**. Interestingly, the iodo-derivative, **147**, had an apparently different mode of action compared with the chloro- and bromo-derivatives in inhibiting parasite respiration [48]. A series of 5-alkenylbenzofuroxan derivatives were also developed (**169-193**, Table **8**), and were evaluated *in vitro*, and shown to have activity against different strains/clones of *T. cruzi*, i.e. Tulahuen 2, CL Brener, Y and Colombiana [46, 49]. For some of the most potent trypanocidal derivatives, the toxicity against mammalian cells was evaluated using THP-1 human macrophages (see Table **8**) obtaining values for selectivity indexes (SI) (mammal/parasite) since 9 for Tulahuen 2 strain. In this study **Tbf** (**57**) and Ketoconazole (**198**, **Ktz**, (±)-*cis*-1-acetyl-4-{*p*-[2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy}phenylpiperazine, Fig. (**1**)) were included as reference trypanocidal compounds. As observed previously, the absence of the *N*-oxide moiety, derivatives **194-197**, produces inactive compounds. Clearly, the substituent of the olefin modifies the trypanocidal activity in that, in general, the phenyl substituted with an electron withdrawing group is less active than one with an electron donating group. Aliphatic chains in the alkene-substitution thus result in derivatives with low activity. Parent compounds **148** and **149** and derivatives **176**, **180** and **181** were investigated in a pre-clinical study in which aspects related to scale-up [50], analytical procedures [51], *in vivo* effects [52], metabolism and mutagenicity studies were analyzed. The two last studies indicated are currently in progress. From *in vivo* studies, the parent compounds appear to be excellent for treatment of *T. cruzi* infections from different origins. Regarding the scale-up studies, we found a convenient, efficient, safe, and cheap method for developing these benzofuroxans as excellent candidates for drug development.

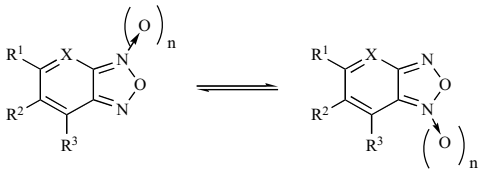
Table 6. First Generation Benzofuroxan and Benzofurazan Derivatives Tested as Trypanocidal Agents



Ref.	n	-R^1	-R^2	PGI (%)[a,b]
107	1	-CH=NNHCONHCH$_2$(CH$_2$)$_2$CH$_3$	-H	45.0
108	1	-CH=NNHCONHCH$_2$(CH$_2$)$_4$CH$_3$	-H	55.0
109	1	-CH=NNHCONH-3-(dimethylamino)propyl	-H	11.0
110	1	-CH=NNHCONH-3-(diethylamino)propyl	-H	8.0
111	1	-CONHCH$_2$(CH$_2$)$_2$CH$_3$	-H	90.0
112	1	-CONHCH$_2$(CH$_2$)$_4$CH$_3$	-H	79.0
113	1	-CONH-3-(dimethylamino)propyl	-H	15.0
114	1	-CONH-3-(diethylamino)propyl	-H	17.0
115	1	-CONH-3-(4-morpholinyl)propyl	-H	19.0
116	1	-CONH-3-(1-piperidinyl)propyl	-H	45.0
117	1	-CH$_2$NHCH$_2$(CH$_2$)$_2$CH$_3$	-H	21.0
118	1	-CH$_2$NNHCONHCH$_2$(CH$_2$)$_2$CH$_3$	-H	55.0
119	0	-CH=NNHCONHCH$_2$(CH$_2$)$_2$CH$_3$	-H	0.0
120	0	-CONHCH$_2$(CH$_2$)$_2$CH$_3$	-H	0.0
121	0	-CONHCH$_2$(CH$_2$)$_4$CH$_3$	-H	10.0
122	1	-CONHCH$_2$CH$_2$OCH$_3$	-H	0.0
123	1	-CO-4-morpholinyl	-H	1.0
124	1	-CONHPh-4-Cl	-H	35.0
125	1	-CH=CHCONHCH$_2$(CH$_2$)$_2$CH$_3$	-H	0.0
126	1	-CSNHCH$_2$(CH$_2$)$_2$CH$_3$	-H	6.0
127	1	-SO$_2$NH$_2$	-H	7.0
128	1	-CH=NNHCONHCH$_2$CH$_2$OCH$_3$	-H	0.0
129	1	-CH=NNHCONHCH$_2$CH$_2$Ph	-H	0.0
130	1	-CH=NNHCO$_2$CH$_3$	-H	5.0
131	1	-CH=NNHCONH$_2$	-H	0.0
132	1	-CH=NNHCSNH$_2$	-H	5.0
133	1	-CH=NNHCONHCH$_2$(CH$_2$)$_2$CH$_3$	-Br	5.0

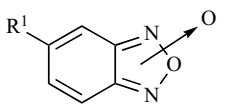
[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain.

Table 7. Benzofuroxan and Benzofurazan Derivatives using Hansch Series Design Methodology and Corresponding *T. cruzi* Antiproliferative Activities



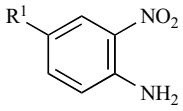
Ref.	n	X	-R^1	-R^2	-R^3	PGI (%)[a,b]
134	1	CH	-NO_2	-H	-H	89.0
135	1	CH	-CHO	-H	-H	67.0
136	1	CH	-CN	-H	-H	9.0
137	1	CH	-CH=$C(CN)_2$	-H	-H	66.0
138	1	CH	-*E*-CH=$CHNO_2$	-H	-H	92.0
139	1	CH	-CO_2H	-H	-H	1.0
140	1	CH	-CH=NOH	-H	-H	38.0
141	1	CH	-CH_2OH	-H	-H	21.0
142	1	CH	-H	-H	-H	35.0
143	1	CH	-CH_3	-H	-H	22.0
144	1	CH	-Cl	-H	-H	2.0
145	1	CH	-Br	-H	-H	5.0
146	1	CH	-CH_2Cl	-H	-H	97.0
147	1	CH	-CH_2I	-H	-H	93.0
148	1	CH	-*E*-CH=CHPh	-H	-H	93.0
149	1	CH	-*Z*-CH=CHPh	-H	-H	100.0
150	1	CH	-Ph	-H	-H	31.0
151	1	CH	-OCH_3	-H	-H	10.0
152	1	CH	-$N(CH_3)_2$	-H	-H	26.0
153	1	CH	-OCH_3	-OCH_3	-H	0.0 [c]
154	1	CH	-CHO	-H	-Br	0.0
155	1	CH	-CH_3	-H	-Br	0.0
156	1	CH	-CH_2Br	-H	-Br	100.0
157	1	N	-H	-H	-H	53.0
158	1	CH	-CON_3	-H	-H	34.0
159	1	CH	-N=C=O	-H	-H	8.0
160	1	CH	-$NHCO_2$-*t*-butyl	-H	-H	18.0
161	0	CH	-Br	-H	-H	0.0
162	0	CH	-CH_2Cl	-H	-H	28.0
163	0	CH	-(*E/Z*)-CH=CHPh	-H	-H	10.0
168	1	CH	-CH_2Br	-H	-H	100.0

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain. [c] Data not published.



hemine transformation

?



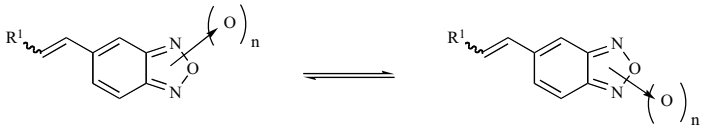
-R^1= -H, **142**
-CH_3, **143**
-(*E/Z*)-CH=CHPh, **148/149**
-Ph, **150**

	PGI [a]
-R^1= -H, **163**	26.0
-CH_3, **164**	0.0
-(*E/Z*)-CH=CHPh, **165**	0.0
-Ph, **166**	41.0

[a] Percentage of growth inhibition, at 5th day, of Tulahuen 2 epimastigote treated with 25 μM

Fig. (3). Can aminonitroaromatic derivatives possibly be the active species of benzofuroxan?

Table 8. Arylethenylbenzofuroxans of Second Generation and their Anti-*T.cruzi* Activities



Ref.	n	isomer	-R^1	IC_{50} (μM)[a,b]	SI[c,d]
169	1	*Z*	-Ph-*p*-NO_2	7.2	ns[e]
170	1	*E*	-Ph-*o*-NO_2	26.4	ns
171	1	*Z*	-Ph-*o*-NO_2	24.4	ns
172	1	*E*	-Ph-*p*-CN	>> 25.0	ns
173	1	*Z*	-Ph-*p*-CN	>> 25.0	ns
174	1	*E*	-Ph-*p*-CO_2H	>> 25.0	ns
175	1	*Z*	-Ph-*p*-CO_2H	>> 25.0	ns
176	1	*E/Z*[f]	-Ph-*p*-Cl	4.3	15.5
177	1	*E*	-Ph-*p*-Br	8.7	ns
178	1	*E/Z*[f]	-Ph-*m*-F	2.5	ns
179	1	*E/Z*[f]	-Ph-*p*-CF_3	9.8	ns
180	1	*E*	-Ph-3,4-(OCH_2O)	5.3	41.5
181	1	*Z*	-Ph-3,4-(OCH_2O)	2.9	41.4
182	1	*E*	-Ph-*p*-OCH_3	8.0	ns
183	1	*E*	-Ph-*p*-$N(CH_3)_2$	15.7	> 25.0
184	1	*E*	-Ph-*p*-OH	10.2	ns
185	1	*Z*	-Ph-3-NO_2-4-$NHCOCH_3$	24.0	ns
186	1	-	-H	>> 25.0	ns
187	1	*E/Z*[f]	-$CH_2(CH_2)_2CH_3$	19.7	ns
188	1	*E/Z*[f]	-cyclohexyl	13.6	ns
189	1	*E*	-CO_2CH_3	>> 25.0	ns

(Table 8. Contd….)

Ref.	n	isomer	-R[1]	IC_{50} (μM)[a,b]	SI[c,d]
190	1	*E*	-2-thienyl	0.6	ns
191	1	*E*	-5-NO_2-2-thienyl	6.0	ns
192	1	*Z*	-5-benzofurazanyl	>> 25.0	ns
193	1	*E*	-4-pyridinyl	15.0	ns
194	0	*Z*	-Ph-*o*-NO_2	>> 25.0	ns
195	0	*E*	-Ph-3,4-(OCH_2O)	>> 25.0	ns
196	0	*Z*	-Ph-3,4-(OCH_2O)	>> 25.0	< 40.0
197	0	*E*	-Ph-*p*-$N(CH_3)_2$	>> 25.0	ns
57 (Tbf)	-	-	-	17.1	19.3
198 (Ktz)	-	-	-	10.0	4.4

[a] IC_{50}: doses, in μM, that produces 50 % of parasite growth inhibition respect to untreated microorganism. [b] Conditions: fifth day of *in vitro* treatment, epimastigote Tulahuen 2 strain. [c] SI: selectivity index, defined as the ratio $IC_{50,THP\text{-}1\text{-}macrophages}$ / $IC_{50,T.\ cruzi}$. [d] For parent compounds, SI_{148}: 10.2, SI_{149}: 8.9. [e] ns: not studied. [f] Studied as 1:1 *E:Z*-mixture.

Currently, we are developing hybrid compounds which have the benzofuroxanyl system, as parasite-OS generator, and another moiety with a recognized parasite specific enzyme inhibitory activity. The vinylsulfone pharmacophore was included in the benzofuroxan structure to provide cruzain inhibitory activity (derivatives **199-207**, Table **9**) [53]. Although, the compounds were not enzymatic inhibitors, per se, they were effective in reducing or eliminating the parasite in animals with fully established *T. cruzi* infections. Other approaches involved the incorporation of a hydrazone or an allylamine moiety, to serve as TR or MSB inhibitory pharmacophores, respectively (**208-214**, Table **9**). The *in vitro* results showed that the compounds were moderate active but none of them actually inhibited the corresponding biochemical pathway[1,2].

3.2. Quinoxaline *N,N'*-dioxide Derivatives and Related Compounds

In the first study [38] in which we were looking for *N*-oxide derivatives with trypanocidal activity, quinoxaline *N,N'*-dioxides were developed including those with a semicarbazone moiety, like prototype **5**, and in structures of derivatives (**215-218**, Table **10**). These first derivatives were not trypanocidal, nevertheless in a collaborative research program between our laboratory and the group of Dr. Antonio Monge, about thirty quinoxaline dioxide and quinoxaline from the Monge compound-library were evaluated biologically (**219-251**, Table **10**) [54]. In this group, derivatives **219**-**220**, **229**, **230**, **247**, and **248** were excellent *in vitro T. cruzi* growth inhibitors. 2D-QSAR analysis indicated that both lipophilicity and LUMO energy could play a role in the bioactivity. Some derivatives, like **228**, and **238-242**, have, in the biological medium, significant solubility problems that could explain the low activity observed. Derivatives **229**, **230**, **247** and **248** are currently being evaluated *in vivo* in a murine Chagas' disease model.

From these quinoxaline-prototypes a second group of more than fifty dioxides were evaluated and derivatives **252** and **253** (Table **11**) were identified as excellent *in vitro* trypanocidal agents. These derivatives also displayed excellent *in vitro* activities against the *T. cruzi* CL Brener clone as well as the Colombiana strain[3]. A third approach is currently in development in which we are combining the quinoxaline dioxide system and allylamine moiety (**254-260**, Table **11**)[4]. In this last approach only compounds **256** and **259** showed *in vitro* activities.

Recently, two new families of *N*-oxide containing quinoxalines, selected from our in-house-chemical library, were evaluated as *in vitro* growth inhibitors of *T. cruzi* [55]. Derivatives from pyrimido[1,2-*a*]quinoxaline 6-oxide family had poor activity while phenazine 5,10-dioxide derivatives had good to excellent anti-*T. cruzi* activities, and are suitable for further structural modification (**261-285**, Table **12**). The anti-*T. cruzi* activity of phenazine derivatives is related to substituent electronic descriptors, σ_p^-.

3.3. Imidazole and Benzimidazole *N*-Oxide Derivatives and Related Compounds

The structure of **Bnz** (**2**, Fig. (**1**)), a nitroimidazole, prompted us to investigate the trypanocidal activity of some related *N*-oxides (Fig. (**4**)). Consequently, we have prepared some selected derivatives of imidazole *N*-oxide and benzimidazole *N*-oxide and have evaluated them biologically against *T. cruzi* [59,60]. The first structural modifications done in the benzimidazole system, i.e. *N*-oxidation (**286-293**, Table **13**), 1-oxidation-3-hydroxylation (**294-299**, Table **13**), and *N-O*-alkylation (**300-303**, Table **13**), did not produce active compounds. Derivatives **294-299** had low activity that could

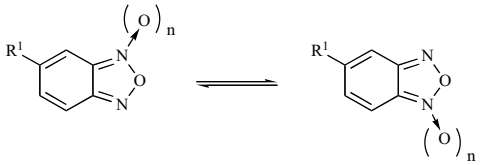
[1] Porcal, W.; Cerecetto, H.; González, M. Unpublished data.

[2] Gerpe, A.; Cerecetto, H.; González, M. Unpublished data.

[3] Monge, A.; Cerecetto, H.; González, M. Unpublished data.

[4] Gerpe, A.; Cerecetto, H.; González, M. Unpublished data.

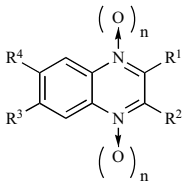
Table 9. Benzofuroxans Designed as Inhibitors of Strategic Enzymatic Targets of *T. cruzi* and their Biological Behavior



Ref.	n	-R^1	IC_{50} (μM)[a,b]	SI[c]
199	1	-*E*-CH=CHSPh	2.6	ns[d]
200	1	-*Z*-CH=CHSPh	3.8	ns
201	0	-*E*-CH=CHSPh	> 25.0	ns
202	1	-*E*-CH=CHSOPh	2.6	47.7
203	1	-*Z*-CH=CHSOPh	0.7	150.0
204	1	-*E*-CH=CHSO_2Ph	1.6	255.6
205	1	-*Z*-CH=CHSO_2Ph	1.1	99.1
206	1	-$CH_2SO_2CH_2CH_2OH$	> 25.0	< 12.0
207	1	-$CH_2SO_2CH=CH_2$	14.6	18.8
Ref.	**n**	**-R^1**	**PGI (%)[e,f]**	**IC_{50} (μM)[g]**
208	1	-CH_2OPh-4-CHO	98.0	nd[h]
209	1	-CH_2OPh-4-CH=NNHCONH_2	25.0	> 25.0
210	1	-CH_2OPh-4-CH=NNHCSNH_2	70.0	19.8
211	1	-CH_2OPh-4-CH=NNHC(NH)NH_2	40.0	38.2
212	1	-CH_2-piperazinyl-CH_2CH=CHPh	14.0	>> 25.0
213	1	-$CH_2N(CH_3)CH_2$CH=CHPh	56.0	16.9
214	1	-CH=NNHCSNHCH_2CH=CH_2	33.0	> 25.0

[a] IC_{50}: doses, in μM, that produces 50 % of parasite growth inhibition respect to untreated microorganism. [b] Conditions: fifth day of *in vitro* treatment, epimastigote Tulahuen 2 strain. [c] SI: selectivity index, defined as the ratio $IC_{50,THP\text{-}1\text{-}macrophages}$ / $IC_{50,T.\ cruzi}$. [d] ns: not studied. [e] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [f] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain. [g] IC_{50}, in μM, at fifth day of *in vitro* treatment, CL Brener clone. [h] nd: not determined.

Table 10. Quinoxalines Tested for *In Vitro* Trypanocidal Activity

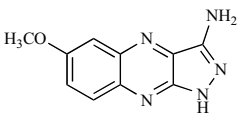


Ref.	n	-R^1	-R^2	-R^3	-R^4	PGI (%)[a,b]
215	1	-CH_3	-CH_3	-H	-CH=NNHCONH-*n*-butyl	0.0
216	1	-H	-H	-H	-CH=NNHCONH-*n*-butyl	23.0
217	1	-CN	-NH_2	-H	-CH=NNHCONH-*n*-butyl	17.0
218	1	-CH_3	-CH=NNHCONH-*n*-butyl	-H	-H	6.0

(Table 10. Contd….)

Ref.	n	-R[1]	-R[2]	-R[3]	-R[4]	PGI (%)[a,b]
219	1	-CN	-4-(Ph-2-OCH_3)piperazinyl	-H	-Cl	100.0
220	1	-CN	-4-(Ph-3-CF_3)piperazinyl	-H	-Cl	94.0
221	1	-CN	-4-(Ph-4-NO_2)piperazinyl	-H	-Cl	3.0
222	1	-CN	-4-(CH_2Ph-3,4-(OCH_2O))piperazinyl	-H	-Cl	33.0
223	1	-CN	-NHPh-4-CH_3	-H	-Cl	19.0
224	1	-CN	-NHPh	-H	-Cl	63.0
225	1	-CN	-$NH(CH_2)_3N(CH_3)_2$	-H	-Cl	16.0
226	1	-CH_3	-$COCH_3$	-H	-Cl	51.0
227	1	-CN	-4-(Ph-4-NO_2)piperazinyl	-Cl	-OCH_3	6.0
228	1	-CN	-NH_2	-Cl	-Cl	25.0
229	1	-CH_3	-SPh	-Cl	-Cl	93.0
230	1	-CH_3	-COPh	-Cl	-Cl	91.0
231	1	-CH_3	-CO(4-Ph-piperazinyl)	-Cl	-Cl	47.0
232	1	-CN	-NH_2	-H	-OCH_3	25.0
233	1	-CN	-4-(Ph-4-NO_2)piperazinyl	-H	-OCH_3	19.0
234	1	-CN	-4-(Ph-4-NO_2)piperazinyl	-CH_3	-CH_3	0.0
235	1	-CN	-$NH(CH_2)_3N(CH_3)_2$	-CH_3	-CH_3	1.0
236	1	-CH_3	-4-(Ph-4-NO_2)piperazinyl	-CH_3	-CH_3	1.0
237	1	-CH_3	-4-Ph-piperazinyl	-CH_3	-CH_3	0.0
238	1	-CN	-NH_2	-H	-H	25.0
239	1	-CN	-NH_2	-H	-CH_3	5.0
240	1	-CN	-NH_2	-H	-CH=$NNHCO_2CH_3$	19.0
241	1	-CN	-NH_2	-H	-CH=$NNHCONH(CH_2)_2$Ph	11.0
242	1	-CN	-NH_2	-H	-CH=$NNHCONH(CH_2)_2OCH_3$	20.0
243	1	-CH_3	-CH_3	-H	-CH=$NNHCO_2CH_3$	28.0
244	1	-CH_3	-CH_3	-H	-CH=$NNHCONH(CH_2)_2$Ph	17.0
245	1	-CH_3	-$COCH_3$	-H	-F	22.0
246	0	-CN	-NH_2	-H	-CH_3	0.0
247	0	-CN	-Cl	-H	-Cl	100.0
248	0	-CN	-Cl	-CH_3	-CH_3	81.0
249	c					0.0
250						0.0
251						31.0

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain. [c] Structures:



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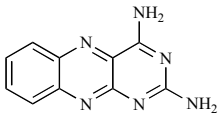
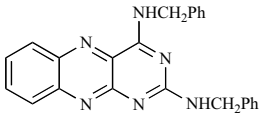
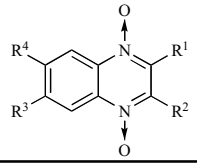


250



251

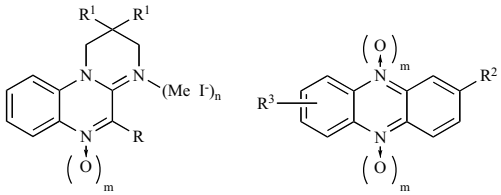
Table 11. Generation of Quinoxaline Dioxides and Tests for *In Vitro* Trypanocidal Activity



Ref.	-R^1	-R^2	-R^3	-R^4	PGI (%)[a,b]
252	-CO_2Et	-Ph	-Cl	-Cl	99.0
253	-CO_2Et	-Ph	-H	-Cl	88.0
254	-CO_2Et	-Ph	-F	-F	100.0
255	-COPh	-CH_3	-F	-F	92.0
256	-CO_2Et	-Ph	-F	-F	100.0
257	-$COCH_3$	CH_3	-F	-F	100.0
258	-CN	-NH_2	-H	-CH=NNHC(S)$NHCH_2CH=CH_2$	6.0
259	-CO_2Et	-Ph			83.0
260	-CONHPh	-CH_3			0.0

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain.

Table 12. Quinoxaline Derived Compounds: pyrimido[1,2-*a*]quinoxaline 6-oxides and phenazine 5,10-dioxides tested as *In Vitro* Anti-*T. cruzi* Agents



Ref.	n	m	-R	-R^1	PGI (%)[a,b]	Ref.	m	-R^2	-R^3	Ratio *7*-isomer / *8*-isomer[c]	PGI (%)[a,b]
261	0	1	-Ph	-H	36	274	1	-NH_2	-Br	54 : 46	81
262	0	1	-(*p*-OCH_3)Ph	-H	36	275	1	-NH_2	-Cl	63 : 37	61
263	0	1	-(*p*-Cl)Ph	-H	14	276	1	-NH_2	-OCH_3	95 : 5	38
264	0	1	-(*p*-NO_2)Ph	-H	9	277	1	-NH_2	-CH_3	70 : 30	37
265	0	1	-(*o*-Cl)Ph	-H	14	278	1	-$NHCOCH_2Cl$	[d]	55 : 45	79
266	0	1	-(*o*-NO_2)Ph	-H	12	279	1	-OH	-Br	64 : 36	0
267	0	1	-(*p*-OCH_3)Ph	-Me	13	280	1	-OH	-H	-	0
268	0	1	-CH_2Ph	-H	13	281	1	-OH	-OCH_3	100 : 0	0
269	0	1	-CH_3	-H	22	282	1	-OH	-CH_3	76 : 24	43
270	0	0	-Ph	-H	29	283	1	-OH		35 : 65	0
271	1	1	-Ph	-H	20	284	0	-OH	-Br	70 : 30	0
272	1	1	-(*p*-OCH_3)Ph	-H	9	285	0	-OH	-CH_3	56 : 44	45
273	1	1	-(*p*-NO_2)Ph	-H	20						

[a] PGI: percentage of growth inhibition. [b] Inhibition of epimastigotes growth, 25 μM doses. [c] Determined by ^{1}H NMR from the reaction mixture [56-58]. The compounds were evaluated as mixture of isomers. [d] –R^3= -(1,3-dioxolan-2-yl).

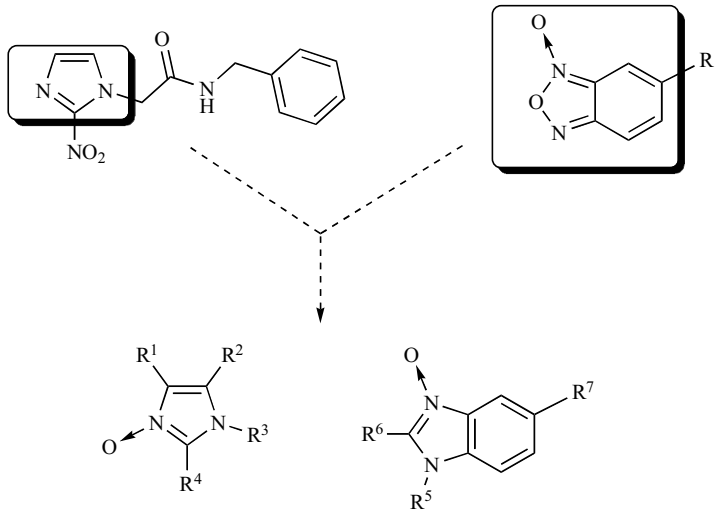
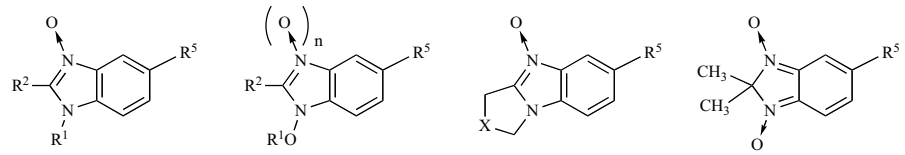


Fig. (4). Strategy for design of imidazole and benzimidazole derivatives.

Table 13. Benzimidazole *N*-Oxide Derivatives Developed as Trypanocidal Agents



Subfamily **A** **B** **C** **D**

Ref.	Subfamily	n	-R^1	-R^2	-R^5	-X-	PGI (%)[a,b]
286	A	-	-H	-H	$-NO_2$	-	< 4.0
287	A	-	-H	$-CONH(CH_2)_3CH_3$	$-NO_2$	-	9.0
288	A	-	-H	$-CO_2Et$	$-NO_2$	-	< 4.0
289	A	-	-H	$-CO_2CH(CH_3)_2$	$-NO_2$	-	< 4.0
290	A	-	-H	$-CO_2(CH_2)_3CH_3$	$-NO_2$	-	5.0
291	A	-	-H	$-CO_2(CH_2)_5CH_3$	$-NO_2$	-	< 4.0
292	A	-	-H	$-CO_2Et$	$-CO_2Et$	-	< 4.0
293	A	-	-H	$-CO_2Et$	$-CH_3$	-	< 4.0
294	A	-	-OH	$-CH_3$	-H	-	< 4.0
295	A	-	-OH	$-CH_3$	$-CH_3$	-	< 4.0
296	A	-	-OH	$-CH_3$	$-OCH_3$	-	< 4.0
297	A	-	-OH	$-CH_3$	-Br	-	< 4.0
298	A	-	-OH	$-CH_3$	-Cl	-	< 4.0
299	A	-	-OH	$-CH_3$	-*E*-CH=CHPh	-	19.0
300	B	0	$-CH_3$	$-CO_2Et$	$-NO_2$	-	< 4.0
301	B	0	$-CO_2Et$	$-CO_2Et$	$-NO_2$	-	5.0
302	B	0	$-CO_2$*i*But	$-CO_2Et$	$-NO_2$	-	9.0
303	B	0	$-CO_2CH_2CCl_3$	$-CO_2Et$	$-NO_2$	-	10.0
304	B	1	$-CH_2CO_2Et$	$-CH_3$	-H	-	< 4.0

(Table 13. Contd….)

Ref.	Subfamily	n	-R^1	-R^2	-R^5	-X-	PGI (%)[a,b]
305	B	1	-CH_2CO_2Et	-CH_3	-CH_3	-	25.0
306	B	1	-CH_2CO_2Et	-CH_3	-*E*-CH=CHPh	-	8.0
307	B	1	-$(CH_2)_4CH_3$	-CH_3	-H	-	< 4.0
308	C	-	-	-	-NO_2	-CH_2-	< 4.0
309	C	-	-	-	-NO_2	-$(CH_2)_2$-	< 4.0
310	C	-	-	-	-NO_2	-CH_2O-	< 4.0
311	C	-	-	-	-NO_2	-CH_2S-	< 4.0
312	D	-	-	-	-H	-	87.0
313	D	-	-	-	-CH_3	-	85.0
314	D	-	-	-	-OCH_3	-	52.0
315	D	-	-	-	-Br	-	77.0
316	D	-	-	-	-*E*-CH=CHPh	-	100.0
317	D	-	-	-	-*Z*-CH=CHPh	-	100.0
318	D	-	-	-	-CH=NOH	-	72.0
319	D	-	-	-	[c]	-	69.0
320	D	-	-	-	[d]	-	59.0

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain. [c] –R^3= -CH=NNHCONH-*n*-hexyl. [d] –R^3= -(1,3-dioxolan-2-yl).

have resulted from low solubility in the biological medium, however the low activities of derivatives **286-293** could be the result of benzimidazole *N*-oxide derivatives which exist as the mixture of *N*-oxide and *N*-hydroxy tautomers and the proportion of both depends on the polarity of the solvent [61], consequently they are not "true" *N*-oxides. For these reasons, we prepared non-tautomerizable benzimidazole *N*-oxides (**304-320**, Table **13**), in finding that benzimidazole 1,3-dioxide derivatives were very soluble in water and had excellent *in vitro* activity against two different *T. cruzi* strains, Tulahuen 2 and Y as well as the CL Brener clone [60]. Derivatives **318** and **320** were not cytotoxic against J774 murine macrophages, at the doses assayed, and have adequate *in vivo* behavior promoting better animals' survival percentages than reference compounds (**Bnz**) and diminution of anti-*T. cruzi* antibodies level's [60]. A second generation of benzimidazole 1,3-dioxide derivatives is currently under study (**321-330**, Table **14**) where modifications in positions 2- and 5- are being done in order to investigate the contribution to activity of substituents at these positions [62][5,6,7]. In general benzimidazole 1,3-dioxides were more active than the corresponding benzofuroxan analogues (compare activities of benzofuroxans **212-214**, Table **9**, to benzimidazoles activities, **328-330** Table **14**).

On the other hand, we have studied imidazole derivatives. In the first synthetic approach 4-methylimidazole N^3-oxide derivatives were prepared with good yields (**331-337**, Table **15**) [59,63]. Secondly, we tried to prepare 4-alkyloxycarbonylimidazole N^3-oxide derivatives however imidazole-2-one **338-341** were obtained as main products as a result of *N*-oxide rearrangements [64]. All of these derivatives were inactive, < 4 % of parasite inhibition at 25 μM, against epimastigote Tulahuen 2 strain of *T. cruzi*.

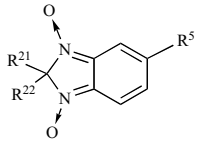
One strategy in the search for new anti-*T. cruzi* agents involves the blockage/modification of parasite's polyamines synthesis. Some essential polyamines, like spermidine, the precursor for the biosynthesis of trypanothione, is not actively supplied by a known mechanism in *T. cruzi*. *T. cruzi* epimastigotes are unable to synthesize significant amounts of putrescine and cadaverine *de novo*, but take up polyamines from the medium. After conversion into spermidine and aminopropylcadaverine, these polyamines were used to synthesize trypanothione [65]. Consequently, inhibitors/modifiers of polyamines biochemistry could be a promising approach for the development of new anti-*T. cruzi* drugs. In this sense we evaluated a series of imidazolidines that could be seen as carriers of *N,N'*-disubstituted ethylenediamines (Fig. (**5**)) [66]. The imidazolidines **342-386** (Table **16**) were the most anti-*T. cruzi* active derivatives found, while the bisimidazolidines **387-394** (Table **17**) were inactive at 25 μM. We also studied the biological activities of three ethylenediamines that were potentially produced by hydrolysis

[5] Gerpe, A.; Cerecetto, H.; González, M. Unpublished data.

[6] Boiani, M.; Cerecetto, H.; González, M. Unpublished data.

[7] Merlino, A.; Cerecetto, H.; González, M. Unpublished data.

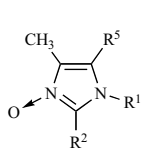
Table 14. Second Generation of Benzimidazole *N,N'*-Dioxide Derivatives Evaluated as Trypanocidal Agents

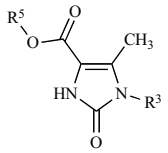


Ref.	-R^{21}	-R^{22}	-R^5	PGI (%)[a,b]
321	-CH_3	-CH_2Ph	-H	48.0
322	-CH_3	-CH_2Ph-4-OCH_3	-H	52.0
323	-CH_3	-CH_2Ph-4-Cl	-H	0.0
324	-CH_3	-CH_2Ph-4-NO_2	-H	100.0
325	-CH_3	-$(CH_2)_4CH_3$	-H	95.0
326	-$CH_2CH_2CH_2CH_2$-		-H	90.0
327	-$CH_2CH_2CH_2CH_2$-		-CH=NOH	74.0
328	-CH_3	-CH_3	-CH_2-piperazinyl-CH_2CH=CHPh	50.0
329	-CH_3	-CH_3	-$CH_2N(CH_3)CH_2CH$=CHPh	57.0
330	-CH_3	-CH_3	-CH=NNHCSNHCH_2CH=CH_2	72.0

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain.

Table 15. Imidazole Derivatives Found to be Inactive Against *T. cruzi* Proliferation





Ref.	-R^1	-R^2	-R^5	Ref.	-R^3	-R^5
331	-Ph-4-CH_3	-H	-H	**338**	-Ph-4-CH_3	-CH_2CH_3
332	-Ph-4-CH_3	-H	-CH_3	**339**	-$CH_2CH_2OCH_3$	-CH_3
333	-Ph-4-CH_3	-CH_3	-CH_3	**340**	-CH_2CH_2Ph	-CH_2CH_3
334	-Ph-4-OCH_3	-H	-CH_3	**341**	-CH_2furyl	-CH_3
335	-CH_2CH_2Ph	-H	-CH_3			
336	-CH_2CH_2Ph	-CH_3	-CH_3			
337	-CH_2furyl	-CH_3	-CH_3			

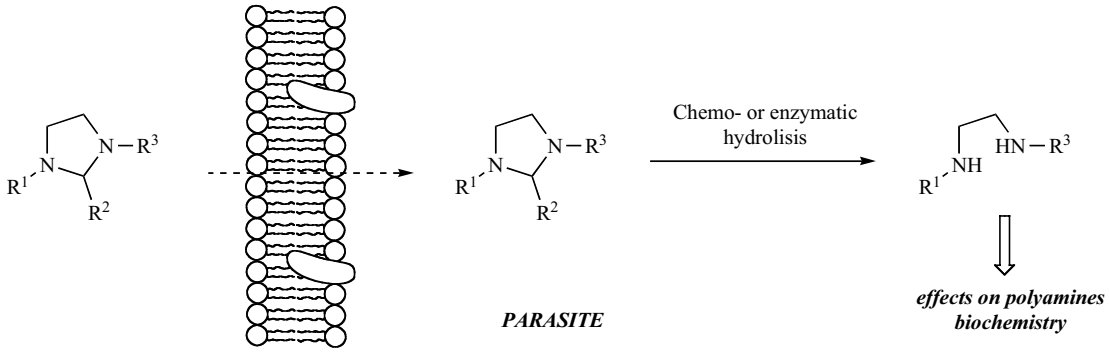
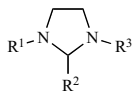


Fig. (5). Postulated mechanism of activation of imidazolidines.

Table 16. ***In Vitro*** **Antiproliferative Activities of Imidazolidine Derivatives Against** ***T. cruzi***



Ref.	-R^1	-R^2	-R^3	PGI (%)[a,b]
342	-Ph	-H	-Ph	12.3
343	-Ph	-H	-CH_2Ph	17.2
344	-Ph-4-Me	-H	-CH_2Ph	44.2
345	-Ph-4-Cl	-H	-CH_2Ph	64.7
346	-CH_2Ph-4-Cl	-H	-CH_2Ph-4-Cl	100.0
347	-CH_2Ph-3-Cl	-H	-CH_2Ph-3-Cl	100.0
348	-CH_2Ph-3,4-diCl	-H	-CH_2Ph-3,4-diCl	100.0
349	-Ph	-Me	-Ph	12.4
350	-CH_2Ph-4-OMe	-Me	-CH_2Ph-4-OMe	40.0
351	-Ph	-Ph	-Me	6.6
352	-Ph-4-Me	-Ph	-Me	8.9
353	-Ph	-Ph	-Ph	39.6
354	-Ph	-Ph	-CH_2Ph	45.1
355	-Ph-4-Me	-Ph	-CH_2Ph	46.0
356	-Ph-4-Cl	-Ph	-CH_2Ph	70.4
357	-CH_2Ph	-Ph	-CH_2Ph	63.0
358	-CH_2Ph-4-OMe	-Ph	-CH_2Ph-4-OMe	75.3
359	-CH_2Ph-2-OH	-Ph	-CH_2Ph-2-OH	4.8
360	-CH_2Ph-4-Me	-Ph	-CH_2Ph-4-Me	100.0
361	-CH_2Ph-4-Cl	-Ph	-CH_2Ph-4-Cl	94.5
362	-CH_2Ph-3-Cl	-Ph	-CH_2Ph-3-Cl	100.0
363	-CH_2Ph-3,4-diCl	-Ph	-CH_2Ph-3,4-diCl	100.0
364	-CH_2Ph-4-Cl	-2-furyl	-CH_2Ph-4-Cl	89.4
365	-CH_2Ph-3,4-diCl	-2-furyl	-CH_2Ph-3,4-diCl	93.9
366	-CH_2Ph-4-OMe	-CH=CHPh	-CH_2Ph-4-OMe	98.6
367	-CH_2Ph-4-Cl	-CH=CHPh	-CH_2Ph-4-Cl	100.0
368	-CH_2Ph-4-Cl	-Ph-4-OMe	-CH_2Ph-4-Cl	100.0
369	-CH_2Ph-4-Cl	-Ph-3-OH	-CH_2Ph-4-Cl	97.4
370	-CH_2Ph-4-Cl	-Ph-2-OH	-CH_2Ph-4-Cl	86.8
371	-CH_2Ph-4-OMe	-Ph-4-Cl	-CH_2Ph-4-OMe	100.0
372	-CH_2Ph-4-Me	-Ph-4-Cl	-CH_2Ph-4-Me	100.0
373	-CH_2Ph	-Ph-4-Cl	-CH_2Ph	60.0
374	-CH_2Ph-4-Cl	-Ph-4-Cl	-CH_2Ph-4-Cl	100.0
375	-CH_2Ph-3,4-diCl	-Ph-4-Cl	-CH_2Ph-3,4-diCl	50.0

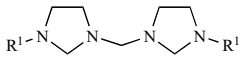
(Table 16. Contd….)

Ref.	-R^1	-R^2	-R^3	PGI (%)[a,b]
376	-CH_2Ph-4-OMe	-Ph-3-Cl	-CH_2Ph-4-OMe	95.0
377	-CH_2Ph-4-Me	-Ph-3-Cl	-CH_2Ph-4-Me	99.0
378	-CH_2Ph-4-Cl	-Ph-3-Cl	-CH_2Ph-4-Cl	100.0
379	-CH_2Ph-4-OMe	-Ph-2-Cl	-CH_2Ph-4-OMe	49.0
380	-CH_2Ph-4-Cl	-Ph-2-Cl	-CH_2Ph-4-Cl	100.0
381	-CH_2Ph-4-OMe	-Ph-3,4-diCl	-CH_2Ph-4-OMe	91.0
382	-CH_2Ph-4-Cl	-Ph-3,4-diCl	-CH_2Ph-4-Cl	92.0
383	-CH_2Ph-3,4-diCl	-Ph-3,4-diCl	-CH_2Ph-3,4-diCl	30.0
384	-CH_2Ph-4-OMe	-Ph-4-NO_2	-CH_2Ph-4-OMe	83.0
385	-CH_2Ph-4-Cl	-Ph-4-NO_2	-CH_2Ph-4-Cl	100.0
386	-CH_2Ph-4-OMe	-Ph-3-NO_2	-CH_2Ph-4-OMe	93.0

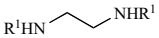
[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain.

from the parent imidazolidines (**395-397**, Table **17**). Diamines **395** and **396** were as active as the corresponding imidazolidines while ethylenediamine **397** was inactive under the assay conditions used, and was like the corresponding heterocyclic derivative, imidazolidine **359**. These results suggested that the imidazolidine activities could be the result of the corresponding diamines activities. The use of 2D-QSAR allows one to investigate if lipophilicity plays an important role in bioactivity being the most hydrophobic compounds the most active against *T. cruzi*.

Table 17. ***In Vitro*** **Antiproliferative Activities of Bisimidazolidines and *N,N'*-disubstituted-ethylenediamines Against *T. cruzi***



387-394



395-397

Ref.	-R^1	PGI (%)[a,b]
387	-Ph-4-OMe	0.0
388	-Ph-4-Me	0.0
389	-Ph	0.0
390	-Ph-4-Cl	0.0
391	-Ph-3-Cl	8.0
392	-Ph-4-Br	0.0
393	-Ph-3-Br	10.8
394	-Ph-4-NO_2	0.0
395	-CH_2Ph-4-OMe	100.0
396	-CH_2Ph-4-Cl	100.0
397	-CH_2Ph-2-OH	0.0

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain.

3.4. Indazole *N*-Oxide Derivatives and Related Compounds

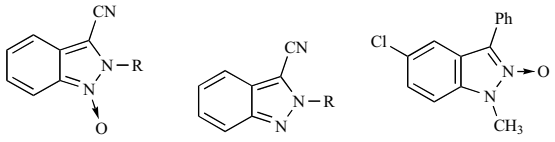
The fourth group of *N*-oxide heterocyclic compounds studied as anti-*T. cruzi* agents were indazole derivatives [67]. These derivatives were partially active, with compound **403** being the most effective against the *T. cruzi* epimastigote form (Table **18**), while **399** was the most active against the *T. cruzi* trypomastigote form at 100 μg/mL. Again the reduced derivatives (**411-413**, Table **18**) were less active than the corresponding *N*-oxide. ESR experiments using *T. cruzi* and derivative **402** were done demonstrating that this indazole is able to generate free radicals within the parasitic cells, in that the free radical $OH^{\cdot}$ was detected. Indazole *N*-oxides appear to have a small if any effect on parasite oxygen uptake, and inhibition was evident in a concentration dependent manner without oxygen recycling.

Another approach was been the development of 5-nitroindazole derivatives where the *N*-oxide OS-producer is substituted by the bioreducible nitro-moiety [68]. Some of the first series derivatives (**415-427**, Table **19**) had excellent *in vitro* activity, derivative **419** being non-toxic against J774 macrophages at the doses assayed. Electrochemical studies have been done in to better understand the mechanism of bioreduction by this family of compounds [69,70]. On the one hand, we are currently working with some structural modifications that involve the substituent in position 1- and the nitro-moiety (**428-440**, Table **19**)[8,9]. The lateral flexible chain and its substitution could play a role in the anti-*T. cruzi* activity (see activity of tricyclic derivatives **438** and **439**, Table **19**) and the absence of nitro-moiety affects the

[8] Gerpe, A.; Cerecetto, H.; González, M. Unpublished data.

[9] Rodríguez, J.; Olea-Azar, C.; Cerecetto, H.; González, M. Unpublished data.

Table 18. ***In Vitro*** **Antiproliferative Activities of Indazole *N*-oxides and Reduced Analogues Against *T. cruzi***

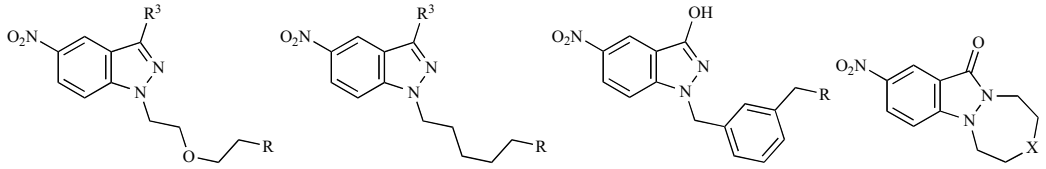


398-410 **411-413** **414**

Ref.	-R	PGI (%)[a,b]	Ref.	-R	PGI (%)[a,b]	Ref.	PGI (%)[a,b]
398	-Ph	5.0	411	-Ph-4-OH	33.0	414	21.0
399	-Ph-4-OH	25.0	412	-Ph-4-I	17.0		
400	-Ph-4-CH_3	5.0	413	-Ph-4-NO_2	34.0		
401	-Ph-4-Cl	53.0					
402	-Ph-4-I	51.0					
403	-Ph-4-NO_2	70.0					
404	-Ph-3-NO_2	52.0					
405	-1-naphthyl	44.0					
406	-2-naphthyl	25.0					
407	-CH_2furyl	49.0					
408	-$(CH_2)_3CH_3$	13.0					
409	-CH_2CH_2Cl	0.0					
410	-CH_2CH_2Ph	23.0					

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote CL Brener clone.

Table 19. ***In Vitro*** **Antiproliferative Activities of 5-nitroindazole Derivatives Against *T. cruzi***



Subfamily A **B** **C** **D**

Ref.	Subfamily	-R	-R^3	-X-	PGI (%)[a,b]	Unspecific Toxicity[c,d]
415	A	-$N(CH_3)_2$	-OH	-	3.0	Non
416	A	-1-piperidinyl	-OH	-	9.0	Non
417	A	-$N(CH_3)_2$	-OCH_3	-	17.0	Non
418	A	-1-piperidinyl	-OCH_3	-	25.0	Non
419	A	-$N(CH_3)_2$	-OCH_2Ph	-	80.0	Non
420	A	-1-piperidinyl	-OCH_2Ph	-	24.0	Non
421	B	-$N(CH_3)_2$	-OH	-	6.0	Non
422	B	-1-piperidinyl	-OH	-	8.0	Non
423	B	-1-piperidinyl	-OCH_3	-	33.0	Yes

(Table 19. Contd….)

Ref.	Subfamily	-R	-R^3	-X-	PGI (%)[a,b]	Unspecific Toxicity[c,d]
424	B	-$N(CH_3)_2$	-OCH_2Ph	-	100.0	Yes
425	B	-1-piperidinyl	-OCH_2Ph	-	100.0	Yes
426	C	-$N(CH_3)_2$	-	-	10.0	Non
427	C	-1-piperidinyl	-	-	11.0	Non
428	A	-$N^+O^-(CH_3)_2$	-OCH_2Ph	-	0.0	ns[e]
429	A	-1-(1-oxide)piperidinyl	-OCH_2Ph	-	6.0	ns
430	A	-4-thiomorpholinyl	-OH	-	0.0	ns
431	A	-4-thiomorpholinyl	-OCH_2Ph	-	20.0	ns
432	B	-$NH(CH_2)_3CH_3$	-OCH_3	-	58.0	ns
433	B	-$NH(CH_2)_3CH_3$	-OCH_2Ph	-	94.0	ns
434	A	-$NH(CH_2)_3CH$=CHPh	-OCH_2Ph	-	100.0	Yes
435	A	-piperazinyl-CH_2CH=CHPh	-OCH_3	-	100.0	Yes
436	A	-piperazinyl-CH_2CH=CHPh	-OCH_2Ph	-	100.0	Yes
437	B	-piperazinyl-CH_2CH=CHPh	-OCH_2Ph	-	97.0	Yes
438	D	-	-	-O-	6.0	ns
439	D	-	-	-CH_2-	4.0	ns
440	B[f]	1-piperidinyl	-OCH_2Ph	-	18.0	ns

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote CL Brener clone. [c] Unspecific toxicity was evaluated on J774 macrophages at 24 and 48 h. [d] Non: non toxic up to 100 μg/mL; Yes: toxic up to 100 μg/mL. [e] ns: not studied. [f] Without 5-nitro-substituent.

observed anti-*T. cruzi* activity (compare derivative **440** activity to **425** activity). The profiles of accumulated membrane sterols and precursors seen in tested *T. cruzi* were not consistent with derivatives, **434-437**, developed as *T. cruzi* MBS inhibitors, specifically as inhibitors of SE[8]. On the other hand, we are working with an *in vivo* murine model of Chagas' disease with the most promising derivatives, **418**, and **419**.

A theoretical systematic approach was used to model the anti-*T. cruzi* activity of our four series of active *N*-oxide containing heterocycles, benzofuroxan, benzimidazole *N*-oxide, indazole *N*-oxide, and quinoxaline *N,N'*-dioxide derivatives [71]. The theoretical descriptors, quantum chemical (AM1) global descriptors and properties coded by radial distribution function (RDF), pointed to the relevance of specific electronic properties. The local-RDF identified an electrophilic center at 4.1-4.9 Å from the oxygen atom of the *N*-oxide moiety, although, other observations are still required to explain the biological activity. The ability of these descriptors to distinguish among activity types was assessed using Kohonen neural networks and the best clustering descriptors have been later used for model building. The best results have been obtained using *k*-nearest neighbors (*k*-NN) and decision tree (J48) methods combined with global descriptors. Since tree-based methods are easily translated into classification rules, the J48 model is a useful tool in the *de novo* construction of new *N*-oxide containing heterocycle lead structures.

4. METALLIC COMPLEXES

The many activities of metal ions in biology stimulated, in the past decades, the development of metal based chemotherapeutics in different fields of medicine. Even though emphasis had been mainly on cancer treatment as a result of the great success of cisplatin, recent studies also included parasitic diseases [72]. The postulated metabolic similarity between tumor cells and *T. cruzi* allowed for the study of classical antitumoral metallic complexes as anti-parasite agents [73]. In this sense, we have worked using two different approaches, one was the synthesis of complexes using ligands bearing antitrypanosomal activity, and the other one was the use of pharmacologically active metals. In the next sections we discuss each group according to the class of ligand used in the syntheses of the different families of complexes.

4.1. 5-Nitrofuran as Ligand

Using the 5-nitrofuryl moiety as a pharmacophoric scaffold we developed four different series of metallic complexes where the central metal atom was rhenium (Re), ruthenium (Ru), palladium (Pd) or platinum (Pt), respectively. Our first approach was the development of Re and Ru com-

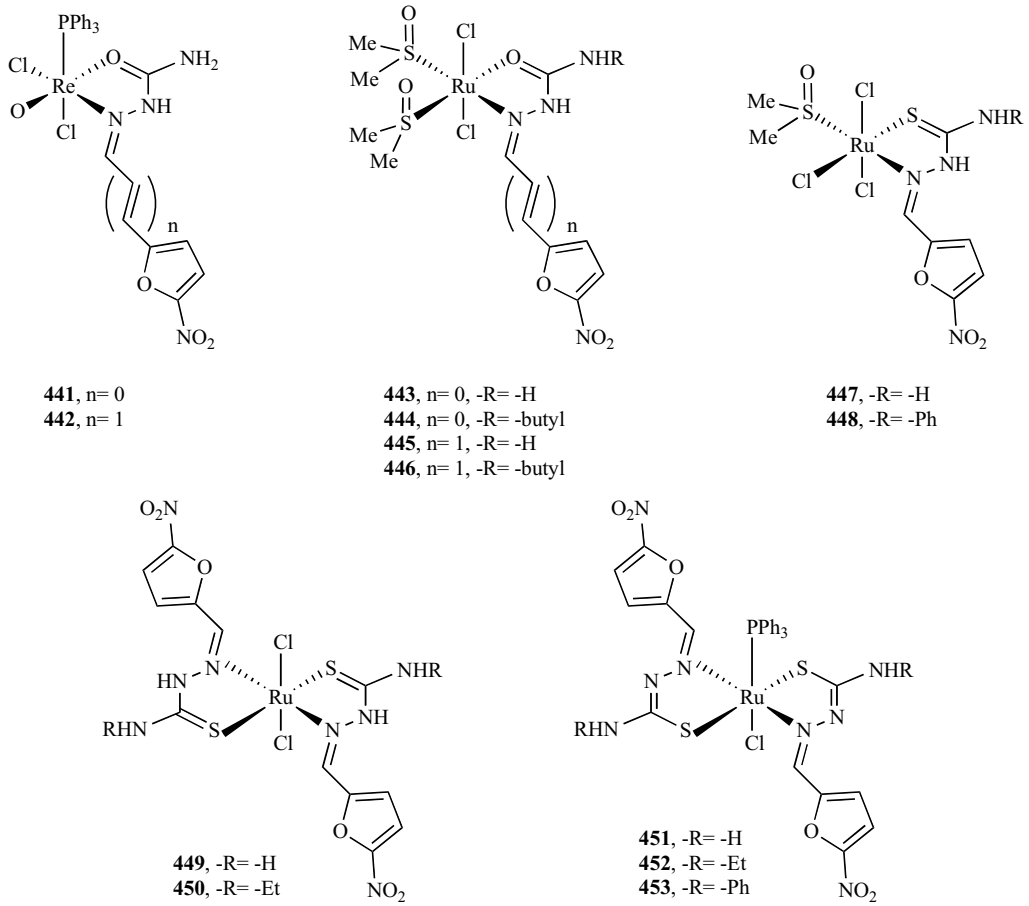


Fig. (6). Re and Ru complexes with 5-nitrofuryl derivatives developed as anti-*T. cruzi* agents.

plexes, using the semicarbazones **4**, **5**, **17** and **18** as ligand, producing derivatives of the type $[Re^{V}OCl_2(PPh_3)$(nitrofuryl-derivative)] and $[Ru^{II}Cl_2(DMSO)_2$(nitrofuryl-derivative)], (**441-446**, Fig. (**6**)) [74,75]. Re complexes were unstable in aqueous solution, and were as active as the free ligand [76]. Complexation to Ru of the 5-nitrofurylsemicarbazones led to the absence of antiprotozoa activity even though, according to the ESR studies, free radical production and redox cycling induction were detected when the compounds were incubated in presence of *T. cruzi* cells [76]. In addition, complexes **443-446** had the ability to bind non-covalently to the DNA minor groove and to nick the DNA upon covalent interaction *via* an oxidative mechanism [77]. The observed lack of anti-trypanosomal activity could be explained on the basis of the ability of Ru-complex derivatives to bind protein and their high hydrophilicity. More lipophilic Ru complexes were obtained using triphenylphosphine as co-ligand[10] and thiosemicarbazones **31**, **33** and **34** as ligand (Table **2**). Some improvements in the activity were observed with these new Ru-complexes (**447-453**, Fig. (**6**)) having PGI values between 20 to 53 % (Tulahuen 2 strain, 25 μM doses).

The thiosemicarbazone-ligands were also used for the preparation of Pd and Pt complexes. Some derivatives of the first series, Pd(II) complexes with the formula $[PdCl_2$(nitrofuryl-derivative)] and [Pd(deprotonated nitrofuryl-derivative)$_2$], had excellent *in vitro* activity (**454-469**, Table **20**) [78,79]. In most cases, the activity of the ligand was maintained or even increased as a result of Pd complexation. These complexes had strong DNA-binding ability, were able to generate an OS for the parasite and irreversibly inhibited TR [78]. The oxygen radical scavenger ability of this family of compounds was analyzed and was found not to correlate with anti-parasite activity [80]. In the series of Pt(II) complexes (**470-477**, Table **20**), with a potential dual mechanism of action involving producing of toxic free radicals and DNA interaction, the activity was lower than the corresponding Pd-complexes [81][11]. Mammal toxicity studies should be done in order to select the family of complexes, Pd or Pt, for further *in vivo* studies.

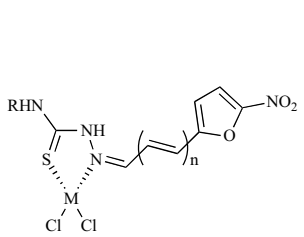
4.2. 3-Aminoquinoxaline-2-Carbonitrile *N,N'*-Dioxides and other *N*-Oxides as Ligands

Recently, we reported the synthesis, characterization, and biological properties of insulin-mimetic and hypoxia-cytotoxic agents. The compounds constitute a series of novel vanadyl complexes with bidentate 3-aminoquinoxaline-2-carbonitrile *N,N'*-dioxide derivatives as ligands [82,83]. One of the most important observed physicochemical features of these complexes was their improved solubility in hydrophilic media. Consequently we thought that these kinds of com-

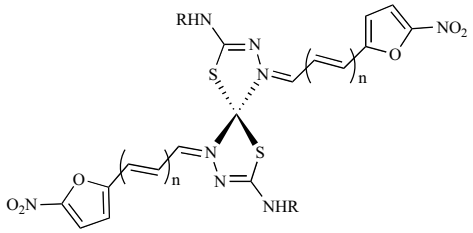
[10] Otero, L.; Gambino, D.; Cerecetto, H.; González, M. Unpublished data.

[11] Vieites, M.; Gambino, D.; Cerecetto, H.; González, M. Unpublished data.

Table 20. ***In Vitro*** **Antiproliferative Activities of Nitrofurylthiosemicarbazone Containing Pd and Pt Complexes Against** ***T. cruzi***



Subfamily A



B

Ref.	M	Subfamily	n	-R	IC_{50} (μM)[a,b]	Ref.	M	Subfamily	n	-R	IC_{50} (μM)[a,b]
454	Pd	A	0	-H	2.4	**470**	Pt	A	0	-H	>> 25.0
455		A	1	-H	6.4						
456		B	0	-H	4.5	**471**		B	0	-H	>> 25.0
457		B	1	-H	4.3						
458		A	0	$-CH_3$	4.3	**472**		A	0	$-CH_3$	13.1
459		A	1	$-CH_3$	2.7						
460		B	0	$-CH_3$	4.7	**473**		B	0	$-CH_3$	6.9
461		B	1	$-CH_3$	4.3						
462		A	0	$-CH_2CH_3$	5.9	**474**		A	0	$-CH_2CH_3$	27.5
463		A	1	$-CH_2CH_3$	2.4						
464		B	0	$-CH_2CH_3$	>> 25.0	**475**		B	0	$-CH_2CH_3$	0.8
465		B	1	$-CH_2CH_3$	5.2						
466		A	0	-Ph	>> 25.0	**476**		A	0	-Ph	15.0
467		A	1	-Ph	>> 25.0						
468		B	0	-Ph	>> 25.0	**477**		B	0	-Ph	>> 25.0
469		B	1	-Ph	>> 25.0						

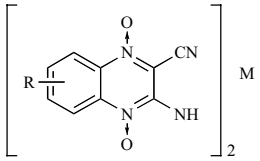
[a] IC_{50}: doses, in μM, that produces 50 % of parasite growth inhibition respect to untreated microorganism. [b] Conditions: fifth day of *in vitro* treatment, epimastigote Tulahuen 2 strain.

plexes could improve the anti-*T. cruzi* activity of the corresponding ligands which had difficulties with solubility (see above, Table **10**). Moreover the inhibition of the *T. cruzi* acidocalcisome, an organelle with specific functions in parasite homeostasis, by vanadate was recently described [84]. Having these two ideas in mind, we studied the complexation of 3-amino-2-carbonitrile-quinoxaline *N,N'*-dioxide derivatives with vanadium (V) in an effort to develop novel anti-*T. cruzi* agents (**482-488**, Table **21**) [85]. $V^{IV}O$(acetylacetonate)$_2$, **489**, was included in the biological study to better understand the trypanocidal effect of V, as vanadyl entity. According to the 2D-QSAR studies, the biological response of this family of complexes depended on both the lipophilic properties, the most lipophilic complex (**488**) being the least active, and the electronic effect of the quinoxaline substituents, producing the halogen-substituted complexes (**485-487**) the greatest activity. The low bioactivity of precursor **489** and some complexes, like **488**, indicated that vanadyl *per se* did not have anti-*T. cruzi* activity, and behaving in a different sense compared with the vanadate anion. Using the same kinds of ligands other metals were used to form complexes. Copper (Cu) and Pd were used yielding complexes of Cu(II) and Pd(II) (**490-493**, Table **21**). In general, they were as active as the analogues V(IV) complex analogs in improving the ligands anti-*T. cruzi* activities[12].

Another approach in the complexation of *N*-oxide and the effect on the anti-*T. cruzi* bioactivity was the study of Pd or Pt derivatives of the bioactive ligand sodium pyridine-2-thiolate *N*-oxide (**494**, Fig. (**7**)), a well known parasite-specific NADH-fumarate reductase inhibitor [86]. Both complexes (**495** and **496**, Fig. (**7**)) were excellent growth inhibitors *in vitro*, with IC_{50} values in the nanomolar range, and with low non-specific cytotoxicity on mammalian cells

[12] Torre, M.; Gambino, D.; Cerecetto, H.; González, M. Unpublished data.

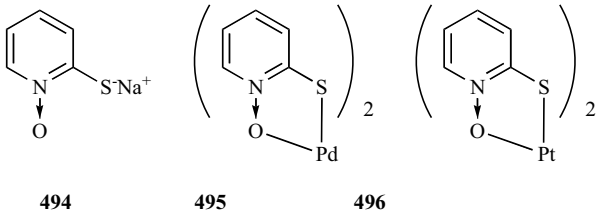
Table 21. ***In Vitro*** **Antiproliferative Activities of Quinoxaline Dioxide Complexes and the Corresponding Ligands Against** ***T. cruzi***



V^{IV} complexes M = V=O **Cu^{II} complexes** M = Cu **Pd^{II} complexes** M = Pd

Ligands			V^{IV} complexes		Cu^{II} complexes		Pd^{II} complexes	
Ref.	**-R**	**PGI (%)[a,b]**	**Ref.**	**PGI (%) (IC_{50}[c], μM)**	**Ref.**	**PGI (%)**	**Ref.**	**PGI (%)**
232	$-OCH_3$	25.0	**482**	43.5 (35.0)				
238	-H	25.0	**483**	59.5 (20.0)	**490**	63.0	492	41.0
239	$-CH_3$	5.0	**484**	47.5 (27.0)	**491**	14.0	493	54.0
478	-F	5.0	**485**	84.0 (19.9)				
479	-Cl	0.0	**486**	95.0 (16.8)				
480	-Br	31.5	**487**	91.5 (12.8)				
481	$-CF_3$	0.0	**488**	15.5 (> 25.0)				
			489	13.5 (> 25.0)				

[a] PGI: *in vitro* percentage of growth inhibition respect to untreated parasite. [b] Conditions: 25 μM doses, fifth day of treatment, epimastigote Tulahuen 2 strain. [c] IC_{50}: doses, in μM, that produces 50 % of parasite growth inhibition respect to untreated microorganism.



494 **495** **496**

	IC_{50} [a]	SI [b]
494	0.190	4.5
495	0.067	4.9
496	0.200	ÒÒ 10.0

[a] IC_{50}: doses, in μM, that produces 50 % of parasite growth inhibition of Tulahuen 2 epimastigote. [b] SI: selectivity index, defined as the ratio $IC_{50,J774\text{-macrophages}}$ / $IC_{50,T.\ cruzi}$.

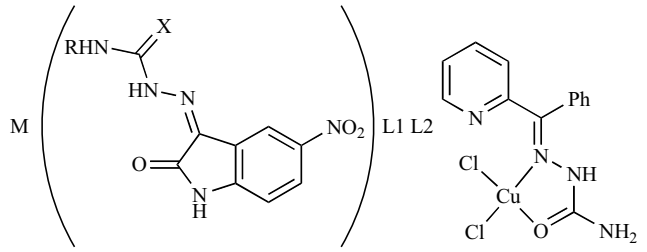
Fig. (7). Pd and Pt complexes with mercaptopyridine *N*-oxide with excellent anti-*T.cruzi* activity.

[87]. The trypanocidal mode of action was studied and the data suggest that these complexes, like the ligand, act as inhibitors of the parasite-specific enzyme NADH-fumarate reductase.

4.3. Complexes with Pharmacologically Active Metals

Other groups of complexes, derived from pharmacologically active metals, were evaluated biologically in our laboratory, and some of them were inactive against Tulahuen 2 strain of *T. cruzi* (**497-500**, Fig. (**8**))[13]. However, certain Cu(II) complexes derived from 4-nitroacetophenone thiosemicarbazones (**504** and **505**, Table **22**) showed excellent anti-*T. cruzi* activity and high mammal/parasite selectivity indexes [88]. Cu(II) complexes anti-*T. cruzi* activities were higher than the corresponding activities of thiosemicarbazone ligands although the former had better SI values (Table **22**). The effect of Cu(II), as acetate ($Cu^{II}(OAc)_2$, **507**) or sulfate ($CuSO_4$, **508**), was evaluated and the low bioactivity of these compounds and complex **506** could indicate that Cu(II) species do not possess anti-*T. cruzi* activity *per se*. The significant enhancement of activity upon complexation could be due to either changes in lipophilicity, to the rigid conformation of the ligand in the complex, which could facilitate its interaction with the biological target, or to a greater stability of the thiosemicarbazone in the complex or to redox effects involving the thiosemicarbazone and Cu. However, our results suggest the differences in activity between the three thiosemicarbazones and the corresponding complexes cannot be attributable to variations in their electrochemical behaviors [88]. Comprehensive studies on the *in vivo* behaviors of ligand **500** and complex **503** might be done in the future.

[13] Beraldo, H.; Cerecetto, H.; González, M. Unpublished data.

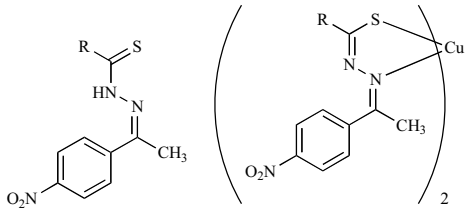


497, X= O, -R= -H, M= Cu, L1= Cl, L2= H_2O
498, X= S, -R= -CH_3, M= Ni, L1= Cl_2, L2= H_2O
499, X= S, -R= -Ph, M= Ni, L1= Cl, L2= Cl

500

Fig. (8). Inactive Cu and Ni complexes against *T. cruzi.*

Table 22. *In Vitro* Activities of Cu(II) Complexes and the Corresponding Ligands Against *T. cruzi* and Mammals Cells



501, -R= -$NHCH_3$
502, -R= -$N(CH_3)_2$
503, -R= -4-piperidinyl

504, -R= -$NHCH_3$
505, -R= -$N(CH_3)_2$
506, -R= -4-piperidinyl

Ref.	IC_{50} (μM)[a,b]	SI[c]
501	> 25.0	> 16.0
502	0.28	> 1428.0
503	>> 25.0	ns[d]
504	2.0	2.0
505	0.056	893.0
506	>> 25.0	ns[d]
507	>> 2.0	ns[d]
508	>> 2.0	ns[d]

[a] IC_{50}: doses, in μM, that produces 50 % of parasite growth inhibition respect to untreated microorganism. [b] Conditions: fifth day of *in vitro* treatment, epimastigote Tulahuen 2 strain. [c] SI: selectivity index, defined as the ratio $IC_{50,THP\text{-}1\text{-}macrophages}$ / $IC_{50,T.\ cruzi}$. [d] ns: no studied.

5. CONCLUDING REMARKS

The information amassed from our large experience in the evaluation of a wide range of different chemical families against *Trypanosoma cruzi* parasite has resulted in the identification and ultimately the potential use of compounds in the clinic as antichagasic drugs. However, new structural modifications, derived from the most active compounds or structurally new ones, or more comprehensive pharmacological studies could be done to produce more efficient clinical agents.

ACKNOWLEDGEMENT

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