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Artigo

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Derivados 1,4-di-*N*-óxido Quinoxalinas: O Interesse no Tratamento da Doença de Chagas

Resumo: Mais de 100 milhões de pessoas estão em risco de contrair a doença de Chagas. Estima-se que em 2008, mais de 10.000 pessoas morreram devido a esta patologia. Apesar de a doença ter sido descoberta há mais de 100 anos, ainda é preciso encontrar tratamento seguro e eficaz. Portanto, novos fármacos eficazes contra a doença de Chagas necessitam urgentemente serem descobertos. Derivados de quinoxalina apresentam interessantes propriedades biológicas (anti-infecciosa, citotóxica, anti-candida, anti-protozoário) e a avaliação de suas propriedades farmacológicas ainda está em andamento. Com respeito a isso, nós estamos há mais de uma década em busca de agentes anti-chagásicos. Nesta revisão mostramos nosso trabalho em andamento para identificação de novos agentes anti- *T. cruzi com o* padrão estrutural quinoxalina-di-*N*-óxido e apresentamos a relação estrutura-atividade observada entre as diferentes substituições nas posições C-2, C-3, C-6 e C-7 do anel da quinoxalina.

Palavras-chave: Doença de Chagas; *Trypanosoma cruzi*; 1,4-di-N-óxido Quinoxalinas.

Abstracts

More than 100 million people are at risk of contracting Chagas disease. It is estimated that in 2008, Chagas disease was responsible for the death of more than 10,000 people. Despite the fact that there are more than 100 years since the disease was discovered, safe and effective treatments still have to be found. Therefore, new drugs active against Chagas disease are urgently required. Quinoxaline derivatives show very interesting biological properties (anti-infective, cytotoxic, anticandida, antiprotozoal) and evaluation of their medicinal chemistry is still in progress. In this regard, we have spent more than a decade in the search for anti-Chagasic agents. In this review, we summarize our on-going work to identify new anti-*T. cruzi* agents with the quinoxaline-di-*N*-oxide scaffold. We present the structure-activity relationship observed among the different substitutions in C-2, C-3, C-6 and C-7 position of the quinoxaline ring.

Keywords: Chagas disease; *Trypanosoma cruzi*; Quinoxaline 1,4-di-*N*-oxide.

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Quinoxaline 1,4-di-*N*-oxide Derivatives: Interest in the Treatment of Chagas Disease

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1. Introduction

Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite, *Trypanosoma cruzi* (*T. cruzi*). It is mainly found in Latin America, where it is mostly transmitted to humans through the feces of triatomine bugs. According to the World Health Organization, 8 million people are infected with *T. cruzi* worldwide, with the majority of the cases occurring in Latin America, where Chagas disease is endemic.[1,2] However, it has now spread to other continents due to migration patterns; in the past few decades, the cases of Chagas disease has increased in the United States of America and Canada as well as in many European and some Western Pacific countries. More than 100 million people are at risk of the disease. It is estimated that in 2008, Chagas disease killed more than 10,000 people.

Despite the fact that it has been more than 100 years since the disease was discovered, safe and effective treatments have yet to be found. Currently, vector control is the most useful method for preventing Chagas disease in Latin America. Chagas disease is curable if treatment is initiated soon after infection.[3-6] Up to 30% of chronically infected people develops cardiac alterations and up to 10% develop digestive, neurological or mixed alterations for which specific treatment may be necessary. Only two drugs have been commercialized, Nifurtimox (Nfx) and Benznidazole (Bzn), and both drugs have some of limitations, such as low activity in the chronic phase of the disease, emergence of resistance, and toxicity (Figure 1). Therefore, there is an

urgent need to develop new, safe and effective therapeutic alternatives that are also cost-effective.

The quinoxaline scaffold is present in several compounds that show very interesting biological properties and there is active interest in their research concerning medicinal chemistry. More importantly, its oxidized form, 1,4-di-*N*-oxide (QDO) derivatives, greatly increases the biological properties of the structure.[7] In fact, the QDO are known as being anti-infective agents and they are cytotoxic against hypoxic cells present in solid tumours, with anticandida, antiprotozoal and mutagenic properties, depending on specific chemical features.[8-16]

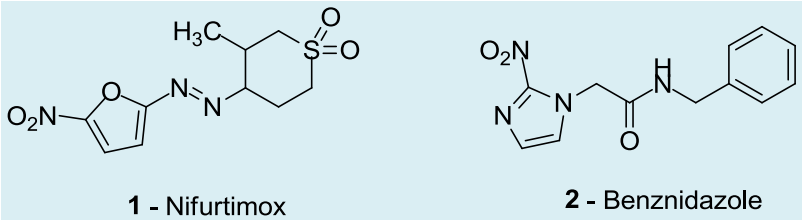


Figure 1. Nifurtimox and Benznidazole structures

Furthermore, it is well-known that some QDO are species that suffer a bioreductive process under hypoxic conditions, producing hydroxyl radical and quinoxaline. Similar to the nitro pharmacophore of antitrypanosomal drugs, the *N*-oxide moiety of the quinoxaline could produce parasitic damage through the production of radical species affecting the redox metabolism.[15] As previously described,[17] the absence of the *N*-oxide moiety produces a decrease in the anti-epimastigote activity, thereby confirming that this group plays a key role in the mechanism of QDO antitrypanosomal activity.[18]

Based on this idea, and in the search for more selective and less toxic anti-Chagas drugs, our synthesis group, led by professor Monge, has synthesized different series of quinoxaline-1,4-di-*N*-oxide derivatives, with a great variety of substituents in positions 2, 3, 6 and 7 (Figure 2). Here is a summary of more than one decade of work carried out in close collaboration with the University of Uruguay, whose research personnel have conducted the biological activity assays.

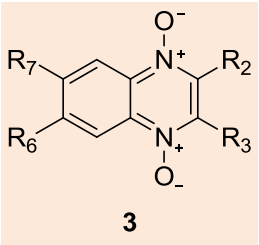


Figure 2. General structure of quinoxaline 1,4-di-*N*-oxide (QDO)

2. Background

Trypanothione reductase (TR) is the most thoroughly studied enzyme of the trypanothione redox metabolism. Despite 40% identity in their primary sequences, TR and human glutathione reductase are exclusive towards their respective substrate trypanothione and glutathione. So, TR is a key enzyme of the parasite anti-oxidant defense system; however, it does not occur

in the mammalian host. Therefore, TR emerged as one of the most promising targets in the development of new anti-trypanosomatid agents.[19-22] Mepacrine, **4** (in the US, known as quinacrine), was the first tricyclic compound identified as a competitive inhibitor of *T. cruzi* TR, but not of human glutathione reductase (GR). Molecular modeling of the active center of TR led to the design of phenothiazines and other tricyclic compounds as potent inhibitors of TR (Figure 3).[23-25]

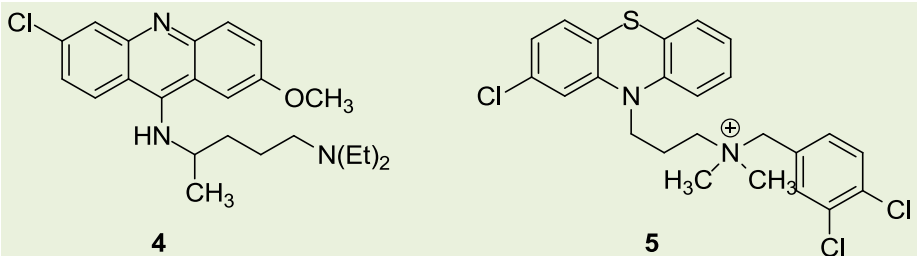


Figure 3. Inhibitors of *T. Cruzi* Trypanothione reductase

Some quinoxaline derivatives developed by Monge and coworkers resembled the skeleton of these inhibitors.[23-27] The activity of some of the most powerful TR inhibitors is not only due to the capability for inhibition, but also due to their redox activity.[28,29] In this sense, when the QDO scaffold is considered, the resulting compounds are capable of acting as substrate of reductive enzymes, generating reactive oxygen species. These two facts and the ability of QDO to act as anti-infective agents towards a great number of microorganisms encouraged us to evaluate some selected quinoxalines for their anti-trypanosomatid activity.

The first contribution regarding quinoxaline derivatives with antichagas activity goes back to the year 2002. Thirty-three quinoxaline derivatives were selected taking into account their volume, polarity, lipophilicity and electrochemical behavior (Figure 4). The selected compounds were evaluated for their *in vitro* activity against *T. cruzi* Tulahuen strain. Moreover, the IC_{50} in Tulahuen and Brener strains were determined for six derivatives showing a PGI (Percentage of Growth Inhibition) greater than 80%.[23,27]

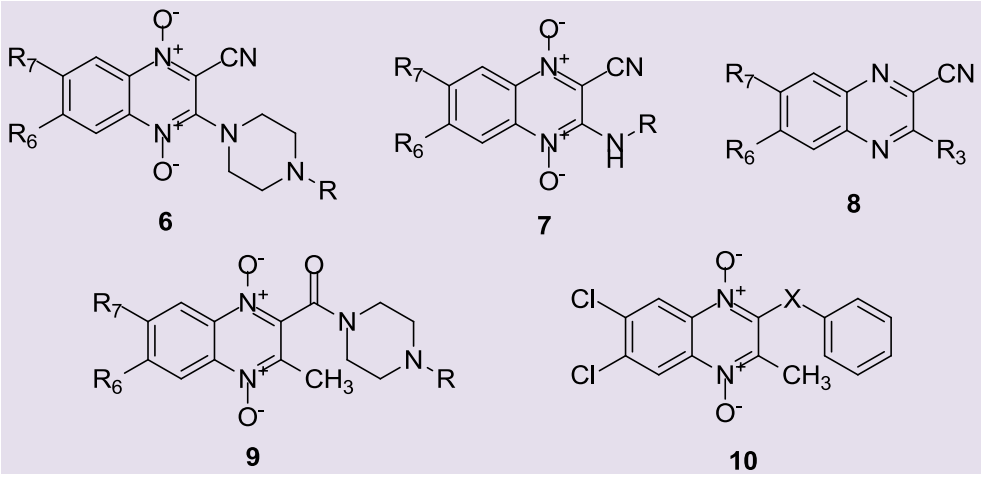


Figure 4. General structures of some selected QDO derivatives

In this first approach, five compounds (**11-15**) showed very promising results and emerged as an excellent starting point for future structural modifications of the QDO scaffold in an effort to develop new anti-trypanosomatid agents (**Figure 5**).

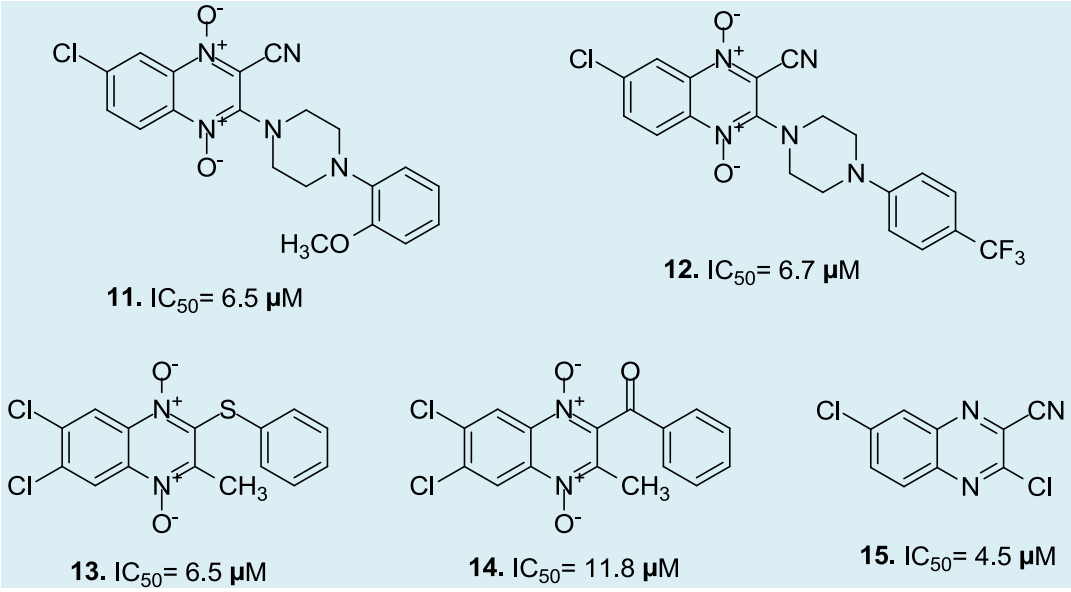


Figure 5. The most potent quinoxaline derivatives evaluated against *T. cruzi* in the first screening. IC_{50}: concentration (in µM) that inhibits 50% of epimastigote form of *T. Cruzi* growth

3. Ten years of research

These *in vitro* results indicated that QDO hold promise for the treatment of Chagas disease. Therefore, a study considering the quinoxaline scaffold for the search of new anti-trypanosomatid agents was developed.

3.1. Vanadium complexes

With the aim of improving the bioavailability of quinoxaline derivatives, some vanadyl complexes of 3-aminoquinoxalin-2-carbonitrile 1,4-di-*N*-oxide were prepared.[30]

Seven complexes were prepared (Figure 6**,** general structure **17**) and their *in vitro* activity against the epimastigote form of *T. Cruzi* (Tulahuen 2) was studied. The results revealed that 3 of the complexes showed a PGI higher than 80% and all of them improved the activity compared with their corresponding free ligands.

Moreover the lipophilicity of the new complexes was studied and some structure-activity relationships were performed. The studies revealed that vanadium complexation improves the solubility in hydrophilic media of the quinoxaline scaffold.

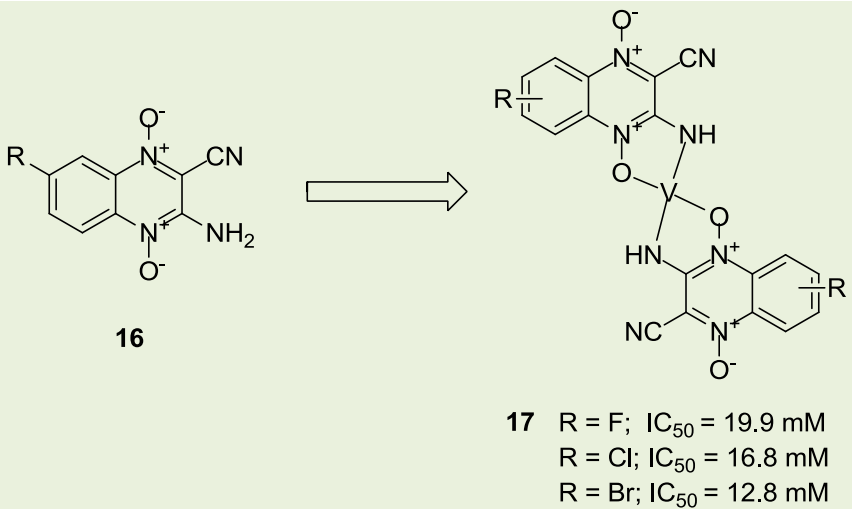


Figure 6. General structure of quinoxaline free ligands and their corresponding vanadyl complexes

3.2. *N*-acylhydrazones

In collaboration with LASSBio research laboratory located in Rio de Janeiro, we have designed, synthesized, performed docking studies and evaluated the trypanocidal activity of novel quinoxaline-*N*-acylhydrazone derivatives.[31] The idea of synthesizing this type of compounds originates from the publication of some *N*-acylhydrazone derivatives as bioactive compounds with anti-trypanosome activity.[32,33] We studied the ability of the new derivatives to inhibit the parasite growth at the doses of 25 μM. Two out of the sixteen synthesized compounds (**19, 20**) presented IC_{50} values of the same order of magnitude as the standard drug Nfx (IC_{50}=7.7μM). (Figure 7). The activity data and their low cytotoxicity revealed these compounds as promising lead compounds.

In an attempt to theoretically explain the difference found in the trypanocidal activity of the new quinoxaline *N*-acylhydrazone derivatives, docking studies using the cruzain enzyme were performed.

These studies showed that a reasonable correlation exists in the molecular design of the quinoxaline derivatives so that they have affinity for the cruzain enzyme. The prediction values *in silico* (AG bind values), that were obtained by docking studies, pointed out compounds **19** and **20** as the most promising inhibitors, assuming that the molecular mechanism of these compounds involves this cruzain target. These studies also suggest that low bioactivity may be due to a lack of adequate distances to the sulphur atom of the Cys25 of cruzain.

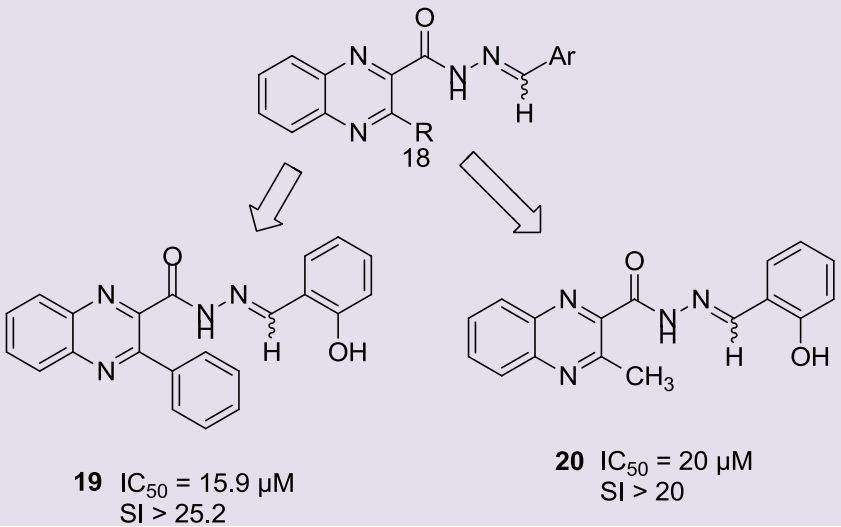


Figure 7. General structure of quinoxaline (*E/Z*)-*N*-acylhydrazones and the most active compounds

3.3. 2-Amide-3-carbonitrilederivatives

Another example of our research group in an attempt to obtain a new hybrid QDO derivative with potential anti-tubercular and anti-*T. cruzi* activity is described here.[34] First of all, nitrofuryl moiety is present in Nfx (compound **1** in Figure 1) and in a large number of anti-*T. cruzi* agents acting via a nitro-reduction process.[18] In 2004, Tangallapally and coworkers[35] published that nitrofuryl derivatives have the requirements for optimum inhibition of UDP-galactosemutase (compound **24** in Figure 8), responsible for the biosynthesis of galactofuranose, an essential component of the mycobacterial cell wall. In addition, we have QDO with good *in vitro* selectivity against *M. tuberculosis* (compounds **21** and **22** in **Figure 8**) and against *T. cruzi* (compound **23** in **Figure 8**). Therefore, we decided to design a new series of QDO amide derivatives with dual anti-tubercular and anti-chagas activity (Figure 8).

Although the hybridization process, pharmacophore QDO plus pharmacophore nitrofuran, does not produce dual active compounds. The derivatives **26** and **27** showed interesting antichagas activity. Moreover, it was observed that the activity increases for the compounds that have the most electron withdrawing groups.

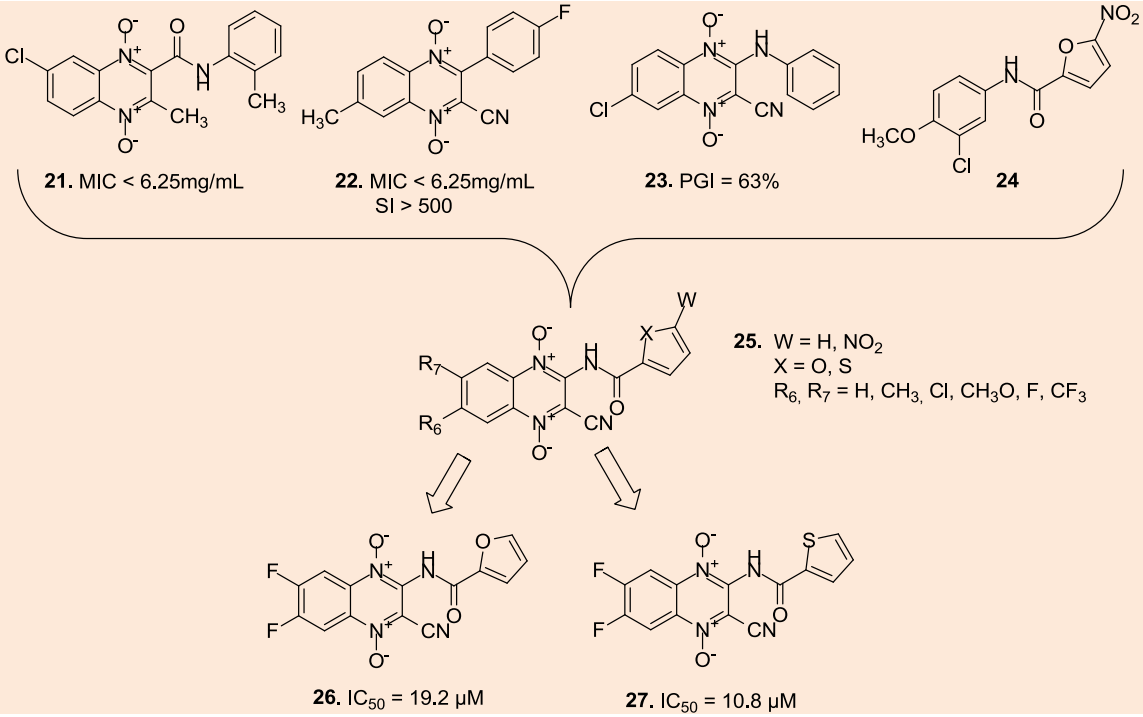


Figure 8. Design of new heterocyclic-2-carboxylic acid (3-cyano-1,4-di-*N*-oxidequinoxalin-2-yl)amide derivatives and the most active compounds **26** and **27**, against *T. cruzi* Tulahuen 2 strain. MIC: Minimum inhibitory concentration against *M. tuberculosis* H37Rv; PGI: Percentage of growth inhibition of epimastigote form of Tulahuen 2 strain at 25 µM doses; IC_{50}: concentration (in µM) that inhibits 50% of epimastigote form of *T. Cruzi* growth

3.4. 3-aryl- 2-carbonitrile derivatives

In a continuing effort to identify new active compounds that can combat Chagas disease and other neglected diseases, our research group prepared 23 new 3-arylquinoxaline-di-*N*-oxide derivatives.[36]

Several structural modifications were introduced at the lead compounds **11, 12, 26** and **27** (Figure 9) by applying the isosteric and homologous strategies (for instance elimination of the piperazinyl ring or by replacement of a methyl with a phenyl group) attempting to increase their lipophilic properties. We synthesized two series, maintaining the carbonitrile group linked to C-2 and with the aromatic group directly linked in C-3. Almost all of the compounds were active against the Tulahuen 2 strain, inhibiting growth > 50% at doses 25 µM. The most active compounds **29-30** and **33-34** are showed in Figure 9.

In view of the results achieved and in a further attempt to improve the biological profile of these QDO, we applied the QSAR models that we had developed in order to estimate the antitrypanosomal parameters of new possible antichagasic candidates. The proposed structures are presented in Figure 10.

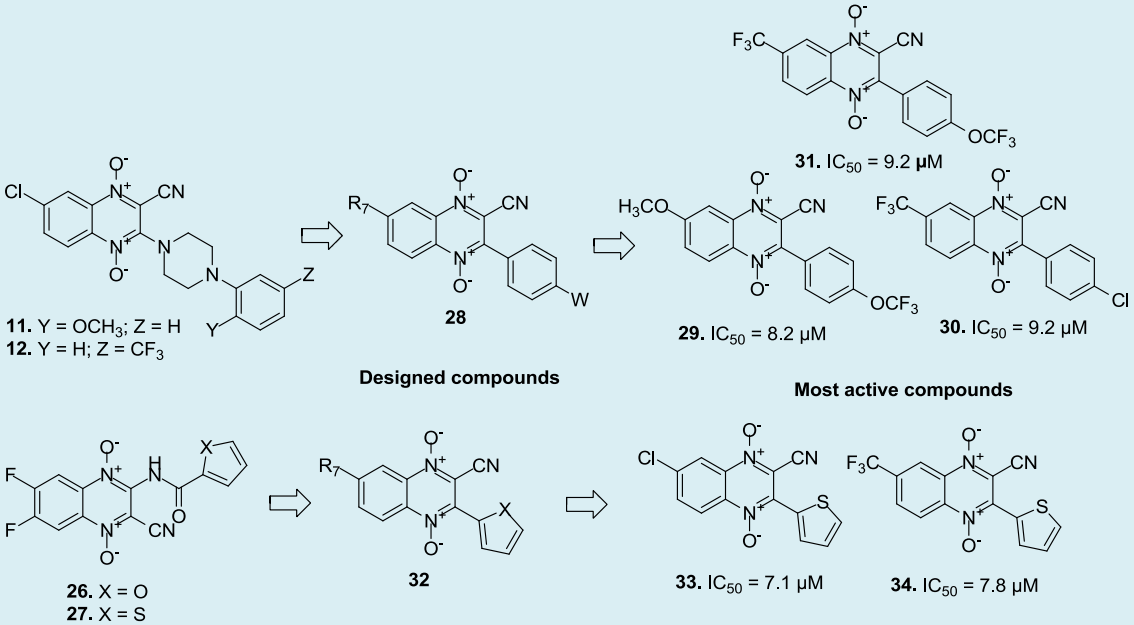


Figure 9. Design of 3-arylquinoxaline-2-carbonitrile di-*N*-oxide as anti-trypanosomal drugs from previous lead compounds with structural modifications and the most active compounds obtained

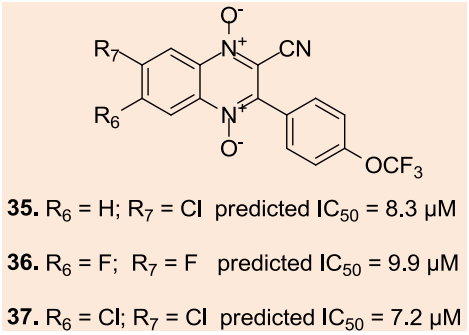


Figure 10. Structure of the three best resulting anti-trypanosomal derivatives, as predicted by QSAR among the 49 newly proposed 3-arylquinoxalines-2-carbonitrile di-*N*-oxide

3.5. Ketone derivatives

In spite of the good results obtained from the 2-carbonitrile derivatives, in view of the toxicity data[27, 37] a decision was made to substitute the carbonitrile group in C-2 of the quinoxaline ring for a carbonyl group. Taking into account the activity of the previously described compound **14**,[23] a new family consisting of more than forty QDO derivatives[38] was synthesized in order to analyze its *in vitro* activity against the epimastigote form of three different straws and one clone of *T. cruzi*. Optimal anti-*T. cruzi* QDO were identified from a structural point of view (Figure 11). First of all, we were able to determine the importance of the presence of a good electrophilic center in C-2. The derivatives in which the carbonyl group in C-2 was replaced by a methylene (CH_2) group were completely inactive at the doses assayed (**14** *vs*. **39**), showing the importance of the derivative **14**. We were also able to confirm the importance of *N*-oxides because the optimal activity was found in the dioxidized form of the quinoxaline and its reduction led to compounds that were equally inactive (**42** *vs*. **43**). Derivatives with CF_3 in C-3 were the most active in

comparison with derivatives with CH_3 or phenyl group in this position (**41** *vs.* **40** and **45** *vs.* **44**). Moreover, the addition of good electron-withdrawing substituents in positions 3, 6 and 7 increased the antichagas activity of the compounds.

The leader compound was derivative **45**, with anti-Tulahuen 2 activity nearly 20 times more active than the reference drug Nfx., with the best selectivity index data of the series (macrophage/*T. cruzi*) and not mutagenic in the Ames test.[39] This compound contained all the structural requirements observed for other derivatives: a good electrophilic center in position C-2 and three excellent electron-withdrawing moieties in C-3, C-6 and C-7 positions.

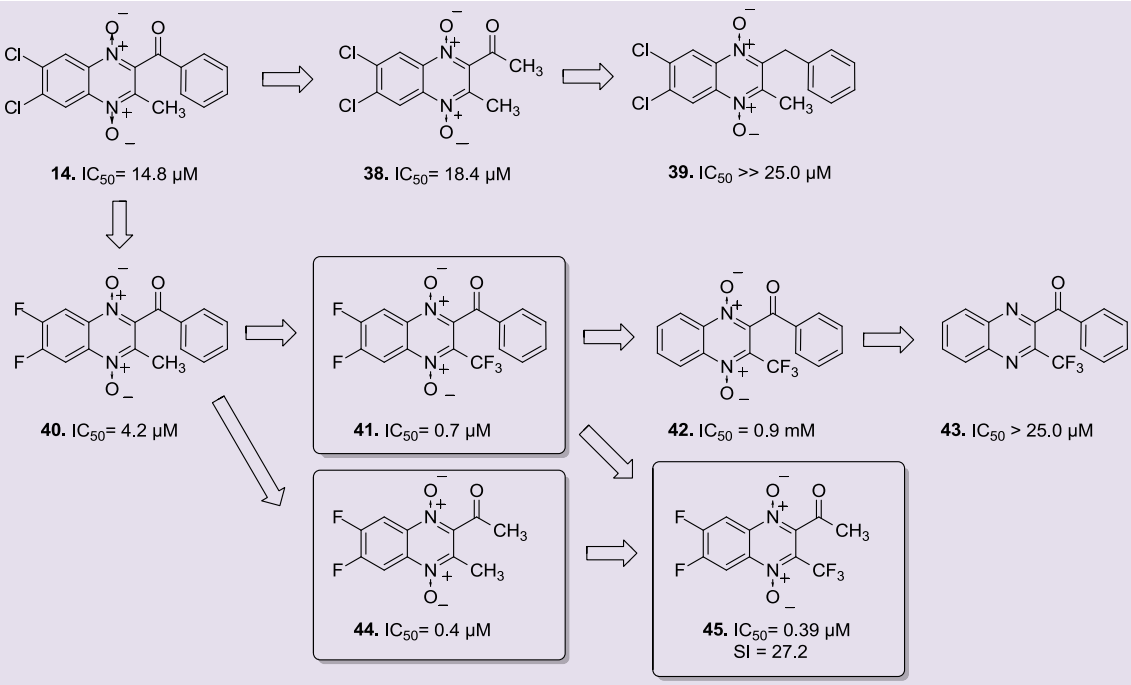


Figure 11. Structural anti-*T. cruzi* activity for the studied quinoxalines. The IC_{50} refer to Tulahuen 2 strain

3.6. Ester derivatives

Continuing with the optimization based on the structure-activity relationships schemes planned by our working group, thirty quinoxaline 1,4-di-*N*-oxide derivatives were prepared. The synthesized derivatives were modified at C-2 of the quinoxaline ring, possessing a methyl or ethyl ester group instead of the ketone moiety.[38,40]

The new derivatives showed excellent *in vitro* biological activity against Tulahuen 2 strain of *T. cruzi*. Sixteen out of the eighteen compounds with CF_3 in C-3 were more potent than the reference drug Nfx. Compound **53** was identified as the most active, showing an IC_{50} value of 0.4 µM, 18 times more active than Nfx (7.7 µM). (**Figure 12**).In general, the ester derivatives improve the biological activity of the corresponding ketone in every case with the exception of substituents CF_3 at C-3 and F at C-6 and C-7. With regard to the C-2 substituent, the influence of a methyl or ethyl group in determining the biological activity is not clear. Compound **53**, which was substituted with trifluoromethyl in C-3 and fluoro groups at the C-6 and C-7 of the quinoxaline ring, was the most active and selective in the cytotoxicity assay (**Figure 12**).

Once again, it can be confirmed that the most decisive structural characteristic, in

terms of *in vitro* biological activity, is the presence of substituents with an electron withdrawing character in positions 3, 6 and 7 of the quinoxaline ring, especially fluorine atoms.

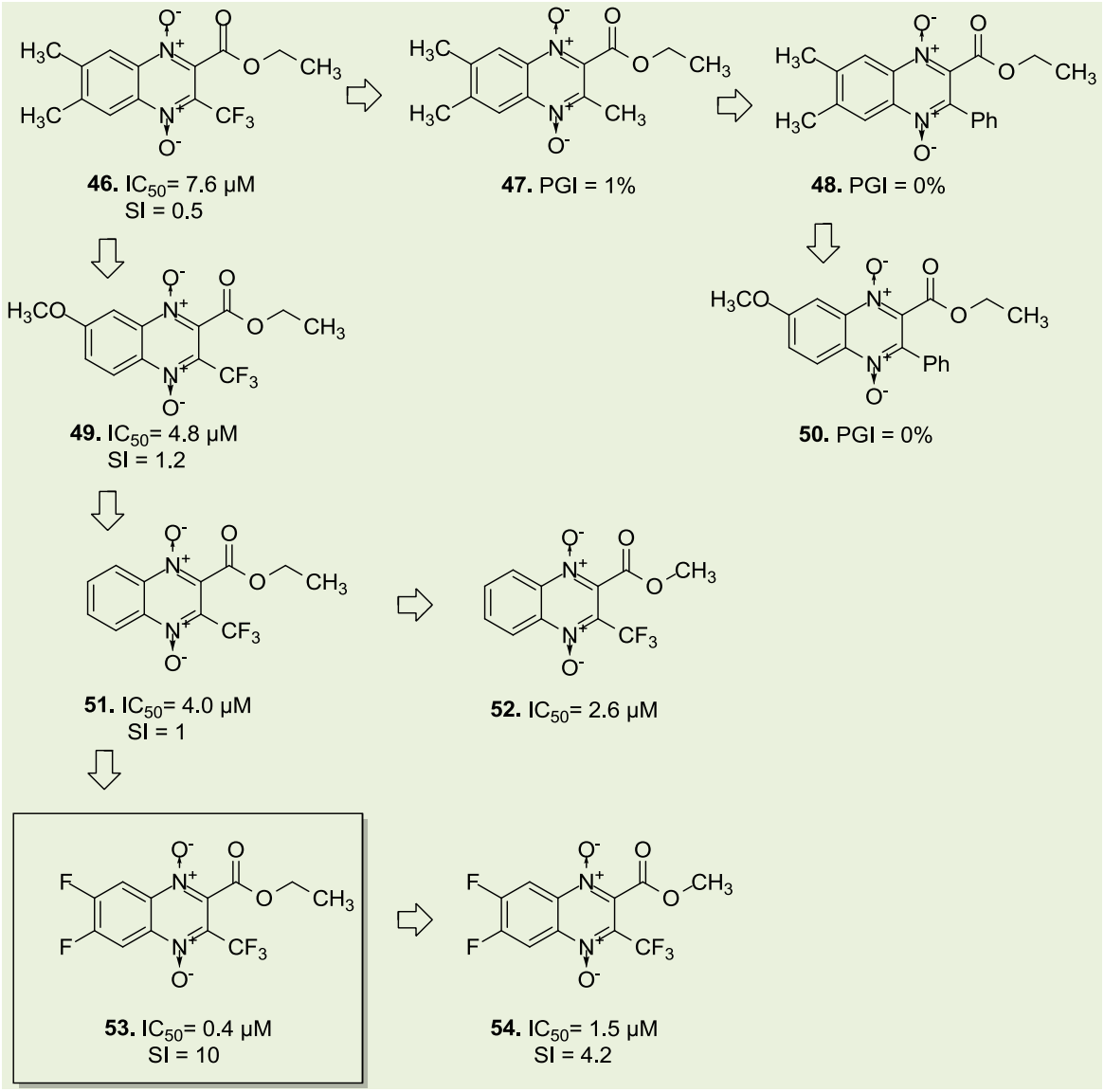


Figure 12. Structural anti-*T. cruzi* activity for the studied quinoxalines. The IC_{50} and PGI% refer to Tulahuen 2 strain

4. Complementary assays

In previous work, the presence of the *N*-oxide moiety significantly potentiated the activity against *T. cruzi*, which may indicate the importance of a bioreduction or metabolization process of these groups by specific enzymes within the activity of related with these types of derivatives.[8,9,38] However, the mechanism of action through which these derivatives carry out their activity is still under investigation. We present some studies performed in an attempt to go insight into the mechanism of action of QDO derivatives.

4.1. Effect on mitochondrial dehydrogenase activity

As mentioned in the introduction of this paper, some QDO compounds are species that suffer a bioreductive process under hypoxic conditions, producing hydroxyl radical and quinoxaline. This led us to believe that our quinoxalines could produce parasitic damage through the production of radical species affecting the redox metabolism. It has been demonstrated that mitochondrial dehydrogenases could be involved in the mechanism of action of *N*-oxide containing heterocycles, such as furoxans and benzofuroxans.[41,42] In order to better understand the anti-*T. cruzi* mechanism of action of our compounds, we studied the effect of some selected QDO derivatives: compound **13** (Figure 5) and compounds **41**, **42** and **45** (Figure 11), on the mitochondrial dehydrogenase activity in comparison with the reference drug, Nfx.[40] The results show that Nfx. does not affect the mitochondrial dehydrogenase activity, while the quinoxaline derivatives (compound **13**, **41**, **42** and **45**) produce a decrease in a time-dependent manner (**Figure 13**). With this assay, we were able to demonstrate that mitochondria are affected when these derivatives are used.

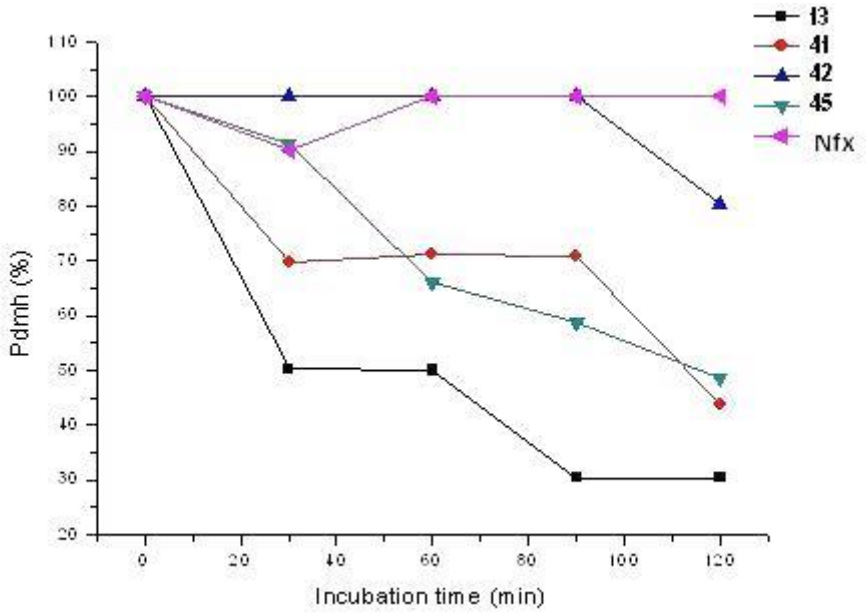


Figure 13. Variation of the percentage of mitochondrial dehydrogenase activity (Pmdh), produced by the compounds **13**, **41**, **42** and **45** with respect to time, compared to the untreated control of *T. cruzi* epimastigote, Y strain, using the colorimetric MTT assay with a described procedure[43]

4.2. Cyclic voltammetry studies

In previous studies, an inhibition of mitochondrial dehydrogenases was demonstrated and it was also observed that benzofuroxan derivatives containing *N*-oxide cause a mitochondrial membrane depolarization in *T. cruzi*.[38, 41, 42] In order to shed some light on this subject, a study was conducted which involved the measuring of the reduction potentials of the *N*-oxide groups in different synthesized derivatives and selected analogs. The experimental goal of these studies was to determine the reduction potentials for two series of QDO: ketone[44] and ester[40] derivatives, in order to obtain evidence of the relationship between

their electrochemical behaviour, their structure, and their anti-*T. cruzi* activity. The electrochemical properties of these compounds were studied using cyclic voltammetry and first derivative cyclic voltammetry in DMF with $(Bu_4N)ClO_4$ as supporting electrolyte. All reductions were diffusion-controlled, as indicated by fairly constant current functions at varying scan rates for each derivative.[45,46]

With respect to the mechanism, the heterocyles di-*N*-oxide as anti-Chagasic agents that possess the di-iminio structure are expected to suffer a reduction by means of an electron in order to form a radical anion (Figure 14). In a first phase, the QDO could be activated through a process of bioreduction in order to form a reactive anion radical, which could later lead to the superoxide ion or other toxic reactive oxygen species, or to direct interactions with macromolecules in a subsequent phase. If this mechanism occurs for these compounds, the activation process by bioreduction would generally be expected to be simpler for those compounds that can be reduced more easily or in other words, for those compounds that show less negative reduction potentials.

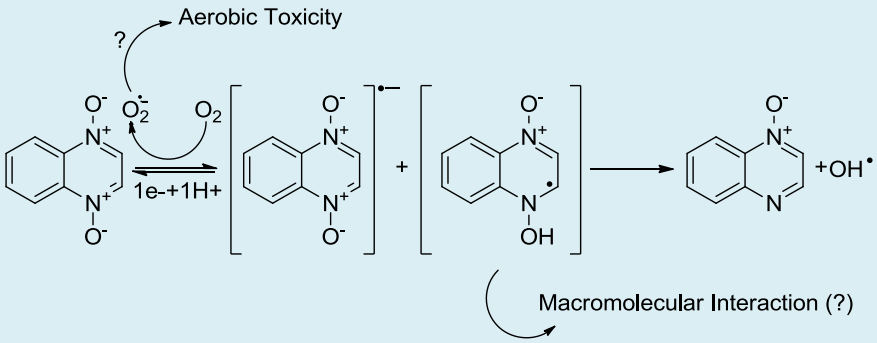
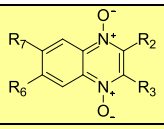


Figure 14. One electron reduction of QDO to form a radical anion

In the current study, several voltammmetric waves were observed during cyclic voltammetry of the QDO derivatives between -0.4 and 2.3 V. The first reduction process observed in the voltamogrames may be attributed to reduction of the nitrone functionality, forming a radical anion.

In this way, it was found that the structure influences both the reduction potentials and the activities of the studied compounds; therefore, a relationship between reduction potential and activity for the described quinoxaline derivatives is possible (Table 1). The presence of electron-withdrawing substituents in C-3, C-6 and C-7 of the quinoxaline ring resulted in a positive shift in reduction potential. Compounds with less negative values of reduction potential generally showed greater efficacy against the parasite. These results suggested that a bioreduction process of the *N*-oxides might be taking place in the mechanism of action of these types of derivatives.

Table1. Structure, cyclic voltammmetric data and anti-Chagas activity of the QDO. [a]$E_{1/2}$= Half-wave potentials for ferrocene. [b]Percentage of growth inhibition of Tulahuen 2 strain, dose = 25□M. [c]IC_{50}= concentration that inhibits 50% of epimastigote form of *T. cruzi* growth

	R_2	R_3	R_6/R_7	Cyclic voltammetry	Anti-Chagas activity	
				$E_{1/2}$[V][a]	*PGI* [%][b]	IC_{50} [μM][c]
43	-Ph	$-CF_3$	F/F	-1.188	100	0.7
44	-Ph	$-CF_3$	H/H	-1.308	100	0.9
47	$-CH_3$	$-CF_3$	F/F	-1.179	100	0.39
49	$-OCH_2CH_3$	$-CH_3$	CH_3/CH_3	-1.677	1	-
51	$-OCH_2CH_3$	$-CF_3$	H/OCH_3	-1.357	100	4.8
53	$-OCH_2CH_3$	$-CF_3$	H/H	-1.281	100	4.0
54	$-OCH_3$	$-CF_3$	H/H	-1.313	100	2.6
55	$-OCH_2CH_3$	$-CF_3$	F/F	-1.161	100	0.4
56	$-OCH_3$	$-CF_3$	F/F	-1.160	100	1.5
57	$-CH_2CH_3$	$-CF_3$	Cl/Cl	-1.093	100	0.78
58	$-OCH_3$	$-CH_3$	Cl/Cl	-1.366	66	-
59	$-OCH_2CH_3$	$-CH_3$	Cl/Cl	-1.370	32	-
60	$-OCH_3$	$-CF_3$	Cl/Cl	-1.076	100	10.8
61	$-OCH_2CH_3$	$-CF_3$	Cl/Cl	-1.081	100	2.9

The information obtained will be useful for the design of new derivatives in the future with greater biological activities against *T. cruzi* and better toxicity profiles.

4.3. Metabolites

In an attempt of elucidating the mechanism of action of QDO and taking into account the results previously obtained in the mitochondrial dehydrogenase activity assay, some derivatives were selected for studying the modification of excreted metabolites by ^{1}H-NMR. Studying the changes in the biochemical pathway is a very useful tool in the elucidation of mechanism of action; therefore, cell-free medium of quinoxaline-treated parasite where compared to the untreated *T. cruzi*-free medium.[38,41,47]

The changes in the most relevant modified metabolites were studied (Figure 15). It can be observed that the end-metabolite concentration is lower when parasites are treated with compound **13** in comparison with **41**-treated parasites. This fact could be explained considering that compound **13** is a better mitochondrial dehydrogenase inhibitor as it can be observed in Figure 15.

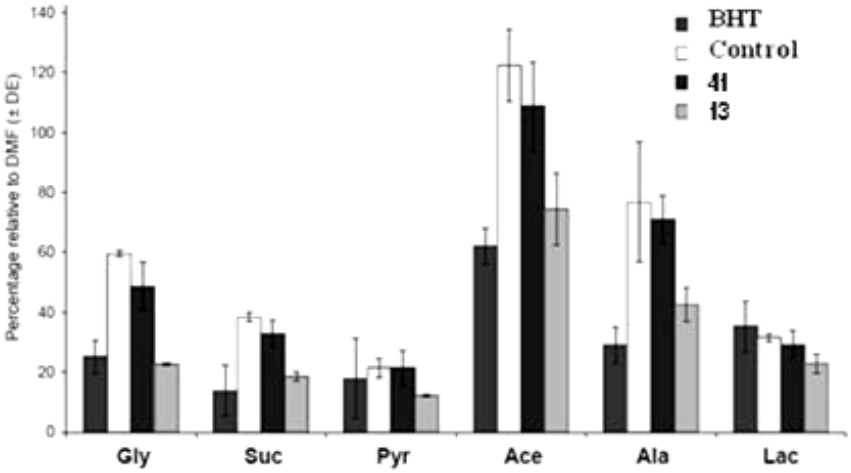


Figure 15. Percentage of the end-metabolite excreted to the medium by *T. cruzi* (Y strain), in different treatments

4.4. *In vivo* assays

Considering the promising results of some of the studied quinoxalines, derivatives **41** and **45** were selected for *in vivo* assays.[38] These derivatives had previously showed good selectivity, were active against trypomastigote form of *T. cruzi* and were not mutagenic in the Ames test.

The selected compounds were evaluated in murine models of acute Chagas disease. Three different experiments were carried out and weekly parasitemia, animal survival percentages and anti-*T. cruzi* antibody levels after the infection were studied to evaluate the *in vivo* activity. The studies revealed greater survival rates at the end of the experiments when the animals were treated with derivative **41**.

In one of the experiments animals were infected with Y-trypomastigotes and compounds **41** and **45** were administered at 10 or 30 and 10 (mg/kg bw) day, respectively. The study revealed that both derivatives avoided the second parasitemia maximum which is shown if treated with Bzn. Animals treated with derivative **41** presented lower numbers of trypomastigotes in a non-dose dependence, while animals treated with derivative **45** did not improve the parasitemia profile (Figure 16a).The observed antibody levels for **41** and Bzn-treated animals were lower than the values for the control group (PBS), although no significant differences between **41** and **45**-treated were found at day 90 after infection (Figure 16b).

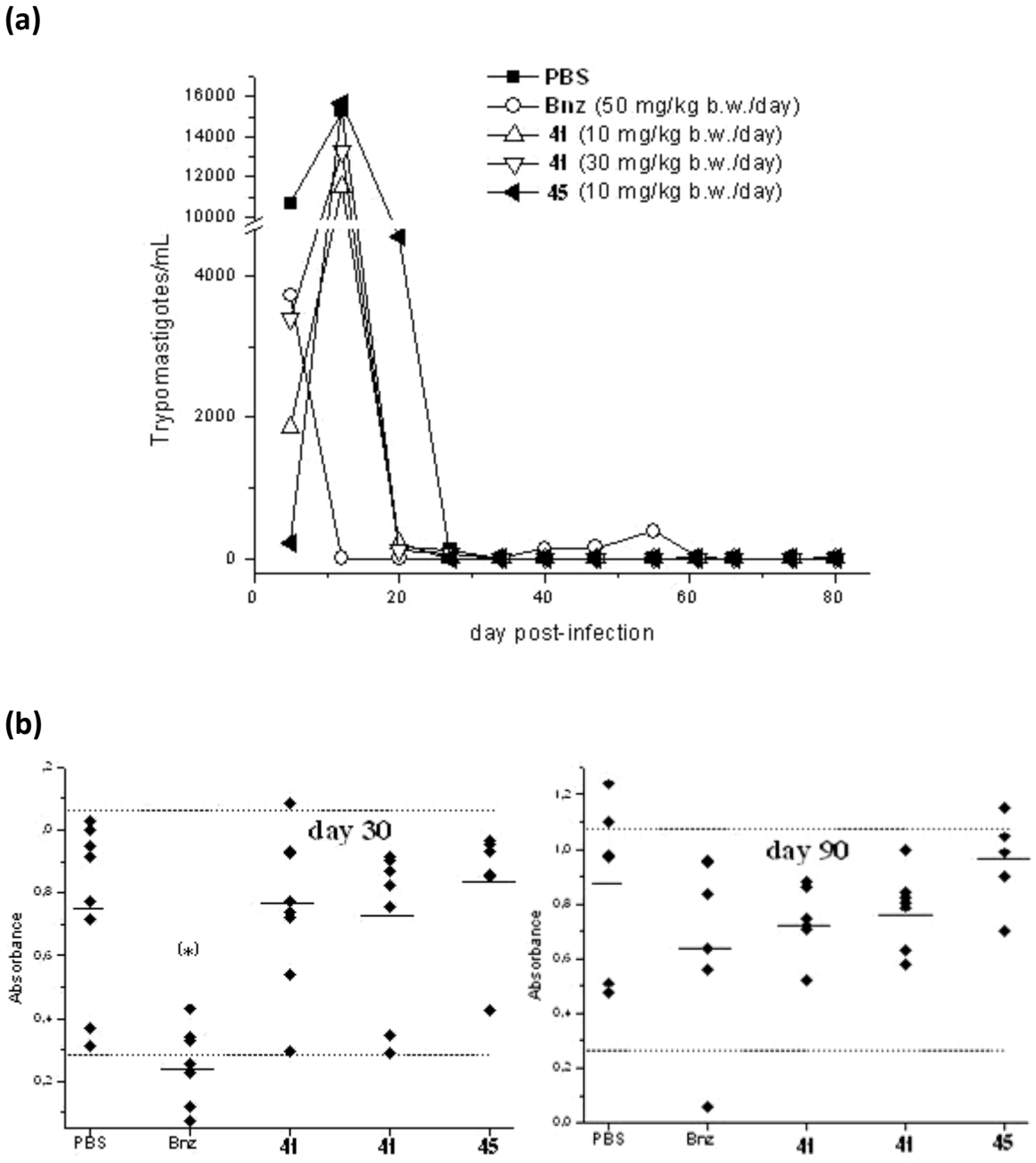


Figure 16. Y-trypomastigotes infected animals. a) Parasitemia in mice. b) Anti-*T. cruzi* antibody levels

Two experiments were carried out in animals infected with CL Brener trypomastigotes. **45**-treated animals showed a better parasitemia profile in agreement with the antibodies values that were lower than when treated with derivative **41** (Figure 17).

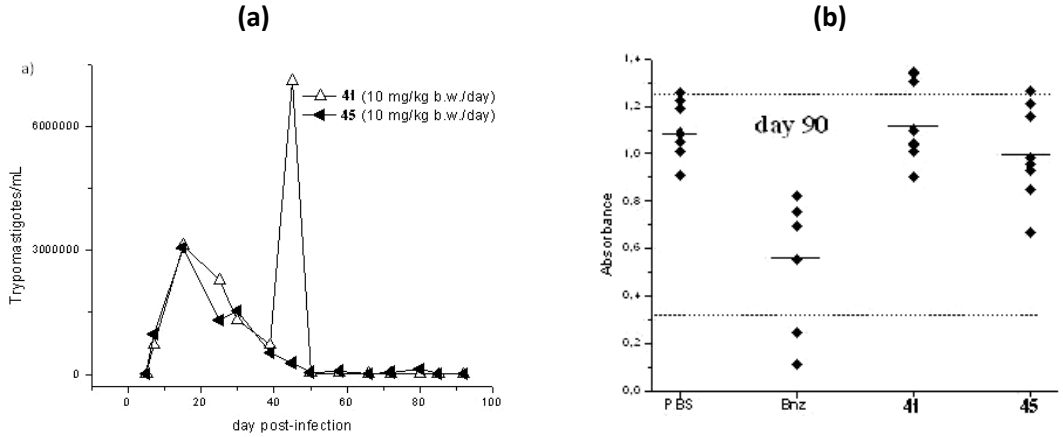


Figure 17. CL Brener trypomastigotes infected animals. a) Parasitemia in mice. b) Anti-*T. cruzi* antibody levels

5. Conclusions

During the last decade QDO have shown activity against *T. cruzi*. A continuous evaluation of more than 200 quinoxaline derivatives as anti-trypanosomatid agents has allowed us to establish some structural requirements highlighting the relevance of *N*-oxide moiety and electro withdrawing substituents on the quinoxaline ring. Moreover, it has been established that mitochondria are affected when using QDO derivatives. The voltammetric results provide some pieces of information that may be important for understanding the mechanism of anti-*T-cruzi* activity for these compounds and suggest a possible relationship between ease of reduction and activity. In addition, due to the *in vitro* activity against *T. cruzi* and the lack of mutagenesis properties, derivatives **41** and **45** were moved on to preliminary *in vivo* assays showing very interesting profiles. These promising results make them valid as new leads for synthesizing new derivatives which could improve the anti *T. cruzi* activity. Additional studies are currently being carried out with ester derivatives.

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