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Imidazolidines as new anti-*Trypanosoma cruzi* agents: Biological evaluation and structure–activity relationships

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Abstract—Imidazolidine derivatives were studied as anti-*Trypanosoma cruzi* agents. Imidazolines can be considered as ethylenediamine/carbonyl precursors and therefore interfere with the biosynthesis of polyamines into the parasite. Some of the derivatives were found to have high and selective activity against the proliferative stages of the parasite, with IC_{50} values against the epimastigote form in the low micromolar range as the reference drug Nifurtimox. The imidazolidines demonstrated to be stable after five days of incubation in buffer glucose, pH 7, indicating that diamines were not obtained in these conditions. But it was found that two of the studied diamine precursors were as active as the parent compounds. Probably, the imidazolidines affect the mitochondrial integrity according to the excreted end-products found in the NMR studies. The QSAR studies indicated that the bioactivities are correlated with the lipophilicities. In conclusion, we have described a new and relevant bioactivity for imidazolidines. The results support further in vivo studies of some of these imidazolidine derivatives.

1. Introduction

Chagas disease remains the major parasitic disease burden in Latin America, despite recent advances in the control of its vectorial and transfusional transmission.[1–3] Chemotherapy to control this parasitic infection remains unsatisfactory. Despite significant progress in our understanding of the biochemistry and physiology of its etiological agent, *Trypanosoma cruzi* (*T. cruzi*),[4,5] which has resulted in the validation of several metabolic steps essential for parasite survival as potential chemotherapeutic targets,[5,6] current specific treatments are based on old and quite unspecific drugs, associated with long-term treatments that may give rise to severe side effects. In fact, although nifurtimox (Nfx, Fig. 1) and benznidazole (Bnz, Fig. 1), the only two drugs available for clinical treatment of this disease, are able to eliminate patent parasitemia and to reduce serological titers in acute and early chronic infections, they are not active against all *T. cruzi* strains and have significantly lower efficacy in long-term chronic infections.[5,6]

Both drugs act via the reduction of the nitro group. In the case of Nfx, reduction generates an unstable nitro anion radical which produces highly toxic reduced oxygen species, whereas Bnz involves covalent modification of macromolecules by nitro reduction intermediates.[7] The side effects of these drugs result from the oxidative or reductive damage in the host's tissues and are thus inextricably linked to its anti-parasitic activity.

The search of new anti-*T. cruzi* agents has been based on different strategies,[4] that is, inhibition of specific parasite enzymes, actions on parasite DNA, and oxidative stress damage. Other possibility has been the blockage/modification of some biochemical pathways, like polyamine syntheses. Some essential polyamines, like spermidine the precursor for the synthesis of trypanothione, are

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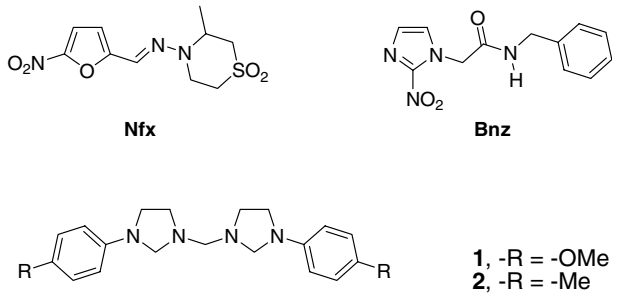


Figure 1. Nifurtimox, benznidazole, and *bis*imidazolidines with bactericidal activity.

not supplied by a uniform mechanism in *T. cruzi*. *T. cruzi* epimastigotes are unable to synthesize de novo significant amounts of putrescine and cadaverine, but take up polyamines from the medium. After conversion into spermidine and aminopropylcadaverine, these polyamines are used to synthesize trypanothione.[8] Consequently, inhibitors/modifiers of polyamine biochemistry could be a promising approach for the development of new anti-*T. cruzi* drugs.[9]

On the other hand, imidazolidines (tetrahydroimidazol derivatives) are cyclic aminals of pharmacological interest due to the bioactivity shown by some members, which is closely related to the substitution patterns. For example, *N*,*N*′-dibenzyl-2-arylimidazolidines showed antibacterial and antiamebic activity.[10] Fungicide, bactericide, and antiviral activities had also been reported for *N*,*N*′-bisaminoalkylimidazolidines and *N*,*N*′-dihydroxyphenylimidazolidines.[11] Recently, we have described some *bis*imidazolidines (*bis*(3-arylimidazolidinyl-1)methanes) with interesting bactericidal activity (i.e., compounds **1** and **2**, Fig. 1).[12] On the other hand, due to the hydrophobic nature of imidazolidines they can be used to increase the bioavailability of biologically active precursors. Thus, they had been employed as carriers of ethylenediamines[13] or carbonyl compounds.[14] They are also used as model of the coenzyme *N*,*N*-methylenetetrahydrofolic acid, which participates in single carbon transfer at the oxidation level of formaldehyde.[15]

In this work, we describe the synthesis and in vitro anti-*T. cruzi* proliferative activity of extracellular epimastigotes of a series of imidazolidines which includes terms such as bisimidazolidines (**1**–**8**), symmetrical 1,3-diaryl substituted (**9**, **16**, **20**), 1-aryl-3-methylimidazolidines (**18**, **19**), 1-aryl-3-benzylimidazolidines (**10**–**12**, **21**–**23**), and 1,3-dibenzylimidazolidines (**13**–**15**, **17**, **24**–**53**) (Table 1). The use of the epimastigote form of *T. cruzi* as biological model is done in view of: the existence of the epimastigote form as an obligate mammalian intracellular stage has been revisited and confirmed recently.[16] Moreover, we have recently demonstrated that there is an excellent relationship between anti-proliferative activity against epimastigotes and in vivo active anti-Chagas agents. We found that different families of compounds with excellent in vitro anti-*T. cruzi* epimastigote activity have displayed very good in vivo anti-*T. cruzi* activities.[17]

Taking into account that imidazolidines could act as ethylenediamines/carbonyl precursors we describe stability in buffer glucose, pH 7, of some imidazolidines and the activity of some ethylenediamine reactants, **54**–**56** (Table 3). Mammal cytotoxicities of the most active imidazolines and changes in the *T. cruzi* excreted metabolites by one of the most active imidazolidine derivatives are described. A structure–activity relationship analysis is also discussed in order to identify the structural requirements for optimum activity. The results indicate that such derivatives are good candidates for in vivo chemotherapeutic studies in appropriate animal models for Chagas' disease.

2. Methods and results

2.1. Synthesis

*Bis*imidazolidines (*bis*(3-arylimidazolidinyl-1)methanes) **1**–**8** (Table 1 and Scheme 1) were synthesized by a condensation reaction between *N*-arylethylenediamines with an excess of formaldehyde.[12] Imidazolidines **9**–**53** were synthesized by the classical method involving condensation between *N*,*N*′-disubstituted ethylenediamines with a variety of aldehydes in equimolecular conditions or an excess of aqueous formaldehyde (37%) (Table 1 and Scheme 1). *N*,*N*′-Disubstituted ethylenediamine precursors were prepared following literature procedures, those having benzyl substituents were synthesized by condensation of ethylenediamine with aromatic aldehydes employing a microwave-assisted solvent-free reaction developed previously[28] and further reduction of the generated Schiff bases with sodium borohydride. All new compounds, derivatives, were characterized by NMR (^{1}H, NOESY, and COSY) and mass spectrometry. Purity was established by microanalysis (C, H, N). 1,2,3-Trisubstituted compounds display a preferential conformation with a transoid orientation of the N-1, N-3, and C-2 substituents.[19]

2.2. Biological characterization

2.2.1. *Trypanosoma cruzi* growth inhibition experiments. Some of the studied compounds had a dose-dependent effect on the growth of extracellular *T. cruzi* epimastigotes (Tulahuen 2 strain) in BHI-Tryptose medium at 28 °C. The percentage of growth inhibition (PGI) at 25 μM is gathered in Table 1. For the most active derivatives IC_{50} were evaluated (Table 2).[20] The selectivity index ($IC_{50(T.\ cruzi)}/IC_{50(macrophages)}$)[21] was also studied for derivatives **25**, **28**, **30**–**32**, **35**–**37**, and **45** and it was >10 being the $IC_{50(macrophages)} > 50$ μM.

2.2.2. Ethylenediamines as bioactive entities. In order to study if the ethylenediamines are the bioactive entities after a potential biological hydrolysis of the imidazolidine carriers,[13,14] two different studies were performed.

In one hand, we have studied the biological activities of three ethylenediamines potentially produced by hydrolysis from the parent imidazolines (Table 3). We selected them as examples of ethylenediamines coming from imi-

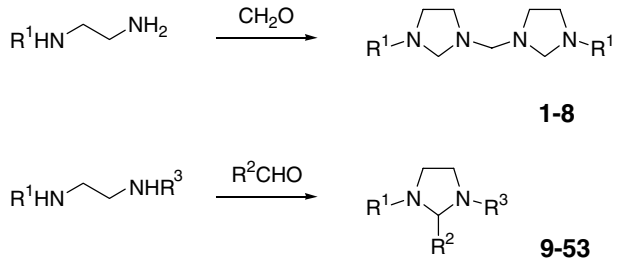
Table 1. Anti-proliferative activities of imidazolines against *T. cruzi*

Compound	–R[1a]	–R[2a]	–R[3a]	PGI[b]
1	4-$CH_3OC_6H_4$	—	—	0.0
2	4-$CH_3C_6H_4$	—	—	0.0
3	C_6H_5	—	—	0.0
4	4-ClC_6H_4	—	—	0.0
5	3-ClC_6H_4	—	—	8.0
6	4-BrC_6H_4	—	—	0.0
7	3-BrC_6H_4	—	—	10.8
8	4-$NO_2C_6H_4$	—	—	0.0
9	C_6H_5	H	C_6H_5	12.3
10	C_6H_5	H	$C_6H_5CH_2$	17.2
11	4-$CH_3C_6H_4$	H	$C_6H_5CH_2$	44.2
12	4-ClC_6H_4	H	$C_6H_5CH_2$	64.7
13	4-$ClC_6H_4CH_2$	H	4-$ClC_6H_4CH_2$	100.0
14	3-$ClC_6H_4CH_2$	H	3-$ClC_6H_4CH_2$	100.0
15	3,4-$Cl_2C_6H_3CH_2$	H	3,4-$Cl_2C_6H_3CH_2$	100.0
16	C_6H_5	CH_3	C_6H_5	12.4
17	4-$CH_3OC_6H_4CH_2$	CH_3	4-$CH_3OC_6H_4CH_2$	40.0
18	C_6H_5	C_6H_5	CH_3	6.6
19	4-$CH_3C_6H_4$	C_6H_5	CH_3	8.9
20	C_6H_5	C_6H_5	C_6H_5	39.6
21	C_6H_5	C_6H_5	$C_6H_5CH_2$	45.1
22	4-$CH_3C_6H_4$	C_6H_5	$C_6H_5CH_2$	46.0
23	4-ClC_6H_4	C_6H_5	$C_6H_5CH_2$	70.4
24	$C_6H_5CH_2$	C_6H_5	$C_6H_5CH_2$	63.0
25	4-$CH_3OC_6H_4CH_2$	C_6H_5	4-$CH_3OC_6H_4CH_2$	75.3
26	2-$OHC_6H_4CH_2$	C_6H_5	2-$OHC_6H_4CH_2$	4.8
27	4-$CH_3C_6H_4CH_2$	C_6H_5	4-$CH_3C_6H_4CH_2$	100.0
28	4-$ClC_6H_4CH_2$	C_6H_5	4-$ClC_6H_4CH_2$	94.5
29	3-$ClC_6H_4CH_2$	C_6H_5	3-$ClC_6H_4CH_2$	100.0
30	3,4-$Cl_2C_6H_3CH_2$	C_6H_5	3,4-$Cl_2C_6H_3CH_2$	100.0
31	4-$ClC_6H_4CH_2$	2-Furyl	4-$ClC_6H_4CH_2$	89.4
32	3,4-$Cl_2C_6H_3CH_2$	2-Furyl	3,4-$Cl_2C_6H_3CH_2$	93.9
33	4-$CH_3OC_6H_4CH_2$	–CH=CHC_6H_5	4-$CH_3OC_6H_4CH_2$	98.6
34	4-$ClC_6H_4CH_2$	–CH=CHC_6H_5	4-$ClC_6H_4CH_2$	100.0
35	4-$ClC_6H_4CH_2$	4-$CH_3OC_6H_4$	4-$ClC_6H_4CH_2$	100.0
36	4-$ClC_6H_4CH_2$	3-OHC_6H_4	4-$ClC_6H_4CH_2$	97.4
37	4-$ClC_6H_4CH_2$	2-OHC_6H_4	4-$ClC_6H_4CH_2$	86.8
38	4-$CH_3OC_6H_4CH_2$	4-ClC_6H_4	4-$CH_3OC_6H_4CH_2$	100.0
39	4-$CH_3C_6H_4CH_2$	4-ClC_6H_4	4-$CH_3C_6H_4CH_2$	100.0
40	$C_6H_5CH_2$	4-ClC_6H_4	$C_6H_5CH_2$	60.0
41	4-$ClC_6H_4CH_2$	4-ClC_6H_4	4-$ClC_6H_4CH_2$	100.0
42	3,4-$Cl_2C_6H_3CH_2$	4-ClC_6H_4	3,4-$Cl_2C_6H_3CH_2$	50.0
43	4-$CH_3OC_6H_4CH_2$	3-ClC_6H_4	4-$CH_3OC_6H_4CH_2$	95.0
44	4-$CH_3C_6H_4CH_2$	3-ClC_6H_4	4-$CH_3C_6H_4CH_2$	99.0
45	4-$ClC_6H_4CH_2$	3-ClC_6H_4	4-$ClC_6H_4CH_2$	100.0
46	4-$CH_3OC_6H_4CH_2$	2-ClC_6H_4	4-$CH_3OC_6H_4CH_2$	49.0
47	4-$ClC_6H_4CH_2$	2-ClC_6H_4	4-$ClC_6H_4CH_2$	100.0
48	4-$CH_3OC_6H_4CH_2$	3,4-$Cl_2C_6H_3$	4-$CH_3OC_6H_4CH_2$	91.0
49	4-$ClC_6H_4CH_2$	3,4-$Cl_2C_6H_3$	4-$ClC_6H_4CH_2$	92.0
50	3,4-$Cl_2C_6H_3CH_2$	3,4-Cl C_6H_3	3,4-$Cl_2C_6H_3CH_2$	30.0
51	4-$CH_3OC_6H_4CH_2$	4-$NO_2C_6H_5$	4-$CH_3OC_6H_4CH_2$	83.0
52	4-$ClC_6H_4CH_2$	4-$NO_2C_6H_5$	4-$ClC_6H_4CH_2$	100.0
53	4-$CH_3OC_6H_4CH_2$	3-$NO_2C_6H_5$	4-$CH_3OC_6H_4CH_2$	93.0
Nfx	—	—	—	100.0

[a] According to Scheme 1.

[b] PGI: percentage of parasite growth inhibition (%) at 25 μM. The results are means of three different experiments with a SD less than 10% in all cases.

dazolines with varied bioactivities. Ethylenediamines **55** and **54** could be obtained from two of the most active 2-phenylimidazolines, **28** (IC_{50} = 5 μM) and **25** (IC_{50} = 1.1 μM), respectively, and ethylenediamine **56** could be obtained from one of the inactive 2-phenylimidazolines (**26**, IC_{50} > 50 μM). Clearly, two of the diamines, **54** and **55**, were as active as the corresponding imidazolines, that is, **13**, **17**, **25**, **28**, **31**, **33**–**38**, **41**, **43**, **45**–**49**, and **51**–**53**, while the ethylenediamine **56** was inactive in the assayed conditions like the corresponding



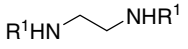
Scheme 1. Synthetic procedures used for the development of derivatives **1**–**53**.

Table 2. IC_{50} values of the most active imidazoline derivatives

Compound	IC_{50}[a] (μM)	Compound	IC_{50} (μM)
13	10.3	**38**	14.9
14	9.7	**39**	9.0
15	15.1	**40**	23.5
17	37.0	**41**	8.9
25	1.1	**42**	25.0
27	12.5	**43**	14.0
28	5.0	**44**	13.4
29	14.7	**45**	4.7
30	6.4	**46**	25.3
31	4.3	**47**	11.0
32	4.1	**48**	16.0
33	10.0	**49**	9.0
34	10.2	**51**	16.5
35	5.0	**52**	14.5
36	4.6	**53**	23.0
37	4.3	Nfx	7.7

[a] IC_{50}: concentration that causes a 50% reduction in parasite growth. The results are means of three different experiments with a SD less than 10% in all cases.

Table 3. IC_{50} values for three selected diamines



Compound	$-R^1$	IC_{50}[a] (μM)
54	4-$CH_3OC_6H_4CH_2$	16.2
55	4-$ClC_6H_4CH_2$	2.0
56	2-$OHC_6H_4CH_2$	Inactive[b]

[a] IC_{50}: concentration that causes a 50% reduction in parasite growth. The results are means of three different experiments with a SD less than 10% in all cases.

[b] Percentage of growth inhibition, at 25 μM, was 0.

heterocyclic derivative, namely **26**. These results showed that the heterocyclic activities could be the result of the corresponding diamine activities.

On the other hand, we have studied the stability of some of the most relevant imidazolines, **35**, **38**, and **43**, in the biological conditions. In order to probe a heterocyclic masking role, we have analyzed the presence of the ethylenediamines after different treatment time in buffer glucose, pH = 7.0, at 28 °C. Accordingly, after five days treatment no diamines' presences were observed. If the diamines are the biological active entities this result indicates that the imidazolines-diamines transformations occur into the parasite probably by specific enzymes.

Table 4. Increment in the amount of the end-products excreted to the medium with respect to the untreated control

Compound	Percentages (%) of amount of increment of the excreted metabolites[a]				
	Lac	Ala	Ace	Gly	Other metabolites[a]
32	10	30	10	15	Pyr, Suc[b]
36	30	40	60	60	Pyr, Suc, EtOH[c]

[a] With respect to untreated cells.

[b] Excreted in lower amount than 9% with respect to untreated cells. Suc, succinate.

[c] EtOH/ethanol.

2.2.3. Changes in the excreted metabolites' profile. In order to study the changes in the biochemical pathways promoted by the active imidazolines, we have studied the modifications in the excreted metabolites by ^1H NMR spectroscopy. This kind of studies has probed to be a useful tool in the mechanism of action elucidation.[22] For that, we have selected two of the most active imidazolines, namely **32** and **36**, and using ^1H NMR spectroscopy we compared the spectra of the cell-free medium of imidazoline-treated parasites with those of the untreated *T. cruzi*-free medium as control. We have focused mainly in the changes of the excreted salts of the carboxylic acids, lactate (Lac) and acetate (Ace), and the aminoacids, alanine (Ala) and glycine (Gly), being them the most relevant modified metabolites. The studied heterocycles produced an increment of the excretion of Lac, Ala, Ace, and Gly (Table 4) with respect to these metabolites in the medium with the parasite without treatment (Table 4). These results could indicate some changes in the mitochondrion integrity[23] being the mitochondrion partly oxidized end-product Ace and the metabolic products from pyruvate (Pyr), Ala and Lac, increased. Normally, a part of Pyr enters to the mitochondrion and participates in the *T. cruzi* Krebs cycle and other part is transaminated to Ala[24] or transformed into the secondary metabolite Lac. The presence of increasing amount, with respect to control, of Ala and Lac could indicate that Pyr is not involved in the mitochondrion metabolism maybe the function of this organ is affected by the studied compounds. Also, the increased presence of Gly could be the result of an Ace transamination increment due to a higher concentration of this end-product in the cytosol as the result of a higher release from the mitochondrion.

2.3. Structure–activity relationships

Molecular modeling studies were performed on the developed imidazoline derivatives by calculating some stereoelectronic properties in order to understand the mechanism of action. These properties were determined using semiempirical AM1 calculations.[25,26] A detailed conformational search for each of the molecules was performed, using MM methods, to find the minimum energy and highest abundance conformer. The geometry of this conformer was fully optimized by applying AM1

in the gas phase. Then, single point AM1 calculation was performed. The properties determined and examined in this study were imidazoline carbon-2 Mulliken charge (Q2), magnitude of dipolar moment (μ), HOMO's and LUMO's energies, gap ($E_{LUMO} - E_{HOMO}$), and the logarithm of the partition coefficient of the non-ionized molecules ($\log P$). Theoretical $\log P$ ($\mathrm{clog}\,P$) was calculated using Villar method, implemented in PC SPARTAN 04 package[26] at the AM1 semiempirical level. In the equations and models, n represents the number of data points, r^2 is the correlation coefficient, s is the standard deviation of the regression equation, the F value is related to the F-statistic analysis (Fischer test), and r^2 adj defines the cross-validated correlation coefficient. Activity used in the structure–activity relationship studies was the inhibitory effect (compounds **3**–**5**, **9**–**53**) on the growth of *T. cruzi* expressed as percentage of growth inhibition at day 5 and 25 μM, PGI. First, one-variable and multivariable regressions between both activities and the physicochemical properties were studied. Only structure–activity models having a value of r^2 adj above 0.5 were considered. One of the best equations was obtained when we analyzed the correlation between activity and the independent $\mathrm{clog}\,P$, $(\mathrm{clog}\,P)^2$ and the magnitude of dipolar moment, μ (Eq. 1, Table 5 and Fig. 2). The best statistical correlation was obtained when μ was omitted in Eq. 1 (Eq. 2, Fig. 2). Some outliers compounds that not adjust in these equations were identified, that is, derivatives **14**, **15**, **19**, **20**, **26**, **42**, and **50**. Probably, for these compounds other physicochemical properties modulate its anti-*T. cruzi* activities. Important orthogonality was observed between the other analyzed descriptors, that is, E_{LUMO} with $\mathrm{clog}\,P$ and with μ, or Q2 with $\mathrm{clog}\,P$, so they were not included together in the analysis.[27] No correlations were observed when gap or E_{HOMO} was included in the analysis. Besides, the correlation matrix for the used physicochemical descriptors was performed and cross-correlations between the descriptors used in each equation were not obtained.[27] These parameters are therefore orthogonal, a fact that affords their use in the multilinear regression procedure.[28]

3. Discussion

The results indicate that the in vitro activity of the studied imidazolidines against *T. cruzi* epimastigotes is superior in most cases to that of Nfx, the drug used for the treatment of this disease. In particular, derivatives **25**, **28**, **31**, **32**, **35**–**37**, and **45** display low micromolar IC_{50} values (see Table 2), with appreciable selectivity indexes. Except derivatives **25** and **32** the most active imidazolidines have in common the structural motif 4-chlorobenzyl 1,3-*bis*substitution. The ethylenediamine **55**, which potentially could be generated from these imidazolidines into the parasite, was also the most active among the evaluated diamines (see Table 3). The rest of the imidazolidines did not display relevant bioactivities, specially the *bis*imidazolidines (**1**–**8**) with antibacterial activities[12] were not active against the protozoan *T. cruzi*. According to QSAR equations the studied compounds' lipophilicities play a relevant role in the bioactivity being the most hydrophobic compounds the most active against *T. cruzi*. If these derivatives are precursors of the active diamines the hydrophobic characters are related to its capability

Table 5. Summary of QSAR results

Eq.	Statistical parameters						Expressions
	r^2 adj	r^2	s	p	F value	n	
1	0.8308	0.8167	14.80	<0.0001	58.91	40	$\mathrm{PGI} = -41.6(\pm16.4) + 0.7(\pm1.7)\mu + 29.6(\pm9.1)\mathrm{clog}\,P - 1.0\ (\pm1.2)(\mathrm{clog}\,P)^2$
2	0.8300	0.8208	14.63	<0.0001	90.34	40	$\mathrm{PGI} = -41.1(\pm16.2) + 29.8(\pm8.9)\mathrm{clog}\,P - 1.0(\pm1.1)(\mathrm{clog}\,P)^2$

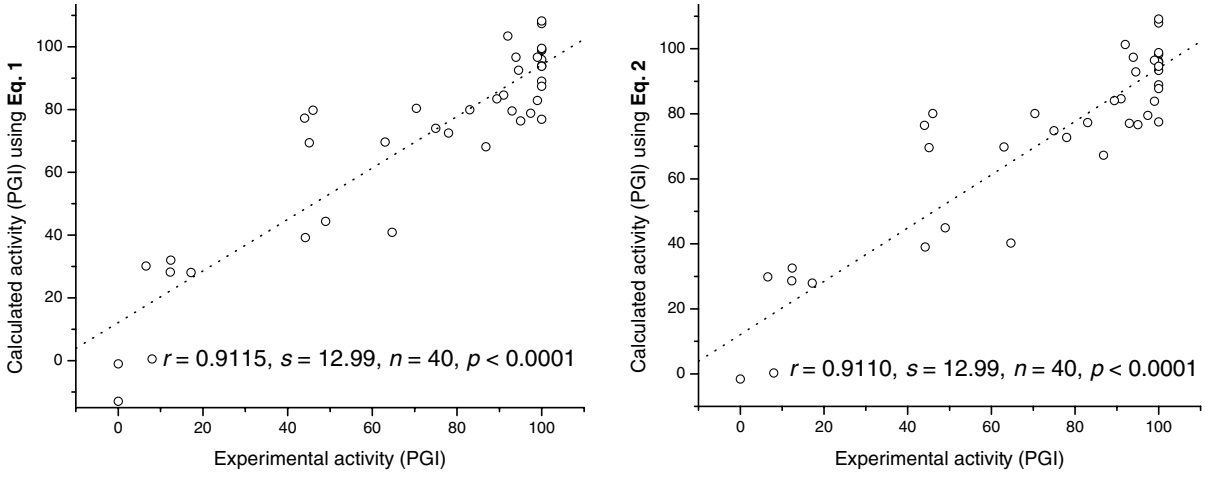


Figure 2. Plot of PGI at 25 μM experimental versus calculated values from Eqs. 1 and 2.

to cross throughout the biological membranes. The effects on the metabolic pathways, studied by ^{1}H NMR, showed that these compounds could act in some manner on the mitochondrion however more studies would be performed.

4. Conclusions

In conclusion, we have studied a new scaffold, imidazolidine, as anti-*T. cruzi* entity. These compounds may serve as leads for further exploration of the structural requirements of these anti-parasitic activities and the promising in vitro results warrant further in vivo studies in animal models of Chagas disease.

5. Experimental

5.1. Chemistry

All starting materials were commercially available research-grade chemicals and used without further purification. All solvents were dried and distilled prior to use. All the reactions were carried out in a nitrogen atmosphere. Imidazolidines **1**–**8**, **9**, **11**–**13**, **15**–**20**, **22**, **23**–**26**, **28**, **30**, **31**, **33**–**35**, **37**, **38**, **40**, **41**, **43**, **45**–**49**, **51**, and **53** were prepared applying previously described methodology.[12,18,19a,29–38] Melting points were determined with a Büchi capillary apparatus and are uncorrected. NMR spectra were recorded on a Bruker MSL 300 MHz spectrometer using deuteriochloroform as the solvent. Chemical shifts are reported in ppm relative to TMS as an internal standard. Splitting multiplicities are reported as singlet (s), doublet (d), double doublet (dd), triplet (t), and multiplet (m). Mass spectra (EI) were recorded with a GC-MS Shimadzu QP-1000 spectrometer operating at 20 eV. Column chromatography was performed on silica gel 60 (0.063–0.200 mesh) with typically 30–50 g of stationary phase per gram substance. TLC analyses were carried out on aluminum sheets coated with silica gel 60 F_{254}.

5.2. General procedure for the synthesis of imidazolidines 10, 14, 21, 27, 29, 32, 36, 39, 42, 44, 50, and 52

Imidazolidine derivatives were obtained by reaction of the corresponding *N*,*N*′-disubstituted ethylenediamines (0.01 mol) and aldehydes (0.01 mol) or aqueous formaldehyde (37%, excess) in ethanol (20 mL) under reflux during 1.5 h. Compounds **10**, **21**, **27**, **29**, **32**, **36**, **39**, **42**, **44**, **50**, and **52** were precipitated by cooling the mixture and were recrystallized from ethanol. Compound **14** was extracted with chloroform and purified by column chromatography (benzene/methanol, 9:1). Melting points, yields, spectroscopic data, and elemental analysis of the new compounds are given below.

5.2.1. 1-Benzyl-3-phenylimidazolidine (10). Mp 68.0–70.0 °C; 72%. ^{1}H NMR δ ppm: 6.80–7.45 (m, 10$H_{arom.}$), 4.00 (s, 2H, NCH_2N), 3.75 (s, 2H, CH_2Ar), 3.45 (t, 2H, CH_2N), 3.01 (t, 2H, CH_2N). MS: *m*/*z* 238 ($M^{+\cdot}$). Anal. Calcd for $C_{16}H_{18}N_2$: C, 80.67; H, 7.56; N, 11.76. Found: C, 80.45; H, 7.62; N, 11.61.

5.2.2. 1,3-Di-(3-chlorobenzyl)imidazolidine (14). This compound was obtained as oil; 78%. ^{1}H NMR δ ppm: 7.65 (m, 2$H_{arom.}$), 7.30–7.50 (m, 6$H_{arom.}$), 3.81 (s, 4H, CH_2Ar), 3.50 (s, 2H, NCH_2N), 2.85 (s, 2H CH_2N). MS: *m*/*z* 320 ($M^{+\cdot}$), 322 ($M^{+\cdot}$+2). Anal. Calcd for $C_{17}H_{18}Cl_2N_2$: C, 63.56; H, 5.65; N, 8.72. Found: C, 63.62; H, 5.59; N, 8.70.

5.2.3. 1-Benzyl-2,3-diphenylimidazolidine (21). Mp 113.0–114.0 °C; 70%. ^{1}H NMR δ ppm: 7.20–7.40 (m, 10$H_{arom.}$), 7.10–7.20 (dd, 2$H_{arom.}$), 6.60 (t, 1$H_{arom.}$), 6.50 (d, 2$H_{arom.}$), 5.05 (s, 1H, NC*H*N), 3.70 (d, 1H, CH_2Ar), 3.50 (d, 1H, CH_2Ar), 3.20 (m, 2H, CH_2N), 2.90 (m, 2H, CH_2N). MS: *m*/*z* 314 ($M^{+\cdot}$). Anal. Calcd for $C_{22}H_{22}N_2$: C, 84.08; H, 7.01; N, 8.92. Found: C, 84.16; H, 7.12; N, 8.82.

5.2.4. 1,3-Di-(4-methylbenzyl)-2-phenylimidazolidine (27). Mp 82.0–83.0 °C; 82%. ^{1}H NMR δ ppm: 7.70 (d, 2$H_{arom.}$), 7.30–7.50 (m, 3$H_{arom.}$), 7.15 (dd, 4$H_{arom.}$), 7.05 (dd, 4$H_{arom.}$), 3.85 (s, 1H, NC*H*N), 3.72 (d, 2H, CH_2Ar), 3.10–3.20 (m, 4H, CH_2Ar and CH_2N), 2.49 (m, 2H, CH_2N), 2.30 (s, 6H, CH_3). MS: *m*/*z* 356 ($M^{+\cdot}$). Anal. Calcd for $C_{25}H_{28}N_2$: C, 84.23; H, 7.92; N, 7.86. Found: C, 84.30; H, 7.87; N, 7.93.

5.2.5. 1,3-Di-(3-chlorobenzyl)-2-phenylimidazolidine (29). Mp 74.0–75.0 °C; 88%. ^{1}H NMR δ ppm: 7.62 (d, 2$H_{arom.}$), 7.30–7.50 (m, 3$H_{arom.}$), 7.21 (s, 2$H_{arom.}$), 7.00–7.15 (m, 6$H_{arom.}$), 3.81 (s, 1H, NC*H*N), 3.69 (d, 2H, CH_2Ar), 3.10–3.25 (m, 4H, CH_2Ar and CH_2N), 2.50 (m, 2H, CH_2N). MS: *m*/*z* 396 ($M^{+\cdot}$), 398 ($M^{+\cdot}$+2). Anal. Calcd for $C_{23}H_{22}Cl_2N_2$: C, 69.52; H, 5.58; N, 7.05. Found: C, 69.59; H, 5.50; N, 7.13.

5.2.6. 1,3-Di-(3,4-dichlorobenzyl)-2-(2-furyl)imidazolidine (32). Mp 87.0–89.0 °C; 79%. ^{1}H NMR δ ppm: 7.20–7.50 (m, 5$H_{arom.}$), 7.10 (d, 2$H_{arom.}$), 6.35 (dd, 2$H_{arom.}$), 4.15 (s, 1H, NC*H*N), 3.71 (d, 2H, CH_2Ar), 3.35 (d, 2H, CH_2Ar), 3.10 (m, 2H, CH_2N), 2.60 (m, 2H, CH_2N). MS: *m*/*z* 454 ($M^{+\cdot}$), 456 ($M^{+\cdot}$+2), 458 ($M^{+\cdot}$+4). Anal. Calcd for $C_{21}H_{18}Cl_4N_2O$: C, 55.29; H, 3.98; N, 6.14. Found: C, 55.32; H, 4.10; N, 6.20.

5.2.7. 1,3-Di-(4-chlorobenzyl)-2-(3-hydroxyphenyl)imidazolidine (36). Mp 110.0–112.0 °C; 70%. ^{1}H NMR δ ppm: 10.81 (br s, 1H, OH), 7.20–7.30 (m, 9$H_{arom.}$), 7.15 (d, 1$H_{arom.}$), 7.00 (d, 1$H_{arom.}$), 6.80 (t, 1$H_{arom.}$), 4.05 (s, 1H, NC*H*N), 3.90 (d, 2H CH_2Ar), 3.20 (d, 2H, CH_2Ar), 3.12 (m, 2H, CH_2N), 2.49 (m, 2H, CH_2N). MS: *m*/*z* 412 ($M^{+\cdot}$), 414 ($M^{+\cdot}$+2). Anal. Calcd for $C_{23}H_{22}Cl_2N_2O$: C, 66.83; H, 5.36; N, 6.78. Found: C, 66.76; H, 5.43; N, 6.84.

5.2.8. 1,3-Di-(4-methylbenzyl)-2-(4-chlorophenyl)imidazolidine (39). Mp 108.0–109.0 °C; 75%. ^{1}H NMR δ ppm: 7.60 (d, 2$H_{arom.}$), 7.35 (d, 2$H_{arom.}$), 7.00–7.20 (m, 8$H_{arom.}$), 3.82 (s, 1H, NC*H*N), 3.70 (d, 2H, CH_2Ar), 3.10–3.20 (m, 4H, CH_2Ar and CH_2N), 2.50 (m, 2H, CH_2N), 2.30 (s, 6H, CH_3). MS: *m*/*z* 390 ($M^{+\cdot}$), 392 ($M^{+\cdot}$+2). Anal. Calcd

for $C_{25}H_{27}ClN_2$: C, 76.81; H, 6.96; N, 7.17. Found: C, 76.75; H, 6.99; N, 7.25.

5.2.9. 1,3-Di-(3,4-dichlorobenzyl)-2-(4-chlorophenyl)imidazolidine (42). Mp 114.0–115.0 °C; 85%. ^{1}H NMR δ ppm: 7.50 (d, 2H$_{arom.}$), 7.20–7.40 (m, 6H$_{arom.}$), 7.05 (d, 2H$_{arom.}$), 3.80 (s, 1H, NC*H*N), 3.65 (d, 2H, CH_2Ar), 3.05–3.20 (m, 4H, CH_2Ar and CH_2N), 2.45 (m, 2H CH_2N). MS: *m*/*z* 498 ($M^{+\cdot}$), 500 ($M^{+\cdot}$+2), 502 ($M^{+\cdot}$+4). Anal. Calcd for $C_{23}H_{19}Cl_5N_2$: C, 55.18; H, 3.82; N, 5.60. Found: C, 55.23; H, 3.78; N, 5.55.

5.2.10. 1,3-Di-(4-methylbenzyl)-2-(3-chlorophenyl)imidazolidine (44). Mp 97.0–98.0 °C; 79%. ^{1}H NMR δ ppm: 7.65 (s, 1H$_{arom.}$), 7.50 (m, 1H$_{arom.}$), 7.28–7.30 (m, 2H$_{arom.}$), 7.06 (d, 4H$_{arom.}$), 7.13 (d, 4H$_{arom.}$), 3.81 (s, 1H, NC*H*N), 3.71 (d, 2H, CH_2Ar), 3.21 (d, 2H, CH_2Ar), 3.15 (m, 2H, CH_2N), 2.50 (m, 2H, CH_2N), 2.31 (s, 6H, CH_3). MS: *m*/*z* 390 ($M^{+\cdot}$), 392 ($M^{+\cdot}$+2). Anal. Calcd for $C_{25}H_{27}ClN_2$: C, 76.81; H, 6.96; N, 7.17. Found: C, 76.86; H, 7.02; N, 7.25.

5.2.11. 1,3-Di-(3,4-dichlorobenzyl)-2-(3,4-dichlorophenyl)-imidazolidine (50). Mp 64.0–65.0 °C; 73%. ^{1}H NMR δ ppm: 7.62 (s, 1H$_{arom.}$), 7.40–7.50 (m, 2H$_{arom.}$), 7.20–7.38 (m, 4H$_{arom.}$), 7.00–7.08 (m, 2H$_{arom.}$), 3.79 (s, 1H, NC*H*N), 3.60 (d, 2H, CH_2Ar), 3.10–3.30 (m, 4H, CH_2Ar and CH_2N), 2.50 (m, 2H, C*H* $_2$N). MS: *m*/*z* 532 ($M^{+\cdot}$), 534 ($M^{+\cdot}$+2), 536 ($M^{+\cdot}$+4). Anal. Calcd for $C_{23}H_{18}Cl_6N_2$: C, 51.62; H, 3.39; N, 5.23. Found: C, 51.55; H, 3.45; N, 5.17.

5.2.12. 1,3-Di-(4-chlorobenzyl)-2-(4-nitrophenyl)imidazolidine (52). Mp 122.0–124.0 °C; 90%. ^{1}H NMR δ ppm: 8.31 (d, 2H$_{arom.}$), 7.72 (d, 2H$_{arom.}$), 7.20 (dd, 4H$_{arom.}$), 7.10 (dd, 4H$_{arom.}$), 3.95 (s, 1H, NCHN), 3.62 (d, 2H, CH_2Ar), 3.29 (2H, CH_2Ar), 3.12 (m, 2H, CH_2N), 2.51 (m, 2H, CH_2N). MS: *m*/*z* 441 ($M^{+\cdot}$), 443 ($M^{+\cdot}$+2). Anal. Calcd for $C_{23}H_{21}Cl_2N_3O_2$: C, 62.45; H, 4.79; N, 9.50. Found: C, 62.70; H, 4.72; N, 9.63.

5.3. Biology

5.3.1. Anti-*T. cruzi* in vitro evaluation

5.3.1.1. Trypanocidal (epimastigotes) in vitro test. *Trypanosoma cruzi* epimastigotes (Tulahuen 2 strain) were grown at 28 °C in an axenic medium (BHI-Tryptose) as previously described,[39] complemented with 5% fetal bovine serum (FBS). Cells from a 10-day-old culture (stationary phase) were inoculated into 50 mL of fresh culture medium to give an initial concentration of 1×10^6 cells/mL. Cell growth was followed by measuring everyday the absorbance of the culture at 600 nm. Before inoculation, the media were supplemented with the indicated amount of the drug from a stock solution in DMSO. The final concentration of DMSO in the culture media never exceeded 0.4% and the control was run in the presence of 0.4% DMSO and in the absence of any drug. No effect on epimastigote growth was observed by the presence of up to 1% DMSO in the culture media. The percentage of inhibition was calculated as follows: % = $\{1 - [(A_p - A_{0p})/(A_c - A_{0c})]\} \times 100$, where $A_p = A_{600}$ of the culture containing the drug at day 5; $A_{0p} = A_{600}$ of the culture containing the drug just after addition of the inocula (day 0); $A_c = A_{600}$ of the culture in the absence of any drug (control) at day 5; $A_{0c} = A_{600}$ in the absence of the drug at day 0. To determine IC_{50} values, 50% inhibitory concentrations, parasite growth was followed in the absence (control) and presence of increasing concentrations of the corresponding drug. At day 5, the absorbance of the culture was measured and related to the control. The IC_{50} value was taken as the concentration of drug needed to reduce the absorbance ratio to 50%.

5.3.2. Cytotoxicity to human macrophages. THP-1 human macrophages were seeded (100,000 cells/well) in 96-well flat bottom microplates (Nunclon) with 200 μL of RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum. Cells were allowed to attach for 48 h in a humidified 5% CO_2/95% air atmosphere at 37 °C. Then, cells were exposed to the compounds (25–1000 μM) for 48 h. Afterward, the cells were washed with PBS and incubated (37 °C) with 0.4 mg/mL 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma) for 3 h. Then, formazan was dissolved with DMSO (180 μL), and optical densities were measured. Each concentration was assayed three times, and six growth controls were used in each test. Cytotoxicity percentages (%*C*) were determined as follows: %*C* = [100 − (ODd − ODdm)/(ODc − ODcm)]_100, where ODd is the mean of OD_{595} of wells with macrophages and different concentrations of the compounds, ODdm is the mean of OD_{595} of wells with different compound concentrations in the medium, ODc is the growth control, and ODcm is the mean of OD_{595} of wells with medium only.

5.3.3. ^{1}H NMR study of the excreted metabolites. For the spectroscopic studies, 5 mL of a 2-day-treated *T. cruzi* (Y strain) with each studied imidazolidine was centrifuged at 1500*g* for 10 min at 4 °C. The pellet was discarded, and the parasite-free supernatant was stored at −20 °C until used. The chemical displacements used to identify the respective metabolites were confirmed by both adding each analyzed metabolite to the studied supernatant and by the study of a control solution with 4 μg/mL of each metabolites in buffer phosphate, pH = 7.4.

5.4. QSAR studies

The compounds were built with standard bond lengths and angles using the Spartan'04, 1.0.1 version,[24] suite of programs and the geometry of each molecule was fully optimized by applying semiempirical AM1 method in gas phase from the most stable conformer obtained using molecular mechanics (MMFF) methods. Then, a single point calculation using AM1 was used. Lipophilic properties of the compounds were included into the analyses, as clog *P* (log *P* calculated by Villar method, AM1).

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27. Squared correlation matrix of descriptors used in the QSAR study.

E_{LUMO}	1			
clog *P*	−0.4773	1		
Q2	−0.1330	0.5803	1	
μ	−0.7413	0.3123	0.1189	1
r	E_{LUMO}	clog *P*	Q2	μ

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