Accepted Manuscript

Structural design, synthesis and pharmacological evaluation of thiazoles against *Trypanosoma cruzi*

Gevanio Bezerra de Oliveira Filho, Marcos Veríssimo de Oliveira Cardoso, José Wanderlan Pontes Espíndola, Dayane Albuquerque Oliveira e Silva, Rafaela Salgado Ferreira, Pollyanne Lacerda Coelho, Pâmela Silva dos Anjos, Emanuelle de Souza Santos, Cássio Santana Meira, Diogo Rodrigo Magalhaes Moreira, Milena Botelho Pereira Soares, Ana Cristina Lima Leite

PII: S0223-5234(17)30762-6

DOI: 10.1016/j.ejmech.2017.09.047

Reference: EJMECH 9762

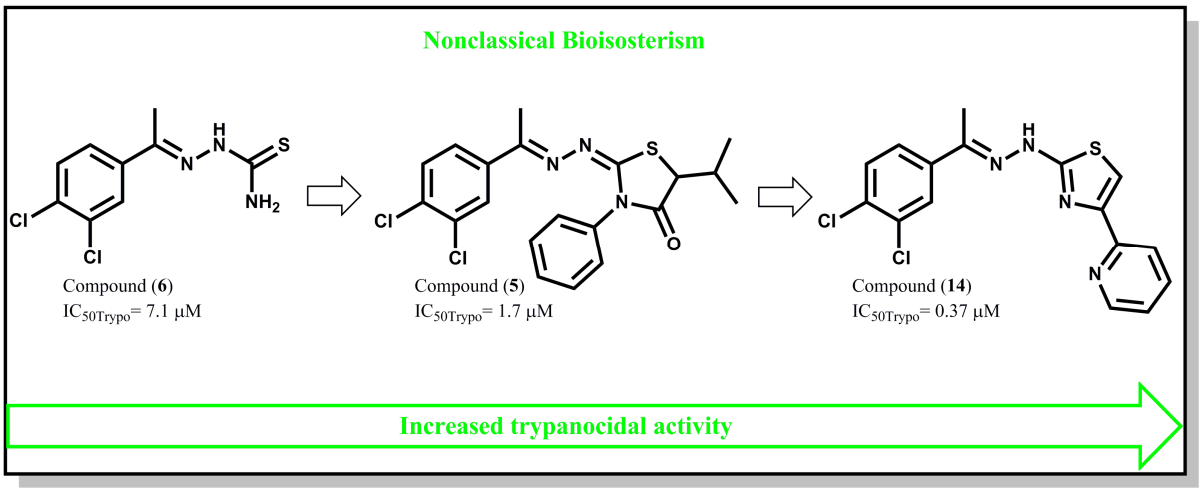
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 5 July 2017

Revised Date: 29 August 2017

Accepted Date: 21 September 2017

Please cite this article as: G.B. de Oliveira Filho, Marcos.Verí. de Oliveira Cardoso, José.Wanderlan.Pontes. Espíndola, D.A. Oliveira e Silva, R.S. Ferreira, P.L. Coelho, Pâ. Silva dos Anjos, E. de Souza Santos, Cá.Santana. Meira, D.R.M. Moreira, M.B.P. Soares, A.C.L. Leite, Structural design, synthesis and pharmacological evaluation of thiazoles against *Trypanosoma cruzi*, *European Journal of Medicinal Chemistry* (2017), doi: 10.1016/j.ejmech.2017.09.047.



Structural Design, Synthesis and Pharmacological Evaluation of Thiazoles against *Trypanosoma cruzi*

Gevanio Bezerra de Oliveira Filho,[a,b,*] Marcos Veríssimo de Oliveira Cardoso,[c] José Wanderlan Pontes Espíndola,[a] Dayane Albuquerque Oliveira e Silva,[a] Rafaela Salgado Ferreira,[d] Pollyanne Lacerda Coelho,[d] Pâmela Silva dos Anjos,[e] Emanuelle de Souza Santos,[e] Cássio Santana Meira,[e] Diogo Rodrigo Magalhaes Moreira,[e] Milena Botelho Pereira Soares,[e,f] Ana Cristina Lima Leite[a,*]

[a] Laboratório de Planejamento em Química Medicinal - LpQM, Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Pernambuco - UFPE, 50740-520, Recife, PE, Brazil.

[b] Faculdade de Integração do Sertão - FIS, Rua João Luiz de Melo, 2110 - COHAB, Serra Talhada – PE

[c] Colegiado de Nutrição, Universidade de Pernambuco, CEP 56328-903, Petrolina, PE, Brazil.

[d] Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, CEP 31270-901, Belo Horizonte, MG, Brazil.

[e] Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, CEP 40296-710, Salvador, BA, Brazil.

[f] Centro de Biotecnologia e Terapia Celular, Hospital São Rafael, Salvador, BA, Brazil.

*To whom correspondence should be addressed: phone +55 81-21268511, fax +55 81-21268510; e-mails: gev_filho@hotmail.com and acllb2003@yahoo.com.br

Abstract

Chagas disease is one of the most significant health problems in the American continent. Benznidazole (BDZ) and nifurtimox (NFX) are the only drugs approved for treatment and exhibit strong side effects and ineffectiveness in the chronic stage, besides different susceptibility among *T. cruzi* DTUs (Discrete Typing Units). Therefore, new drugs to treat this disease are necessary. Thiazole compounds have been described as potent trypanocidal agents. Here we report the structural planning, synthesis and anti-*T. cruzi* evaluation of a new series of 1,3-thiazoles (**7-28**), which were designed by placing this heterocycle instead of thiazolidin-4-one ring. The synthesis was conducted in an ultrasonic bath with 2-propanol as solvent at room temperature. By varying substituents attached to the phenyl and thiazole rings, substituents were observed to retain, enhance or greatly increase their anti-*T. cruzi* activity. In some cases, methyl at position 5 of the thiazole (compounds **9**, **12** and **23**) increased trypanocidal property. The exchange of phenyl for pyridinyl heterocycle resulted in increased activity, giving rise to the most potent compound against the trypomasigote form (**14**, $IC_{50trypo}$= 0.37 μM). Importantly, these new thiazoles were toxic for trypomastigotes without affecting macrophages and cardiomyoblast viability. The compounds were also evaluated against cruzain, and five of the most active compounds against trypomastigotes (**7**, **9**, **12**, **16** and **23**) inhibited more than 70 % of enzymatic activity at 10 μM, among which compound **7** had an IC_{50} in the submicromolar range, suggesting a possible mechanism of action. In addition, examination of *T. cruzi* cell death showed that compound **14** induces apoptosis. We also examined the activity against intracellular parasites, revealing that compound **14** inhibited *T. cruzi* infection with potency similar to benznidazole. The antiparasitic effect of **14** and benznidazole in combination was also investigated against trypomastigotes and revealed that they have synergistic effects, showing a promising profile for drug combination. Finally, in mice acutely-infected with *T. cruzi*, **14** treatment significanty reduced the blood parasitaemia and had a protective effect on mortality. In conclusion, we report the identification of compounds (**7**), (**12**), (**15**), (**23**) and (**26**) with similar trypanocidal activity of benznidazole; compounds (**9**) and (**21**) as trypanocidal agents equipotent with BDZ, and compound **14** with potency 28 times better than the reference drug without affecting macrophages and cardiomyoblast viability. Mechanistically, the compounds inhibit cruzain, and **14** induces *T. cruzi* cell death by an apoptotic process, being considered a good starting point for the development of new anti-Chagas drug candidates.

Keywords: Chagas disease; thiosemicarbazones; thiazoles; *Trypanosoma cruzi*.

Introduction

One of the most significant health problems in the American continent in terms of human health (i.e., number of people infected with and dying from it), socioeconomic impact and geographic distribution is the Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) [1]. In Brazil , losses of over US$ 1.3 billion in wages and industrial productivity are extimated due to workers with Chagas disease [2]. Regardless of the decreased incidence of new infections in Brazil and other countries due to urbanization and improved living conditions, an estimated number of 6-8 million people remains infected [1–3].

Benznidazole (BDZ) and Nifurtimox (NFX), launched in the early 1970s, are the only drugs approved for human treatment. Both compounds share some characteristics: better tolerance by children, higher effectiveness during the acute phase of *T. cruzi* infection, higher toxicity in adults, and different susceptibility among *T. cruzi* DTUs (Discrete Typing Units). Private-funded laboratories lack interest on Chagas disease research, while equipment and technological restrictions in public laboratories hamper the development of new drugs and treatment strategies. This scenario generates the current situation where the available drugs are the same as in 1970, have strong side effects and ineffectiveness in the chronic phase of the disease [1,4]. Thus, new drugs to treat Chagas disease are necessary.

Trypanosoma cruzi contains cysteine, serine, threonine and metallo proteinases. The most abundant among these enzymes is cruzipain, a cysteine proteinase expressed as a complex mixture of isoforms by the major developmental stages of the parasite, including some membrane-bound isoforms. This enzyme is an immunodominant antigen in human chronic Chagas disease and seems to be important in the host/parasite relationship. Inhibitors of cruzipain kill the parasite and cure infected mice, thus making the enzyme a very promising target for the development of new drugs against Chagas disease [5–12].

The importance of this enzyme has recently been emphasized in a study by Sbaraglini et al., whose computer-guided drug repositioning strategy led to the discovery of the trypanocidal effects of clofazimine and benidipine. These compounds showed inhibitory effects on cruzipain, on different parasite stages and in a murine model of acute Chagas disease. Benidipine and clofazimine were able to reduce the parasite burden in cardiac and skeletal muscles of chronically infected mice compared with untreated mice as well as diminish the inflammatory process in these tissues [12].

Regarding the identification of cruzain inhibitors, most of the efforts have been conducted through the investigation of peptides and peptide-like compounds, such as ureas [13,14], hydrazones [15–17], triazoles [18,19], thiosemicarbazones [20–25], thiazolidin-4-ones [26–28] and 1,3-thiazoles [29–31].

Recently, our research group identified the thiazolidin-4-one (**5**) [32], a cyclic bioisoster of the potent cruzain inhibitor 3,4-dichlorophenyl thiosemicarbazone (**6**) [20],. Compound (**5**) was less active against cruzain than thiosemicarbazone (**6**). However, the antiparasitic activity against Y strain of *T. cruzi* trypomastigotes and host cell cytotoxicity in J774 macrophages revealed that compound (**5**) is a stronger and more selective antiparasitic agent than thiosemicarbazone (**6**). In *T. cruzi* infected mice treated orally with 100 mg/kg of compound (**5**), a decreased of parasitemia was observed [32].

Thiazoles compounds (**1, 2, 3 and 4**) have been described as potent trypanocidal agents [28–31], then this work devised a series of 1,3-thiazoles for bioisosteric exchange thiazolidin-4-one ring through thiazole heterocycle (**Figure 1**). The compounds of this series (**7-28**) were designed in such a way as to vary the group linked to position 4 of the thiazole ring, through insertion of alkyl substituents and aromatic rings with different substitutions in *ortho*, *meta* and *para*.

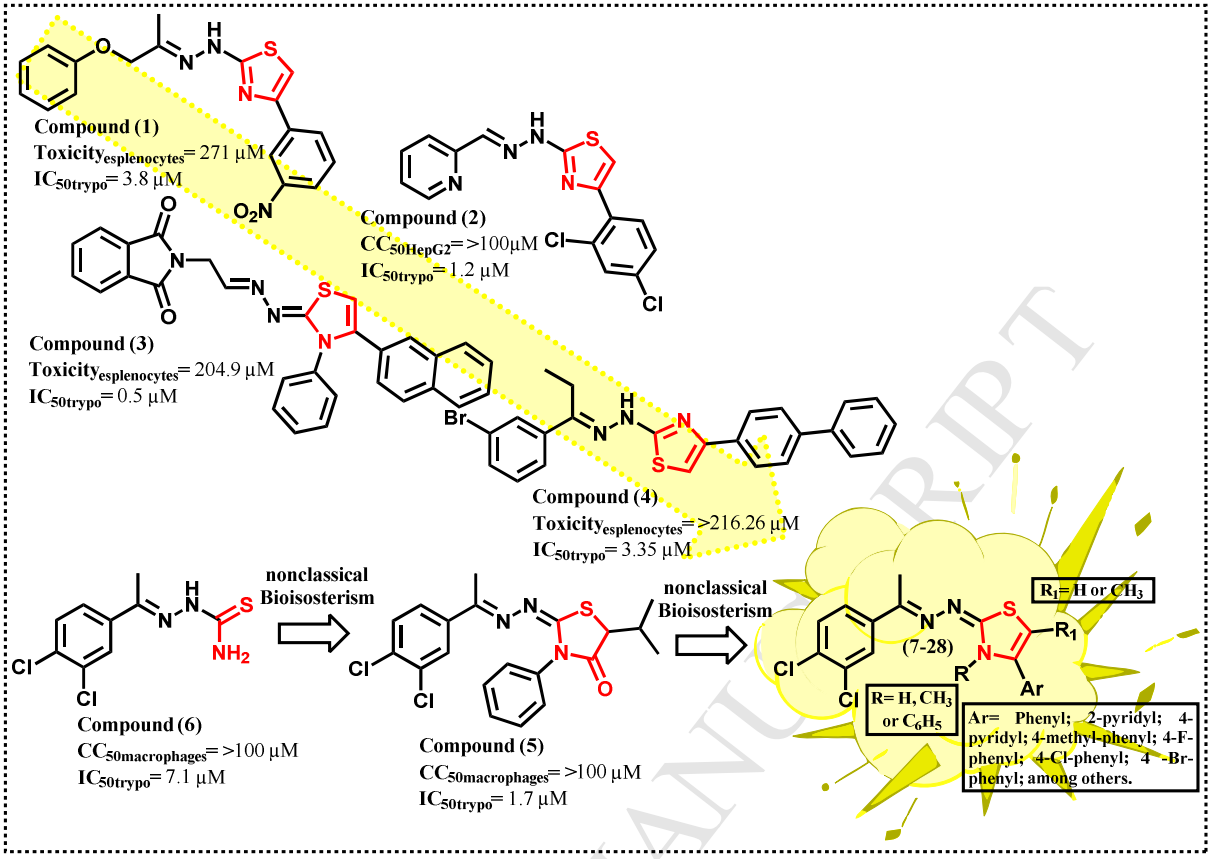
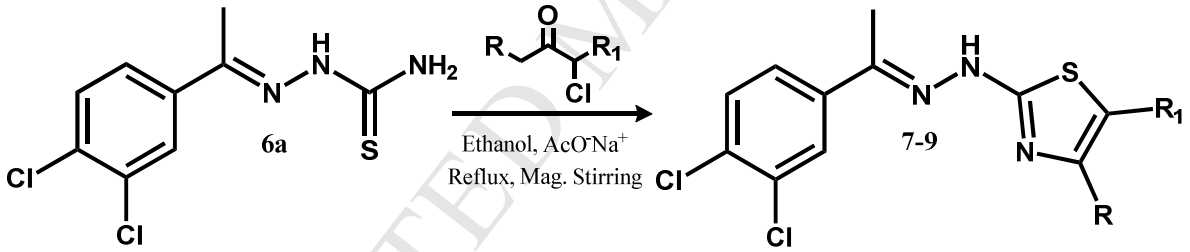


Figure 1: Planning structural of 1,3-thiazoles serie **7-28**. Replace the thiazolidin-4-one heterocycle by other structurally equivalent (1,3-thiazole).

Results and Discussion

Synthesis and chemical characterization

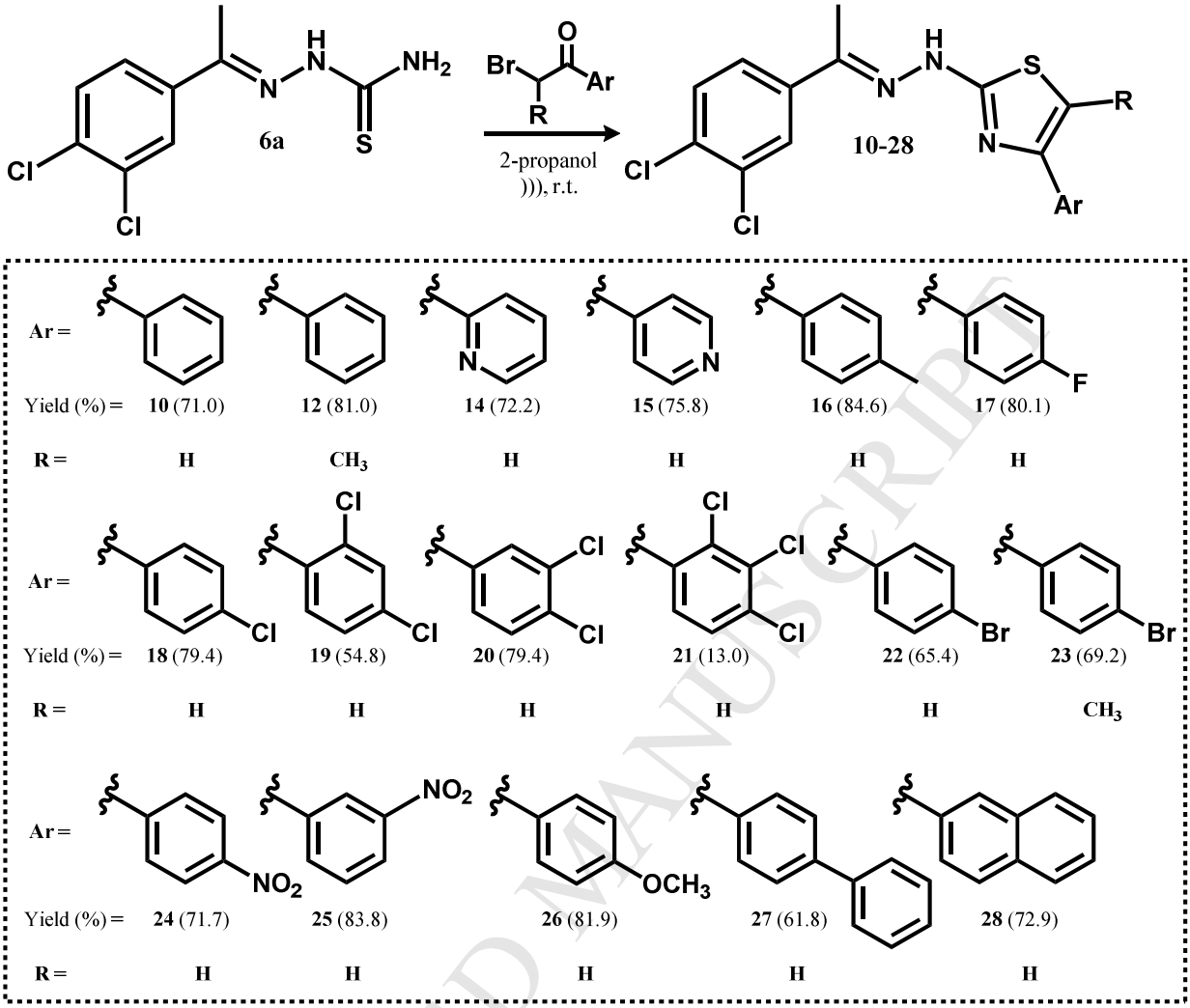
The general route to prepare thiazoles (**7-28**) is shown in **Scheme 1, 2** and **3**. Firstly, thiosemicarbazones (**6a-c**) were prepared by reacting commercially available 3′,4′-dichloroacetophenone with the appropriate thiosemicarbazide in an ultrasound bath in the presence of catalytic H_2SO_4 (or HCl in the case of thiosemicarbazone **6c**). Thiazoles (**7**–**9**) were prepared according to the methodology described by Hantzsch [33], by reacting the respective aryl thiosemicarbazone with commercially available chloroacetones (**Scheme 1**). These reactions were carried out in the presence of an excess of anhydrous NaOAc under reflux, affording compounds (**7**–**9**) in variable yields (46-88 %) and acceptable purity (> 95 %).



Comp	R	R_1	Yield (%)
7	CH_3	H	78.9
8	CH_2Cl	H	88.4
9	CH_3	CH_3	46.0

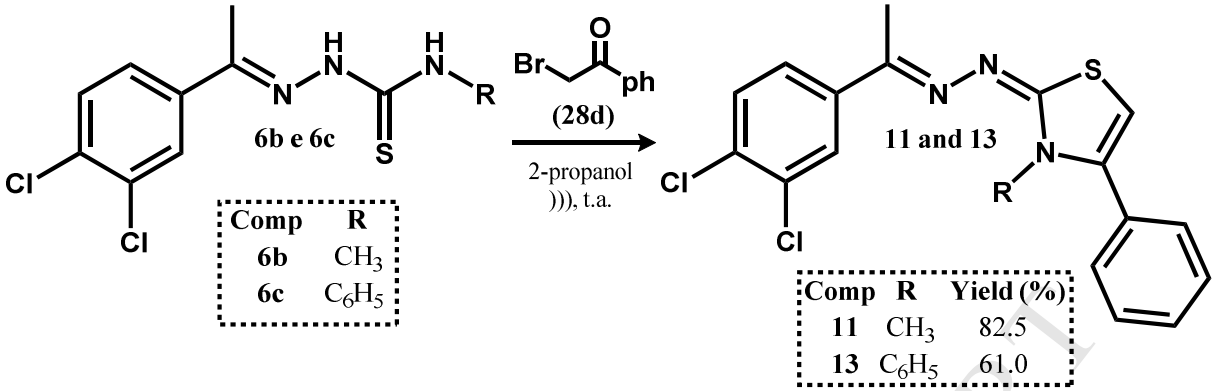
Scheme 1. Synthetic procedures for thiazoles (**7-9**).

For the series of thiazoles (**10-28**), due to previous experience of our research group [28–31] and literature reports [34], the synthesis was conducted in an ultrasonic bath (40 MHz, 30-120 min) with 2-propanol as solvent at room temperature. For these conditions the reaction proceeded faster and with similar yields to the Hantzsch method. The 2-bromoacetophenones used were purchased from commercial sources (**Scheme 2**).



Scheme 2. Synthetic procedures for thiazoles (**10-28**).

To investigate the effect of substituents inserted on the N3 position for pharmacological activity, we prepared the aryl-thiazoles (**11**) and (**13**) (**Scheme 3**), in the same manner as compounds (**10-28**).



Scheme 3. Synthetic procedures for thiazoles (**11** and **13**).

The chemical structures of compounds were determined by nuclear magnetic resonance (N.M.R., ^{1}H and ^{13}C), infrared (I.R.) and mass spectra (HR-MS), while purity was determined by elemental analysis (E.A.). The analysis of ^{1}H and ^{13}C NMR spectra of compounds (**7-28**) has highlighted the signals relating to the methine located at the C-5 position in the heterocyclic ring, which are diagnostic for this class of compounds. In NMR ^{13}C, the carbon referring to this methine is easy to locate, in δ= 101-117 ppm. In IR, the presence of the band relating iminics links as well as the absence of absorption in the carbonyl region are also suggestive of the formation of compounds (**7-28**).

Another important structural feature of these compounds is the position of the double bond involving the C2 carbon of the heterocycle ring. Depending on imino-lactam tautomerism, the C2 carbon double bond present in compounds (**7-10, 12, 14-28**) can be endocyclic or exocyclic in relation to the heterocycle (**Figure 2**). Compounds **11** and **13** posses a methyl or phenyl group respectively in N-4 of thiazole nucleus, thus they presented a exocyclic bond.

In the NMR ^{1}H of compounds (**7-10, 12, 14-28**), the chemical shift of the N-H ranges between 10.5-11.5 ppm (DMSO-d6), while in thiosemicarbazone (**6a-c**), the chemical shift of hidrazinic protons varies between 10.29-10.66 ppm. The data from literature for amidic proton of lactams, thiazolidin-4-ones and thiazolidin-2,4-diones show the chemical shift in the range

of 11.4-12.3 ppm [35,36]. In view of these reports, we can suggest that the N-H signal in the compounds (**7-10, 12, 14-28**) is characteristic of hidrazinic protons (**Figure 2**, form I).

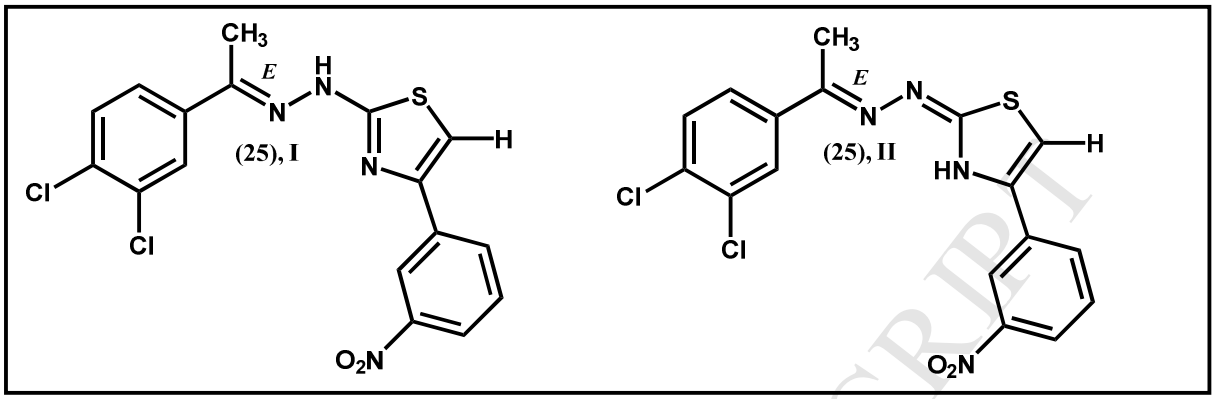


Figure 2: Position of the C = N bond at the C2 carbon of the compound (25)

For an unambiguous assignment of this connection, we try to obtain crystals of the compounds (**7-28**) suitable for the diffraction of X-rays, but without success. Recently, Cardoso et al. (2014) [29] identified by X-ray crystallography that the position of the double bond involving the C2 carbon of the heterocycle ring of 2-pyridyl thiazoles is endocyclic.Based on this, we can suggest this is also true for compounds (**7-10, 12, 14-28**) (**Figure 2**, form I).

Another important structural feature of these compounds is the isomerism *Z* or *E* in hydrazine double-bond C2=N2. Based on compounds previously crystallized by our group to define the relative configuration, we suggest that the major isomer formed presents the *E* configuration in hydrazine double-bond C2=N2 (**Figure 3**) [26–29,37,38]. Besides, a representative NMR-^{1}H spectrum of compound **25** is presented in Supplementary Material.

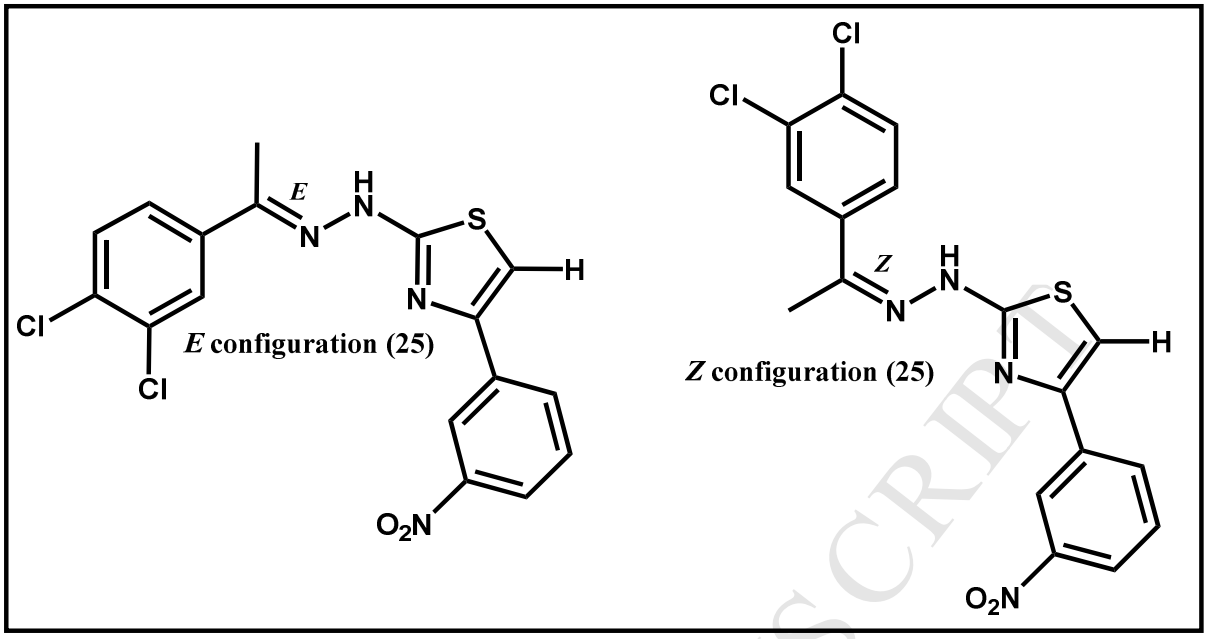
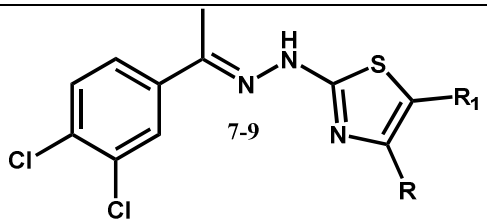


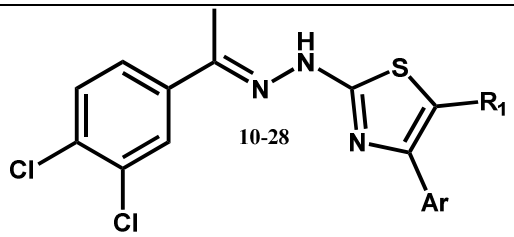
Figure 3: Isomerism *Z* or *E* in hydrazine double-bond C2=N2.

Pharmacological evaluation

We assessed host cell cytotoxicity in J774 macrophages and H9c2 rat cardiomyoblasts, while *in vitro* anti-*T. cruzi* activity was determined against bloodstream trypomastigotes of Y strain (**Table 1**). Compounds that showed IC_{50} values comparable to benznidazole were considered active.

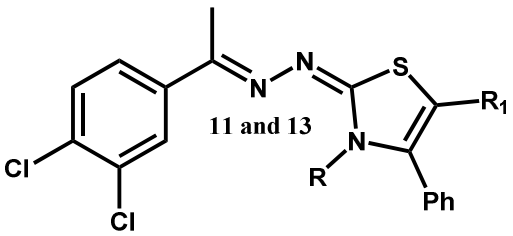
Table 1. Anti-*T. cruzi* activity and cytotoxicity effects of thiazoles **7-28**.

Cpd.	Ar/R	R_1	Trypomastigotes, Y strain *T. cruzi* IC_{50} ± S.D. (μM) [a]	Macrophages CC_{50} ± S.D. (μM)[b]	H9c2 CC_{50} ± S.D. (μM)[c]
			 7-9		
7	CH_3	H	17.1 (± 0.7)	33.71 (± 0.63)	26.12 (± 0.55)
8	CH_2Cl	H	>50	>50	>50
9	CH_3	CH_3	10.5 (± 1.5)	41.76 (± 2.98)	>50



10-28

10	Ph	H	>50	>50	>50
12	Ph	CH_3	17.8 (± 2.1)	>50	>50
14	2-Py	H	0.37 (± 0.1)	10.06 (± 1.18)	10.18 (± 2.52)
15	4-Py	H	16.8 (± 0.7)	39.81 (± 0.03)	41.94 (± 1.52)
16	4-CH_3Ph	H	47.74 (± 2.38)	>50	>50
17	4-FPh	H	>50	>50	>50
18	4-ClPh	H	>50	>50	>50
19	2.4-*di*ClPh	H	>50	>50	>50
20	3.4-*di*ClPh	H	>50	>50	>50
21	2.3.4-*tri*ClPh	H	6 (± 1.52)	21.28 (± 1.98)	16.97 (± 1.84)
22	4-BrPh	H	>50	>50	>50
23	4-BrPh	CH_3	19.1 (± 0.4)	>50	>50
24	4-NO_2Ph	H	>50	>50	>50
25	3-NO_2Ph	H	>50	>50	>50
26	4-OCH_3Ph	H	17.1 (± 1.68)	>50	>50
27	4-PhPh	H	>50	>50	>50
28	2-Naphtyl	H	>50	>50	>50



11 and 13

11	CH_3	H	>50	>50	>50
13	Ph	H	>50	>50	>50
Bdz	-	-	10.61 (± 0,87)	>50	>50
GV	-	-	NT	0.45 (± 0.04)	1.5 (± 0.7)

[a] Determined after 24h of incubation in the presence of compounds. [b] Determined in J774 cells for 72h after incubation. [c] Determined in H9c2 rat cardiomyoblast cells for 72h after incubation. NT = not tested. Bdz = Benznidazole. GV = Gentian violet. S.D. = standard deviation.

Regarding cytotoxicity of the compounds (**7-28**) in J774 macrophages, five compounds (**7, 9, 14, 15, and 21**) were toxic at concentrations less than 50 µM, and for H9c2 rat cardiomyoblast cells, only four compounds (**7, 14, 15 and 21**) were toxic at concentrations less than 50 µM. Beside these compounds, other thiazoles did not affect the cell viability of macrophages or cardiomyoblast at concentrations below 50 µM. The most toxic congener (**14**) was about 22 (J774) and 7 (H9c2) times less toxic than the reference inhibitor (gentian violet).

Compound (**7**, $IC_{50trypo}$= 17.1 µM), which has a methyl in position 4 of the thiazole ring, was one of the most potent compounds in the series, showed trypanocidal property similar to that of benznidazole ($IC_{50trypo}$= 10.6 µM). Similarly, compound (**9**, $IC_{50trypo}$= 10.5 µM), which differs to (**7**) only by one methyl at position 5 of the thiazole ring, demonstrated increased trypanocidal property, being equipotent to benznidazole. Also, compared to the compound unsubstituted in the phenyl ring (**10**, $IC_{50trypo}$= >50 µM) that did not show trypanocidal activity, inserting a methyl at position 5 of the thiazole ring (**12**), greatly increased pharmacological activity, to give a compound (**12**, $IC_{50trypo}$= 17.8 µM) equipotent to benznidazole. This suggests that substitution of alkyl groups at position 4 and 5 of the thiazole ring are beneficial for trypanocidal activity, corroborating the recent work of de Moraes Gomes [30,31]. On the other hand, the insertion of a chloromethyl (**8**, $IC_{50trypo}$= >50 µM) at position 4 of the thiazole ring has been shown deleterious for trypanocidal activity.

The mono-*para*-substituted compound with methoxy (**26**, $IC_{50trypo}$= 17.1 µM) was also equipotent to benznidazole. In the same position, substitution with methyl (**16**, $IC_{50trypo}$= 47.74 µM), fluorine (**17**, $IC_{50trypo}$= >50 µM), chlorine (**18**, $IC_{50trypo}$= >50 µM) or bromine (**22**, $IC_{50trypo}$= >50 µM) was deleterious for trypanocidal activity. Insertion of nitro groups in the *meta* and *para* positions, (**24**) and (**25**) also were not beneficial for pharmacological activity.

Interestingly, the simple insertion of a methyl at position 5 of the thiazole ring, generating compound (**23**, $IC_{50trypo}$= 19.1 µM) mono-*para*-bromo-substituted, produced a

compound with similar trypanocidal activity of benznidazole, which corroborates results of compound (**9**) and (**12)**, wherein inserting alkyl substituents at the 5-position of the heterocycle increased pharmacological activity, and should be explored by our research group. The tri-substituted 2',3',4'-tri-chloro compound (**21**, $IC_{50trypo}$= 6 µM) also showed similar potency compared to benznidazole, while the simple substitution for dichlorinated compounds at the 2',4'- (**19**) and 3', 4'- (**20**) was not beneficial for trypanocidal activity. We tried to evaluate the influence of exchange of H by methyl (**11**) or (**13**) phenyl in the N3 position of the thiazole ring, but neither insertion was beneficial for trypanocidal activity, in agreement with the recent work of de Moraes Gomes [30,31].

The exchange of phenyl (**10**, $IC_{50trypo}$= >50 µM) for heterocycle 2-pyridyl (**14**, $CC_{50trypo}$= 0.37 µM) and 4-pyridyl (**15**, $IC_{50trypo}$= 16.8 µM) resulted in increased activity, giving rise to the most potent compound of the series (**14**, $IC_{50trypo}$= 0.37 µM), which reminds us of the importance of the 2-pyridyl heterocycle, which might facilitate binding with a possible biological target, since it posses a hydrogen bond acceptor. Compound **14** was approximately 28-fold more potent than benznidazole. On the other hand, exchange for phenyl-phenyl (**27**) or naphthyl (**28**) did not result in increased trypanocidal activity.

Altogether, results obtained for the series (**7-28**) corroborate literature reports [28–31,39] that thiazoles are compounds with potent trypanocidal activity and low toxicity to mammalian cells, as observed also in the work of da Silva et al., where compounds **4d** and **4k** presented the best activities $IC_{50trypo}$ = 1.2 and 1.6 µM, respectively [40]. Compounds **4d** and **4k** have a 2-pyridyl group as a structural feature similar to compound **14** described herein. This leads us to note the importance of this group. Compound **14** was approximately 4-fold more potent than **4d** and **4k** (**Figure 4**).

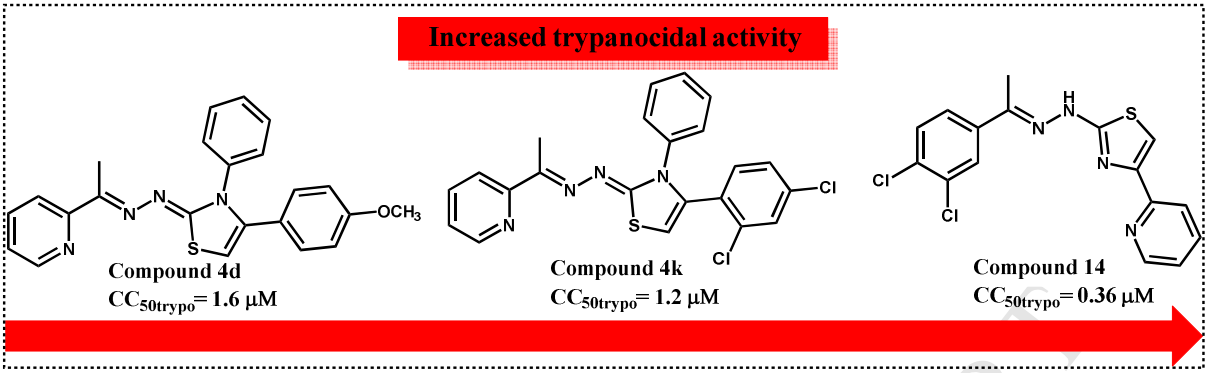


Figure 4: Summary of SAR for thiazole compounds.

Finally, it is noteworthy that although the molecular target involved in trypanocidal properties of 1,3-thiazoles (**7-28**) is not known, the pharmacological properties profile identified is relevant. Screening of the series revealed compounds (**7**), (**12**), (**15**), (**23**) and (**26**) with trypanocidal activity similar to benznidazole; compounds (**9**) and (**21**) as trypanocidal agents equipotent with the reference drug (BDZ), and compound **14** about 28 times more potent than benznidazole.

The summary of structure activity relationship (SAR) is presented in **Figure 5.**

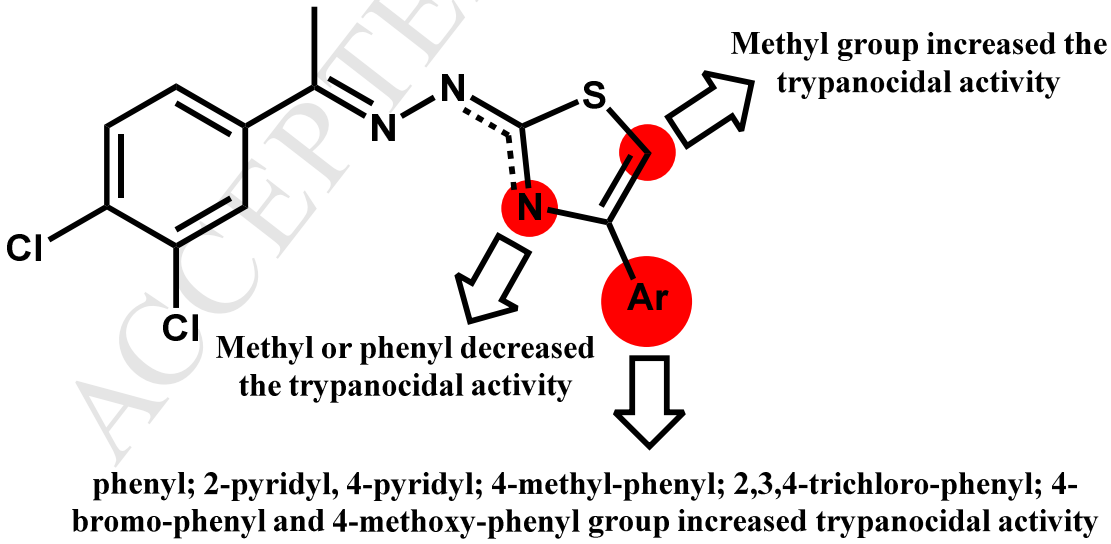


Figure 5: Summary of SAR of trypanocidal activity.

The inhibitory activity for thiazoles (**7-28**) against cruzain was also investigated. We measured cruzain enzymatic activity inhibition by using an assay based on competition with the substrate Z-Phe-Arg-aminomethylcoumarin (Z-FR-AMC). Compounds were screened at 10 µM, the maximum concentration at which they were soluble in the assay buffer (**Table 2**) [41].

As proposed by Du et al.[20], the thioamide moiety has an important role in the cruzain inhibition mechanism, however it has been demonstrated that cyclic derivatives, as 1,3-thiazoles [29], thiazolidin-4-ones [28] and 2,4-oxadiazoles [42], may also inhibit this enzyme. In this work, most cyclic derivatives present only modest inhibitory activity of cruzain, however, five of most active compounds for trypomastigote form (**7**, **9**, **12**, **16** and **23**) inhibit this enzyme by more than 70%, corroborating with the literature [29]. Compounds **7** and **9** were most active, respectively with IC_{50} values of 0.6 and 9 µM, suggesting a possible mechanism of action these compounds. Compound **14** (the most active of all series), did not inhibit cruzain, suggesting that it kills the parasite by acting on another target.

Table 2: Cruzain inhibition of thiazoles **7-28**.

Cpd.	% cruzain inhibition ± S.D. at 10 µM[a]	IC_{50} (µM)[b]
7	97 ± 1	0.6 ± 0.2
8	50 ± 5	ND
9	84 ± 5	9 ± 3
10	59 ± 8	ND
11	12 ± 5	ND
12	76 ± 4	ND
13	31 ± 4	ND
14	17 ± 1	ND
15	32 ± 2	ND
16	75± 5	ND
17	32 ± 3	ND
18	31 ± 2	ND
19	35 ± 9	ND
20	ND	ND

21	38 ± 8	ND
22	43 ± 3	ND
23	74 ± 4	ND
24	ND	ND
25	33 ± 7	ND
26	41 ± 2	ND
27	44 ± 8	ND
28	44 ± 5	ND

[a] Determined in a competition assay with ZFR-AMC, average for two screens, each in triplicate. [b] Means relating to at least two IC_{50} determinations. All errors calculated by the formula: $\sigma/\sqrt{n}$, at where σ = standard deviation and n= number of experiments. S.D. = standard deviation. ND = Not determined.

To understand how compound **14** affects parasite cells, we performed a staining with Rhodamine 123, to detect changes in the mitochondrial membrane potential in *T. cruzi* trypomastigotes. As seen in **Figure 6,** treatment with **14,** at its IC_{50} or two times the IC_{50}**,** induced mithocondria despolarization evidenced by a decrease in Rhodamine 123 fluorescence intensity, which typically lead to apoptosis.

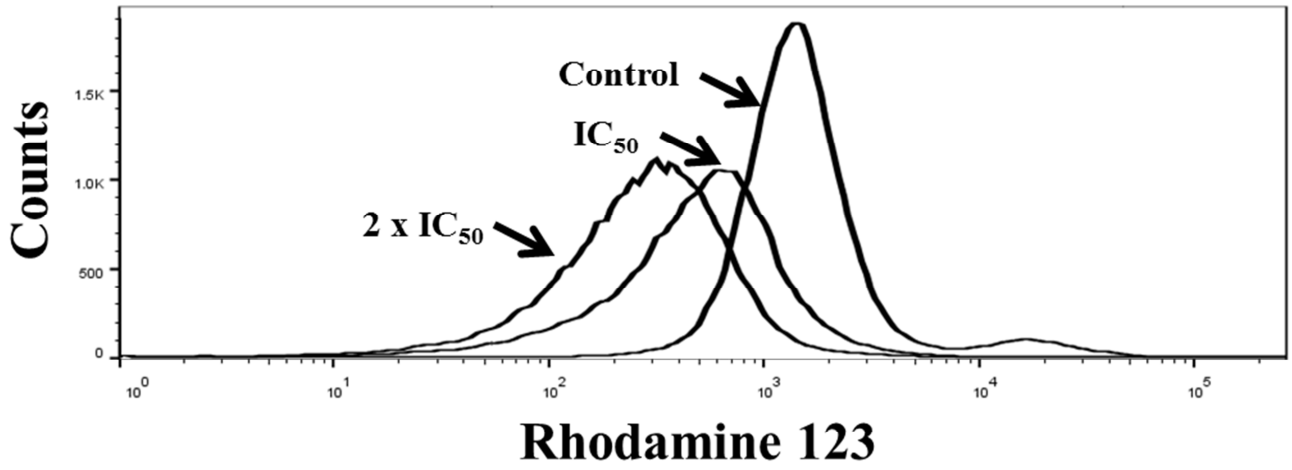
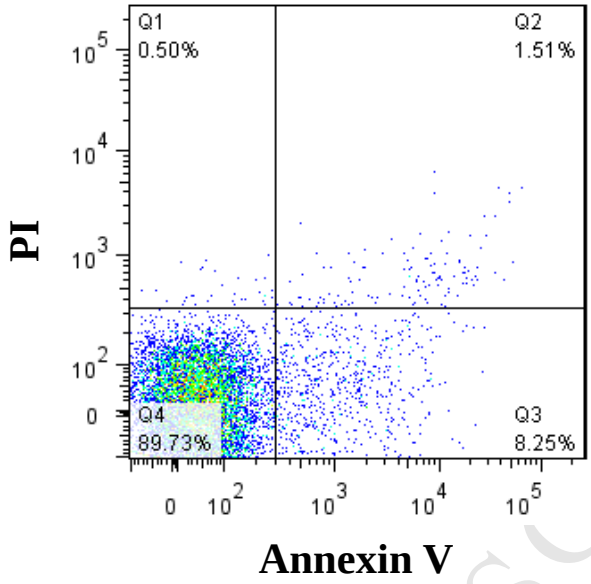


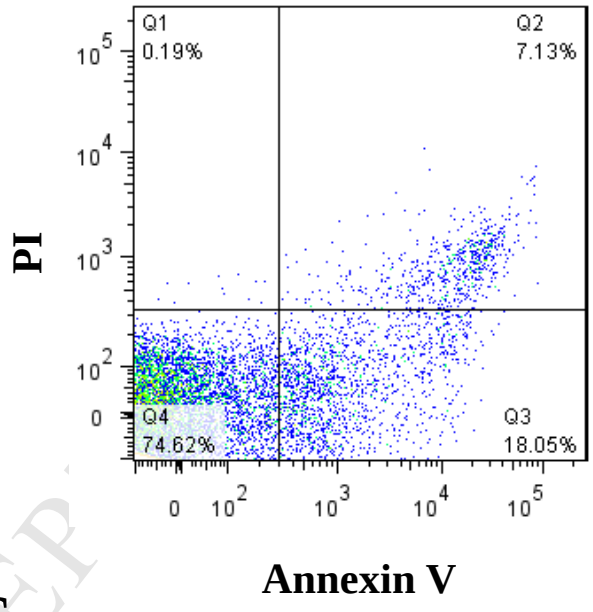
Figure 6. Effects of **14** on the mitochondrial membrane potential. Overlay flow cytometric histograms of the control and the 14- treated parasites labeled with Rhodamine 123. The reduction of Rhodamine 123 fluorescence intensity, mainly at the 2x IC_{50} of **14**, indicates the depolarization of the mitochondrial membrane.

To confirm the hypothesis of cell death by apoptosis a double staining with annexin V and propidium iodide was performed for flow cytometry analysis. Eventual phosphatidylserine flipping in the membrane surface is an alteration observed in the apoptotic process, while the collapse of the cell, making it permable to propidium iodide, is an alteration observed in the process of necrosis [43]. As shown in **Figure 7**, in untreated cultures, most trypomastigotes were negative for annexin V and PI staining, demonstrating cell viability. In comparison to untreated parasites, a significant increase in the number of Annexin V-positive parasites was observed under treatment with **14** (0.4 μM) for 24h, indicating parasite cell death through apoptosis induction.

A


Q1
0.50%
Q2
1.51%
Q4
89.73%
Q3
8.25%
PI
Annexin V

B


Q1
0.19%
Q2
7.13%
Q4
74.62%
Q3
18.05%
PI
Annexin V

C

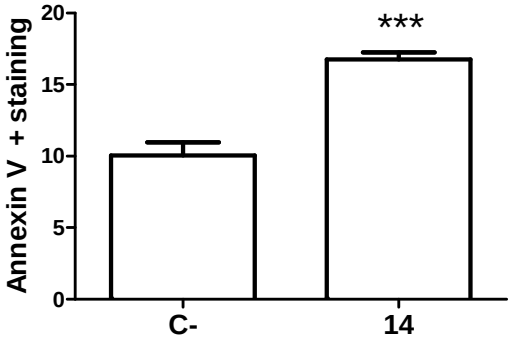

Annexin V + staining
***
C-
14

Figure 7. **Flow cytometry analysis of trypomastigotes treated with 14 and stained with annexin V**. (A) Untreated trypomastigotes; (B) trypomastigotes treated with **14** (0.4 µM); (C) Percentage of stained cells with annexin V after 24 h of treatment with **14**. Values represent the means ± SEM of six determinations. ***P* < 0.01.

Given the selectivity of these compounds against bloodstream trypomastigotes of *T. cruzi*, we also examined their activity against intracellular parasites. To this end, we assessed an *in vitro* model of parasite infection using mouse macrophages infected with Y strain trypomastigotes. Three days after infection, 15–20 % of the untreated macrophages were infected, and a high number of amastigotes per 100 macrophages were observed. Treatment with 5 µM of benznidazole reduced the number of infected cells and the number of intracellular amastigotes (*P*< 0.001). We then tested the most potent compound, thiazole (**14**), at concentrations of 8, 2 and 0.5 µM. As shown in **Figure 8**, this compound inhibited *T. cruzi* infection in a concentration dependent manner, being equipotent to benznidazole at concentration of 2 µM, and showing higher potency than benznidazole at concentration of 8 µM (*P* < 0.001).

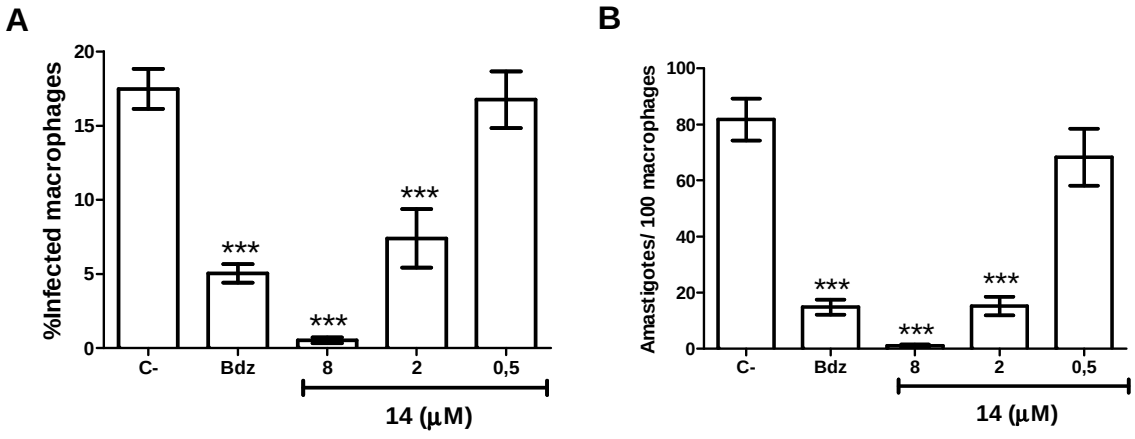


Figure 8. Thiazole **14** affects intracellular parasite development. The percentage of infected macrophages (**A**) and the relative number of amastigotes per 100 macrophages (**B**) are higher

in untreated controls than in cultures treated with test compound **14** or Bdz. Infected macrophages were treated for 3 days with compounds and then analyzed in Perkin-Elmer Opera confocal microscope after staining with hoeschst. Values represent the means ± SEM of six determinations. ***P < 0.001; **P < 0.01; *P< 0.05. Bdz (benznidazole; 5 μM) was used as a positive control.

The antiparasitic effect of **14** and benznidazole in combination was also investigated against trypomastigotes. Such experiment is important because combination therapies can be a valuable tool to improve treatment efficacy and reduce dose levels and toxicity, as well as to prevent the potential development of resistance, which may be advantages for the treatment of parasitic diseases [44,45]. The combination index value of 0.37±0.09 associated with a concave isobologram reveals that **14** demonstrates synergistic effects with benznidazole against the bloodstream parasites *in vitro*, showing a promising profile for drug combination (**Figure 9**).

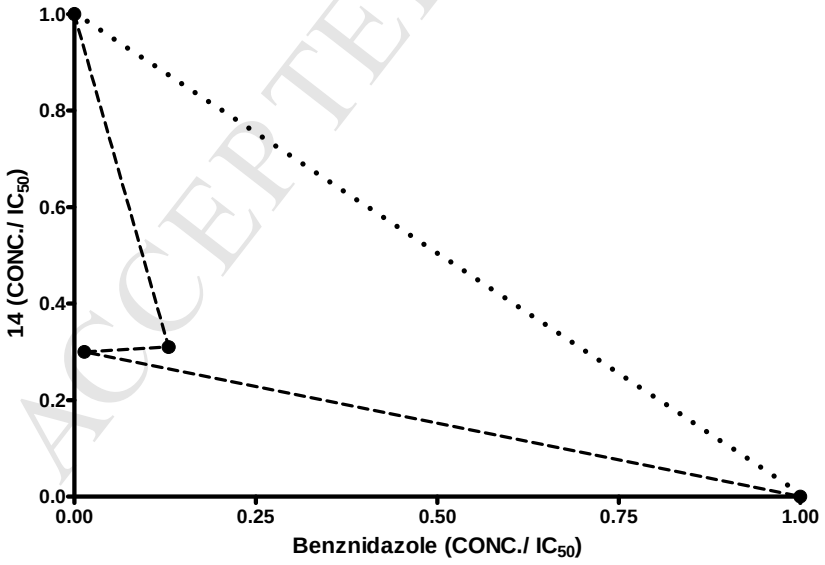


Figure 9. Isobologram describing the synergistic effects of 14 and benznidazole on trypomastigotes viability. Broken lines correspond to the predicted positions of the

experimental points for additive effects. A combination index of 0.37±0.09 was calculated according to Chou & Talalay [46].

Next, *in vivo* studies to evaluate the effects of **14** against *T. cruzi* infection in mice (acute phase) were performed. As shown in **Figure 10**, treatment with compound **14** significantly ($P < 0.001$) reduced blood parasitaemia when compared with mice treated with vehicle (**Figure 10**). At dose of 25 mg/kg, administration of compound **14** caused a reduction in blood parasitaemia of 54.6 and 64.1 % at days 8 and 12 post infection respectively (**Table 3**). In the group treated with benznidazole, it was observed > 99% of inhibition of blood parasitaemia, indicating that eradication of infection was achieved. Treatment with **14**, similar to the treatment with benznidazole, had a protective effect on mortally (**Table 3**). Mice from benznidazole or **14** treated groups did not show any behavioral alteration or signs of toxicity (data not shown).

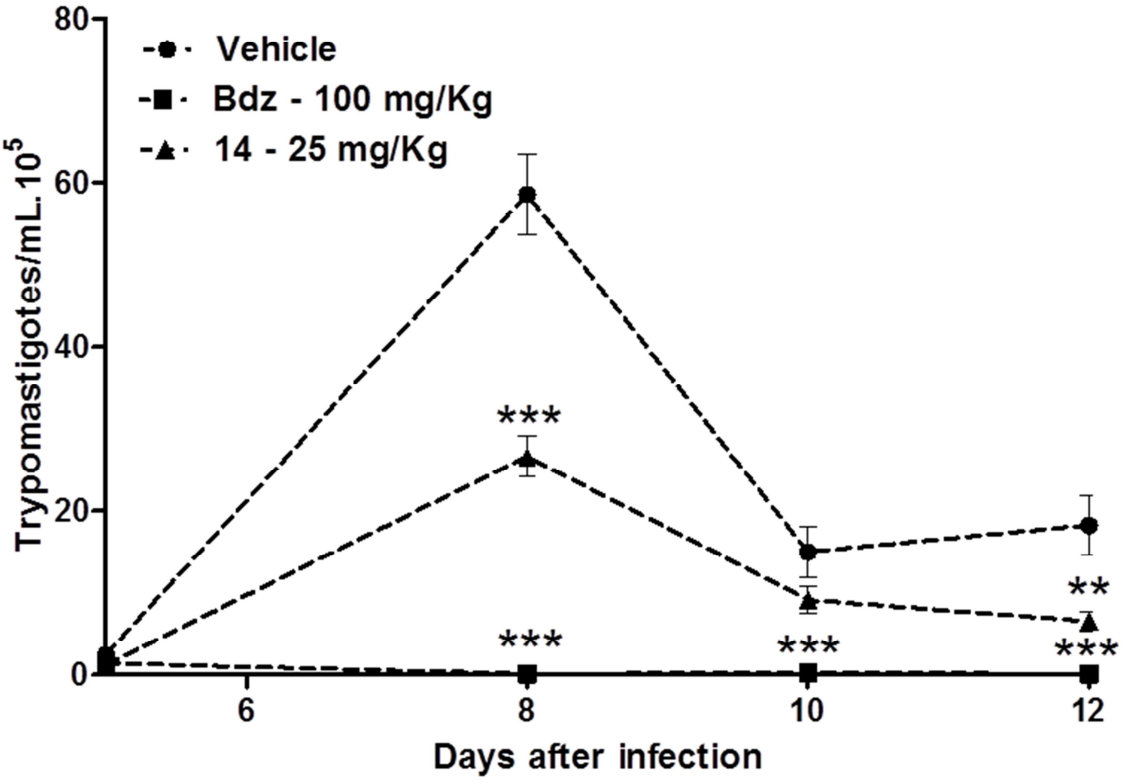


Figure 10. Parasitaemia of BALB/c mice infected with *T. cruzi* and treated with **14**. Female BALB/c mice were infected with 10^4 Y strain trypomastigotes. Five days after infection, mice were treated orally with **14** (25 mg/kg) or benznidazole (100 mg/kg) once a day during five consecutive days. Parasitemia was monitored by counting the number of trypomastigotes in fresh blood samples. Values represent the mean±SEM of 6 mice per group. ** $P < 0.01$; *** $P < 0.001$ compared to untreated-infected group (vehicle).

Table 3. Parasitemia and mortality evaluation in mice infected with Y strain *T. cruzi* and treated daily with **14** or benznidazole for 5 days.

Sample	Dose (mg/Kg)	% Blood parasitemia reduction in mice[a]		Mortality[b]
		8dpi	12 dpi	
14	25	54.6%	64.1	1/6
BDZ	100	>99	>99	0/6
Vehicle	-	-	-	5/6

[a] Calculated as ([average vehicle group − average treated group)/average vehicle group] × 100%). [b]Mortaly was monitored until 30 days after treatment. Dpi = days post-infection. BDZ = benznidazole. Vehicle = untreated and infected group.

Finally, we evaluated if the compounds synthesized that properties within the Lipinski's Rule of Five, which are important for pharmacokinetics and drug development. Compound obeying at least three of the four criteria are to considered to adhere to the Lipinski Rule [47]. Other interest property is the polar surface area (PSA), since compounds with a low PSA ($\leq$ 140 Å^2) tend to have higer oral bioavailability [48,49]. All compounds synthetized, except **24** and **25**, are compatible with Lipinski Rule and present appropriate PSA (**Table 4**).

Table 4: Physicochemical properties calculated for the thiazoles.

Compd.	MW (g/mol)	C log P	H bond donors	H bond acceptors	Criteria met	PSA Å^2
Desirable Value	**<500**	**<5**	**<5**	**<10**	**3 at least**	**≤140**
7	300.2	4.33	1	3	All	37.28
8	334.64	4.86	1	4	All	37.28
9	314.23	4.97	1	3	All	37.28
10	362.27	6.22	1	3	3	37.28
11	376.3	4.84	0	3	All	27.96
12	376.3	6.87	1	3	3	37.28
13	438.37	6.51	0	3	3	27.96
14	363.26	4.29	1	4	4	50.17
15	363.26	5.01	1	4	3	50.17
16	376.3	6.74	1	3	3	37.28
17	380.26	6.37	1	3	3	37.28
18	396.71	6.83	1	3	3	37.28
19	431.16	7.44	1	3	3	37.28
20	431.16	7.44	1	3	3	37.28
21	465.6	8.04	1	3	3	37.28
22	441.17	7.00	1	3	3	37.28
23	455.2	7.64	1	3	3	37.28
24	407.27	6.17	1	5	2	80.42
25	407.27	6.17	1	5	2	80.42
26	392.3	6.07	1	4	3	46.51
27	438.37	7.88	1	3	3	37.28
28	412.33	7.22	1	3	3	37.28

Considering the aim to identify a new antitrypanosomatid, the analysis of the results depicted in **Table 1**, allowed the selection of compound **14** as a trypanosomicidal agent.

Therefore, its physicochemical and ADME properties were calculated using the SwissADME (a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules) and the results were compared to those obtained for benznidazole (**Table 5**).

Table 5: Physico-chemistry properties and ADME profile of compounds **14** and benznidazole calculated using the Program SwissADME.

Predicted properties	**Compounds**	
	14	**Benznidazole**
MW (g/mol)	363.23	260.25
H-Donors	1	1
H-Acceptors	4	4
Rotatable Bonds	4	6
LogP	4.29	0.66
Solubility	1.07 mg/mL	2.27 mg/mL
GI absorption	High	High
Bioavailability Score	0.55	0.55

As demonstrated in **Table 5**, the druglikeness of compound **14** was very similar to benznidazole, with no violations of Lipinsky's rule of 5 [47]. Regardless of the poor solubility (predicted in buffer at pH of 6.5), compound **14** was shown to be highly permeable based on gastrointestinal absorption (GI), according to the BOILED-Egg predictive model (Brain Or IntestinaL EstimateD permeation method). These results were similar to benznidazole. Compound **14** was expected to have an oral bioavailability score of 0.55 as well as benznidazole (**Table 5**). Taken together, this data, suggests a good *in silico* druglikeness profile and great chemical stabilities for compound **14**. In fact, the *in vivo* efficacy of **14** reinforces this ideia.

Conclusions

The 1,3-thiazoles were structurally designed by employing the non-classical bioisosteric exchange of a thiazolidin-4-one ring by a thiazole heterocycle. This led to the synthesis and chemical characterization of compounds **7**-**28**, which were evaluated concerning their anti-*T. cruzi*, cytotoxicity and cruzain inhibition activities. The pharmacological evaluation led to the identification of thiazoles (**7, 9, 12, 14, 15, 21, 23** and **26**) as potents anti-*T. cruzi* agent. Concerning their mechanism of action, some of these compounds inhibit cruzain and compound **14** was observed to induce parasite cell death through an apoptotic process, as indicated by the induced depolarization of the mitochondrial membrane in Rhodamine 123 labeling assay. Compound **14** was also able to inhibit in vitro infection by *T. cruzi*, showed synergistic effect with the reference drug, showing a promising profile for drug combination, and at dose of 25 mg/kg caused a reduction in blood parasitaemia of 54.6 and 64.1 % at days 8 and 10 post infection respectively. These results suggest that the strategies used are promising to obtain novel potent and selective antiparasitic agents.

Reagents and spectra analysis

All reagents were used as purchased from commercial sources (Sigma-Aldrich, Acros Organics, Vetec, or Fluka). Progress of the reactions was followed by thin-layer chromatography (silica gel 60 F254 in aluminum foil). Purity of the target compounds was confirmed by combustion analysis (for C, H, N, S) performed by a Carlo-Erba instrument (model EA 1110). IR was determined in KBr pellets. For NMR, we used a Bruker AMX-300 MHz (300 MHz for ^{1}H and 75.5 MHz for ^{13}C) instruments. DMSO-d_6 or D_2O were purchased from CIL. Chemical shifts are reported in ppm, and multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (double doublet), and coupling constants (*J*) in hertz. NH signals were localized in each spectrum after the addition of a few drops of D_2O. Structural assignments were corroborated by DEPT analysis. Mass spectrometry experiments were performed on a Q-TOF spectrometer LC-IT-TOF (Shimadzu). When otherwise specified, ESI was carried out in the positive ion mode. Reactions in a ultrasound bath were carried out under frequency of 40 kHz (180 W) and without external heating.

General procedure for the synthesis of thiosemicarbazones (**6-c**). Example for compound (**6a**): Synthesis of 1-(3,4-dichlorophenyl)-ethylidenethiosemicarbazone (**6a**). Under magnetic stirring: in a 100 mL round-bottom flask, 1.06 mmol (0.097 g) of thiosemicarbazide, 1.06 mmol (0.2 g) of 3',4'-dichloroacetophenone, 4 drops of H_2SO_4 and 20 mL of ethanol were added and maintained under magnetic stirring and reflux for 20 h. After cooling back to rt, the precipitate was filtered in a Büchner funnel with a sintered disc filter, washed with cold ethanol, and then dried over SiO_2. Product was purified by recrystallization in hot toluol. Colorless crystals, m.p.: 187-189 ºC; yield: 0.10 g (38%).

Under ultrasound irradiation: in a 10 mL round-bottom flask, 1.06 mmol (0.097 g) of thiosemicarbazide, 1.06 mmol (0.2 g) of 3',4'-dichloroacetophenone, 4 drops of H_2SO_4 and 20 mL of ethanol were added and maintained in a ultrasound bath for 30 min. After cooling back to rt, the precipitate was filtered in a Büchner funnel with a sintered disc filter, washed with cold ethanol, and then dried over SiO_2. Product was purified by recrystallization in hot toluol. Colorless crystals, m.p.: 187-189 ºC; yield: 0.22 g (80%); R_f= 0.56 (toluol / ethyl acetate 6:4). IR (KBr): 3427 (NH_2), 3399 (NH), 3138 (C-H), 1591 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 3H, CH_3), 7.58 (d, 1H, *J* 9.37 Hz, Ar), 7.86 (dd, 1H, *J* 9.37 and 3.75 Hz, Ar), 8.18 (s, 1H, NH_2), 8.26 (d, 1H, *J* 3.75 Hz, Ar), 8.36 (s, 1H, NH_2), 10.29 (s, 1H, NH). Signals at δ 8.18, 8.36 and 10.29 ppm disapear after adding D_2O. ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 13.8 (CH_3), 126.7 (CH, Ar), 128.2 (CH, Ar), 130.2 (CH, Ar), 131.3 (C, Ar), 131.6 (3ClC, Ar), 138.2 (4ClC, Ar), 145.312 (C=N), 179.0 (C=S). HRMS (ESI): 259.9565 $[M-H]^+$. Anal. calcd for $C_9H_9Cl_2N_3S$: C, 41.23; H, 3.46; N, 16.03; S, 12.23. Found: C, 41,21; H, 3,61; N, 16,36; S, 12,78.

1-(3,4-dichlorophenyl)ethylideno-4-methylthiosemicarbazone (**6b**). Recrystallization in hot toluol afforded colorless crystal, m.p.: 195-197 ºC; yield: 0.24 g (84%); R_f= 0.7 (toluol / ethyl acetate 6:4). IR (KBr): 3343 e 3219 (NH), 1547 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H, CH_3), 3.04 (d, 3H, *J* 6 Hz, N–CH_3, coupling with N–H), 7.63 (d, 1H, *J* 8.57 Hz, Ar), 7.88 (dd, 1H, *J* 8.57 and 2.14 Hz, Ar), 8.23 (d, 1H, *J* 2.14 Hz, Ar), 8.61 (broad d, 1H, *J* 6 Hz, CH_3–N<u>H</u>), 10.33 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 13.8 (CH_3), 31.1 (N–CH_3), 126.7 (CH, Ar), 128.1 (CH, Ar), 130.2 (CH, Ar), 131.3 (C, Ar), 131.5 (3ClC, Ar), 138.3 (4ClC, Ar), 145.0 (C=N), 178.6 (C=S). HRMS (ESI): 275.9475 $[M+H]^+$. Anal. calcd for $C_{10}H_{11}Cl_2N_3S$: C, 43.49; H, 4.01; N, 15.21; S, 11.61. Found: C, 43.34; H, 3.98; N, 15.55; S, 11.74.

1-(3,4-dichlorophenyl)ethylidene)-4-phenylthiosemicarbazone (**6c**). Recrystallization in hot toluol afforded colorless crystal, m.p.: 200-202 ºC; yield: 0.32 g (89%); R_f= 0.73 (toluol / ethyl acetate 6:4). IR (KBr): 3309 and 3239 (NH), 3046 (C-H), 1523 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.36 (s, 3H, CH_3), 7.23 (t, 1H, *J* 7.21 Hz, Ar), 7.38 (t, 2H, *J* 7.21 Hz, Ar), 7.51 (d, 2H, *J* 7.21 Hz, Ar), 7.64 (d, 1H, *J* 9.09 Hz, Ar), 7.97 (dd, 1H, *J* 9.09 and 2.72 Hz, Ar), 8.34 (d, 1H, *J* 2.72 Hz, Ar), 10.18 (s, 1H, NH), 10.66 (s, 1H, ArNH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 14.2 (CH_3), 125.6 (CH, Ar), 126.5 (CH, Ar), 127.0 (CH, Ar), 128.1 (CH, Ar), 128.4 (CH, Ar), 130.2 (CH, Ar), 131.3 (C, Ar), 131.8 (3ClC, Ar), 138.1 (4ClC, Ar), 139.2 (C–N, Ar), 146.3 (C=N), 177.3 (C=S). HRMS (ESI): 337.9678 $[M+H]^+$. Anal. calcd for $C_{15}H_{13}Cl_2N_3S$: C, 53.26; H, 3.87; N, 12.42; S, 9.48. Found: C, 53.23; H, 3.84; N, 12.31; S, 9.32.

General procedure for the synthesis of 1,3-thiazoles (**7**-**9**): Example for compound (**7**): Synthesis of 2-[1-(3,4-dichlorophenyl)ethylidenohydrazinyl]-4-methyl-1,3-thiazole (**7**). In a 50 mL round-bottom flask, 0.76 mmol (0.2 g) of thiosemicarbazone (**6a**) was dissolved in 30 mL of ethanol followed by addition of 3.04 mmol (0.25 g) of sodium acetate. After stirring and heating for 15 min., 1.14 mmol (0.105 g) of 2-chloroacetone was added in portions and the mixture was maintained under stirring and reflux for 5 h. After cooling back to rt, the precipitate was filtered off and the solvent was evaporated under reduced pressure. The crude mixture was crystallized in hot water, affording brown crystals, m.p.: 102-104 ºC; yield: 0.18 g (78%). R_f: 0.57 (toluol / ethyl acetate 6:4). IR (KBr): 3190 (NH), 1568 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.88 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 6.30 (s, 1H, CH thiazole), 7.63 (d, 1H, *J* 8.4 Hz, Ar), 7.71 (dd, 1H, *J* 8.4 and 1.8 Hz, Ar), 7.90 (d, 1H, *J* 1.8 Hz, Ar), 11.99 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 16.4 (CH_3), 21.4 (CH_3), 101.8 (CH thiazole), 125.5 (CH, Ar), 127.0 (CH, Ar), 130.5 (CH, Ar), 130.7 (C, Ar), 131.2 (3ClC, Ar), 138.8 (4ClC, Ar), 144.5 (C, thiazole), 169.5 (C=N), 172.2 (S–C=N). HRMS (ESI): 300.0168

$[M+H]^+$. Anal. calcd for $C_{12}H_{11}Cl_2N_3S$: C, 48.01; H, 3.69; N, 14.00; S, 10.68. Found: C, 48.07; H, 3.96; N, 14.07; S, 10.65.

4-(chloromethyl)-2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-thiazole (**8**). Recrystallization in hot ethanol afforded brown crystals, m.p.: 124-126 ºC; yield: 1.12 g (88%); R_f= 0.64 (toluol / ethyl acetate 6:4). IR (KBr): 3065 (C-H), 1561 (C=N) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 2.68 (s, 3H, CH_3), 4.64 (s, 2H, CH_2), 6.96 (s, 1H, CH thiazole), 7.15 (dd, 1H, *J* 8.4 and 2.1 Hz, Ar), 7.28 (d, 1H, *J* 2.1 Hz, Ar), 7.42 (d, 1H, *J* 8.4 Hz, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 15.8 (CH_3), 46.5 (CH_2), 111.6 (CH thiazole), 126.3 (CH, Ar), 130.2 (CH, Ar), 131.1 (CH, Ar), 132.4 (C, Ar), 132.6 (C, Ar), 133.2 (C, Ar), 147.8 (C, Ar), 151.6 (C=N), 162.2 (S-C=N). HRMS (ESI): 333.9613 $[M-H]^+$. Anal. calcd for $C_{12}H_{10}Cl_3N_3S$: C, 43.07; H, 3.01; N, 12.56; S, 9.58. Found: C, 43.07; H, 3.00; N, 12.42, S, 9.69.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4,5-dimethylthiazole (**9**). Recrystallization in hot ethanol afforded yellow crystals, m.p.: 157-159 ºC; yield: 0.55 g (46%); R_f= 0.51 (toluol / ethyl acetate 6:4). IR (KBr): 3197 (NH), 2918 (C-H), 1546 (C=N) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 2.04 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 7.63 (d, 1H, *J* 8.4 Hz, Ar), 7.71 (dd, 1H, *J* 8.4 and *J* 2.1 Hz, Ar), 7.90 (d, 1H, *J* 2.1 Hz, Ar), 11.52 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 11.3 (CH_3), 13.8 (CH_3), 21.5 (CH_3), 112.0 (S-C-CH_3 thiazole), 125.9 (CH, Ar), 127.3 (CH, Ar), 130.9 (CH, Ar), 130.98 (C, Ar), 131.6 (3ClC, Ar), 139.4 (4ClC, Ar), 145.2 (N-C-CH_3, thiazole), 167.4 (C=N), 172.5 (S–C=N). HRMS (ESI): 313.9612 $[M+H]^+$. Anal. calcd for $C_{13}H_{13}Cl_2N_3S$: C, 49.69; H, 4.17; N, 13.37; S, 10.20. Found: C, 49.35; H, 4.28; N, 13.27, S, 10.30.

General procedure for the synthesis of 1,3-thiazoles (**10-28**): Example for compound (**10**): Synthesis of 2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-phenylthiazole (**10**). Under ultrasound irradiation: in a 100 mL round-bottom flask, 3.81 mmol (0.76 g) of 2-bromoacetophenone and 25 mL of isopropanol were added and maintained in a ultrasound

bath, the mixture was sonicated at room temperature until complete solubilization of the reagents. Then it was added 3.81 mmol (1.0 g) of the compound (**6a**). Immediately after addition, there was complete solubilisation and change of color of the reaction mixture from light yellow to gray. The mixture was kept under ultrasonic bath at room temperature for 2 hours. Thereafter, the precipitate formed was filtered, washed with isopropanol and dried in over SiO_2. The product was recrystallised in hot ethanol / methanol 2: 1, affording gray crystals, m.p.: 259-261 ºC; yield: 0.98 g (71%). R_f: 0.62 (toluol / ethyl acetate 6:4). IR (KBr): 2918 (CH), 1612 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.31 (s, 3H, CH_3), 7.30-7.43 (m, 4H, Ar), 7.65-7.94 (m, 5H, Ar), 11.25 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 11.5 (CH_3), 102.1 (CH thiazole), 123.2 (CH, Ar), 123.4 (CH, Ar), 124.9 (CH, Ar), 125.3 (CH, Ar), 126.3 (CH, Ar), 128.2 (CH, Ar), 130.98 (C, Ar), 131.6 (3ClC, Ar), 135.0 (C, Ar), 139.4 (4ClC, Ar), 143.9 (C, Ar), 150.0 (C=N), 170.0 (S–C=N). HRMS (ESI): 361.9579 $[M+H]^+$. Anal. calcd for $C_{17}H_{13}Cl_2N_3S$: C, 56.36; H, 3.62; N, 11.60; S, 8.85. Found: C, 56.34; H, 3.69; N, 11.51; S, 8.84.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazono]-3-methyl-4-phenyl-2,3-dihydrothiazole (**11**). Recrystallization in hot ethanol afforded yellow crystals, m.p.: 247-248 ºC; yield: 1.12 g (82%); R_f= 0.93 (toluol / ethyl acetate 6:4). IR (KBr): 3035 (C-H), 1593 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.40 (s, 3H, CH_3), 3.40 (s, 3H, N-CH_3), 6.59 (s, 1H, CH thiazole), 7.51 (m, 5H, Ar), 7.67 (d, 1H, *J* 8.4 Hz, Ar), 7.80 (d, 1H, *J* 8.4 Hz, Ar), 7.98 (s, 1H, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 14.2 (CH_3), 34.1 (N-CH_3), 101.8 (CH thiazole), 125.9 (CH, Ar), 127.4 (CH, Ar), 128.8 (CH, Ar), 129.4 (CH, Ar), 130.0 (CH, Ar), 130.6 (C, Ar), 131.3 (3ClC, Ar), 138.8 (4ClC, Ar), 141.0 (N-C-C, thiazole), 152.5 (C=N), 170.1 (S–C=N). HRMS (ESI): 375.9459 $[M+H]^+$. Anal. calcd for $C_{18}H_{15}Cl_2N_3S$: C, 57.45; H, 4.02; N, 11.17; S, 8.52. Found: C, 57.51; H, 3.98; N, 11.43; S, 8.55.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-5-methyl-4-phenylthiazole (**12**). Recrystallization in hot ethanol afforded beige crystals, m.p.: 234-236 ºC; yield: 1.16 g (81%); R_f= 0.54 (toluol / ethyl acetate 6:4). IR (KBr): 3022 (C-H), 1619 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.35 (s, 3H, CH_3), 2.38 (s, 3H, N-CH_3), 7.42-7.62 (m, 5H, Ar), 7.69 (d, 1H, *J* 8.4 Hz, Ar), 7.83 (d, 1H, *J* 8.4 Hz, Ar), 8.65 (s, 1H, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 12.4 (CH_3), 14.7 (CH_3), 117.9 (S-C-CH_3 thiazole), 126.6 (CH, Ar), 127.6 (CH, Ar), 128.0 (CH, Ar), 128.1 (CH, Ar), 128.9 (CH, Ar), 131.0 (C, Ar), 131.8 (3ClC, Ar), 132.0 (4ClC, Ar), 138.4 (N-C-C, thiazole), 165.9 (C=N), 167.2 (S–C=N). HRMS (ESI): 375.9648 $[M+H]^+$. Anal. calcd for $C_{18}H_{15}Cl_2N_3S$: C, 57.45; H, 4.02; N, 11.17; S, 8.52. Found: C, 57.34; H, 3.95; N, 11.21; S, 8.58.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazono]-3,4-diphenyl-2,3-dihydrothiazole (**13**). Recrystallization in hot toluol afforded beige crystals, m.p.: 170-172 ºC; yield: 0.23 g (61%); R_f= 0.96 (toluol / ethyl acetate 6:4). IR (KBr): 1597 and 1515 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.16 (s, 3H, CH_3), 6.67 (s, 1H, CH thiazole), 7.16-7.52 (m, 10H, Ar), 7.65 (d, 1H, *J* 8.4 Hz, Ar), 7.76 (dd, 1H, *J* 8.4 and 1.8 Hz, Ar), 7.95 (d, 1H, *J* 1.8 Hz, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 14.1 (CH_3), 102.1 (CH thiazole), 125.6 (CH, Ar), 125.8 (CH, Ar), 126.5 (CH, Ar), 127.0 (CH, Ar), 127.3 (CH, Ar), 127.7 (CH, Ar), 128.1 (CH, Ar), 128.2 (CH, Ar), 128.4 (CH, Ar), 128.7 (CH, Ar), 130.2 (CH, Ar), 130.5 (CH, Ar), 130.6 (CH, Ar), 131.1 (C, Ar), 131.2 (C, Ar), 137.5 (C, Ar), 138.9 (C, Ar), 139.6 (C, Ar), 153.2 (C=N), 169.9 (S–C=N). HRMS (ESI): 438.0271 $[M+H]^+$. Anal. calcd for $C_{23}H_{17}Cl_2N_3S$: C, 63.02; H, 3.91; N, 9.59; S, 7.31. Found: C, 62.45; H, 4.08; N, 9.63; S, 6.86.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-(pyridin-2-yl)thiazole (**14**). Recrystallization in hot ethanol afforded green crystals, m.p.: 227 ºC; yield: 1.0 g (72%); R_f= 0.46 (toluol / ethyl acetate 6:4). IR (KBr): 3360 (NH), 3165 (CH), 1615 and 1597 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.35 (s, 3H, CH_3), 7.68-7.79 (m, 3H, Ar), 7.97-8.10 (m, 2H,

Ar), 8.24-8.36 (m, 2H, Ar), 8.64-8.72 (m, 1H, Ar), 11.00 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 14.0 (CH_3), 117.1 (CH thiazole), 124.3 (CH, Ar), 125.8 (CH, Ar), 127.3 (CH, Ar), 130.6 (CH, Ar), 131.3 (CH, Ar), 138.2 (C, Ar), 138.9 (C, Ar), 139.6 (C, Ar), 145.5 (C, Ar), 153.2 (C=N), 169.9 (S–C=N). HRMS (ESI): 362.9710 $[M+H]^+$. Anal. calcd for $C_{16}H_{12}Cl_2N_4S$: C, 52.90; H, 3.33; N, 15.42; S, 8.83. Found: C, 52.85; H, 3.38; N, 15.72; S, 8.88.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-(pyridin-4-yl)thiazole (**15**). Recrystallization in hot ethanol afforded green crystals, m.p.: 197 ºC; yield: 1.05 g (75%); R_f= 0.5 (toluol / ethyl acetate 6:4). IR (KBr): 1630 and 1561 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.31 (s, 3H, CH_3), 7.63 (d, 1H, *J* 7.8 Hz), 7.71 (d, 1H, *J* 7.8 Hz, Ar), 7.89 (s, 1H, Ar), 8.36 (d, 2H, *J* 5.7 Hz, Ar), 8.72 (s, 1H, CH thiazole), 8.93 (d, 2H, *J* 5.7 Hz, Ar), 11.72 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 14.0 (CH_3), 117.0 (CH thiazole), 122.0 (CH, Ar), 125.7 (CH, Ar), 127.3 (CH, Ar), 130.6 (CH, Ar), 131.3 (CH, Ar), 138.1 (C, Ar), 142.1 (C, Ar), 145.1 (C, Ar), 145.9 (C, Ar), 149.1 (C, Ar), 153.2 (C=N), 170.3 (S–C=N). HRMS (ESI): 363.7868 $[M+H]^+$. Anal. calcd for $C_{16}H_{12}Cl_2N_4S$: C, 52.90; H, 3.33; N, 15.42; S, 8.83. Found: C, 52.70; H, 3.37; N, 15.41; S, 8.88.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-(p-tolyl)thiazole (**16**). Recrystallization in hot ethanol afforded beige crystals, m.p.: 256-258 ºC; yield: 1.21 g (84%); R_f= 0.75 (toluol / ethyl acetate 6:4). IR (KBr): 3030 (CH), 1608 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 7.19-7.26 (m, 3H, Ar), 7.63-7.76 (m, 4H, Ar), 7.94 (s, 1H, Ar), 11.50 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 13.9 (CH_3), 20.8 (CH_3), 103.6 (CH thiazole), 125.6 (CH, Ar), 125.8 (CH, Ar), 127.3 (CH, Ar), 129.2 (CH, Ar), 130.6 (CH, Ar), 131.2 (C, Ar), 131.3 (C, Ar), 137.1 (C, Ar), 138.3 (C, Ar), 144.8 (C, Ar), 149.4 (C, Ar), 169.3 (C=N), 172.2 (S–C=N). HRMS (ESI): 375.9742 $[M+H]^+$. Anal. calcd for $C_{18}H_{15}Cl_2N_3S$: C, 57.45; H, 4.02; N, 11.17; S, 8.52. Found: C, 57.15; H, 4.00; N, 10.86; S, 8.42.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-(4-fluorophenyl)thiazole (**17**). Recrystallization in hot ethanol afforded beige crystals, m.p.: 267-269 ºC; yield: 0.85 g (80%); R_f= 0.66 (toluol / ethyl acetate 6:4). IR (KBr): 2923 (CH), 1615 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 7.20-7.25 (m, 2H, Ar), 7.31 (s, 1H, CH thiazole), 7.62 (d, 1H, *J* 8.4 Hz, Ar), 7.72 (d, 1H, *J* 8.4 Hz, Ar), 7.85-7.89 (m, 2H, Ar), 7.91 (s, 1H, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 13.9 (CH_3), 104.3 (CH thiazole), 115.4 (CH, Ar), 115.7 (CH, Ar), 125.7 (CH, Ar), 127.3 (CH, Ar), 127.6 (CH, Ar), 127.7 (CH, Ar), 130.6 (CH, Ar), 131.2 (C, Ar), 131.3 (C, Ar), 138.3 (C, Ar), 144.8 (C, Ar), 148.6 (C=N), 160.1 and 163.3 (d, C–F, Ar), 169.5 (S–C=N). HRMS (ESI): 379.9738 $[M+H]^+$. Anal. calcd for $C_{17}H_{12}Cl_2FN_3S$: C, 53.69; H, 3.18; N, 11.05; S, 8.43. Found: C, 53.53; H, 3.52; N, 10.95; S, 8.42.

4-(4-chlorophenyl)-2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]thiazole (**18**). Recrystallization in hot ethanol afforded beige crystals, m.p.: 266-268 ºC; yield: 1.2 g (79%); R_f= 0.72 (toluol / ethyl acetate 6:4). IR (KBr): 3047 (CH), 1611 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.28 (s, 3H, CH_3), 7.38 (s, 1H, CH thiazole), 7.43 (d, 2H, *J* 8.1 Hz, Ar), 7.61 (d, 1H, *J* 8.4 Hz, Ar), 7.70 (d, 1H, *J* 8.4 Hz, Ar), 7.84 (d, 2H, *J* 8.1 Hz, Ar), 7.90 (s, 1H, Ar), 10.59 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 13.9 (CH_3), 105.3 (CH thiazole), 125.7 (CH, Ar), 127.3 (CH, Ar), 128.4 (CH, Ar), 128.6 (CH, Ar), 128.7 (CH, Ar), 129.5 (CH, Ar), 130.6 (CH, Ar), 131.2 (C, Ar), 131.3 (C, Ar), 132.1 (C, Ar), 133.1 (C, Ar), 138.3 (C, Ar), 144.6 (N–C–C, Ar), 148.6 (C=N), 169.5 (S–C=N). HRMS (ESI): 395.9470 $[M+H]^+$. Anal. calcd for $C_{17}H_{12}Cl_3N_3S$: C, 51.47; H, 3.05; N, 10.59; S, 8.08. Found: C, 51.35; H, 3.22; N, 10.58; S, 7.93.

4-(2,4-dichlorophenyl)-2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]thiazole (**19**). Recrystallization in hot ethanol afforded gray crystals, m.p.: 244-246 ºC; yield: 0.9 g (54%); R_f= 0.65 (toluol / ethyl acetate 6:4). IR (KBr): 2923 (CH), 1623 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.31 (s, 3H, CH_3), 7.44 (s, 1H, CH thiazole), 7.51 (dd, 1H, *J* 8.4 and 2.1

Hz, Ar), 7.69 (d, 1H, *J* 8.4 Hz, Ar), 7.70-7.74 (m, 1H, Ar), 7.77 (d, 1H, *J* 2.1 Hz, Ar), 7.94-7.95 (m, 2H, Ar), 11.51 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 15.8 (CH_3), 119.0 (CH thiazole), 128.3 (CH, Ar), 128.4 (CH, Ar), 128.6 (CH, Ar), 128.7 (CH, Ar), 129.5 (CH, Ar), 132.6 (CH, Ar), 131.2 (C, Ar), 131.3 (C, Ar), 132.1 (C, Ar), 133.1 (C, Ar), 138.3 (C, Ar), 144.6 (C, Ar), 151.6 (C=N), 170.1 (S–C=N). HRMS (ESI): 429.9246 $[M+H]^+$. Anal. calcd for $C_{17}H_{11}Cl_4N_3S$: C, 47.36; H, 2.57; N, 9.75; S, 7.44. Found: C, 47.60; H, 2.61; N, 9.71; S, 7.50.

4-(3,4-dichlorophenyl)-2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]thiazole (**20**). Recrystallization in hot ethanol afforded beige crystals, m.p.: 266-268 ºC; yield: 1.2 g (79%); R_f= 0.76 (toluol / ethyl acetate 6:4). IR (KBr): 3043 (CH), 1616 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 7.56 (s, 1H, CH thiazole), 7.64 (d, 2H, *J* 8.4 Hz, Ar), 7.72 (dd, 1H, *J* 8.4 and 1.8 Hz, Ar), 7.83 (dd, 1H, *J* 8.4 and 1.8 Hz, Ar), 7.91 (d, 1H, *J* 1.8 Hz, Ar), 8.08 (d, 1H, *J* 1.8 Hz, Ar), 9.20 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 14.5 (CH_3), 107.3 (CH thiazole), 126.2 (CH, Ar), 126.3 (CH, Ar), 127.9 (CH, Ar), 130.3 (CH, Ar), 131.2 (CH, Ar), 131.5 (CH, Ar), 131.8 (C, Ar), 132.0 (C, Ar), 132.0 (C, Ar), 132.1 (C, Ar), 135.8 (C, Ar), 139.0 (C, Ar), 144.9 (C, Ar), 148.6 (C=N), 170.3 (S–C=N). HRMS (ESI): 429.8872 $[M+H]^+$. Anal. calcd for $C_{17}H_{11}Cl_4N_3S$: C, 47.36; H, 2.57; N, 9.75; S, 7.44. Found: C, 47.34; H, 2.30; N, 9.71; S, 7.43.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-(2,3,4-trichlorophenyl)thiazole (**21**). Recrystallization in hot ethanol afforded orange crystals, m.p.: 214-217 ºC; yield: 0.23 g (13%); R_f= 0.55 (toluol / ethyl acetate 6:4). IR (KBr): 2922 (CH), 1621 and 1567 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.74 (s, 3H, CH_3), 7.12 (s, 1H, CH thiazole), 7.18 (dd, 1H, *J* 8.1 and 1.8 Hz, Ar), 7.26 (d, 1H, *J* 8.7 Hz, Ar), 7.29 (d, 1H, *J* 1.8 Hz, Ar), 7.40 (d, 1H, *J* 8.7 Hz, Ar), 7.50 (d, 1H, *J* 8.1 Hz, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 15.0 (CH_3), 119.5 (CH thiazole), 124.2 (CH, Ar), 126.3 (CH, Ar), 129.2 (CH, Ar), 130.2 (CH, Ar), 131.1 (CH, Ar), 131.7 (C, Ar), 132.4 (C, Ar), 132.6 (C, Ar), 132.2 (C, Ar), 133.4 (C, Ar), 137.5 (C, Ar), 143.5

(C, Ar), 152.0 (C=N), 170.1 (S–C=N). HRMS (ESI): 465.8882 $[M+H]^+$. Anal. calcd for $C_{17}H_{10}Cl_5N_3S$: C, 43.85; H, 2.16; N, 9.02; S, 6.89. Found: C, 43.90; H, 1.94; N, 9.06; S, 6.81.

4-(4-bromophenyl)-2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]thiazole (**22**). Recrystallization in hot ethanol afforded gray crystals, m.p.: 265-267 ºC; yield: 1.10 g (65%); R_f= 0.71 (toluol / ethyl acetate 6:4). IR (KBr): 2925 (CH), 1616 (C=N) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 2.30 (s, 3H, CH_3), 5.19 (s, 1H, NH), 7.42 (s, 1H, CH thiazole), 7.59 (d, 2H, *J* 8.4 Hz, Ar), 7.65 (d, 1H, *J* 8.4 Hz, Ar), 7.73 (dd, 1H, *J* 8.4 and 1.8 Hz, Ar), 7.81 (d, 2H, *J* 8.4 Hz, Ar), 7.92 (d, 1H, *J* 1.8 Hz, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 13.8 (CH_3), 105.3 (CH thiazole), 120.6 (C, Ar), 125.7 (CH, Ar), 127.3 (CH, Ar), 127.6 (CH, Ar), 129.8 (CH, Ar), 130.6 (CH, Ar), 131.1 (C, Ar), 131.3 (CH, Ar), 131.5 (CH, Ar), 133.7 (C, Ar), 138.4 (C, Ar), 144.2 (C, Ar), 149.1 (C=N), 169.5 (S–C=N). HRMS (ESI): 437.9016 $[M-H]^+$. Anal. calcd for $C_{17}H_{12}BrCl_2N_3S$: C, 46.28; H, 2.74; N, 9.52; S, 7.27. Found: C, 46.29; H, 2.92; N, 8.55; S, 7.30.

4-(4-bromophenyl)-2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-5-methylthiazole (**23**). Recrystallization in hot ethanol afforded beige crystals, m.p.: 270 ºC; yield: 1.20 g (69%); R_f= 0.65 (toluol / ethyl acetate 6:4). IR (KBr): 2923 (CH), 1622 (C=N) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 2.31 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 7.56 (d, 1H, *J* 8.7 Hz, Ar), 7.64 (d, 2H, *J* 3.3 Hz, Ar), 7.67 (d, 2H, *J* 3.3 Hz, Ar), 7.76 (dd, 1H, *J* 8.7 and 1.5 Hz, Ar), 7.97 (d, 1H, *J* 1.5 Hz, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 12.1 (CH_3), 14.0 (CH_3), 118.4 (C5 thiazole), 120.8 (C, Ar), 125.9 (CH, Ar), 127.4 (CH, Ar), 130.2 (CH, Ar), 130.5 (CH, Ar), 131.3 (CH, Ar), 131.5 (CH, Ar), 133.7 (C, Ar), 138.2 (C, Ar), 144.2 (C, Ar), 149.1 (C=N), 165.5 (S–C=N). . Anal. calcd for $C_{18}H_{14}BrCl_2N_3S$: C, 47.49; H, 3.10; N, 9.23; S, 7.04. Found: C, 47.52; H, 3.02; N, 9.83; S, 7.05.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-(4-nitrophenyl)thiazole (**24**). Recrystallization in hot ethanol afforded orange crystals, m.p.: 226-228 ºC; yield: 1.11 g

(71%); R_f= 0.61 (toluol / ethyl acetate 6:4). IR (KBr): 3304 (NH), 1597 and 1565 (C=N) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 2.32 (s, 3H, CH_3), 7.69 (d, 1H, *J* 8.7 Hz, Ar), 7.70 (s, 1H, CH thiazole), 7.76 (dd, 1H, *J* 8.7 and 2.1 Hz, Ar), 7.95 (d, 1H, *J* 2.1 Hz, Ar), 8.13 (d, 2H, *J* 8.7 Hz, Ar), 8.29 (d, 2H, *J* 8.7 Hz, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 16.2 (CH_3), 106.5 (CH thiazole), 121.4 (CH, Ar), 123.4 (CH, Ar), 127.0 (CH, Ar), 128.6 (CH, Ar), 131.6 (CH, Ar), 132.7 (C, Ar), 132.9 (C, Ar), 137.6 (C, Ar), 139.8 (C, Ar), 144.5 (C, Ar), 148.6 (C, Ar), 158.6 (C=N), 171.1 (S–C=N). HRMS (ESI): 404.9496 $[M-H]^+$. Anal. calcd for $C_{17}H_{12}Cl_2N_4O_2S$: C, 50.13; H, 2.97; N, 13.76; S, 7.87. Found: C, 50.12; H, 3.00; N, 13.69; S, 8.07.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-(3-nitrophenyl)thiazole (**25**). Recrystallization in hot ethanol afforded orange crystals, m.p.: 234-236 ºC; yield: 1.30 g (83%); R_f= 0.63 (toluol / ethyl acetate 6:4). IR (KBr): 3089 (C-H), 1614 and 1565 (C=N) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 2.32 (s, 3H, CH_3), 7.64 (d, 1H, *J* 8.4 Hz, Ar), 7.67 (s, 1H, CH thiazole), 7.69-7.74 (m, 2H, Ar), 7.91 (d, 1H, *J* 2.1 Hz, Ar), 8.13 (dd, 1H, *J* 8.4 and 2.1 Hz, Ar), 8.30 (d, 1H, *J* 7.8 Hz, Ar), 8.69 (s, 1H, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 14.2 (CH_3), 107.5 (CH thiazole), 120.4 (CH, Ar), 122.4 (CH, Ar), 126.0 (CH, Ar), 127.6 (CH, Ar), 130.6 (CH, Ar), 130.9 (CH, Ar), 131.5 (CH, Ar), 131.7 (C, Ar), 131.9 (C, Ar), 136.6 (C, Ar), 138.8 (C, Ar), 144.5 (C, Ar), 148.6 (C=N), 170.1 (S–C=N). HRMS (ESI): 406.9958 $[M+H]^+$. Anal. calcd for $C_{17}H_{12}Cl_2N_4O_2S$: C, 50.13; H, 2.97; N, 13.76; S, 7.87. Found: C, 50.33; H, 2.87; N, 13.77; S, 7.88.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-(4-methoxyphenyl)thiazole (**26**). Recrystallization in hot ethanol afforded yellow crystals, m.p.: 216-218 ºC; yield: 1.22 g (81%); R_f= 0.6 (toluol / ethyl acetate 6:4). IR (KBr): 2914 (C-H), 1611 (C=N) cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 2.31 (s, 3H, CH_3), 3.77 (s, 3H, O–CH_3), 6.97 (d, 2H, *J* 8.7 Hz, Ar), 7.17 (s, 1H, CH thiazole), 7.66 (d, 1H, *J* 8.4 Hz, Ar), 7.75 (d, 2H, *J* 8.7 Hz, Ar), 7.78 (d, 1H, *J* 8.4 Hz, Ar), 7.95 (s, 1H, Ar), 10.29 (s, 1H, NH). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 13.8

(CH_3), 55.1 (O–CH_3), 102.2 (CH thiazole), 114.0 (CH, Ar), 125.7 (CH, Ar), 126.9 (CH, Ar), 127.2 (CH, Ar), 130.6 (CH, Ar), 131.1 (C, Ar), 131.3 (C, Ar), 138.4 (C, Ar), 144.4 (C, Ar), 149.5 (C, Ar), 158.9 (C=N), 169.3 (S–C=N). HRMS (ESI): 392.0213 $[M+H]^+$. Anal. calcd for $C_{18}H_{15}Cl_2N_3OS$: C, 55.11; H, 3.85; N, 10.71; S, 8.17. Found: C, 55.09; H, 3.62; N, 10.50; S, 8.23.

4-([1,1'-biphenyl]-4-yl)-2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]thiazole (**27**). Recrystallization in hot toluol afforded beige crystals, m.p.: 269-271 ºC; yield: 1.15 g (61%); R_f= 0.78 (toluol / ethyl acetate 6:4). IR (KBr): 2925 (C-H), 1608 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.32 (s, 3H, CH_3), 7.33-7.48 (m, 5H, Ar), 7.66-7.78 (m, 6H, Ar), 7.93-7.96 (m, 3H, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 15.0 (CH_3), 104.7 (CH thiazole), 125.8 (CH, Ar), 126.2 (CH, Ar), 126.5 (CH, Ar), 126.9 (CH, Ar), 127.3 (CH, Ar), 127.5 (CH, Ar), 128.8 (CH, Ar), 129.0 (CH, Ar), 130.6 (C, Ar), 131.2 (C, Ar), 131.3 (C, Ar), 133.2 (C, Ar), 138.4 (C, Ar), 139.2 (C, Ar), 139.6 (C, Ar), 150.0 (C=N), 169.4 (S–C=N). . Anal. calcd for $C_{23}H_{17}Cl_2N_3S$: C, 63.02; H, 3.91; N, 9.59; S, 7.31. Found: C, 63.08; H, 3.98; N, 9.55; S, 7.21.

2-[1-(3,4-dichlorophenyl)ethylidene-hydrazinyl]-4-(naphthalen-2-yl)thiazole (**28**). Recrystallization in hot toluol afforded beige crystals, m.p.: 259-260 ºC; yield: 1.14 g (72%); R_f= 0.68 (toluol / ethyl acetate 6:4). IR (KBr): 3047 (C-H), 1621 (C=N) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.32 (s, 3H, CH_3), 7.49 (m, 3H, Ar), 7.62-7.75 (m, 2H, Ar), 7.88-8.00 (m, 5H, Ar), 8.38 (s, 1H, CH, Ar). ^{13}C NMR (75.5 MHz, DMSO-d_6): δ 13.9 (CH_3), 105.3 (CH thiazole), 123.9 (CH, Ar), 124.2 (CH, Ar), 125.7 (CH, Ar), 126.1 (CH, Ar), 126.5 (CH, Ar), 127.3 (CH, Ar), 127.6 (CH, Ar), 128.1 (CH, Ar), 130.6 (C, Ar), 131.1 (C, Ar), 131.3 (C, Ar), 131.6 (C, Ar), 132.4 (C. Ar), 133.1 (C, Ar), 138.4 (C, Ar), 144.6 (C, Ar), 149.7 (C=N), 169.5 (S–C=N). HRMS (ESI): 411.9917 $[M+H]^+$. Anal. calcd for $C_{21}H_{15}Cl_2N_3S$: C, 61.17; H, 3.67; N, 10.19; S, 7.78. Found: C, 61.18; H, 3.94; N, 10.21; S, 7.99.

Cruzain inhibition: Cruzain activity was measured by monitoring the cleavage of the fluorogenic substrate Z-FR-AMC, as previously described [50]. Assays were performed in 0.1 M sodium acetate buffer pH 5.5, in the presence of 1 mM beta-mercaptoethanol and 0.01 % Triton X-100, to a final volume of 200 μL and at 25 ^{o}C. The final concentrations of cruzain was 0.5 nM, and the Z-FR-AMC substrate concentration was 2.5 μM (K_m = 1 μM). In all assays, the enzyme was pre-incubated with the compounds for 10 min before adding a solution containing the Z-FR-AMC substrate. Enzyme kinetic was followed by continuous reading for 5 min at 12s intervals, employing a Synergy 2 (Biotek) from the Center of Flow Cytometry and Fluorimetry at the Biochemistry and Immunology Department (UFMG). The filters employed were 340 nM for excitation and 440 nM for emission. Activity was calculated based on initial velocity rates, compared to a DMSO control, since all compound stocks were prepared in DMSO. All compounds were evaluated at 10 μM and inhibition was measured in at least two independent experiments, in each case in triplicate. IC_{50} curves were determined based on at least seven compound concentrations, employing the nonlinear regression analysis of "log (inhibitor) vs response with variable slope – four parameters" in the software GraphPad Prism 5.0. The reported values refer to the average and standard deviation between the values obtained for at least two curves, in which each concentration was evaluate in triplicate.

Animals: Female BALB/c mice (6–8 weeks old) were supplied by the animal breeding facility at Centro de Pesquisas Gonçalo Moniz (Fundação Oswaldo Cruz, Bahia, Brazil) and maintained in sterilized cages under a controlled environment, receiving a balanced diet for rodents and water *ad libitum*. All experiments were carried out in accordance with the recommendations of Ethical Issues Guidelines, and were approved by the local Animal Ethics Committee (Approved number: L-IGM-016/13).

Parasites: Bloodstream trypomastigotes forms of *T. cruzi* were obtained from supernatants of LLC-MK2 cells previously infected and maintained in Dulbecco's modified Eagle medium (DMEM; Life Technologies, GIBCO-BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS; GIBCO), and 50 µg/mL of gentamycin (Novafarma, Anápolis, GO, Brazil) at 37 °C and 5% CO_2.

Host cell toxicity: Cytotoxicity of the compounds was determined initially in the rat cardiomyoblast cell line H9c2 and the murine macrophage cell line J774. Cells were seeded into 96-well plates at a cell density of 1 x 10^4 cells/well in Dulbecco's modified Eagle medium supplemented with 10% FBS, and 50 µg/mL of gentamycin and incubated for 24 h at 37°C and 5% CO_2. After that time each compound, dissolved in DMSO was added at six concentrations (0.41 to 100 µM) in triplicate and incubated for 72 h. Cell viability was determined by AlamarBlue assay (Invitrogen, Carlsbad, CA, USA) according to the manufacturer instructions. Colorimetric readings were performed after 6 h at 570 and 600 nm. Cytotoxic concentration to 50% (CC_{50}) was calculated using data-points gathered from three independent experiments. Gentian violet (Synth, São Paulo, Brazil) was used as positive control. The final concentration of DMSO was less than 1% in all *in vitro* experiments.

Toxicity for Y strain trypomastigotes: trypomastigotes collected from the supernatant of LLC-MK2 cells were dispensed into 96-well plates at a cell density of $4x10^5$ cells/well. Test inhibitors, were diluted into five different concentrations and added into their respective wells, and the plate was incubated for 24h at 37 º C and 5% of CO_2. Aliquots of each well were collected and the number of viable parasites, based on parasite motility, was assessed in a Neubauer chamber. The percentage of inhibition was calculated in relation to untreated cultures. IC_{50} calculation was also carried out using non-linear regression with Prism 4.0 GraphPad software. Benznidazole was used as the reference drug.

Propidium iodide and annexin V staining: Trypomastigotes 1 x 10^7/mL in 24 well-plates were treated with **14 (**0.4 μM**)** in DMEM supplemented with FBS at 37°C for 24 h and labeled for propidium iodide (PI) and annexin V using the annexin V-FITC apoptosis detection kit (Sigma), according to the manufacturer's instructions. Acquisition and analyses was performed using a FACS Calibur flow cytometer (Becton Dickinson, San Diego, CA), with FlowJo software (Tree Star, Ashland, OR). A total of 10,000 events were acquired in the region previously established as that corresponding to trypomastigotes forms of *T. cruzi*.

Rhodamine 123 staining: Trypomastigotes 1 x 10^7/mL in 24 well-plates were treated or not with **14 (**0.4 or 0.8 μM**)** in DMEM supplemented with FBS at 37°C for 24 h. Then, cells were washed twice with PBS solution and resuspended in 0.5 mL PBS containing 5 μg/mL of Rhodamine 123 (Sigma) for 15 minutes. After staining, the parasites were washed twice with PBS solution and immediately analyzed using a FACS Calibur flow cytometer (Becton Dickinson, San Diego, CA), with FlowJo software (Tree Star, Ashland, OR). A total of 10,000 events were acquired in the region previously established as that corresponding to trypomastigotes forms of *T. cruzi*.

Intracellular parasite development: Peritoneal exudate macrophages ($5x10^4$ cells/well) obtained from BALB/c mice were seeded in a 96 well-plate in DMEM supplemented with 10% FBS and incubated for 24 h. Cells were then infected with trypomastigotes (10:1) for 2 h. Free trypomastigotes were removed by successive washes using saline solution and the cells were incubated for 24 h to allow full internalization and differentiation of trypomastigotes to amastigotes. Next, cultures were incubated in complete medium alone or with the thiazole **14** (8, 2 and 0.5 μM) or benznidazole (5 μM) for 72 h. Cells were fixed in formaldehyde 4% and stained with hoeschst (100 μg/mL; Invtrogen, Carlssbad, CA). Plates were then imaged and anaylzed in a Perkin-Elmer Opera confocal microscope to quantifiy the number of infected cells and the number of amastigotes per 100 cells, as previously described

[51]. The one-way ANOVA and Bonferroni for multiple comparisons were used to determine the statistical significance of the group comparisons.

Drug combination: For *in vitro* drug combinations**,** doubling dilutions of each drug (**14** and benzinidazole), used alone or in fixed combinations were incubated with trypomastigotes followed the protocol described above. The analysis of the combined effects was performed by determining the combination index (CI), used as cutoff to determine synergism, by using Chou-Talalay CI method [46] and through the construction of isobologram using the fixed ratio method, as described previously [52].

Infection in mice

Female BALB/c mice (18-22 g) were infected with bloodstream trypomastigotes by intraperitoneal inoculation of 10^4 parasites in 100 µL of saline solution and then mice were randomly divided in three groups (six animals per group). After the day 5 of infection, treatment with 25 mg/kg weight of **14** was given orally for five consecutive days. For the control group, benznidazole was given orally at dose of 100 mg/kg weight. Saline containing 20% DMSO was used as a vehicle and administrated on untreated and infected group. Animal infection was monitored daily by counting the number of motile parasites in 5 µL of fresh blood sample drawn from the lateral tail veins, as recommended by standard protocol [53]. Survival was monitored for 30 days after treatment. The one-way ANOVA and Bonferroni for multiple comparisons were used to determine the statistical significance of the group comparisons.

Acknowledgement

This work received support from CNPq (grant reference 471461/2011-3 to A.C.L.L.), FAPESB (PRONEX program, grant reference 6596 to M.B.P.S.), FAPEMIG and CAPES. G.B.O.F. received a FACEPE Masters scholarship. A.C.L.L., M.B.P.S., R.S.F. and D.R.M.M.

hold a CNPq fellowship. We are thankful to the Center of Flow Cytometry and Fluorimetry at the Biochemistry and Immunology Department (UFMG) for access to the fluorimeter. We also thank the Department of Fundamental Chemistry - Federal University of Pernambuco (DQF-UFPE) and Center for Strategic Technologies of the Northeast (CETENE) for recording the ^{1}H NMR, ^{13}C NMR, LCMS and IR spectra of all compounds.

References

[1] J. Bermudez, C. Davies, A. Simonazzi, J.P. Real, S. Palma, J. Pablo Real, S. Palma, Current drug therapy and pharmaceutical challenges for Chagas disease, Acta Trop. 156 (2016) 1–16. doi:10.1016/j.actatropica.2015.12.017.

[2] DNDI, About Chagas Disease – DNDi, (2017). https://www.dndi.org/diseases-projects/chagas/ (accessed May 22, 2017).

[3] WHO, WHO | Chagas disease (American trypanosomiasis), WHO. (2014). http://www.who.int/mediacentre/factsheets/fs340/en/ (accessed November 18, 2015).

[4] I. Molina, F. Salvador, A. Sánchez-Montalvá, Actualización en enfermedad de Chagas, Enferm. Infecc. Microbiol. Clin. 34 (2016) 132–138. doi:10.1016/j.eimc.2015.12.008.

[5] J.A. Urbina, R. Docampo, Specific chemotherapy of Chagas disease: controversies and advances, Trends Parasitol. 19 (2003) 495–501. doi:10.1016/j.pt.2003.09.001.

[6] J.H. McKerrow, P.S. Doyle, J.C. Engel, L.M. Podust, S.A. Robertson, R. Ferreira, T. Saxton, M. Arkin, I.D. Kerr, L.S. Brinen, C.S. Craik, Two approaches to discovering and developing new drugs for Chagas disease., Mem. Inst. Oswaldo Cruz. 104 Suppl (2009) 263–9. http://www.ncbi.nlm.nih.gov/pubmed/19753483.

[7] J. Clayton, Chagas disease: pushing through the pipeline., Nature. 465 (2010) S12-5. doi:10.1038/nature09224.

[8] E. Chatelain, Chagas Disease Drug Discovery: Toward a New Era, J Biomol Screen. 20 (2015) 22–35. doi:10.1177/1087057114550585.

[9] J.A. Urbina, Specific chemotherapy of Chagas disease: Relevance, current limitations and new approaches, Acta Trop. 115 (2010) 55–68. doi:10.1016/j.actatropica.2009.10.023.

[10] N. Shang, Q. Li, T.-P. Ko, H.-C. Chan, J. Li, Y. Zheng, C.-H. Huang, F. Ren, C.-C. Chen, Z. Zhu, M. Galizzi, Z.-H. Li, C.A. Rodrigues-Poveda, D. Gonzalez-Pacanowska,

P. Veiga-Santos, T.M.U. de Carvalho, W. de Souza, J.A. Urbina, A.H.-J. Wang, R. Docampo, K. Li, Y.-L. Liu, E. Oldfield, R.-T. Guo, Squalene Synthase As a Target for Chagas Disease Therapeutics, PLoS Pathog. 10 (2014) e1004114. doi:10.1371/journal.ppat.1004114.

[11] R.J. Neitz, C. Bryant, S. Chen, J. Gut, E. Hugo Caselli, S. Ponce, S. Chowdhury, H. Xu, M.R. Arkin, J.A. Ellman, A.R. Renslo, Tetrafluorophenoxymethyl ketone cruzain inhibitors with improved pharmacokinetic properties as therapeutic leads for Chagas' disease, Bioorg. Med. Chem. Lett. 25 (2015) 4834–4837. doi:10.1016/j.bmcl.2015.06.066.

[12] M.L. Sbaraglini, C.L. Bellera, L. Fraccaroli, L. Larocca, C. Carrillo, A. Talevi, C.D. Alba Soto, Novel cruzipain inhibitors for the chemotherapy of chronic Chagas disease, Int. J. Antimicrob. Agents. 48 (2016) 91–95. doi:10.1016/j.ijantimicag.2016.02.018.

[13] X. Du, E. Hansell, J.C. Engel, C.R. Caffrey, F.E. Cohen, J.H. McKerrow, Aryl ureas represent a new class of anti-trypanosomal agents, Chem. Biol. 7 (2000) 733–742. doi:10.1016/S1074-5521(00)00018-1.

[14] H.J. Wiggers, J.R. Rocha, W.B. Fernandes, R. Sesti-Costa, Z.A. Carneiro, J. Cheleski, A.B.F. da Silva, L. Juliano, M.H.S. Cezari, J.S. Silva, J.H. McKerrow, C.A. Montanari, Non-peptidic Cruzain Inhibitors with Trypanocidal Activity Discovered by Virtual Screening and In Vitro Assay, PLoS Negl. Trop. Dis. 7 (2013) e2370. doi:10.1371/journal.pntd.0002370.

[15] J.M. dos Santos Filho, A.C.L. Leite, B.G. de Oliveira, D.R.M. Moreira, M.S. Lima, M.B.P. Soares, L.F.C.C. Leite, Design, synthesis and cruzain docking of 3-(4-substituted-aryl)-1,2,4-oxadiazole-N-acylhydrazones as anti-Trypanosoma cruzi agents, Bioorg. Med. Chem. 17 (2009) 6682–6691. doi:10.1016/j.bmc.2009.07.068.

[16] N.C. Romeiro, G. Aguirre, P. Hernández, M. González, H. Cerecetto, I. Aldana, S.

Pérez-Silanes, A. Monge, E.J. Barreiro, L.M. Lima, Synthesis, trypanocidal activity and docking studies of novel quinoxaline-N-acylhydrazones, designed as cruzain inhibitors candidates, Bioorg. Med. Chem. 17 (2009) 641–652. doi:10.1016/j.bmc.2008.11.065.

[17] T. Franklim, L. Freire-de-Lima, J. de Nazareth Sá Diniz, J. Previato, R. Castro, L. Mendonça-Previato, M. de Lima, Design, Synthesis and Trypanocidal Evaluation of Novel 1,2,4-Triazoles-3-thiones Derived from Natural Piperine, Molecules. 18 (2013) 6366–6382. doi:10.3390/molecules18066366.

[18] K. Brak, P.S. Doyle, J.H. McKerrow, J.A. Ellman, Identification of a New Class of Nonpeptidic Inhibitors of Cruzain, J. Am. Chem. Soc. 130 (2008) 6404–6410. doi:10.1021/ja710254m.

[19] K. Brak, I.D. Kerr, K.T. Barrett, N. Fuchi, M. Debnath, K. Ang, J.C. Engel, J.H. McKerrow, P.S. Doyle, L.S. Brinen, J.A. Ellman, Nonpeptidic Tetrafluorophenoxymethyl Ketone Cruzain Inhibitors as Promising New Leads for Chagas Disease Chemotherapy, J. Med. Chem. 53 (2010) 1763–1773. doi:10.1021/jm901633v.

[20] X. Du, C. Guo, E. Hansell, P.S. Doyle, C.R. Caffrey, T.P. Holler, J.H. McKerrow, F.E. Cohen, Synthesis and Structure−Activity Relationship Study of Potent Trypanocidal Thio Semicarbazone Inhibitors of the Trypanosomal Cysteine Protease Cruzain, J. Med. Chem. 45 (2002) 2695–2707. doi:10.1021/jm010459j.

[21] D.C. Greenbaum, Z. Mackey, E. Hansell, P. Doyle, J. Gut, C.R. Caffrey, J. Lehrman, P.J. Rosenthal, J.H. McKerrow, K. Chibale, Synthesis and Structure−Activity Relationships of Parasiticidal Thiosemicarbazone Cysteine Protease Inhibitors against Plasmodium falciparum , Trypanosoma brucei , and Trypanosoma cruzi, J. Med. Chem. 47 (2004) 3212–3219. doi:10.1021/jm030549j.

[22] R. Siles, S.-E. Chen, M. Zhou, K.G. Pinney, M.L. Trawick, Design, synthesis, and biochemical evaluation of novel cruzain inhibitors with potential application in the treatment of Chagas' disease, Bioorg. Med. Chem. Lett. 16 (2006) 4405–4409. doi:10.1016/j.bmcl.2006.05.041.

[23] N. Beatriz Huaman Lozano, V. Goncalves Maltarollo, K. Cacilda Weber, K. Maria Honorio, R. Victorio Carvalho Guido, A. Defini Andricopulo, A. Borges Ferreira Da Silva, Molecular Features for Antitrypanosomal Activity of Thiosemicarbazones Revealed by OPS-PLS QSAR Studies, Med. Chem. (Los. Angeles). 8 (2012) 1045–1056. doi:10.2174/157340612804075043.

[24] D.R. Magalhaes Moreira, A.D.T. de Oliveira, P.A. Teixeira de Moraes Gomes, C.A. de Simone, F.S. Villela, R.S. Ferreira, A.C. da Silva, T.A.R. dos Santos, M.C.A. Brelaz de Castro, V.R.A. Pereira, A.C.L. Leite, Conformational restriction of aryl thiosemicarbazones produces potent and selective anti-Trypanosoma cruzi compounds which induce apoptotic parasite death, Eur. J. Med. Chem. 75 (2014) 467–478. doi:10.1016/j.ejmech.2014.02.001.

[25] J.W.P. Espíndola, M.V. de O. Cardoso, G.B. de O. Filho, D.A. Oliveira e Silva, D.R.M. Moreira, T.M. Bastos, C.A. de Simone, M.B.P. Soares, F.S. Villela, R.S. Ferreira, M.C.A.B. de Castro, V.R.A. Pereira, S.M.F. Murta, P.A. Sales Junior, A.J. Romanha, A.C.L. Leite, Synthesis and structure–activity relationship study of a new series of antiparasitic aryloxyl thiosemicarbazones inhibiting Trypanosoma cruzi cruzain, Eur. J. Med. Chem. 101 (2015) 818–835. doi:10.1016/j.ejmech.2015.06.048.

[26] A.C.L. Leite, D.R. de M. Moreira, M. V. de O. Cardoso, M.Z. Hernandes, V.R. Alves Pereira, R.O. Silva, A.C. Kiperstok, M. da S. Lima, M.B.P. Soares, Synthesis, Cruzain Docking, and in vitro Studies of Aryl-4-Oxothiazolylhydrazones AgainstTrypanosoma cruzi, ChemMedChem. 2 (2007) 1339–1345. doi:10.1002/cmdc.200700022.

[27] M. Zaldini Hernandes, M. Montenegro Rabello, A. Cristina Lima Leite, M. Veríssimo Oliveira Cardoso, D. Rodrigo Magalhaes Moreira, D. José Brondani, C. Alberto Simone, L. Campos Reis, M. Assis Souza, V. Rego Alves Pereira, R. Salgado Ferreira, J. Hobson McKerrow, M.Z. Hernandes, M.M. Rabello, A.C.L. Leite, M.V.O. Cardoso, D.R.M. Moreira, D.J. Brondani, C.A. Simone, L.C. Reis, M.A. Souza, V.R.A. Pereira, Studies toward the structural optimization of novel thiazolylhydrazone-based potent antitrypanosomal agents, Bioorg. Med. Chem. 18 (2010) 7826–7835. doi:10.1016/j.bmc.2010.09.056.

[28] D.R.M. Moreira, S.P.M. Costa, M.Z. Hernandes, M.M. Rabello, G.B. de Oliveira Filho, C.M.L. de Melo, L.F. da Rocha, C.A. de Simone, R.S. Ferreira, J.R.B. Fradico, C.S. Meira, E.T. Guimarães, R.M. Srivastava, V.R.A. Pereira, M.B.P. Soares, A.C.L. Leite, Structural Investigation of Anti- Trypanosoma cruzi 2-Iminothiazolidin-4-ones Allows the Identification of Agents with Efficacy in Infected Mice, J. Med. Chem. 55 (2012) 10918–10936. doi:10.1021/jm301518v.

[29] M.V. de O. Cardoso, L.R.P. de Siqueira, E.B. da Silva, L.B. Costa, M.Z. Hernandes, M.M. Rabello, R.S. Ferreira, L.F. da Cruz, D.R. Magalhães Moreira, V.R.A. Pereira, M.C.A.B. de Castro, P. V. Bernhardt, A.C.L. Leite, 2-Pyridyl thiazoles as novel anti-Trypanosoma cruzi agents: Structural design, synthesis and pharmacological evaluation, Eur. J. Med. Chem. 86 (2014) 48–59. doi:10.1016/j.ejmech.2014.08.012.

[30] P.A.T. de Moraes Gomes, M. de Oliveira Barbosa, E. Farias Santiago, M.V. de Oliveira Cardoso, N.T. Capistrano Costa, M.Z. Hernandes, D.R.M. Moreira, A.C. da Silva, T.A.R. dos Santos, V.R.A. Pereira, F.A. Brayner dos Santosd, G.A. do Nascimento Pereira, R.S. Ferreira, A.C.L. Leite, New 1,3-thiazole derivatives and their biological and ultrastructural effects on Trypanosoma cruzi, Eur. J. Med. Chem. 121 (2016) 387–398. doi:10.1016/j.ejmech.2016.05.050.

[31] P.A.T. de M. Gomes, A.R. Oliveira, M.V. de O. Cardoso, E. de F. Santiago, M. de O. Barbosa, L.R.P. de Siqueira, D.R.M. Moreira, T.M. Bastos, F.A. Brayner, M.B.P. Soares, A.P. de O. Mendes, M.C.A.B. de Castro, V.R.A. Pereira, A.C.L. Leite, L. Siqueira, D.R.M. Moreira, T.M. Bastos, F.A. Brayner, M.B.P. Soares, A.P. de O. Mendes, M. Castro, V.R.A. Pereira, A.C.L. Leite, Phthalimido-thiazoles as building blocks and their effects on the growth and morphology of Trypanosoma cruzi, Eur. J. Med. Chem. 111 (2016) 46–57. doi:10.1016/j.ejmech.2016.01.010.

[32] G.B. de Oliveira Filho, M.V. de Oliveira Cardoso, J.W.P. Espíndola, L.F.G.R. Ferreira, C.A. de Simone, R.S. Ferreira, P.L. Coelho, C.S. Meira, D.R. Magalhaes Moreira, M.B.P. Soares, A.C. Lima Leite, Structural design, synthesis and pharmacological evaluation of 4-thiazolidinones against Trypanosoma cruzi, Bioorg. Med. Chem. 23 (2015) 7478–7486. doi:10.1016/j.bmc.2015.10.048.

[33] A.W. Erian, S.M. Sherif, H.M. Gaber, The Chemistry of ?-Haloketones and Their Utility in Heterocyclic Synthesis, Molecules. 8 (2003) 793–865. doi:10.3390/81100793.

[34] D.-N. Zhang, J.-T. Li, Y.-L. Song, H.-M. Liu, H.-Y. Li, Efficient one-pot three-component synthesis of N-(4-arylthiazol-2-yl) hydrazones in water under ultrasound irradiation., Ultrason. Sonochem. 19 (2012) 475–8. doi:10.1016/j.ultsonch.2011.10.017.

[35] P. Vicini, A. Geronikaki, K. Anastasia, M. Incerti, F. Zani, Synthesis and antimicrobial activity of novel 2-thiazolylimino-5-arylidene-4-thiazolidinones., Bioorg. Med. Chem. 14 (2006) 3859–64. doi:10.1016/j.bmc.2006.01.043.

[36] C. Pizzo, C. Saiz, A. Talevi, L. Gavernet, P. Palestro, C. Bellera, L.B. Blanch, D. Benítez, J.J. Cazzulo, A. Chidichimo, P. Wipf, S.G. Mahler, Synthesis of 2-hydrazolyl-4-thiazolidinones based on multicomponent reactions and biological evaluation against

Trypanosoma Cruzi., Chem. Biol. Drug Des. 77 (2011) 166–72. doi:10.1111/j.1747-0285.2010.01071.x.

[37] A.C.L. Leite, R.S.R.S. de Lima, D.R. de M. Moreira, M.V. de O. Cardoso, A.C. Gouveia de Brito, L.M. Farias dos Santos, M.Z. Hernandes, A.C. Kiperstok, R.S.R.S. de Lima, M.B.P.P. Soares, Synthesis, docking, and in vitro activity of thiosemicarbazones, aminoacyl-thiosemicarbazides and acyl-thiazolidones against Trypanosoma cruzi, Bioorg. Med. Chem. 14 (2006) 3749–3757. doi:10.1016/j.bmc.2006.01.034.

[38] D.R.M. Moreira, A.C. Lima Leite, M.V.O. Cardoso, R.M. Srivastava, M.Z. Hernandes, M.M. Rabello, L.F. Da Cruz, R.S. Ferreira, C.A. De Simone, C.S. Meira, E.T. Guimaraes, A.C. Da Silva, T.A.R. Dos Santos, V.R.A. Pereira, M.B. Pereira Soares, Structural design, synthesis and structure-activity relationships of thiazolidinones with enhanced anti-Trypanosoma cruzi activity, ChemMedChem. 9 (2014) 177–188. doi:10.1002/cmdc.201300354.

[39] M.E. Caputto, A. Ciccarelli, F. Frank, A.G. Moglioni, G.Y. Moltrasio, D. Vega, E. Lombardo, L.M. Finkielsztein, Synthesis and biological evaluation of some novel 1-indanone thiazolylhydrazone derivatives as anti-Trypanosoma cruzi agents, Eur. J. Med. Chem. 55 (2012) 155–163. doi:10.1016/j.ejmech.2012.07.013.

[40] E.B. da Silva, D.A. Oliveira e Silva, A.R. Oliveira, C.H. da Silva Mendes, T.A.R. dos Santos, A.C. da Silva, M.C.A. de Castro, R.S. Ferreira, D.R.M. Moreira, M.V. de O. Cardoso, C.A. de Simone, V.R.A. Pereira, A.C.L. Leite, Desing and synthesis of potent anti - Trypanosoma cruzi agents new thiazoles derivatives which induce apoptotic parasite death, Eur. J. Med. Chem. 130 (2017) 39–50. doi:10.1016/j.ejmech.2017.02.026.

[41] R.S. Ferreira, C. Bryant, K.K.H. Ang, J.H. McKerrow, B.K. Shoichet, A.R. Renslo,

Divergent modes of enzyme inhibition in a homologous structure-activity series., J. Med. Chem. 52 (2009) 5005–8. doi:10.1021/jm9009229.

[42] J.M. dos Santos Filho, D.R.M. Moreira, C.A. de Simone, R.S. Ferreira, J.H. McKerrow, C.S. Meira, E.T. Guimarães, M.B.P. Soares, Optimization of anti-Trypanosoma cruzi oxadiazoles leads to identification of compounds with efficacy in infected mice, Bioorg. Med. Chem. 20 (2012) 6423–6433. doi:10.1016/j.bmc.2012.08.047.

[43] A.L. Matsuo, L.S. Silva, A.C. Torrecilhas, B.S. Pascoalino, T.C. Ramos, E.G. Rodrigues, S. Schenkman, A.C.F. Caires, L.R. Travassos, In vitro and in vivo trypanocidal effects of the cyclopalladated compound 7a, a drug candidate for treatment of Chagas' disease., Antimicrob. Agents Chemother. 54 (2010) 3318–25. doi:10.1128/AAC.00323-10.

[44] L. Vivas, L. Rattray, L. Stewart, E. Bongard, B.L. Robinson, W. Peters, S.L. Croft, Anti-malarial efficacy of pyronaridine and artesunate in combination in vitro and in vivo., Acta Trop. 105 (2008) 222–8. doi:10.1016/j.actatropica.2007.12.005.

[45] L. de F. Diniz, J.A. Urbina, I.M. de Andrade, A.L. Mazzeti, T.A.F. Martins, I.S. Caldas, A. Talvani, I. Ribeiro, M.T. Bahia, Benznidazole and posaconazole in experimental Chagas disease: positive interaction in concomitant and sequential treatments., PLoS Negl. Trop. Dis. 7 (2013) e2367. doi:10.1371/journal.pntd.0002367.

[46] T.C. Chou, P. Talalay, Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors., Adv. Enzyme Regul. 22 (1984) 27–55. http://www.ncbi.nlm.nih.gov/pubmed/6382953.

[47] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 23 (1997) 3–25. doi:10.1016/S0169-

409X(96)00423-1.

[48] N.A. Meanwell, Improving Drug Candidates by Design: A Focus on Physicochemical Properties As a Means of Improving Compound Disposition and Safety, Chem. Res. Toxicol. 24 (2011) 1420–1456. doi:10.1021/tx200211v.

[49] D.F. Veber, S.R. Johnson, H.-Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, Molecular properties that influence the oral bioavailability of drug candidates., J. Med. Chem. 45 (2002) 2615–23. http://www.ncbi.nlm.nih.gov/pubmed/12036371.

[50] N.C. Fonseca, L.F. da Cruz, F. da Silva Villela, G.A. do Nascimento Pereira, J.L. de Siqueira-Neto, D. Kellar, B.M. Suzuki, D. Ray, T.B. de Souza, R.J. Alves, P.A.S. J?nior, A.J. Romanha, S.M.F. Murta, J.H. McKerrow, C.R. Caffrey, R.B. de Oliveira, R.S. Ferreira, Synthesis of a Sugar-Based Thiosemicarbazone Series and Structure-Activity Relationship versus the Parasite Cysteine Proteases Rhodesain, Cruzain, and Schistosoma mansoni Cathepsin B1, Antimicrob. Agents Chemother. 59 (2015) 2666–2677. doi:10.1128/AAC.04601-14.

[51] J. Alonso-Padilla, I. Cotillo, J.L. Presa, J. Cantizani, I. Peña, A.I. Bardera, J.J. Martín, A. Rodriguez, Automated High-Content Assay for Compounds Selectively Toxic to Trypanosoma cruzi in a Myoblastic Cell Line, PLoS Negl. Trop. Dis. 9 (2015) e0003493. doi:10.1371/journal.pntd.0003493.

[52] Q.L. Fivelman, I.S. Adagu, D.C. Warhurst, Modified fixed-ratio isobologram method for studying in vitro interactions between atovaquone and proguanil or dihydroartemisinin against drug-resistant strains of Plasmodium falciparum., Antimicrob. Agents Chemother. 48 (2004) 4097–102. doi:10.1128/AAC.48.11.4097-4102.2004.

[53] Z. BRENER, Therapeutic activity and criterion of cure on mice experimentally infected with Trypanosoma cruzi., Rev. Inst. Med. Trop. Sao Paulo. 4 (n.d.) 389–96.

Highlights

- Twenty two novel thiazoles were synthesized.
- A biological study was carried against *Trypanosoma cruzi*.
- Most of target compounds are selective and inhibitors of *T. cruzi*.
- The novel hybrids lack of cytotoxicity and display a good drug-likeness.
- Thiazole 14 showed reduction of parasitemia in vitro and in vivo.