Research paper

New 1,3-thiazole derivatives and their biological and ultrastructural effects on *Trypanosoma cruzi*

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ABSTRACT

In previous studies, the compound 3-(bromopropiophenone) thiosemicarbazone was described as a potent anti-*Trypanosoma cruzi* and cruzain inhibitor. In view to optimize this activity, 1,3-thiazole core was used as building-block strategy to access new lead generation of anti *T. cruzi* agents. In this way a series of thiazole derivatives were synthesized and most of these derivatives exhibited antiparasitic activity similar to benznidazole (Bzd). Among them, compounds (**1c**) and (**1g**) presented better selective index (SI) than Bzd. In addition, compounds showed inhibitory activity against the cruzain protease. As observed by electron microscopy, compound (**1c**) treatment caused irreversible and specific morphological changes on ultrastructure organization of *T. cruzi*, demonstrating that this class of compounds is killing parasites.

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1. Introduction

Parasitic diseases continue to take an enormous toll on human health, particularly in tropical regions [1]. Chagas disease, caused by *Trypanosoma cruzi*, represents a serious and alarming health problem [2]. It is considered to be one of the most concerning infectious tropical disease in Latin America [3].

Currently, the only drug in use is nitroheterocyclic benznidazole, which is effective in curing the disease in the acute phase, but is less effective in patients that progressed to the chronic phase [4,5].

Furthermore, benznidazole is less than ideal due to the fact that it causes severe side effects, leading to treatment interruption in a large number of patients [6].

Trypanosomes contain an abundance of cysteine proteases (CPs) which are members of the papain superfamily [7]. The cysteine peptidases present stage-regulated levels, are important virulence factors, modulate mammalian host immune cells and facilitate tissue host invasion, what makes these proteases attractive potential targets for chemotherapy [8]. Cruzain is the major *Trypanosoma cruzi* cysteine protease and it has been identified as a crucial enzyme responsible for parasite invasion, differentiation and proliferation in host cells [10]. Among its functions, cruzain induces the production of the proinflammatory peptide Lys bradikinin directly by proteolysis of kininogen or by activation of

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plasmatic pre-kallikrein [9], therefore contributing to the outcome of the infection [11].

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 [9,10], hydrazones [11–14], triazoles [15,16] and thiosemicarbazones [17–19].

Thiosemicarbazones have been largely investigated as anti-*T. cruzi* agents [17,18,20–24]. Originally, thiosemicarbazones were developed as potential inhibitors of cathepsin-L inhibitors, one of the main proteases involved in cancer development [17]. However, based on the homology and similar biochemical properties between cathepsin-L and cruzain, thiosemicarbazones were investigated as a potential class of cruzain inhibitors. Later on, aryl thiosemicarbazones were found to be a class of anti-*T. cruzi* compounds that inhibits cruzain activity [18,19].

Du and co-workers [17] thus identified twelve potent cruzain inhibitors that presented IC₅₀ values below 200 nM. Of the active aryl-thiosemicarbazones, the target compound 3-(bromopropiophenone) thiosemicarbazone (**1**) was able to inhibit cruzain at a concentration of 100 nM in a time-dependent manner, evidencing the reversible inactivation on the enzyme. Remarkable differences in potency were observed across the congeneric series, probably reflecting the importance of the steric factor in binding to the enzyme. Interestingly, several pyrazoline derivatives seem to be more conformational restricted analogues than aryl-thiosemicarbazones, and have been shown to be as potent as aryl-thiosemicarbazones against cruzain and to act in a similar fashion against the parasite *in vitro*, being a typical example of a classic bioisosteric relationship between cyclic and non-cyclic scaffolds [25]. This successful example of cruzain inhibitor has led to increased efforts to find new compounds structurally related to **1**.

1,3-Thiazole, the cyclic analogue of thiosemicarbazone, is one of the most important scaffolds in heterocyclic chemistry and drug design and discovery. It is widely found in diverse pharmacologically active substances and in some naturally occurring compounds [26]. Thiazole is a versatile building-block for lead generation, and it allows easy access of diverse derivatives for subsequent lead optimization [26]. In recent years, many thiazole derivatives have been synthesized and subjected to varied biological activities [26]. Our efforts toward new antiparasitic drug since 2006 have led us to a variety of thiosemicarbazones and thiazolyl hydrazones as trypanocidal agents [27–31].

Promising results achieved by compounds bearing a 1,3-thiazole ring motivated us to investigate the trypanocidal activity of novel thiazolyl hydrazones derived from 3-(bromopropiophenone) thiosemicarbazone (**1**) firstly identified by Du and co-workers [17]. Thus, in this work, the thiosemicarbazone **1** was converted into a set of 4-phenyl-thiazolyl hydrazine, and the effect of antiparasitic activity of different substituents attached at C4 of 1,3-thiazole was investigated. We also studied the influence of a methyl group at the C5 carbon of the thiazole ring, both in the presence or absence of a substituent at the *para* position of the aromatic ring attached to the 1,3-thiazole and the effect of a methyl or phenyl group at N3 of 1,3-thiazole ring on the antiparasitic activity. (Fig. 1).

2. Results and discussion

2.1. Chemistry

The following thiosemicarbazones 3-(bromopropiophenone)-thiosemicarbazone (**1**), 2-[1-(3-bromophenyl)propylidene]-N-methylhydrazinecarbothioamide (**2**) and 2-[1-(3-bromophenyl)propylidene]-N-phenylhydrazinecarbothioamide (**3**) were prepared by reacting commercially available 3'-bromophenyl-1-propanone with corresponding thiosemicarbazide, under reflux in

the presence of catalytic HCl. These intermediate compounds then react with different α -halogenated ketones, obtaining the **1a-3c** series with yields of 57–72% (Scheme 1). All compounds were identified by infrared (IR) and nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectroscopy, mass spectra (ESI-TOF) and their purity was established by elemental analysis (EA).

NMR data are compatible with the proposed compounds. In theory, two geometrical isomers (*E* and *Z*) about the imine (C=N) double bond are possible for the thiosemicarbazones and the respective thiazoles. However, analysis of the ¹H NMR spectra of the compounds indicated one predominant isomer; the *E* isomer by comparison with known analogues [32]. Intramolecular H-bonding involving the proton attached to N4 (in DMSO) with the imine N-atom leads to a distinctive singlet around 10.2 ppm [32] and this is also seen here.

Once thiosemicarbazones were characterized, the respective 1,3-thiazoles were characterized by usual spectroscopy. As exemplified with the ¹H NMR analysis of (*E*)-4-(biphenyl-4-yl)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}thiazole (**1g**), the triplet peak at δ 1.29 and the quartet peak at δ 2.92 correspond to the ethyl group. For the aromatic protons, singlet, doublets or triplets peaks were observed at δ 7.19–7.87. For the thiazole ring, a singlet at δ 6.76 was found. NH proton appeared as singlet at δ 12.89. In ¹³C NMR spectrum of **1g**, peaks δ 11.1 and 22.4 ppm correspond to carbons of ethyl group. Peaks of the aromatic carbons were found at δ 123.0–140.7 ppm. The presence of peaks at δ 101.1 and 170.0 ppm, confirm the thiazole cyclization.

The ¹H NMR spectra of **2c** and **3c** compounds showed that they are composed by diastereomers. Based on previous crystallized compounds by our group, we suggest that the major isomer formed present the *E-Z* configuration (Fig. 2). Indeed, hydrazine double-bond C2=N2 is commonly assigned as *E* configuration [3,31,33,34]. Concerning the exocyclic double-bond N3=C3, we suggest that the predominant configuration is *Z* [17,18,20–24,27,35]. Besides, a representative ¹H NMR spectrum of compound **2c** is presented in Supplementary Material.

2.2. Cytotoxicity and anti-*T. cruzi* activity

Comparing host cell cytotoxicity between thiosemicarbazone **1** and 1,3-thiazole **1a**, a 2-fold lower cytotoxicity for splenocytes is observed. The insertion of substituents at the *para* position of the aromatic ring attached at C4 (1,3-thiazole) in series **1a-m** reduces the toxicity of eight compounds (**1a-g** and **1k**). Comparing non-substituted compound **1a** with **1b** (4-methyl substituted) it can be observed that the last is 2-fold less toxic. However, substitution at position 3 (**1h**, 3-NO₂) and di-substitutions at positions 2,4 (**1j**, dichloro) or 3,4 (**1i**, dichloro) do not reduce toxicity when compared with compound **1a** (Table 1).

The introduction of a methyl at C5 position of thiazole ring (compounds **1l** and **1m**) do not reduce the toxicity. Moreover, comparing compound **1** with **2** and **3**, it can be seen that the introduction of a methyl (**2**) at N3 of thiosemicarbazone kept the cytotoxicity, however the substitution with phenyl (**3**) lead to a 4-fold reduction of cytotoxicity. When the host cell cytotoxicity was compared to Bzd, eight compounds were less toxic (**1b-c**, **1d-g**, **1k** and **3**), being compounds **1f** (4-NO₂), **1g** (4-Ph-Ph) and **1k** (2-naphthyl) devoid of toxicity in spleen cells (Fig. 3).

Concerning activity against trypomastigotes, the conversion of thiosemicarbazone (**1**) in 1,3-thiazoles increased the activity, which led us to identify five compounds with similar activity to Bzd (**1a**, **1c**, **1g**, **1l** and **1m**). Specifically, compound **1a** showed activity and selectivity index similar to Bzd. In contrast, the substitution of phenyl at C4 (**1a**) by 2-naphthyl (**1k**) led to a reduction of activity against trypomastigote form.

Fig. 1. Structural planning of the proposed series of compounds.

Scheme 1. Global synthesis of compounds 1–3c. Reagents and conditions: (a) corresponding thiosemicarbazide, ethanol, HCl, reflux, 2–3 h; (B) corresponding α -haloketone, 2-propanol, rt, 1 h.

Halogens in *para* position (**1d** and **1e**) of aromatic ring attached to thiazole lead to reduced activity in trypanomastigotes. The presence of a methyl (**2c**) or phenyl (**3c**) substituent at N3 (thiazole) was also not beneficial to antiparasitic activity. Although the presence of methyl at C5 of thiazole ring was beneficial to cytotoxicity in splenocytes, it improved the antiparasitic activity, as seen with compounds **1l** and **1m**.

Derivatives **1c** (4-OMe) and **1g** (4-Ph-Ph) presented better antiparasitic activity, with **1c** showing a selectivity index (cytotoxicity/IC₅₀ trypanomastigote) about two-times better than Bzd and **1**. These results may indicate that the methoxy group present in the molecule **1c** is an important substituent for antiparasitic activity.

The compound **1g** (4-Ph-Ph) showed a parasite selectivity 4-folds great than Bzd and compound **1**. Concerning compound **1k**, which contains a bulky 2-naphthyl group, it did not present good

trypanocidal activity. The main difference about **1g** and **1k** is that **1g** present more flexibility than **1k**, indicating that steric effect and lipophilicity of substituents is not directly related to trypanocidal activity, being mainly flexibility features an important tool to be explored in the future.

Even though thiosemicarbazone (**1**), synthesized firstly by Du et al. presented the lowest IC₅₀ value against trypanomastigote form, compounds **1c** and **1g** showed the highest SI values, (31.3 and 64.5 respectively) being thus promising anti-*T. cruzi* candidates (Fig. 4).

2.3. Cruzain inhibition

In view to investigate a possible biologic target, compounds were tested against the enzyme cruzain of the *T. cruzi*. Enzyme inhibition was measured using a fluorimetric assay with the

Fig. 2. Isomers representation of compounds **2c** and **3c**. **A-** Possible isomers for compounds **2c** and **3c**. **B-** Suggested isomer for compounds **2c** and **3c**.

Table 1

In vitro effect of 3'-(bromopropiophenone)-hydrazinyl-thiazole derivatives on *Trypanosoma cruzi* (trypomastigotes) and toxicity against mouse splenocytes.

Cpd	R1	R2	Ar	Y strain <i>T. cruzi</i> , IC ₅₀ (μM) trypomastigote ^a	Toxicity (μM) ^b	SI ^c

1	H	—	—	2.41	34.94	14.5
2	Me	—	—	36.31	33.31	0.9
3	Ph	—	—	13.33	138.01	10.4

1a	H	H	Ph	5.51	64.71	11.7
1b	H	H	4-Me-Ph	16.64	125.30	7.5
1c	H	H	4-Me-O-Ph	3.84	120.10	31.3
1d	H	H	4-F-Ph	146.05	123.67	0.8
1e	H	H	4-Cl-Ph	140.34	118.83	0.8
1f	H	H	4-NO ₂ -Ph	60.00	>231.85	>3.9
1g	H	H	4-Ph-Ph	3.35	>216.26	>64.5
1h	H	H	3-NO ₂ -Ph	15.05	23.18	1.5
1i	H	H	3,4-diCl-Ph	129.72	54.92	0.4
1j	H	H	2,4-diCl-Ph	59.20	54.92	0.9
1k	H	H	2-Naphthyl	50.98	>229.16	>4.5
1l	H	Me	Ph	4.79	12.49	2.6
1m	H	Me	4-Br-Ph	5.80	20.87	3.6
2c	Me	H	4-Me-O-Ph	76.56	58.09	0.7
3c	Ph	H	4-Me-O-Ph	53.10	50.77	0.9
Bzd	—	—	—	6.26	96.1	15.4

^a Determined 24 h after incubation of trypomastigotes with the compounds. The dose-response curves were determined and the IC₅₀ values (μM) were calculated using at least seven concentrations.

^b Highest non-toxic concentration (>90% incorporation of tritiated thymidine) for mouse splenocytes after 24 h of incubation in the presence of the compounds.

^c Selective Index (SI) = Cytotoxicity/IC₅₀ trypomastigote. Bzd = benznidazole.

Fig. 3. Highest non-toxic concentration for mouse splenocytes.

substrate Z-Phe-Arg-aminomethylcoumarin (Z-FR-AMC) [36]. All compounds were screened at 300 μM with exception of **2**, **2c**, **3** (200 μM) and **3c** (100 μM), which had to be evaluated at lower concentrations due to their lower solubility. Compounds that inhibited cruzain by at least 80% at the screening concentration were selected for IC_{50} determination (Table 2). Overall, 11 compounds had their IC_{50} determined, with values ranging from 0.5 to 45 μM .

Firstly, it was observed that a substitution at N3 for thiosemicarbazones **2** and **3** was deleterious to cruzain inhibition. Comparing compound **1** ($\text{IC}_{50} = 0.04 \pm 0.00 \mu\text{M}$) to unsubstituted thiazole **1a** ($\text{IC}_{50} = 44.6 \pm 2.2 \mu\text{M}$) it is observed that the cyclization was also deleterious for cruzain inhibition, reducing potency 100-fold. In fact, none of the cyclic derivatives **1a-m** were more active than thiosemicarbazone **1**. Comparing substituted compounds to unsubstituted **1a**, it was observed that halogenated substituents (**1d-e** and **1i-j**) at the phenyl ring slightly improved the potency against cruzain (IC_{50} s ranging from 11 to 33 μM). Methoxy derivatives did not present cruzain inhibition, even substitution of methyl (**1b**) and phenyl (**1c**) at N3 did not only reduced but worsens the activity.

Methyl substituted compounds at C5, **1l** and **1m**, present a reasonable potency (IC_{50} s = $0.51 \pm 0.02 \mu\text{M}$ and $3.8 \pm 0.7 \mu\text{M}$, respectively) highlighting compound **1l**, which improved the potency in comparison to compound **1a**, unsubstituted at C5. This improvement can be due a better interaction in the active site of the enzyme. Amongst the cyclic derivatives, the most potent was compound **1l**, however, it shows a 12-fold lower potency than **1** ($\text{IC}_{50} = 0.51 \pm 0.02 \mu\text{M}$ vs $\text{IC}_{50} = 0.04 \pm 0.0 \mu\text{M}$).

Curiously, compounds **1c** and **1g**, which are the most active against *T. cruzi* trypomastigotes (IC_{50} s = 3.8 and 3.4 μM , respectively), did not present high inhibitory activity against cruzain, indicating that their anti-parasite activity occurs through a different target.

As proposed by Du et al. [17], the thioamide moiety has an

Table 2

Inhibitory activity *in vitro* of inhibitory activity against the cysteine protease cruzain of *Trypanosoma cruzi*.

Cpd.	R1	R2	Ar	% of inhibition ^a	IC_{50} (μM) $\pm$ SEM ^b
<p>1-3</p>					
1	H	—	—	93.0 $\pm$ 0.4	0.04 $\pm$ 0.0
2	Me	—	—	11.7 $\pm$ 4.9	ND
3	Ph	—	—	49.5 $\pm$ 1.3	ND
<p>1a-1m, 2c, 3c</p>					
1a	H	H	Ph	80.7 $\pm$ 1.7	44.6 $\pm$ 2.2
1b	H	H	4-Me-Ph	97.7 $\pm$ 0.2	19.2 $\pm$ 4.7
1c	H	H	4-Me-O-Ph	72.0 $\pm$ 0.2	ND
1d	H	H	4-F-Ph	95.8 $\pm$ 0.4	16.5 $\pm$ 0.3
1e	H	H	4-Cl-Ph	90.4 $\pm$ 0.8	32.7 $\pm$ 0.7
1f	H	H	4-NO ₂ -Ph	68.9 $\pm$ 1.1	ND
1g	H	H	4-Ph-Ph	18.1 $\pm$ 3.6	ND
1h	H	H	3-NO ₂ -Ph	85.3 $\pm$ 1.2	14.6 $\pm$ 4.0
1i	H	H	3,4-diCl-Ph	86.8 $\pm$ 1.3	11.3 $\pm$ 3.6
1j	H	H	2,4-diCl-Ph	99.7 $\pm$ 0.4	15.1 $\pm$ 3.6
1k	H	H	2-Naphthyl	88.2 $\pm$ 0.1	9.5 $\pm$ 3.0
1l	H	Me	Ph	99.5 $\pm$ 0.3	0.51 $\pm$ 0.02
1m	H	Me	4-Br-Ph	100.0 $\pm$ 0.4	3.8 $\pm$ 0.7
2c	Me	H	4-Me-O-Ph	33.1 $\pm$ 4.1	ND
3c	Ph	H	4-Me-O-Ph	−34.3 $\pm$ 1.3	ND

^a Compounds were tested at 100 μM (**3c**), 200 μM (**2**, **2c** and **3**) and 300 μM (**1**, **1a-m**) and the percent inhibition of catalytic activity was determined.

^b IC_{50} values (μM) represent the mean $\pm$ standard error of the mean (SEM) of three measurements.

Fig. 4. Summary of SAR of trypanocidal activity and increase of SI of compounds. Selective index (SI) = highest non-toxic concentration in spleen cells of BALB/c mice/ IC_{50} trypomastigotes.

important role in the cruzain inhibition mechanism, however it has been demonstrated that cyclic derivatives, as 1,3-thiazoles [31], 2-thiazolidin-4-ones [27] and 2,4-oxadiazoles [13], may also inhibit this enzyme. In this work, most cyclic derivatives present only modest inhibitory activity of cruzain and none of them were most potent than lead thiosemicarbazone **1**, however, the most active compounds for tripomastigote form (**1c** and **1g**) did not present inhibitory activity of cruzain, indicating that cyclic derivatives may act by different paths in the parasite.

2.4. Docking studies

To define the structural determinants for cruzain inhibition, and in order to understand the mode of binding in such compounds, their interactions with cruzain (PDB ID: 3IUT) were investigated by docking studies. The binding modes were determined as the highest (most negative) score among the possible solutions for each ligand. Fig. 5 shows the superposition of the best docking solutions for compounds (**1a,b**, **1d,e** and **1h-m**), which have IC_{50} values experimentally available for inhibition against the cruzain target. The respective Autodock score values for compounds **1a-1b**, **1d-e** and **1h-m**, are -8.59 , -8.76 , -6.86 , -7.46 , -7.99 , -7.52 , -7.15 , -8.29 , -8.24 and -8.53 kcal/mol, respectively.

In order to identify the molecular reasons for high affinities towards the cruzain target, it was selected the compound with the highest potency, **11** ($IC_{50} = 0.51 \pm 0.02 \mu M$), in addition the one which presents one of the lowest inhibitions, **1d** ($IC_{50} = 16.5 \pm 0.3 \mu M$). Additionally, compound **1d** presents the lowest inhibition ($IC_{50} = 146.05 \mu M$) when tested against the tripomastigotes forms of *T. cruzi* Y strains (see Table 1). A detailed analysis of the intermolecular interactions observed in the docking solutions was performed for these molecules. The differences between these two compounds are: (i) the presence of a 4-fluorophenyl linked to the thiazole ring of molecule **1d**, instead of a phenyl in molecule **11**; (ii) a methyl group linked to thiazole ring in molecule **11**, rather than a hydrogen atom, for molecule **1d**.

The difference between the binding modes of these two molecules is show in detail in Fig. 6 and Table 3. It was found a π - π T-shaped interaction for molecule **11** and a π - π interaction for **1d**. The hydrogen bond found for **11**, with the residue CYS25, is shorter ($2.14 \text{ \AA}$) and stronger than the hydrogen bond found for molecule **1d** ($2.26 \text{ \AA}$) with the residue TRP184. It was also found a weak polar interaction (data not shown) for molecule **11**, with the CYS25

residue ($2.68 \text{ \AA}$) of cruzain. These different intermolecular interactions are responsible for the greater stability of the complex formed with **11** than **1d**, with docking scores of -8.24 and -6.86 kcal/mol, respectively. These findings corroborate with the *in vitro* inhibition potency measured for cruzain target.

2.5. Ultrastructural studies

To investigate the parasite morphological alterations caused by compound **1c**, one of the most potent compounds tested (IC_{50} of $3.84 \mu M$), its effects were evaluated at the ultrastructural level. The ultrastructural effects of **1c** on tripomastigotes after 24 h were analyzed by Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM), in concentrations corresponding to once or twice the IC_{50} value (3.4 and $6.8 \mu M$) (Table 1), and revealed several morphological alterations (Fig. 7).

The control group analyzed by SEM showed typical morphology with elongated body (Fig. 7A), whereas the group treated with **1c** showed contortion of the parasite body with presence of blebs and alteration in morphology and size of the flagellum which also presented blebs (Fig. 7B).

The control group analyzed by TEM showed normal

Fig. 6. Detailed view of the docking solutions for compound **1d** (above) and **11** (below). The cruzain's residues forming hydrophobic contacts (HC) are colored in green, residues forming hydrogen bonds are colored in blue, residues forming π - π T-shaped are colored in red, and residues forming π - π interactions are colored in magenta. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5. Superposition of the docking solutions for compounds **1a-1b**, **1d-e** and **1h-m** (blue), bound to *T. cruzi* cruzain (green), besides the experimental position of the KB2 co-crystallized ligand (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Molecular interactions between the cruzain target and molecules **1d** and **1l**.

Residues (cruzain)	Molecular interactions	
	1d	1l
GLN19	HC	HC
GLY20	HC	—
GLN21	HC	—
GLY23	—	HC
CYS25	—	2.14
TRP26	—	PIT
GLY65	—	HC
ALA141	HC	HC
SER142	HC	—
TRP144	HC	—
ASP161	HC	HC
HIS162	HC	HC
TRP184	2.26 and PI	HC
Autodock Score	−6.86	−8.24

HC = hydrophobic contacts. PIT = π - π T-shaped interaction. HB = Hydrogen bond, with distances [Å] between donor and acceptor. PI = π - π interactions. Autodock Score in kcal/mol.

ultrastructural morphology of organelles such as kinetoplast, nucleus, nucleolus, flagellum, ribosomes and microtubules membrane (Fig. 7E). The groups treated with **1c** analyzed showed intense cytosolic vacuolization, formation of blebs in the membrane, alterations in the reservosomes, swelling of the mitochondrion and abnormal chromatin condensation (Fig. 7G and H). At the highest compound concentration (6.8 μ M) these alterations were emphasized, and alterations of the parasite morphology were induced. Furthermore, the cytosolic vacuolization was significantly more intense, indicating a dose-dependent action of compound **1c** (Fig. 7G and H).

Mitochondrial swelling had already been observed upon treatment with various compounds [33,37,38] and published data demonstrate that *T. cruzi* mitochondrial membranes, in contrast to those of vertebrate cells, are rich in endogenous parasite sterols, which are thought to be required for their energy transducing activities, being an important action target [34].

The intense cytoplasmic vacuolization observed (Fig. 7G and H), has also been reported in the study of several other compounds described in literature [38–40]. Braga et al. [39] reports that under stress conditions, the cell reacts developing a contractile vacuole. Alterations in the reservosomes could also be important in growth inhibition, since they are involved in endocytosis, storage and breakdown of nutrients and could hamper the proliferation process of the parasite [38]. Observation of alterations in the nucleus can also indicate an effect on parasite proliferation [33].

3. Conclusions

New 3-(bromopropiophenone)hydrazinyl-1,3-thiazoles were prepared from the reaction between 3-(bromopropiophenone)-thiosemicarbazone and α -haloketones. The *in vitro* bioassay showed that compounds possess substantial antiparasitic activity against the trypanomastigote form of *T. cruzi*. Most compounds were characterized as cruzain inhibitors. However, some of the most potent trypanocidal compounds did not inhibit this enzyme indicating that other targets are probably important to kill the parasite. The derivatives **1c** and **1g** exhibited improved selectivity for parasites when compared to the reference drug benznidazole. Ultrastructural analysis showed that the compound **1c** caused irreversible lesions and changes in parasite morphology in a dose-dependent manner. Our results pointed out the importance of 1,3-thiazoles core as building-blocks for lead generation and showed that adjustment of conformational flexibility could represent a key

event to be explored in the future.

4. Experimental section

4.1. Chemistry

Reagents were purchased from Acros Organics, Fluka, Sigma-Aldrich or Vetec and solvents from Vetec or Dinâmica. The deuterated solvents (DMSO- d_6 , $CDCl_3$, D_2O) were of the CIL brand (Tédia Brazil). The reactions were monitored in thin layer chromatography (TLC) using silica gel 60 containing fluorescent indicator F₂₅₄. The chromatographic plates were visualized under UV light (at dual wavelength 365 or 254 nm). Melting points were measured using a capillary Thomas Hoover apparatus and the values ($^{\circ}C$) were not subsequently corrected. For all novel compounds, 1H and ^{13}C NMR analyses were performed, and, when necessary, two-dimensional analysis (DEPT) as well as the addition of D_2O for locating NH signals. All compounds were solubilized in DMSO- d_6 . The 1H and ^{13}C NMR spectra were obtained using Unity Plus model Varian instruments (400 MHz for 1H , 100 MHz for ^{13}C) or Bruker AMX (300 MHz for 1H , 75.5 MHz for ^{13}C), using tetramethylsilane (tms) as internal standard. The number of signals in the 1H NMR spectra were designated as follows: s/singlet; d/doublet, t/triplet, dd/double doublet, q/quartet, m/multiplet and the coupling constants, in hertz, as *J*. Infrared spectroscopy was performed on a Bruker instrument (model IFS 66) using KBr pellets.

4.1.1. General procedure for synthesis of compounds 1, 2 and 3

In a round bottom flask of 100 mL, 1-(3-bromophenyl)propan-1-one (5.5 mmol) and the respective thiosemicarbazide (5.5 mmol) were dissolved in ethanol (20 mL) and HCl (cat.). The mixture was maintained under magnetic stirring and heating reflux for 2–3 h. The reaction was monitored by thin-layer chromatographic plate (TLC). The resulting solid was filtered through a sintered funnel and washed with ethanol to yield pure product.

4.1.1.1. (E)-2-[1-(3-bromophenyl)propylidene]hydrazinecarbothioamide (1). 90% yield; mp ($^{\circ}C$): 144–146; IR (KBr, cm^{-1}) 3415.33 (NH), 1507.11 (C=N); 1H NMR (DMSO- d_6 , 300 MHz) δ ppm: 0.99 (t, 3H, *J* = 7.4 Hz, CH_3), 2.86 (q, 2H, *J* = 7.6 Hz, CH_2), 7.34 (t, 1H, *J* = 8.0 Hz, Ar), 7.56 (d, 1H, *J* = 8.0 Hz, Ar), 7.86 (d, 1H, *J* = 7.6 Hz, Ar), 8.08 and 8.15 (2 s, 2H, NH_2), 8.31 (s, 1H, Ar), 10.35 (s, 1H, NH); ^{13}C NMR (DMSO- d_6 , 75.5 MHz) δ ppm: 10.8 (CH_3), 19.1 (CH_2), 122.1 (Ar), 125.7 (Ar), 129.0 (Ar), 130.4 (Ar), 131.8 (Ar), 138.8 (Ar), 150.2 (C=N), 179.0 (C=S). Anal. Calcd for $C_{10}H_{12}BrN_3S$: C, 41.97; H, 4.23; N, 14.68; S, 11.20. found: C, 41.93; H, 4.26; N, 14.62; S, 11.27. HRMS: 284.9940 [$M+H$] $^{+}$.

4.1.1.2. (E)-2-[1-(3-bromophenyl)propylidene]-N-methylhydrazinecarbothioamide (2). 89% yield; mp ($^{\circ}C$): 148–150; IR (KBr, cm^{-1}): 1552.22 (C=N); 1H NMR (DMSO- d_6 , 300 MHz) δ ppm: 1.21 (t, *J* = 7.6 Hz, 3H, CH_3), 2.69 (q, *J* = 7.6 Hz, 2H, CH_2), 3.29 (d, 3H, CH_3), 7.29 (t, *J* = 7.8 Hz, 1H, Ar), 7.53 (d, *J* = 7.8 Hz, 1H, Ar), 7.60 (d, *J* = 7.8 Hz, 1H, Ar), 7.84 (s, 1H, Ar), 8.42 (s, 1H, NH), 8.81 (s, 1H, NH); ^{13}C NMR (DMSO- d_6 , 75.5 MHz) δ ppm: 10.4 (CH_3), 20.1 (CH_2), 31.4 (CH_3), 122.9 (Ar), 124.9 (Ar), 129.2 (Ar), 130.1 (Ar), 132.5 (Ar), 138.4 (Ar), 149.9 (C=N), 178.9 (C=S). Anal. Calcd for $C_{11}H_{14}BrN_3S$: C, 44.01; H, 4.70; N, 14.00; S, 10.68. found: C, 43.93; H, 4.84; N, 14.02; S, 9.83. HRMS: 300.0316 [$M+H$] $^{+}$.

4.1.1.3. (E)-2-[1-(3-bromophenyl)propylidene]-N-phenylhydrazinecarbothioamide (3). 87% yield; mp ($^{\circ}C$): 169–173; IR (KBr, cm^{-1}): 1588.14 (C=N); 1H NMR (DMSO- d_6 , 300 MHz) δ ppm: 1.05 (t, *J* = 7.5 Hz, 3H, CH_3), 2.94 (q, *J* = 7.5 Hz, 2H, CH_2), 7.22 (t, *J* = 7.5 Hz, 1H, Ar), 7.35–7.61 (m, 6H, Ar), 7.95 (d, *J* = 8.1 Hz, 1H, Ar), 8.21 (s, 1H,

Fig. 7. Effect of **1c** on trypomastigotes of *T. cruzi* morphology observed by SEM and TEM. A– SEM of control untreated trypomastigotes showing the typical elongated body. B– SEM of parasite treated with 3.4 μM of **1c** showing contortion (star) of the parasite body and blebs in the flagellum and parasite body. C and D– SEM of parasite treated with 6.8 μM of **1c** showing contortion (star) of the parasite body and alteration in morphology and size of the flagellum. E– TEM of untreated trypomastigotes showing the normal morphology, with kinetoplast (K), nucleus (N), nucleolus (n), flagellum (F), ribosomes (Ri) and Microtubules membrane (Mt). F– TEM of parasite treated with 3.4 μM of **1c** showing alterations in the reservosomes (R), formation of vacuoles (asterisk), bubbles in the membrane (black arrow), abnormal chromatin condensation (Ct), membrane projections (PM) and swelling of the mitochondrion. G and H– TEM of parasite treated with 6.8 μM of **1c** showing alterations of the parasite morphology, abnormal chromatin condensation (Ct), intense vacuolization in the cytoplasm (asterisk), swelling of the mitochondrion, bubbles in the membrane (black arrow) and alterations in the reservosomes (R).

Ar), 10.15 (s, 1H, NH), 10.76 (s, 1H, NH); ^{13}C NMR (DMSO- d_6 , 75.5 MHz) δ_{ppm} : 10.9 (CH₃), 19.5 (CH₂), 122.1 (Ar), 125.5 (Ar), 126.1 (Ar), 126.3 (Ar), 128.1 (Ar), 129.3 (Ar), 130.4 (Ar), 132.0 (Ar), 138.7 (Ar), 139.2 (Ar), 151.1 (C=N), 177.3 (C=S). Anal. Calcd for

$\text{C}_{16}\text{H}_{16}\text{BrN}_3\text{S}$: C, 53.04; H, 4.45; N, 11.60; S, 8.85. found: C, 52.93; H, 4.39; N, 11.56; S, 8.79. HRMS: 361.0602 $[\text{M}+\text{H}]^+$.

4.1.2. General procedure for synthesis of the series (1a–3c)

In round bottom flask, aryl thiosemicarbazone (**1**, **2** or **3**) and the respective 2-bromoacetophenone were dissolved in 2-propanol (20 mL). The reaction mixture was kept at room temperature for 1 h. The reactions were monitored by thin-layer chromatographic plate (TLC). The resulting solid was filtered through sintered funnel with distilled water, yielding the pure product.

4.1.2.1. (E)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-phenylthiazole (1a). 72% yield; mp (°C): 196–199; IR (KBr, cm⁻¹): 1516.36 (C=N); ¹H NMR (DMSO-d₆, 300 MHz) δ ppm: 1.03 (t, J = 6.8 Hz, 3H, CH₃), 2.82 (d, J = 7.2 Hz, 2H, CH₂), 7.28–7.39 (m, 5H, Ar), 7.53 (d, J = 7.2 Hz, 1H, Ar), 7.73 (d, J = 7.2 Hz, 1H, Ar), 7.82 (d, J = 7.2 Hz, 2H, Ar), 7.91 (s, 1H, thiazole), 10.64 (s, 1H, NH); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 10.1 (CH₃), 19.6 (CH₂), 104.4 (CH, thiazole), 122.1 (Ar), 124.9 (Ar), 125.6 (Ar), 127.8 (Ar), 127.9 (Ar), 128.2 (Ar), 128.6 (Ar), 130.7 (Ar), 131.4 (Ar), 134.0 (Ar), 138.9 (C–N, thiazole), 149.8 (C=N), 169.6 (S–C=N, thiazole). *Anal.* Calcd for C₁₈H₁₆BrN₃S: C, 55.96; H, 4.17; N, 10.88; S, 8.30. found: C, 56.02; H, 4.14; N, 10.83; S, 8.27. HRMS: 387.1737 [M+H]⁺.

4.1.2.2. (E)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-p-tolylthiazole (1b). 69% yield; mp (°C): 192–196; IR (KBr, cm⁻¹): 1505.60 (C=N); ¹H NMR (DMSO-d₆, 300 MHz) δ ppm: 1.06 (s, 3H, CH₃), 2.31 (s, 3H, CH₃), 2.86 (d, J = 6.8 Hz, 2H, CH₂), 6.32 (s, 1H, NH), 7.21–7.75 (m, 8H, Ar), 7.94 (s, 1H, thiazole); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 10.6 (CH₃), 19.6 (CH₃), 20.8 (CH₂), 103.4 (CH, thiazole), 122.1 (Ar), 124.8 (Ar), 125.6 (Ar), 127.8 (Ar), 128.2 (Ar), 129.2 (Ar), 130.7 (Ar), 131.3 (Ar), 134.0 (Ar), 137.0 (Ar), 138.9 (C–N, thiazole), 149.0 (C=N), 169.5 (S–C=N, thiazole). *Anal.* Calcd for C₁₉H₁₈BrN₃S: C, 57.00; H, 4.53; N, 10.50; S, 8.01. found: C, 57.07; H, 4.58; N, 10.48; S, 8.05. HRMS: 399.1896 [M+H]⁺.

4.1.2.3. (E)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-(4-methoxyphenyl)thiazole (1c). 62% yield; mp (°C): 197–200; IR (KBr, cm⁻¹): 3115.41 (NH), 2954.46 and 2831.76 (CH Ar), 1509.43 (C=N); ¹H NMR (DMSO-d₆, 300 MHz) δ ppm: 1.06 (t, J = 7.3 Hz, 3H, CH₃), 2.78 (q, J = 7.5 Hz, 2H, CH₂), 3.78 (s, 3H, CH₃), 5.71 (s, 1H, NH), 6.98 (d, J = 8.7 Hz, 2H, Ar), 7.16 (s, 1H, Ar), 7.39 (t, J = 7.8 Hz, 1H, Ar), 7.57 (dd, J = 8.1 Hz, 1H, Ar), 7.78 (d, J = 7.8 Hz, 1H, Ar), 7.79 (d, J = 8.4 Hz, 2H, Ar), 7.95 (s, 1H, thiazole); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 10.7 (CH₃), 19.6 (CH₂), 55.2 (CH₃), 102.3 (CH, thiazole), 114.0 (Ar), 122.2 (Ar), 124.9 (Ar), 125.6 (Ar), 127.0 (Ar), 127.9 (Ar), 128.2 (Ar), 130.8 (Ar), 131.4 (Ar), 137.0 (Ar), 139.0 (C–N, thiazole), 158.9 (C=N), 169.5 (S–C=N, thiazole). *Anal.* Calcd for C₁₉H₁₈BrN₃OS: C, 54.81; H, 4.36; N, 10.09; O, 3.84; S, 7.70. found: C, 54.96; H, 4.20; N, 10.02; S, 7.74. HRMS: 414.8419 [M – H]⁻.

4.1.2.4. (E)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-(4-fluorophenyl)thiazole (1d). 65% yield; mp (°C): 207–210; IR (KBr, cm⁻¹): 1509.35 (C=N); ¹H NMR (DMSO-d₆, 300 MHz) δ ppm: 1.07 (t, J = 7.4 Hz, 3H, CH₃), 2.85 (q, J = 7.4 Hz, 2H, CH₂), 4.28 (s, 1H, NH), 7.22–7.93 (m, 9H, Ar); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 10.6 (CH₃), 19.5 (CH₂), 104.1 (CH, thiazole), 115.3 (Ar), 115.6 (Ar), 122.1 (Ar), 124.8 (Ar), 127.5 (Ar), 127.6 (Ar), 128.1 (Ar), 129.2 (Ar), 130.7 (Ar), 131.3 (Ar), 139.1 (C–N, thiazole), 149.2 (C=N), 169.7 (S–C=N, thiazole). *Anal.* Calcd for C₁₈H₁₅BrFN₃S: C, 53.47; H, 3.74; N, 10.39; S, 7.93. found: C, 54.14; H, 3.69; N, 10.41; S, 7.98. HRMS: 404.0101 [M+H]⁺.

4.1.2.5. (E)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-(4-chlorophenyl)thiazole (1e). 62% yield; mp (°C): 204–209; IR (KBr, cm⁻¹): 3922.26 (NH); ¹H NMR (DMSO-d₆, 300 MHz) δ ppm: 1.09 (t, J = 5.8 Hz, 3H, CH₃), 2.83 (q, J = 5.8 Hz, 2H, CH₂), 6.01 (s, 1H, NH), 7.36–7.93 (m, 9H, Ar); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 10.6

(CH₃), 19.6 (CH₂), 105.2 (CH, thiazole), 122.1 (Ar), 124.8 (Ar), 127.3 (Ar), 128.7 (Ar), 129.6 (Ar), 130.8 (Ar), 131.3 (Ar), 132.0 (Ar), 132.4 (Ar), 133.5 (Ar), 139.1 (C–N, thiazole), 149.2 (C=N), 169.8 (S–C=N, thiazole). *Anal.* Calcd for C₁₈H₁₅BrClN₃S: C, 51.38; H, 3.59; N, 9.99; S, 7.62. found: C, 52.04; H, 3.60; N, 9.97; S, 7.64. HRMS: 421.1562 [M+H]⁺.

4.1.2.6. (E)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-(4-nitrophenyl)thiazole (1f). 63% yield; mp (°C): 193–196; IR (KBr, cm⁻¹): 3332.81 (NH), 1508.37 (C=N); ¹H NMR (DMSO-d₆, 300 MHz) δ ppm: 1.07 (t, J = 7.8 Hz, 3H, CH₃), 2.86 (q, J = 7.8 Hz, 2H, CH₂), 7.39–8.29 (m, 9H, Ar), 11.66 (s, 1H, NH); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 10.5 (CH₃), 19.6 (CH₂), 109.2 (CH, thiazole), 122.1 (Ar), 124.1 (Ar), 124.8 (Ar), 126.3 (Ar), 128.2 (Ar), 130.7 (Ar), 131.3 (Ar), 132.4 (Ar), 133.5 (Ar), 139.0 (Ar), 140.7 (C–N, thiazole), 146.2 (C=N), 170.0 (S–C=N, thiazole). *Anal.* Calcd for C₁₈H₁₅BrN₄O₂S: C, 50.13; H, 3.51; N, 12.99; S, 7.43. found: C, 50.15; H, 3.55; N, 12.97; S, 7.39. HRMS: 430.9228 [M+H]⁺.

4.1.2.7. (E)-4-(biphenyl-4-yl)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}thiazole (1g). 64% yield; mp (°C): 218–221; IR (KBr, cm⁻¹): 1611.72 (C=N); ¹H NMR (DMSO-d₆, 300 MHz) δ ppm: 1.29 (t, J = 7.8 Hz, 3H, CH₃), 2.92 (q, J = 7.8 Hz, 2H, CH₂), 6.76 (s, 1H, Thiazole), 7.19–7.87 (m, 13H, Ar), 12.89 (s, 1H, NH); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 11.1 (CH₃), 22.4 (CH₂), 101.1 (CH, thiazole), 123.0 (Ar), 125.4 (Ar), 125.8 (Ar), 126.0 (Ar), 126.9 (Ar), 128.1 (Ar), 128.2 (Ar), 128.9 (Ar), 129.7 (Ar), 130.2 (Ar), 133.5 (Ar), 136.9 (Ar), 139.5 (Ar), 140.7 (Ar), 143.2 (C–N, thiazole), 159.7 (C=N), 170.0 (S–C=N, thiazole). *Anal.* Calcd for C₂₄H₂₀BrN₃S: C, 62.34; H, 4.36; N, 9.09; S, 6.93. found: C, 62.35; H, 4.38; N, 9.07; S, 6.96. HRMS: 461.9808 [M+H]⁺.

4.1.2.8. (E)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-(3-nitrophenyl)thiazole (1h). 64% yield; mp (°C): 220–223; IR (KBr, cm⁻¹): 1530.66 (C=N); ¹H NMR (DMSO-d₆, 300 MHz) δ ppm: 1.07 (t, J = 7.8 Hz, 3H, CH₃), 2.86 (q, J = 7.8 Hz, 2H, CH₂), 6.83 (s, 1H, NH), 7.39–8.72 (m, 9H, Ar), 11.6 (s, 1H, NH); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 10.5 (CH₃), 19.5 (CH₂), 107.1 (CH, thiazole), 120.0 (Ar), 122.1 (Ar), 124.8 (Ar), 128.2 (Ar), 130.2 (Ar), 130.7 (Ar), 131.3 (Ar), 131.5 (Ar), 133.5 (Ar), 136.2 (Ar), 139.0 (Ar), 140.7 (Ar), 148.3 (C–N, thiazole), 149.3 (C=N), 169.9 (S–C=N, thiazole). *Anal.* Calcd for C₁₈H₁₅BrN₄O₂S: C, 50.13; H, 3.51; N, 12.99; S, 7.43. found: C, 50.09; H, 3.56; N, 12.97; S, 7.45. HRMS: 431.2205 [M+H]⁺.

4.1.2.9. (E)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-(3,4-dichlorophenyl)thiazole (1i). 60% yield; mp (°C): 228–231; IR (KBr, cm⁻¹): 3642.77 and 2971.43 (CH Ar), 1475.20 (C=N); ¹H NMR (DMSO-d₆, 300 MHz) δ ppm: 1.04 (t, J = 7.5 Hz, 3H, CH₃), 2.84 (q, J = 7.5 Hz, 2H, CH₂), 5.29 (s, 1H, NH), 7.35–8.10 (m, 8H, Ar); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 10.6 (CH₃), 19.6 (CH₂), 106.6 (CH, thiazole), 122.2 (Ar), 124.8 (Ar), 125.6 (Ar), 127.2 (Ar), 128.2 (Ar), 129.7 (Ar), 130.8 (Ar), 131.5 (Ar), 135.2 (Ar), 139.0 (Ar), 147.9 (Ar), 149.3 (Ar), 150.4 (C–N, thiazole), 149.3 (C=N), 169.8 (S–C=N, thiazole). *Anal.* Calcd for C₁₈H₁₄BrCl₂N₃S: C, 47.49; H, 3.10; N, 9.23; S, 7.04. found: C, 47.47; H, 3.13; N, 9.26; S, 7.06. HRMS: 451.8046 [M – H]⁻.

4.1.2.10. (E)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-(2,4-dichlorophenyl)thiazole (1j). 66% yield; mp (°C): 174–179; IR (KBr, cm⁻¹): 1518.07 (C=N); ¹H NMR (DMSO-d₆, 400 MHz) δ ppm: 1.06 (t, J = 7.6 Hz, 3H, CH₃), 2.84 (q, J = 7.6 Hz, 2H, CH₂), 4.04 (s, 1H, NH), 7.38–7.94 (m, 8H, Ar); ¹³C NMR (DMSO-d₆, 75.5 MHz) δ ppm: 10.6 (CH₃), 19.6 (CH₂), 109.8 (CH, thiazole), 122.1 (Ar), 124.8 (Ar), 125.6 (Ar), 127.5 (Ar), 128.2 (Ar), 129.7 (Ar), 130.7 (Ar), 131.3 (Ar), 131.6 (Ar), 132.3 (Ar), 132.6 (Ar), 135.2 (Ar), 139.0 (C–N, thiazole),

149.3 (C=N), 168.8 (S=C=N, thiazole). *Anal.* Calcd for $C_{18}H_{14}BrClN_3S$: C, 47.49; H, 3.10; N, 9.23; S, 7.04. found: C, 47.45; H, 3.13; N, 9.28; S, 7.05. HRMS: 451.9214 [M – H][–].

4.1.2.11. (*E*)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-4-(naphthalen-2-yl)thiazole (**1k**). 57% yield; mp (°C): 198–201; IR (KBr, cm^{–1}): 1614.36 (C=N); ¹H NMR (DMSO-*d*₆, 300 MHz) δ ppm: 1.36 (t, *J* = 7.6 Hz, 3H, CH₃), 2.97 (q, *J* = 7.6 Hz, 2H, CH₂), 6.92 (s, 1H, thiazole), 7.31 (t, *J* = 7.5 Hz, 1H, Ar), 7.52–7.59 (m, 3H, Ar), 7.68–7.73 (m, 2H, Ar), 7.82 (t, *J* = 4.6 Hz, 1H, Ar), 7.90–7.98 (m, 3H, Ar), 8.26 (s, 1H, Ar), 13.02 (s, 1H, NH); ¹³C NMR (DMSO-*d*₆, 75.5 MHz) δ ppm: 11.1 (CH₃), 22.4 (CH₂), 101.6 (CH, thiazole), 120.0 (Ar), 122.2 (Ar), 123.0 (Ar), 124.2 (Ar), 125.4 (Ar), 125.6 (Ar), 127.3 (Ar), 127.7 (Ar), 128.9 (Ar), 129.6 (Ar), 129.7 (Ar), 130.3 (Ar), 133.1 (Ar), 133.5 (Ar), 133.8 (Ar), 136.9 (Ar), 140.9 (C–N, thiazole), 159.6 (C=N), 170.1 (S=C=N, thiazole). *Anal.* Calcd for $C_{22}H_{18}BrN_3S$: C, 60.55; H, 4.16; N, 9.63; S, 7.35. found: C, 60.57; H, 4.14; N, 9.59; S, 7.40. HRMS: 436.1018 [M+H]⁺.

4.1.2.12. (*E*)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-5-methyl-4-phenylthiazole (**1l**). 62% yield; mp (°C): 202–206; IR (KBr, cm^{–1}): 1614.94 (C=N); ¹H NMR (DMSO-*d*₆, 300 MHz) δ ppm: 1.32 (t, *J* = 7.6 Hz, 3H, CH₃), 2.46 (s, 3H, CH₃), 2.96 (q, *J* = 7.6 Hz, 2H, CH₂), 7.30 (t, 1H, Ar), 7.45–7.57 (m, 6H, Ar), 7.68 (d, 1H, Ar), 7.93 (s, 1H, Ar), 12.9 (s, 1H, NH); ¹³C NMR (DMSO-*d*₆, 75.5 MHz) δ ppm: 11.1 (CH₃), 12.3 (CH₃), 22.3 (CH₂), 115.9 (C, thiazole), 120.0 (Ar), 122.9 (Ar), 125.3 (Ar), 127.8 (Ar), 129.5 (Ar), 129.6 (Ar), 129.9 (Ar), 130.2 (Ar), 133.3 (Ar), 135.2 (Ar), 137.1 (C–N, thiazole), 159.1 (C=N), 167.6 (S=C=N, thiazole). *Anal.* Calcd for $C_{19}H_{18}BrN_3S$: C, 57.00; H, 4.53; N, 10.50; S, 8.01. found: C, 57.07; H, 4.49; N, 10.45; S, 8.09. HRMS: 400.0076 [M+H]⁺.

4.1.2.13. (*E*)-4-(4-bromophenyl)-2-{2-[1-(3-bromophenyl)propylidene]hydrazinyl}-5-methylthiazole (**1m**). 61% yield; mp (°C): 192–197; IR (KBr, cm^{–1}): 1614.94 (C=N); ¹H NMR (DMSO-*d*₆, 300 MHz) δ ppm: 1.05 (t, *J* = 7.2 Hz, 3H, CH₃), 2.40 (s, 3H, CH₃), 2.83 (q, *J* = 7.2 Hz, 2H, CH₂), 4.64 (s, 1H, NH), 7.38 (t, *J* = 7.8 Hz, 1H, Ar), 7.58 (m, 5H, Ar), 7.76 (d, *J* = 7.2 Hz, 1H, Ar), 7.94 (s, 1H, Ar); ¹³C NMR (DMSO-*d*₆, 75.5 MHz) δ ppm: 10.6 (CH₃), 12.1 (CH₃), 19.6 (CH₂), 118.2 (C, thiazole), 120.6 (Ar), 122.1 (Ar), 124.8 (Ar), 125.6 (Ar), 127.8 (Ar), 128.2 (Ar), 129.2 (Ar), 130.1 (Ar), 130.7 (Ar), 131.3 (Ar), 138.9 (C–N, thiazole), 149.2 (C=N), 165.8 (S=C=N, thiazole). *Anal.* Calcd for $C_{19}H_{17}Br_2N_3S$: C, 47.62; H, 3.58; N, 8.77; S, 6.69. found: C, 47.64; H, 3.59; N, 8.73; S, 6.71. HRMS: 475.5986 [M – H][–].

4.1.2.14. (*E*)-2-[(*Z*)-[1-(3-bromophenyl)propylidene]hydrazono]-4-(4-methoxyphenyl)-3-methyl-2,3-dihydrothiazole (**2c**). 63% yield; mp (°C): 185–190; IR (KBr, cm^{–1}): 1583.39 (C=N); ¹H NMR (DMSO-*d*₆, 300 MHz) δ ppm: 1.80 (t, *J* = 7.6 Hz, 3H, CH₃), 3.97 (q, *J* = 7.4 Hz, 2H, CH₂), 4.42 (s, 3H, CH₃), 4.68 (s, 3H, CH₃), 7.56 (d, *J* = 8.7 Hz, 2H, Ar), 7.81–7.89 (m, 3H, Ar), 8.12 (d, *J* = 7.5 Hz, 1H, Ar), 8.22 (d, *J* = 8.4 Hz, 1H, Ar), 8.46 (s, 1H, Ar), 13.61 (s, 1H, NH); ¹³C NMR (DMSO-*d*₆, 75.5 MHz) δ ppm: 11.4 (CH₃), 24.1 (CH₂), 39.9 (CH₃), 55.5 (CH₃), 106.9 (CH, thiazole), 114.8 (Ar), 119.7 (Ar), 122.8 (Ar), 125.8 (Ar), 130.2 (Ar), 130.8 (Ar), 133.3 (Ar), 136.5 (Ar), 137.6 (Ar), 144.5 (Ar), 161.5 (C–N, thiazole), 163.8 (C=N), 171.7 (S=C–N, thiazole). *Anal.* Calcd for $C_{20}H_{20}BrN_3OS$: C, 55.82; H, 4.68; N, 9.76; S, 7.45. found: C, 55.86; H, 4.63; N, 9.76; S, 7.42. HRMS: 430.0137 [M+H]⁺.

4.1.2.15. (*E*)-2-[(*Z*)-[1-(3-bromophenyl)propylidene]hydrazono]-3,4-diphenyl-2,3-dihydrothiazole (**3c**). 69% yield; mp (°C): 246–250; IR (KBr, cm^{–1}): 1585.32 (C=N); ¹H NMR (DMSO-*d*₆, 400 MHz) δ ppm: 1.80 (t, *J* = 6.0 Hz, 3H, CH₃), 2.58 (q, *J* = 6.0 Hz, 2H, CH₂), 3.86 (s, 3H, CH₃), 6.63 (s, 1H, Thiazole), 7.01 (d, *J* = 6.8 Hz, 2H, Ar), 7.18–7.32 (m, 5H, Ar), 7.58–7.70 (m, 5H, Ar), 7.95 (s, 1H, Ar); ¹³C NMR (DMSO-*d*₆,

75.5 MHz), δ ppm: 55.5 (CH₃), 70.7 (CH₂), 99.0 (CH₃), 115.1 (CH, thiazole), 123.0 (Ar), 125.3 (Ar), 127.3 (Ar), 128.2 (Ar), 128.9 (Ar), 129.0 (Ar), 129.5 (Ar), 129.6 (Ar), 131.0 (Ar), 130.2 (Ar), 130.6 (Ar), 133.5 (Ar), 138.1 (Ar), 144.0 (Ar), 161.0 (C–N, thiazole), 164.1 (C=N), 172.0 (S=C–N, thiazole). *Anal.* Calcd for $C_{24}H_{20}BrN_3S$: C, 62.34; H, 4.36; N, 9.09; S, 6.93. found: C, 62.36; H, 4.33; N, 9.06; S, 6.89. HRMS: 492.1973 [M+H]⁺.

4.2. Biological assays

4.2.1. Toxicity to splenocytes

Splenocytes from BALB/c mice were divided into plate with 96 wells at a density of 5×10^6 cells per wells in RPMI-1640 medium containing 10% inactivated Fetal Bovine Serum (FBS). Each chemical inhibitor was dissolved in DMSO at the concentration of 10 mg/mL and then the sample was serially diluted in RPMI-1640 medium supplemented with 10% FBS at the concentrations of 1.0, 5.0, 10, 25, 50 and 100 μ g/mL, in triplicate. As a positive control, we used saponin in concentration of 0.1 μ g/mL, while as the negative control wells received only an RPMI-1640 medium supplemented with 10% FBS and DMSO. The plate was added 1.0 μ Ci of ³H- thymidine to each well and the plate was incubated for 24 h at 37 °C and 5% CO₂. The plate was then read in the counter beta irradiation (Multilabel Reader, Finland) and tritiated thymidine percent incorporation was determined. For cells that were not treated with drugs (negative control) was calculated as 100% of tritiated thymidine incorporation (100% viable cells). For cells treated with saponin, cell viability was 5%. When the percentage of incorporation was higher than 90%, the concentration of the drug was regarded as nontoxic to splenocytes.

4.2.2. Toxicity to trypomastigotes

Strain Y trypomastigotes were collected from Vero cells supernatant and distributed in a plate with 96 wells for a final density of 4×10^5 cells per well. Each chemical inhibitor was added to the wells in triplicate. Benznidazole were used as positive controls in this assay. The plate was then cultivated for 24 h at 37 °C containing 5% CO₂. After this time, aliquots from each well were collected and the number of viable parasites (ie, with apparent motility) was counted in a Neubauer chamber. To the wells that did not receive the chemical inhibitors, it was assumed as 100% the number of viable parasites. The dose-response curves were determined and the IC₅₀ values (μ M) were calculated using at least seven concentrations (data-points) using nonlinear regression (Prism, version 4.0).

4.2.3. Ultrastructural studies

The parasites were cultured for 24 h in medium RPMI 1640 medium (SigmaAldrich, St. Louis, MO, USA) buffered to pH 7.5, supplemented with HEPES (20 mM), 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μ g/mL) containing the compound **1c** in IC₅₀ concentration and twice the value of IC₅₀. The parasites were collected, washed in PBS and fixed with 2.5% glutaraldehyde, 4% formaldehyde, and 0.1 M cacodylate buffer at pH 6.8. They were then postfixed in 2% osmium tetroxide (OsO₄) in a 0.1 M cacodylate buffer at pH 6.8 and processed for routine transmission electron microscopy (TEM) and scanning electron microscopy (SEM). For SEM analysis, the parasites were dehydration in a graded series of acetone and finally embedded in epon. Sections were stained with uranyl acetate and lead citrate and observed with Tecnai spirit G2 Biotwin microscope.

4.2.4. Cruzain analysis

Recombinant cruzain truncated at the C-terminal was kindly provided by Allison Doak and Brian Shoichet (UCSF, USA). Cruzain activity was monitored as previously reported, based on the fluorescence resulting from the cleavage of the substrate Z-Phe-Arg-aminomethylcoumarin (Z-FR-AMC), with 340/440 nm excitation/emission filters. The assays were carried in a microplate reader Synergy 2 (Biotek®) from the Center of Flow Cytometry and Fluorimetry at the Biochemistry and Immunology Department (UFMG). Fluorescence was measured at 25 °C, for five minutes at 12 s intervals, using BioTek's Gen5™ Reader Control and Data Analysis Software. Reaction rates were calculated from initial velocity rates compared to a DMSO control. Compounds were tested in sodium acetate buffer 0.1 M, pH 5.5, containing 0.01% Triton X-100, 1 mM β -mercaptoethanol, 1 nM cruzain and 2.5 μ M substrate. There was no pre-incubation with the enzyme. All assays were performed in at least twice in independent experiments, each performed in triplicate. When the inhibition was higher than 80% at highest soluble concentration for the compounds evaluated (300, 200 and 100 μ M), IC₅₀ curves were determined based on seven different compound concentrations and calculated using GraphPad Prism 6 (GraphPad, San Diego, USA), employing the nonlinear regression “log (inhibitor) vs response with variable slope – four parameters” analysis.

4.3. Docking studies

The compounds **1a-b**, **1d-e** and **1h-m** were selected for *in silico* studies, because their inhibitory potency (IC₅₀) was measured against the cysteine protease of *Trypanosoma cruzi* (cruzain), as one can see in Table 3. The structures and conformational analysis were obtained through the application of the RM1 semi-empirical approach [41], which is available as part of the SPARTAN 08® program [42], using internal default settings for convergence criteria. The analysis and docking calculation were carried using the *T. cruzi* cruzain target [15], available at the RCSB Protein Data Bank (PDB ID: 3IUT), which is composed of a co-crystallized complex with an inhibitor (referred as KB2). The space for searching of docking solutions was defined to lie within a region of 27.0 Å in direction X, 25.5 Å in direction Y, and 31.5 Å in direction Z, centered on the coordinates based on the reference of the co-crystallized ligand KB2 (X = 2.772; Y = 12.987; Z = 4.357; all in Å). The docking calculations were carried out using the AutoDockTools and AutoDock (v.4.2) programs [43], using the default parameters for all the variables, except for the number of docking runs (50), the maximum number of energy evaluations on Genetic Algorithm-GA (25,000,000), and the maximum number of generations in GA (10,000). Preliminary calculations indicate that these three specific parameter modifications provide significant improvement in the results obtained using the docking procedure, thereby producing more significant solutions. The ligands were then docked using the Genetic Algorithm followed by a Local Search procedure (GA_LS), also known as a Lamarckian Genetic Algorithm (LGA), and the 50 lowest energy structures were stored for further analysis. The residues GLN19, CYS25, SER61, LEU67, MET68, ASN70, ASP161, HIS162 and TRP184 were treated as flexible during the calculations, in order to take into account some induced fit effects. The Binana program [44] was used to analyze the molecular interactions present in the best docking solutions, using default setting, except for hydrogen bond distance, which was changed to a maximum of 3.5 Å. Figures were generated with Pymol software [45].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2016.05.050>.

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