**Anti-Parasite effects of new Thiosemicarbazones and their Products Thiazolidinone including Cellular aspects of Intracellular Elimination of Trypanosoma Cruzi in Vitro**

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**Abstract**

*Trypanosoma cruzi*, agent of Chagas disease in humans, invades and replicates within a wide variety of nucleated mammalian cells until the lysis of the host cells. This study was undertaken to evaluate the cellular features of effect of new compounds of thiosemicarbazone and their thiazolidinone derivate on the multiplication of extra- and intracellular *T. cruzi*. Most of the compounds interrupted epimastigote proliferation at 1 mM, and above 5 mM led to drastic cytoplasmin reduction, nuclear condensation and death. Ultrastructural assays showed that epimastigotes treated with 1 mM of the compounds retained main parasite organelles such as the Golgi complex and the mitochondria, but underwent drastic reduction in cytoplasm volume and led to the formation of blebs on the plasma membrane, suggesting a cell death process by apoptosis. Infected cultures treated for 24 h with the compounds showed similar effects, with drastic decrease in infection and the elimination of intracellular parasite at 1 mM without toxic effects on the host cells. Intracellular amastigotes showed progressive disorganization leading to the rupture and elimination of the parasite. The results strongly suggest that in presence of thiosemicarbazones and their derivate, intracellular amastigotes arrested the proliferation, leading to irreversible ultrastructural disorganization, death, and then elimination. When the intracellular *T. cruzi* elimination process was analysed, it showed that autophagy was present, in intriguing and new features to futures studies. In addition, our results *in vitro* also suggest that these compounds are promising molecules against intracellular *T. cruzi* when compared to the anti-proliferative drugs Hydroxyurea and Benznidazole.

**Keywords:** Anti-parasite drug; Thiazolidinone; Thiosemicarbazone; *Trypanosoma cruzi*

**Introduction**

The kinyto plastid protozoan parasite *Trypanosoma cruzi* is the causative agent of human Chagas’s disease, and it is endemic in regions of Latin America where it has become a major public health problem, constituting one of the largest parasitic disease burdens [1]. *T. cruzi* has a complex life cycle in which different developmental forms are present in both vertebrate and invertebrate hosts [2]. Three forms are well described: 1. amastigotes (dividing form found in the cytoplasm of the vertebrate host cell); 2. trypomastigotes (invasive form); and 3. epimastigotes (extracellular and replicating form found in the invertebrate host). In the vertebrate host, the replication of intracellular *T. cruzi* is responsible for the main pathologies associated with chronic Chagas’s disease [3]. The establishment of the parasite inside the host cells is key to the success of the infection and the progression of the disease. The drugs which are most frequently used for the treatment of Chagas’s disease are nitroheterocyclic compounds: Nitrofurans (Nifurtimox) and nitroimidazoles such as Benznidazole, discovered over three decades ago which induce drastic toxic effects in the vertebrate [1]. New therapeutic compounds are needed, mainly because the major limitation of anti-*T. cruzi* chemotherapeutics in the chronic phase of Chagas’s disease is their low efficiency [4].

It was previously demonstrated the anti-parasitic effects of Hydroxyurea (HU), a semicarbazone anticancer drug, on the intracellular *Toxoplasma gondii*, *Leishmania amazonensis* and *T. cruzi* in vitro [5]. Hydroxyurea, thiosemicarbazones (TSC) and thiazolidinones (TZN) inhibit the cell cycle in the G1/S phase by inactivating the enzyme ribonucleotide reductase and the synthesis of DNA [6,7], but TSC are 1000 times more potent than Hydroxyurea [8].

Recently, it has demonstrated that TSC and analogue compounds, 4-thiazolidinones (4-TZN), decreased *in vitro* infection and induced intracellular elimination of *T. gondii* [9,10]. This study also used TSC substituted at the aryl hydrazone moiety with a nitro substituent at the ortho, meta and para positions, and 4-thiazolidinones (TZN) substituted at the N-3 position with a phenyl, methyl and hydrogen substituent, and with the same groups at the arylhydrazine moiety. These molecular structures have a good cellular perfusion where they target the parasite and interrupt its multiplication. Carvalho et al. [11] showed that after the arrest of intracellular proliferating parasite, the death of *T. gondii* and its digestion by lysosomes fusion occurs.

Thus, the aim of the present study was to evaluate the effects of these TSC and TZN compounds against extra- and intracellular *T. cruzi*. Furthermore, we also report the morphological and ultrastructural features of the parasite in the presence of these drugs.

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Material and Methods

Host cells

Vero cells (kidney fibroblasts of the African monkey) were grown in plastic Falcon flasks (25 cm²) containing medium 1152 (Sigma-Aldrich, St. Louis, MO, USA) with 5% foetal calf serum (FCS; Gibco, Grand Island, NY, USA), and were trypsinized when the cell density approached a semi-confluent monolayer. One day before use in the experiments, approximately 3 x 10⁵ cells were placed on Linbro 24-well tissue plates containing a sterile round coverslip, or were placed in 25 cm² flasks (3-5 x 10⁶/flasks), and maintained at 37°C overnight in a 5% CO₂, 95% air atmosphere.

Parasite cultures and host cell infection

Epimastigotes of T. cruzi (DM28 strain) were cultivated in liver infusion triptase (LIT) medium supplemented with 10% FCS at 28°C. The epimastigotes were incubated at a density of 10⁶ parasites/mL in the compounds for 24-120 h as described below.

Trypomastigotes of T. cruzi (DM28 strain) were obtained from a culture of Vero cells infected for 6-7 days at 37°C. The triatomine bugs were incubated with host cells for 48 h at 37°C in medium supplemented with 5% FCS. All parasites were assumed to have interacted with the cells because the parasite: host cell ratio was 10:1.

Chemistry

The synthesis of TSC (1, 3, 5-9) and TZN (2, 4, 10-14) derivatives followed the method described [12]. The nomenclature of the compounds are the following: compound 1 (benzaldehyde thiosemicarbazone); compound 2 (acid[2-(bephenylidine-hidrazone) -4-oxo -1,3-thiazolidin-5-yl] acetic); compound 3 (para-nitrobenzaldehyde thiosemicarbazone); compound 4 ([(4-chloro-benzylidine-hidrazone)-4-oxo-1,3-thiazolidin-5-yl] acetic acid); compound 5 (4-chlorobenzaldehyde 4-phenyl-3-thiosemicarbazone); compound 6 (3,4-dichlorobenzaldehyde 4-phenyl-3-thiosemicarbazone); compound 7 (2-flurobenzaldehyde 4-phenyl-3-thiosemicarbazone); compound 8 (4-flurobenzaldehyde 4-phenyl-3-thiosemicarbazone); compound 9 (3-chlorobenzaldehyde 4-phenyl-3-thiosemicarbazone); compound 10 (2-[3-(chlorophenyl) methylene] hydrazone) - 4-oxo-3-phenyl-5-thioazolinedione acid); compound 11 (2-[3,4-dichlorophenyl] methylene] hydrazone) - 4-oxo-3-phenyl-5-thioazolinedione acid); compound 12 (2-[3,4-dichlorophenyl] methylene] hydrazone) - 4-oxo-3-phenyl-5-thioazolinedione acid); compound 13 (2-[3-(4-flurophenyl) methylene] hydrazone) - 4-oxo-3-phenyl-5-thioazolinedione acid); compound 14 (2-[3-(4-flurophenyl) methylene] hydrazone) - 4-oxo-3-phenyl-5-thioazolinedione acid) (figure 1). Briefly, the TSCs were obtained by reactions between the thiosemicarbazide and the benzaldehydes substituted in acid medium under reflux (yields 57-98%). These products were cyclized with thiosemicarbazide and the benzaldehydes substituted in acid medium (figure 1). Briefly, the TSCs were obtained by reactions between the thiosemicarbazide and the benzaldehydes substituted in acid medium under reflux (yields 57-98%). These products were cyclized with thiosemicarbazide and the benzaldehydes substituted in acid medium (figure 1). Briefly, the TSCs were obtained by reactions between the thiosemicarbazide and the benzaldehydes substituted in acid medium under reflux (yields 57-98%). These products were cyclized with thiosemicarbazide and the benzaldehydes substituted in acid medium (figure 1). Briefly, the TSCs were obtained by reactions between the thiosemicarbazide and the benzaldehydes substituted in acid medium under reflux (yields 57-98%). These products were cyclized with thiosemicarbazide and the benzaldehydes substituted in acid medium (figure 1).

Morphological analyses and quantification

For morphological analyses, infected and untreated infected cultures were rinsed with PBS, pH 7.2, at 37°C and then fixed with Bouin’s solution for 15 min and stained with Giemsa (10% v/v) for 2 h at room temperature. DMSO solved all drugs and added to DMEM medium. DMSO used at a final concentration of 1% (v/v), which was not toxic for Vero cells or parasites (data not shown). Infected cells were incubated in the presence of HU, BZN and all compounds for 24 h at 37°C. Trypan blue (0.2% w/v) was used to assay the viability of treated and untreated infected cells.

Treatment of parasites

Extracellular parasites, Epimastigotes of T. cruzi (DM28 strain), were cultivated as described above and incubated with the drugs for 24-120 h in a range of concentrations from 0.1 to 10 mM.

For morphological analyses, the epimastigote form of T. cruzi was rinsed with PBS, pH 7.2, at 37°C. Parasites were fixed in a solution containing 1% glutaraldehyde, 4% paraformaldehyde, 5 mM CaCl₂, and 5% sucrose in 0.1 M-cacodylate buffer, pH 7.2 for 15 min and stained with Giemsa (10% v/v) for 2 h at room temperature. All preparations were examined using a Zeiss Axioskop microscope equipped with a 63X objective and showed images obtained with the Analysis System software (USA).

At least 300 cells in the same monolayer scored at 40X magnification for each drug and each time tested. The following parameters were analysed in treated and untreated cultures: a) infected cells containing normal intracellular amastigotes, rounded shape; b) uninfected cells; and c) parasites. Finally, the LD50 values of all compounds for host cells and intracellular parasites obtained after 24 h of exposure at the concentration of 0.1-10 mM.

Statistical analysis was performed by two-way ANOVA (Graph Pad Prism). P values of <0.05 were considered to be significant. The data reported here are representative of three experiments performed in quadruplicate.

In order to observe the acidic compartments, untreated or treated infected cultures were incubated with acridine orange (Sigma Chemical Co.) or Lyso-tracker red (Molecular Probes, Inc. Eugene, OR, USA)- the lysosomes specific probe. Acridine orange (5µg/mL) was added to the medium DMEM 1152 without FCS and added to the cultures for 30 min at 37°C, as previously used by Carvalho and Melo [11]. Lyso-tracker red (50nM) was added in the medium 1152 without FCS and...
added to the cultures for 30 min at 37°C. The cultures were washed with medium 1152 and the cells were examined at a Zeiss Axioplan photomicroscope equipped with a 63X objective and showed images obtained with the Analysis System software (USA), 429 and 577 nm UV excitation filter, respectively.

The selective marker monodansylcadaverine (MDC) (Sigma Aldrich*) was used for autophagic vacuole detection. For MDC staining, the live infected, treated or untreated cells were incubated with 0.05 mM MDC in PBS for 10 min for subsequent observation in a fluorescence microscope Zeiss Axioplan using UV excitation filter (350 nm) [13].

**Ultrastructural analyses**

Transmission Electron Microscopy (TEM). For ultrastructural analyses, untreated or treated epimastigotes, or Vero cells infected with parasites were washed with PBS at 37°C and fixed at room temperature in a solution containing 1% glutaraldehyde, 4% paraformaldehyde, 5 mM CaCl₂, and 5% sucrose in 0.1 M cacodylate buffer, pH 7.2. Cells were postfixed for 1 hour in a solution containing 2% OSO₄, 0.8% potassium ferrocyanide, and 5 mM CaCl₂ in 0.1 M-cacodylate buffer, pH 7.2, rinsed with 0.1 M cacodylate buffer, pH 7.2, dehydrated in acetone and embedded in PolyBed (Polyscience Inc., Germany). Thin sections were stained with uranyl acetate and lead citrate, and were viewed with a Zeiss 900 Transmission Electron Microscope at 80 kV.

**Scanning Electron Microscopy (SEM)**

For scanning electron microscopy the untreated and treated cells, were fixed in 2.5% (v/v) glutaraldehyde, 4% (v/v) formaldehyde, 5 mM CaCl₂, and 5% sucrose dissolved in cacodylate buffer 0.1 M (pH 7.2) at room temperature for 2 h. The samples were postfixed in 1% osmium tetroxide in the same buffer for 1 h, dehydrated in a gradient series of ethanol and immediately critical-point dried in CO₂. The dried samples were positioned on a specimen support prior to sputtering with gold. All micrographs were taken with a ZEISS EVO 40 microscope at 15 kV, employing secondary electrons.

**Results**

**Epimastigote**

Epimastigotes, the extracellular replicative form of *T. cruzi*, was exposed to the compounds using different concentrations (0.1-10 mM) and incubation times, as demonstrated in (Tables 1 and 2) respectively. The analysis of epimastigotes toxicity was assessed by parasite elimination (Figure 2).

The number of epimastigotes decreased after the incubation with the compounds since the concentration of 0.1 mM (Table 1), and led to the almost complete elimination of the parasites at 5 mM (70-80%), with the exception of compound 4. For compounds 2, 3, 5, 7, 8, 10, 11, 12, and 14 the drastic elimination of parasites occurred after the 1 mM concentration. The complete elimination occurred at 10 mM for all compounds. In order to observe when the elimination occurred, the epimastigotes were only incubated at 0.1 mM, but until 120 hours (Table 2). The compounds 1, 6, 9 and 13, arrested parasites proliferation since the first period of incubation (24 hours) and continuously arrested until 72 hours, when the parasite elimination started and the number of parasites decreased in the cultures. This parasite toxic effect (parasite elimination) was similar to BZN. The anti-proliferative and toxic effects (elimination) of TSC and 4-TZN were mainly more effective than HU, where compounds 1, 6, 9 and 13 also showed drastic parasite reduction liked BNZ effects.

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**Table 1:** Epimastigotes of *Trypanosoma cruzi* incubated with compounds (14), hydroxyurea (HU) and benzimidazole (BZN) (96 h) at 28°C and pH 7.2. Each bar represents the standard deviation. Values are mean ± SD (n=3). The toxic effect of treatment was measured by growing the parasites in LIT for 96 h and quantifying cell live.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control</th>
<th>0.1 mM</th>
<th>1 mM</th>
<th>5 mM</th>
<th>10 mM</th>
</tr>
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<tbody>
<tr>
<td>TSC</td>
<td>14 ± 0.7</td>
<td>5 ± 0.2</td>
<td>2 ± 0.2</td>
<td>1 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>4-TZN</td>
<td>11 ± 0.7</td>
<td>5 ± 0.7</td>
<td>2 ± 0.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HU</td>
<td>36 ± 2</td>
<td>33 ± 1.8</td>
<td>7 ± 0.5</td>
<td>3.6 ± 0.5</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>BZN</td>
<td>14 ± 1.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Epimastigotes treated with 0.1 mM for different time (h) (× 10°6)</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
</tr>
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<tbody>
<tr>
<td>Compound</td>
<td>0</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>TSC</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>4-TZN</td>
<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>HU</td>
<td>1.5 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>BZN</td>
<td>10 ± 0.4</td>
<td>10 ± 0.4</td>
<td>10 ± 0.4</td>
<td>10 ± 0.4</td>
<td>10 ± 0.4</td>
<td>10 ± 0.4</td>
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To observe the ultrastructural features of epimastigotes during the actions of the compounds, parasites were incubated with compound 1 at 1 mM for 24 hours (Figure 2). The elongated structure in the untreated parasite observed under light (Figure 2a), transmission (Figure 2e) and scanning electron microscopy (Figure 2b), changed drastically after treatment as can be observed (Figure 2c,d,f). The parasites underwent loss of cytoplasm volume leading to a circular form. However, this drastic morphological effect did not achieve all parasites at the same time, since elongated epimastigotes were still noted (Figure 2c, arrow). Although the flagellum elongation did not affect, several blebs occurred in the plasmatic parasite membrane (Figure 2f), suggesting an apoptosis process.

The Vero cells infected with triompastigotes of *T. cruzi* for 48 hours at 37°C showed proliferating intracellular amastigotes, an established infection (Figure 3a and 3b). Then, these infected cultures were incubated with different concentrations (0.1-10 mM) of TSC and 4-TZN for 24 hours, and quantitative and morphological analyses were performed.
performed to observe the anti-\textit{T. cruzi} action of the drug.

Anti-parasitic effects in the presence of all compounds showed a decrease in infection (Table 3) and elimination of intracellular amastigotes (toxic effects) (Table 3) until 5 mM. At 10 mM, the new compounds, but not HU or BZN, achieved toxic effects on the host cells.

TSC (compounds 1,3, 5, 6, 7) and 4-TZN (compounds 10, 11, 12) incubated during 24 hours showed drastically the percentage of infection at 1 mM (Table 3). The intracellular amastigotes, when present, were disrupted (Figure 3d). On the other hand, the HU and BZN treatments caused a progressive decrease in infection only after incubation at 5 mM. Even though the treatment with BZN slowly reduced the infection and eliminated the intracellular amastigotes, it was accompanied by high vacuolization in the cytoplasm and the death of the host cells.

The important fact was that the TSC and TZN led to parasite elimination at a low dose and after the infection had been established. None of the compounds used here had drastic cytotoxic effects on the host cell until 5 mM, as demonstrated by the Trypan blue test (data not shown).

To observe the stage and cellular features of intracellular parasite elimination, the compound 1 treatment was used.

Acridine orange, a vital probe indicative of pH acidification, showed increase in fluorescent staining in different areas of the infected host cell cytoplasm including the parasite location (Figure 4, arrowheads). In the same way, the staining with the lysosomes specific probe (Lysotracker red) showed an increased fluorescent pattern in these organelles and the parasite site (Figure 5, arrowheads). These results suggested the involvement of a kind of vacuole digestion process with the lysosomes participation.

The analysis of MDC positive staining indicated that in the treated infected host cells, autophagic vacuoles (containing parasite) were present, while in untreated infected cells they did not occur (Figure 6). These results also suggest the involvement of autophagy process with the elimination of intracellular \textit{T. cruzi}.

Transmission electron microscopy showed the progressive effects of the compound 1 on parasite morphology and ultrastructure (Figure 7). In untreated cultures (Figure 7a), the cytoplasm contained normal organelles and proliferative amastigotes in the cytoplasm. In the cultures treated with the compound 1 at 1 mM for 24 hours, some intracellular parasites were located inside vacuoles surrounded by double-membrane (Figure 7b,c) and showed drastic morphological alterations or were disrupted (Figure 7d). This parasite disorganization and elimination occurred in an asynchronous manner, suggesting that the drugs target the parasite at different steps from the proliferative cycle.

However, with BZN, the amastigotes showed variable degrees of alteration, such as cytoplasmic vacuolization, loss of volume in the cytoplasm, and the rupture of the plasma membrane, with drastic and significant consequences to the host cell (data not shown) as also demonstrated [14].

The lethal dose (LD50), the concentration that caused the elimination of 50% of population, was demonstrated (Table 4). Compounds 1, 6, 9, and 13 had similar values to BZN, while compounds 7, 10, 11, 12 had similar lethal dose concentration values to HU. However, compounds 7, 11, and 12 only caused similar lethal dose in extracellular and intracellular parasites.

In addition, the results above showed the importance of position and type of the radical inserted in the benzene ring of each compound to quickly arrest extra and intracellular proliferation and induce toxic effects in the parasites. Compounds 5, 11 and 12 may be of great interest to quickly arrest extra and intracellular proliferation and induce toxic effects in the parasites.

Discussion

The treatment of Chagas disease involves two drugs based on the compounds Nifurtimox and Benznidazole. These compounds are relatively effective to the tripomastigotes and amastigotes forms. However, they have some limitations that include a variable action on chronic phase, cytotoxicity and the induction of parasite resistance.
and intracellular forms of T. cruzi. The TSC scaffold has advantages in the synthesis of new compounds, such as low molecular weight, good hydrogen bond donating and accepting capabilities, and easy, economical synthesis routes. TSC (a Class of Shiff base compounds containing thiourea moiety) are well known for their biological activity as antiparasitics, antibacterial and antimalarial agents [16]. The principal target of TSC involves the inhibition of the ribonucleotide diphosphate reductase (rdr), the arresting DNA synthesis and the cell cycle on G1/S phase [17]. Moreover, TSC can also chelate endogenous metals such Fe (III), forming stable complexes and inhibiting metal-dependent enzymes, such as cysteine proteases [18]. This cysteine proteases inhibition with TSC could be through a covalent modification of the cysteine thiol groups via the electrophilic centre (the thione carbon and/or imine carbon) of the thiosemicarbazone moiety [16].

Recent studies have also demonstrated the efficacy of TSC compounds associated with metals, like platinum, against Trypanosoma cruzi, with potent activity against cruzain [19]. Other studies showed that some TSC, such as metronidazole-thiosemicarbazone compounds, were highly effective against Entamoeba histolytica and Plasmodium spp. [20]. In the present study, it was firstly demonstrated the chemical profile of the TSC as anti-T. cruzi action. Despite the wide pharmacological range of this class of compounds, the communal properties of the drugs used here affected both extra- and intracellular T. cruzi. In these compounds used here, there are H, Cl or NO₂ groups on the arylhydrazone moiety. This chemical structure showed proteases inhibition with TSC could be through a covalent modification depending enzymes, such as cysteine proteases [18]. This cysteine protease inhibition with TSC could be through a covalent modification of the cysteine thiol groups via the electrophilic centre (the thione carbon and/or imine carbon) of the thiosemicarbazone moiety [16].

For the last case, in the last few years a large number of potential targets of chemotherapy for Chagas’ disease have been described [15]. Nevertheless, little is known about TSC or TZN as anti-parasite actions.

In the present study, it was demonstrated the in vitro efficacy of new thiosemicarbazone compounds against proliferative extra-
Demonstrated of the cellular structure target was observed. TSC and TZN have been demonstrated for the last few years, little is known about the death and elimination mechanisms involved with amastigotes. Scale bars: A=2 μm; C=0.5 μm; B,D=100 μm.

Nevertheless, while several anticancer and antiparasitic tests using TSC and TZN have been demonstrated for the last few years, little of the cellular structure target was observed. [5,10]. Demonstrated that HU, TSC and TZN respectively, were efficient at arresting the multiplication of intracellular Toxoplasma gondii, Leishmania amazonensis and Trypanosoma cruzi, they also demonstrated that a drastic morphological parasite disorganization occurred, which led to parasite elimination, without toxic effects on the host cells [5,10]. In case of T. cruzi, a parasite that replicates within the cytoplasm of the host cell does not interage with the endo cytic pathway of the cell, so there is no known mechanism for parasite elimination. [5,10]. These results showed that the anti proliferative action of the compounds lead to a development of a mechanism to eliminate the parasite which involves the formation of autophagic vacuole, followed by lysosomes fusion recognition of endocytic pathway and finally, parasite digestion and elimination from the intracellular environment. In the case of intracellular T. gondii treated with TSC or TZN, the parasite was eliminated by a process that involves the fusion of parasitophorous vacuole with lysosomes, as demonstrated [10]. For Trypanosomes intracellular parasites, however, the mechanisms involved with death and elimination in presence of TSC or TZN should be studied. In case of intracellular T. cruzi it can also occur, since in the presence of a low concentration of the compounds, mainly the compound 1, the parasite elimination is more effective. Although the molecular mechanism of action of HU, TSC and TZN is mainly associated with the arrest of parasite reproduction, these trypanocidal compounds also induce toxic effects on the host cells. Anyway, oxidative damage is not the key action of BNZ, as the corresponding nitroanion radical is not the key action of BNZ, as the corresponding nitroanion radical was only detected at concentrations much higher than those that killed the parasite. The action of BNZ could involve a covalent bond or other interactions of nitroreduction intermediates with parasite components [29], or binding to DNA, lipids and proteins [30]. Some studies have already explored the morphological features associated with Trypanosoma death in the presence of HU, TSC or TZN [5]. Intracellular parasites [5] T. gondii, L. amazonensis and T. cruzi were eliminated in presence of HU. A recent study by our group [12] showed that the disorganization and destruction of intracellular Toxoplasma in the presence of HU, TSC and TZN, using non-macrophage cells, involved fusion between the lysosomes of host cells and the vacuole containing the parasites [12,10]. These cellular aspects demonstrate that the PV becomes permissive to lysosome fusion when the parasite is dead. This event is associated with the elimination of the parasite by the digestive pathway of the host cell-a major molecular mechanism of cell defence against microorganism invasion. In this case, the death and elimination mechanisms involved with amastigotes in the presence of TSC and TZN will be studied.

Taking together, these results indicate that the autophagic process is responsible for the parasite elimination from the host cell.
Overall, the results obtained is considered so far to be promising since they provide interesting data about thio semicarbazones and their specific action on intracellular Trypanosoma cruzi and other trypanosomatids. The efficiency of TSC compounds in interrupting cell multiplication supports the potential extensive application of TSC and TZN to anti-parasite therapy.

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