

## **Do thiazolidine compounds act on intracellular amastigotes of *Trypanosoma cruzi*? A systematic review**

Compostos tiazolidínicos atuam sobre formas amastigotas de *Trypanosoma cruzi*? Uma revisão sistemática

Actuan los compuestos tiazolidínicos sobre los amastigotas intracelulares de *Trypanosoma cruzi*? Una revisión sistemática

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## **Abstract**

Benznidazole (Bdz) are the drug of choice to treat Chagas Disease. However, the drug causes several side effects, and *Trypanosoma cruzi*, the etiological agent of Chagas disease, can be less susceptible to the action of drugs in the chronic phase, due to its reduced metabolism and dormancy in tissues. Thus, using compounds that are lethal to amastigote forms, which are prevalent in the Chagas disease chronic phase, is essential for the success of therapy. We propose to evaluate, through a systematic review, the efficacy of thiazolidine and its imidazolidine derivatives against *T. cruzi* intracellular amastigotes, and to compare the results with those for Bdz. A systematic search was made on eight English Language Systematic Databases - Science Direct, Scopus, Pubmed, Google Scholar, LILACS, Scielo, Trip Database and Cochrane, to collect studies, without a time scale. IC<sub>50</sub> values, cytotoxic effects (CC<sub>50</sub>), a selective index (SI), the mechanism of action of each compound and the length of treatment was included in this review to

evaluate the effectiveness of each compound. The compound 2-Iminothiazolidin-4-one 18 was more effective than Bdz, as it affected intracellular amastigotes by making a structural modification in the parasite, and by inhibiting cruzain, being promising for the antichagasic therapy.

**Keywords:** Alternative treatment; Antichagasic drugs; Benznidazole; Intracellular amastigotes; Thiazolidine compounds.

### Resumo

O Benznidazol (Bdz) é a droga de escolha para o tratamento da doença de Chagas. No entanto, o medicamento possui vários efeitos colaterais, e o *Trypanosoma cruzi*, agente etiológico da doença de Chagas, pode ser menos suscetível à ação de drogas na fase crônica, devido ao seu metabolismo reduzido e dormência nos tecidos. Assim, a utilização de compostos letais às formas amastigotas, prevalentes na fase crônica da doença de Chagas, é essencial para o sucesso da terapia. Nesse contexto, propomos avaliar, através de uma revisão sistemática, a eficácia de compostos tiazóis e seus derivados imidazóis contra amastigotas intracelulares de *T. cruzi*, e comparar esses dados com a eficácia do Bdz. Scopus, Pubmed, Google Scholar, LILACS, Scielo, Trip Database e Cochrane, para coleta de estudos, sem escala de tempo. Valores de IC<sub>50</sub>, efeitos citotóxicos (CC<sub>50</sub>), Índice de seletividade (ISE), o mecanismo de ação de cada composto, e a duração do tratamento foram incluídos neste estudo para avaliar a eficácia de cada composto. O composto 2-iminotiazolidin-4-ona 18 foi mais eficaz que o Bdz, afetando as formas amastigotas intracelulares através de modificação estrutural no parasita, e por inibição da cruzaina, sendo considerado um composto promissor para a terapia antichagásica.

**Palavras-chave:** Tratamento alternativo; Drogas antichagásicas; Benznidazol; Amastigotas intracelulares; Compostos tiazolidínicos.

### Resumen

El benznidazol (Bdz) es el fármaco de elección para tratar la enfermedad de Chagas. Sin embargo, el fármaco provoca varios efectos secundarios, y *Trypanosoma cruzi*, el agente etiológico de la enfermedad de Chagas, puede ser menos susceptible a la acción de los fármacos en la fase crónica, debido a su metabolismo reducido y latencia en los tejidos. En ese contexto, el uso de compuestos letales para las formas de amastigote, que son frecuentes en la fase crónica de la enfermedad de Chagas, es esencial para el éxito de la terapia. Proponemos evaluar, a través de una revisión sistemática, la eficacia de compuestos tiazolidínicos y sus derivados de imidazolidínicos contra los amastigotes intracelulares de *T. cruzi*, y comparar estos datos con los de Bdz. Se realizó una búsqueda sistemática en ocho bases de datos sistemáticas en idioma inglés - Science Direct, Scopus, Pubmed, Google Scholar, LILACS, Scielo, Trip Database y Cochrane, para recolectar estudios, sin escala de tiempo. Los valores IC<sub>50</sub>, los efectos citotóxicos (CC<sub>50</sub>), índice de selectividad (ISE), el mecanismo de acción de cada compuesto y la duración del tratamiento se incluyeron en esta revisión para evaluar la eficacia de cada compuesto. El compuesto 2-Iminothiazolidin-4-one 18 fue más efectivo que Bdz, ya que afectó a los amastigotes intracelulares al realizar una modificación estructural en el parásito, así como inhibiendo la cruzaina, siendo un compuesto promisorio para la terapia antichagásica.

**Palabras clave:** Tratamiento alternativo; Fármacos antichagásicas; Benznidazole; Amastigotes intracelulares; Compuestos tiazolidínicos.

## 1. Introduction

Chagas disease (CD), or American trypanosomiasis, is a neglected tropical disease (NTD) caused by a hemoflagellate protozoan *Trypanosoma cruzi*. The disease is endemic in 21 Latin American countries, and 6 - 7 million people in the world are infected (WHO 2020). The number of cases of CD has been increasing in North America and Europe, due to globalization (Liu et al. 2015, Requena-Méndez et al. 2015). *T. cruzi* transmission occurs congenitally, via organ transplantation, orally, due to the ingestion of parasite-contaminated food or drink, or vectorially (WHO 2018).

Clinically, CD has acute and chronic phases. The acute phase is characterized by a high parasitaemia, with circulating trypomastigotes, and is asymptomatic in most cases. The acute phase may range from being asymptomatic to a severe presentation in <1% of cases, such as fulminant myocarditis or meningoencephalitis (Yeung et al., 2020); on the other hand, intracellular amastigotes are predominant in the chronic phase and concentrate mainly in the muscles, such as myocardium, and ganglion cells (Rassi et al., 2012). In general, 60 - 70% of chronic patients are asymptomatic. However, 30 - 40% will develop serious symptoms of the disease, which include cardiac complications in 94.5% of cases and digestive complications in 4.5% of cases (Meneghelli et al., 1982, Teixeira et al., 2006).

Trypomastigotes and intracellular amastigote forms of *T. cruzi* are the main targets of CD therapy. Nevertheless, knowledge of CD pathogenesis suggests that the chronic phase should be more widely treated, with the following aims: to reduce the quantity of the parasite in the organism until it is totally eliminated, to promote tissue regeneration through parasite inactivation in the tissue foci, as well as to reverse existing fibrosis (Coura et al., 2012).

Currently, Benznidazole (Bdz) is the only etiological treatment commercially available for the disease (Viotti et al., 2009). However, the drug is toxic and poorly effective during the chronic phase of the disease (Assis et al., 2013). Furthermore, *in vitro* studies reported that Bdz has a high neurotoxicity and deleterious effects on the adrenal gland, the colon and breast tissue (Coura et al., 2002).

The World Health Organization reports CD as one of the most important parasite infections in the world, responsible for an estimated US\$ 1.2 billion per year in lost productivity (WHO 2012). Therefore, the development of an effective antichagasic drug is necessary, especially to treat chronic patients, since they can be susceptible to serious complications, such as cardiomyopathy and digestive alterations.

In this context, pre-clinical drugs such as thiazolidine and its imidazolidine derivatives, are available as trypanocidal potentials. Thiazolidines are isosteric imidazolidines with a heterocyclic ring system, and their hybrids have been reported to have promising anticancer and antiviral properties (Silva-Júnior et al., 2016). On the other hand, trypanocidal effects of these compounds are reported (Moreira et al., 2013).

Due to the clinical importance of chronic CD, we proposed to evaluate, through a systematic review, whether thiazolidine compounds can be effective against *T. cruzi* intracellular amastigotes compared to Bdz.

## 2. Methodology

### 2.1 Guidelines

In this systematic review of the literature, the guidelines established by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) were followed (Moher et al., 2009).

### 2.2 Eligibility criteria

The selected articles met the following criteria: original articles that were published in English, without time scale (Moura et al., 2021); *in vitro* studies about the trypanocidal action of thiazolidine and its imidazolidine derivatives against amastigote forms of *T. cruzi*. IC<sub>50</sub> values, cytotoxic effects (CC<sub>50</sub>), the duration of treatment, a selective index (SI) and the mechanism of action were the parameters set to evaluate the effectiveness of these compounds, compared to Bdz. Nifurtimox is insufficiently reported as a control in the evaluation of the trypanocidal action of thiazolidine compounds. Thus, Bdz was the positive control in all the studies selected. The Selective Index (SI) measures how active the compound is against the pathogen without causing damage to the mammalian cells. It is calculated by the ratio between IC<sub>50</sub> and CC<sub>50</sub> values, the latter, the cytotoxic concentration, causing death to 50% of cell lines. In this review, thiazolidine compounds were compared to Bdz, as to their effectiveness in tests *in vitro* against amastigote forms of *T. cruzi*. Non-original articles were excluded.

### 2.3 Source of information and identification of studies

Electronic databases, namely Science Direct, Scopus, Pubmed, LILACS, Scielo, Tripdatabase, Cochrane and Google Scholar, were used for the conduct of a systematic search for articles that evaluate the efficacy of thiazolidine compounds against *T. cruzi* intracellular amastigotes, compared to Bdz. The search string strategy used “Chagas disease” and “Thiazolidine” or “Trypanocidal” or “Benzonidazole” or “Thiazolidine”. The strings used a combination of terms and synonyms. We evaluated the papers independently and thereafter, we met to compare our findings with a view to confirming

them, and to reducing the number of databases and the heterogeneity in the analyses already made. To facilitate data collection and to determine the terms of inclusion and exclusion of papers, our hypothesis was structured using the acronym PICO (Brasil, 2012), in which P (patients) was represented by amastigotes (Chagas Disease); I – intervention (applications of thiazolidine compounds); C – control, which was Bdz; O – outcome: direct effect of the compounds on *T.cruzi* intracellular amastigotes.

#### **2.4 Specific methods for systematic review**

The popular funnel methods for testing publication bias in meta-analysis are inadequate and potentially misleading when the number of studies included is small and the heterogeneity is large (Engels et al., 2000; Macaskill et al., 2001). However, a critical evaluation program called the Checklist of Downs and Black was used to verify both the methodological quality of the original articles, and the bias of individual studies included in the systematic review (Downs & Black., 1998).

The assessment instrument consists of twenty-seven criteria that analyze the following domains: 1) reporting the results; 2) external validity; 3) bias; 4) confusion variable / selection bias; and 5) strengths of the study. If the evaluator identifies them, they will receive a score of “one”. The absence of a criterion corresponds to a “zero” evaluation/ score (Benjamin et al., 2014).

The Downs and Black scale is recognized as “methodologically strong” and is more flexible than other scales, as it allows for a credible assessment of a wider range of study types. It has the advantage of being able to evaluate and highlight the potential strengths and weaknesses of studies under evaluation.

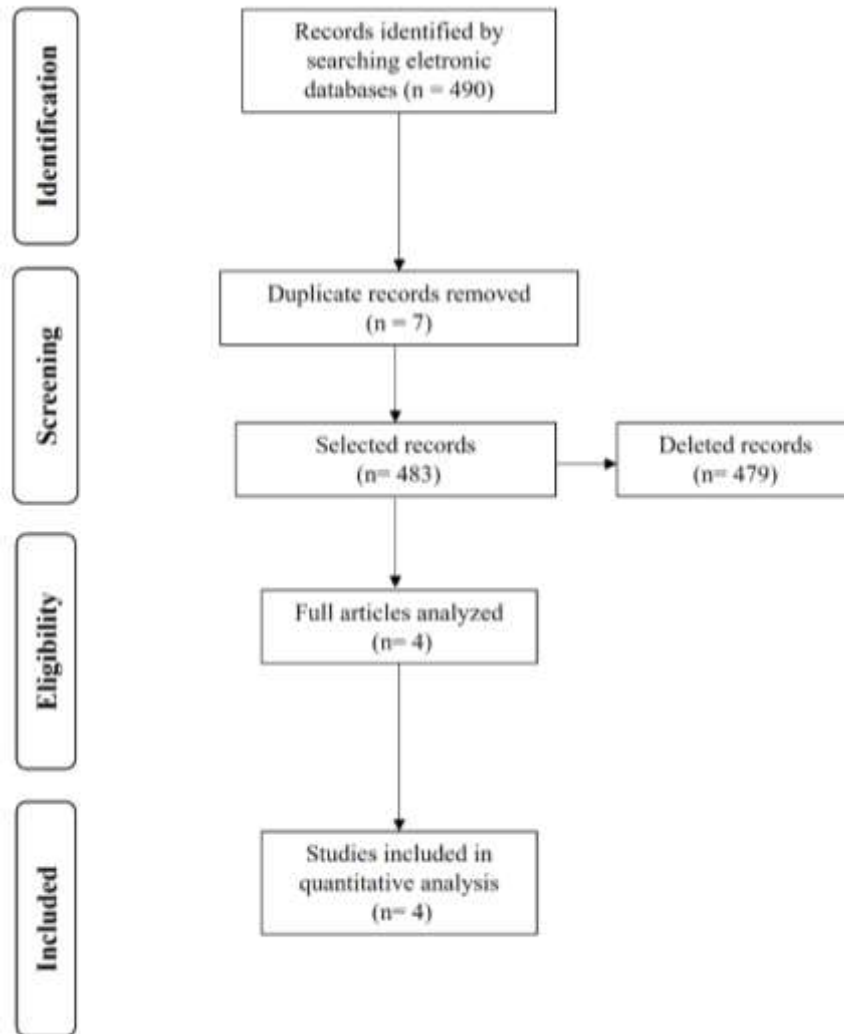
The maximum score that can be achieved by using this instrument is 32 points. Each item that is on the checklist is assigned a score from 0 to 1, with the exception of the item that describes the confounding factors, which can be assigned up to two points, and up to five points can be assigned to the item that evaluates the description of the strengths of the study. However, item 27 was modified (Engers et al., 2016; Samoocha et al., 2010), from the original range of from 0 to 5 points to a possible score of 0 or 1. Thus, a score of 1 was given if the article presented a calculation for strengths and/ or for sample size and 0 if it did not present any of these calculations. After being modified, the checklist now has total scores that can range from 0 to 28 points.

### **3. Results**

#### **3.1 Study selection**

As described in Figure 1, a total of 490 articles was identified in the selected databases as follows: Science Direct (n = 46) 50, Scopus (n = 9), Pubmed (n = 3) and Google Scholar (n = 432). Databases such as LILACS, Scielo, Trip Database and Cochrane databases did not present articles during the search. Seven articles were duplicated and therefore the duplicates were excluded. After analyzing the 483 remaining titles, abstracts and full texts, the articles which did not fit the eligibility criteria were excluded (a total of 479). Four articles met the eligibility criteria determined for qualitative synthesis and for inclusion in the systematic review.

**Figure 1.** Flow diagram for study identification and selection adapted from Moher et al. (2009).



Source: Authors.

### 3.2 Methodological quality

The final checklist score in accordance with the Downs and Black scale of each of the four studies selected, is presented in Table 1 and Supplementary material. According to the quality assessment score (QAS), studies were classified as excellent if they scored 26 to 28 points, good when they scored 20 to 25, fair between 15 to 19, and poor if the total score was 14 or less (Bruinvels & Elbers., 2010). The quality of the papers included varied. The studies carried out by Moreira et al. (2012), Moreira et al. (2013) and Silva-Júnior et al. (2016) were classified as fair (a score of 18, 16 and 17, respectively), while de Oliveira Filho et al. (2017) was classified as poor (score = 14). The latter did not present the principal results regarding the effectiveness of the compound against *T. cruzi* intracellular amastigotes (IC<sub>50</sub> values and SI), and did not use appropriate statistical analysis to determine if the compound is effective or not against the amastigotes.

### 3.3 Characteristics of the studies

Table 1 shows the data regarding the design of each paper analyzed.

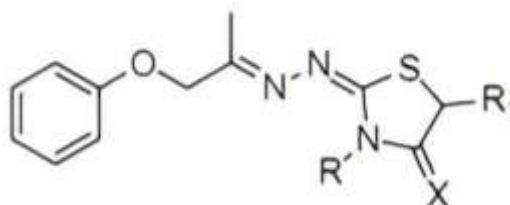
**Table 1.** Summary of the characteristics related to each study analyzed.

Reference	Parasite strain	Evaluated compounds	Compounds concentrations ( $\mu\text{M}$ )	Cell line	Cytotoxic assay
(Moreira et al., 2012)	Y	2-iminothiazolidin-4-one 18	50 – 1.0	Mouse splenocytes	Direct method (Thymidine incorporation)
(Moreira et al., 2013)	Y	*LPSF SF29	8.0 – 0.75	Peritoneal macrophages	Indirect method (Trypan blue)
(Silva-Júnior et al. 2016)	CA-I/72	Thiophene-thiazolidine hybrids 8b	100 – 10	Macrophage J774	Indirect method (MTT)
(de Oliveira Filho et al., 2017)	Y	1,3-thiazole 14	8.0 - 0.5	Macrophage J774	Indirect method (Alamar Blue)

\* 5-(3,4-dichlorobenzylidene)-3-(4-nitrobenzyl)-thiazolidine-2,4-dione (LPSF SF29). Source: Authors.

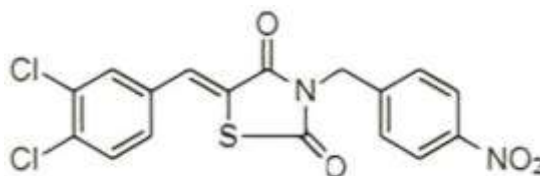
The selected studies contain *in vitro* analysis of thiazolidine compounds against *T. cruzi* amastigote forms. The compounds analyzed were 2-iminothiazolidin-4-one 18 (Moreira et al., 2012) (Figure 2), LPSF SF29 (Moreira et al., 2013) (Figure 3), thiophene-thiazolidine hybrids 8b (Silva-Júnior et al., 2016) (Figure 4) and 1,3-thiazole 14 (de Oliveira Filho et al., 2017) (Figure 5).

**Figure 2.** 2-imidazolidin-4-one-18 chemical structure.



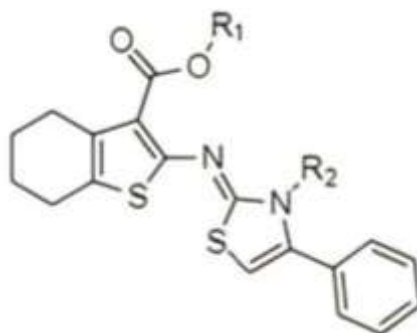
Source: Authors.

**Figure 3.** 5-(3,4-dichlorobenzylidene)-3-(4-nitrobenzyl)-thiazolidine-2,4-dione (LPSF SF29) chemical structure.



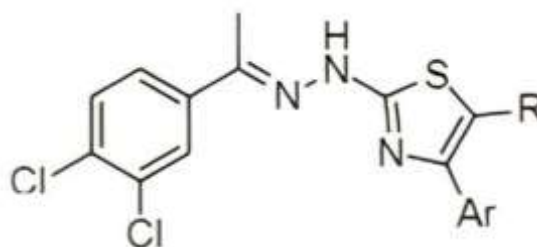
Source: Authors.

**Figure 4.** Thiophene-thiazolidine hybrids 8b chemical structure.



Source: Authors.

**Figure 5.** 1,3-thiazole 14 chemical structure.



Source: Authors.

The Y strain of the parasite was evaluated in three of these studies (Moreira et al., 2012; Moreira et al., 2013; de Oliveira Filho et al., 2017), while the CA-I/72 strain was evaluated by Silva-Júnior et al. (2016). Cytotoxic assays were determined by means of colorimetric methods, in which trypan blue and AlamarBlue (Invitrogen Carlsbad, CA, EUA) were used by Moreira et al. (2013) and de Oliveira Filho et al. (2017), respectively. The MTT method was performed by Silva-Júnior et al. (2016), while Moreira et al. (2012) evaluated the cytotoxic effects of LPSF SF 29 by comparing the percentage of thymidine incorporated in treated cells with that in untreated cells.

In the anti-amastigote assay, peritoneal macrophages were infected by trypomastigotes at a ratio of 10 parasites per cell for 2 h. Free trypomastigotes were removed by successive washes using saline solution, and the number of amastigotes/infected macrophages were determined by manual count under an optical microscope (Moreira et al. 2012., Moreira et al., 2013). On the other hand, detailed data of an anti-amastigote assay are not described by Silva-Júnior et al. (2016) nor by de Oliveira Filho et al. (2017). Antiparasitic activity was quantified by counting the number of infected cells and the number of amastigotes per cell (Silva-Júnior et al., 2016), and by evaluating cell viability based on negative controls (untreated wells) and positive controls (benznidazole at 10  $\mu$ M) (de Oliveira Filho et al., 2017).

### 3.4 Chemical synthesis of compounds

Moreira et al. (2012) obtained the compound 2-iminothiazolidin-4-one 18 by making two modifications in the thiazolidinic ring, namely, by varying substituents at carbon 5 (C5) and by replacing NH with *N*-methyl and *N*-phenyl. Thus, these modifications yielded 2-iminothiazolidin-4-one 5-19.

Moreira et al. (2013) obtained the compound 5-(3,4-dichlorobenzylidene)-3-(4-nitrobenzyl)-thiazolidine-2,4-dione (LPSF SF29) by means of a series of chemical synthesis. Initially, thiazolidine-2,4-dione was reacted with a benzyl halide under basic conditions to obtain 3-(4-nitrobenzyl)-thiazolidine-2,4-dione intermediate. Then, 3-(3,4-dichloro-phenyl)-2-cyano-



2-propenoic acid ethyl ester (LPSF IP17) was synthesized using Knoevenagel condensation between 3,4-dichlorobenzaldehyde and ethyl cyanoacetate. In the end, LPSF SF29 was formed by reacting N-benzyl intermediate with LPSF IP17 in the presence of piperidine.

Thiophene-thiazolidine hybrids 8b (Silva-Júnior et al., 2016), as well as their intermediates (8a to 8d), were formed by using the process of intramolecular cyclization. Initially, 2-aminothiophene analogues were prepared via the Gewald reaction. This consists of a multicomponent synthesis that involves an aldehyde or a ketone. The intermediates were synthesized by treating them with substituted isothiocyanetes (phenyl and allyl), and lastly, thiophen-2-imidothiazolidines were prepared via substitution followed by the intramolecular cyclization. The last compound, 1,3-thiazole 14 (de Oliveira Filho et al., 2016), was synthesized by an ultrasonic bath (40 MHz, 30-120 min) using 2-propanol as a solvent at room temperature.

### 3.5 Anti-amastigote effects

A summary of the parameters evaluated in the trypanocidal action in each compound is given in Table 2.

**Table 2.** Summary of data evaluated for the effectiveness of thiazolidine compounds against *Trypanosoma cruzi* intracellular amastigotes compared to benznidazole and the final checklist of each study, according to the Downs and Black scale.

Compound	IC <sub>50</sub> ( $\mu$ M)	IC <sub>50</sub> ( $\mu$ M)	Selective index (SI)	Length of treatment (h)	Bdz IC <sub>50</sub> ( $\mu$ M)	Length of treatment (h)	Final checklist score (0 - 28)
iminothiazolidin-4-one 18	10.1 $\pm$ 0.09	> 262 $\mu$ M	25	24	13.9 $\pm$ 0.39	24	18
*LPSF SF29	4.5 $\pm$ 1.3	> 32 $\mu$ M	7.1	96	2.6 $\pm$ 0.2	72	16
Thiophene- thiazolidine hybrids 8b	6.03	> 10 $\mu$ M	1.6	72	1.17	72	17
1,3-thiazole 14	**	10.06 $\pm$ 1.8	**	**	**	**	14

\* 5-(3,4-dichlorobenzylidene)-3-(4-nitrobenzyl)-thiazolidine-2,4-dione (LPSF SF29). Source: Authors.

In general, regarding the antiparasitic activity, specifically in accordance with the IC<sub>50</sub> values, all compounds were effective against *T. cruzi* intracellular amastigotes.

The compound 2-iminothiazolidin-4-one 18 was lethal to *T. cruzi* amastigotes at a concentration of 10  $\mu$ M, thus decreasing the number of infected cells more effectively than Bdz. IC<sub>50</sub> values of 2-iminothiazolidin-4-one 18 was 10  $\pm$  0.09  $\mu$ M, while Bdz IC<sub>50</sub> was 13  $\pm$  0.39  $\mu$ M, and the total duration of treatment of the assays performed for both was 24 h. On the other hand, Bdz was more effective than the compound 5-(3,4-dichlorobenzylidene)-3-(4-nitrobenzyl)-thiazolidine-2,4-dione (LPSF SF29), considering the IC<sub>50</sub> values and the length of treatment. Bdz was lethal to *T. cruzi* intracellular amastigotes at 2.6  $\pm$  0.2  $\mu$ M after 72 h of treatment, while LPSF SF29 IC<sub>50</sub> was effective at 4.5  $\pm$  1.3  $\mu$ M, after 96 h of treatment. The compounds of thiophene-thiazolidine hybrids 8b, according to the IC<sub>50</sub> values, were less effective than Bdz, since this drug was lethal to *T. cruzi* intracellular amastigotes in low concentrations (IC<sub>50</sub> = 1.17  $\mu$ M), while the concentration for thiophene-thiazolidine hybrids 8b IC<sub>50</sub> was 6.03  $\mu$ M. The duration of treatment for the assays performed was 72 h.

As for 1,3-thiazole 14, we observed that this compound inhibited amastigote infection in a potentially similar way to Bdz. Concentrations of 8.2  $\mu$ M, 2  $\mu$ M and 0.5  $\mu$ M were tested, and the first, as well as the second concentration, inhibited



amastigotes growth in a dose-dependent manner. However, at 8.2  $\mu\text{M}$ , the 1,3-thiazole 14 was highly lethal to *T. cruzi* amastigotes, while at 2  $\mu\text{M}$ , the compound was equipotent to Bdz. De Oliveira Filho et al. (2016) did not present exact values of the inhibition percentage of Bdz and 1,3-thiazole 14.

The selectivity index (SI) and cytotoxic effects ( $\text{CC}_{50}$ ) were also evaluated. The compound with the highest SI was 2-iminothiazolidin-4-one 18 (the SI was estimated to be above 25), followed by LPSF SF29 (SI approximately 7.1) and thiophene-thiazolidine hybrids 8b (SI = 1.6), in addition to which *in vitro* studies showed that these compounds were non-toxic to mouse splenocytes ( $\text{CC}_{50} > 262 \mu\text{M}$ ), peritoneal macrophages ( $\text{CC}_{50} = 32 \mu\text{M}$ ) and the *J774* macrophage cell line ( $\text{CC}_{50} = 10 \mu\text{M}$ ), respectively. The selectivity index of 1,3-thiazole 14 could not be determined, since  $\text{IC}_{50}$  values of the compound against *T. cruzi* amastigotes were not presented in the study. However, regarding  $\text{CC}_{50}$  values, 1,3-thiazol 14 presented similar cytotoxic effects to those of thiophene-thiazolidine hybrids 8b, both in the *J774* macrophage cell line. The SI of Bdz was not determined in any of these studies.

#### 4. Discussion

In general, the latency of *T. cruzi* intracellular amastigotes in tissues represents a challenge in the search for new therapies for CD. During the chronic phase of the disease, the parasites have a reduced metabolism and can be less susceptible to the action of drugs. Thus, the development of assays with amastigote forms is essential for the success of CD therapy (Villalta et al., 2019).

Bdz and nifurtimox are the drugs available in CD treatment, and both are pro-drugs activated within the parasite by a mitochondrial NADH-dependent type I nitroreductase (TcNTR) (Wilkinson et al., 2008). However, Bdz is the antichagasic of choice, due to its biological effects and acceptable biopharmaceutical performance (Arrúa et al., 2019). In this paper, we analyzed the action of Bdz against *T. cruzi* intracellular amastigotes compared to thiazolidine compounds. Bdz was more effective than LPSF SF29 and thiophene-thiazolidine hybrids 8b, considering parameters such as  $\text{IC}_{50}$  values and the duration of treatment.

The action mechanism of Bdz has not yet been fully established. Hall and Wilkinson suggest that Bdz promotes a reduction in the formation of glyoxal, a cytotoxic compound (Hall & Wilkinson, 2012), while another study demonstrated that this drug can reduce the protein synthesis, and can incorporate RNA precursors and thymidine into DNA molecules (Castro et al., 2006). The major disadvantages of Bdz use are its serious adverse effects, including cutaneous eruption, generalized edema, fever, polyneuropathy, polyneuritis and bone marrow complications (Bern et al., 2011). Moreover, some studies have reported that *T. cruzi* can be resistant to Bdz, especially due to genetic mechanisms (Campos et al., 2014; Zingales et al., 2014).

*In vitro* studies analyzed in this review demonstrated that Bdz is effective against *T. cruzi* intracellular amastigotes, according to  $\text{IC}_{50}$  values. However, due to its reported cytotoxic effects, as well as the parasite being resistant to the drug and the controversies of the drug's effectiveness in the chronic phase of CD, Bdz is insufficient for the treatment of CD in chronic patients, specifically in those who are symptomatic.

In this context, thiazolidines and their derivative compounds may be an alternative, in the future, that can be used in CD treatment. These compounds, in general, are described as having anticancer, anti-inflammatory and antimicrobial activities (Cardoso et al., 2014). However, few studies that report their use for CD chemotherapy were found in the literature (Moreira et al., 2013).

The 2-Iminothiazolidin-4-one 18 presented significant effects against *T. cruzi* amastigotes, according to  $\text{IC}_{50}$  values and the SI. The compound inhibited the activity of cruzain, an enzyme belonging to the family of cysteine proteases that is closely related to cathepsins L and S, which also associated with other pathology in humans (Wiggers et al., 2013). Cruzain is

important for differentiation and proliferation of the parasites in host cells (Duschak et al., 2001; McKerrow et al., 2009), and a major virulence factor, as well as an important chemotherapeutic target for CD, according to pre-clinical validation evidence (Cazzulo et al., 2001; Du et al., 2002; Branquinha et al., 2015).

Cruzain can appear in two locations in *T. cruzi* intracellular amastigotes: on the surface of the parasite, directly in contact with the host cell cytoplasm, as well as in the lysosome-related compartment (Souto-Padron et al., 1990., Engel et al. 2000., Vieira et al., 2005). In general, the literature reports that cruzipain inhibitors, such as diazomethane inhibitors, fluoromethyl ketones, oxygen-containing heterocycles and vinyl sulfones are considered promising agents for the treatment of CD in the chronic phase (Kouznetsov, 2019). These inhibitors can promote some alterations in the morphology of the parasite, and electron microscopy demonstrated that 2-Iminothiazolidin-4-one 18, like other cruzain inhibitors, changed the Golgi complex structure, thereby promoting dilatation of cisternae and the appearance of atypical vacuoles (Engel et al., 1998).

On the other hand, LPSF SF29 can interfere in the polyamine and trypanothione biosynthesis and increases the parasite's sensitivity to oxidative metabolism (Moreira et al., 2013). Transmission electron microscopy (TEM) demonstrated that amastigotes presented vacuoles similar to autophagosomes, as well as an intense mitochondrial swelling, membrane formation inside the mitochondria and cytoplasm vacuolization. Lastly, the trypanocidal action of thiophene-thiazolidine hybrids 8b was attributed to the hydrophobicity of the compound. The study confirms that an *i*-propyl group at R<sub>1</sub> and the phenyl group at R<sub>2</sub> of the molecule structure were the best combinations to increase the activity. This process is related to the hydrophobicity of the compound, and in the structure of thiophene-thiazolidine hybrids 8b, the presence of a phenyl ring at R<sub>5</sub> increases the hydrophobicity and, consequently, the trypanocidal effects (Silva-Júnior et al., 2016). The mechanism of action of 1,3-thiazole 14 against *T. cruzi* amastigotes was not elucidated.

However, we could observe that this compound did not inhibit cruzain, which suggests the need for another target so as to affect the viability of the parasite (de Oliveira Filho et al., 2017).

Since all of the compounds evaluated in this review showed different efficacy against *T. cruzi* intracellular amastigotes, we observed that the chemical composition, as well as some modifications in the structure, can increase or decrease the action mechanism of these compounds, specifically the thiophene-thiazolidine hybrids 8b and 2-iminothiazolidin-4-one 18 ones.

Hydrophobicity was essential to increase the activity of thiophene-thiazolidine hybrids 8b, while chemical alterations at the N3 extremity of 2-iminothiazolidin-4-one 18 have greater effects in the cruzain activity, as well as the trypanocidal action, than alterations at C5. Compounds with an *N*-phenyl, such as 2-iminothiazolidin-4-one 18, were the highest cruzain inhibitors, suggesting that the group *N*-phenyl is essential to increase the activity of cruzain, while other groups, such as NH and *N*-methyl decrease the enzyme activity. Similar chemical effects were also observed in efficacy of the trypanocidal activity (Moreira et al., 2012).

Regarding the need for new antichagasic drugs, in 1997, the World Health Organization (WHO) established some criteria that drugs/compounds should meet prior to being used in CD therapy. These criteria are: parasitological cure in both phases of the disease, specifically in the chronic phase, effectiveness in low concentrations, low cost, without significant side effects and that the parasite does not offer resistance mechanisms (Silva et al., 2007). Bdz is cytotoxic and less effective in chronic patients, specifically in those who are symptomatic (Urbina et al., 2010).

In this review, we observed that the compound 2-Iminothiazolidin-4-one 18, according to *in vitro* assays, was more lethal than Bdz against *T. cruzi* intracellular amastigotes, considering parameters such as IC<sub>50</sub> and the duration of treatment in each assay. However, SI is necessary to determine which drug is more effective and viable for the treatment of the disease, and all of the analyzed studies presented the SI of Bdz.

Furthermore, due to the heterogeneity of methodological parameters, such as the varied duration of treatment in the assays, different strains of the parasite, as well as the different cell lines and concentrations of each compound, it is not appropriate to compare data from the 4 papers analyzed. Nevertheless, according to each study, the evaluated compounds can be promising in the development of new antichagasic drugs, since these compounds are selective to *T. cruzi* amastigotes, which are prevalent in the chronic phase of CD. In this context, preclinical and clinical studies are necessary to determine the efficacy and safety of these drugs in patients who have chronic CD.

## 5. Conclusions

The thiazolidine compounds analyzed in this review showed different effects in *T. cruzi* intracellular amastigotes, according to *in vitro* assays. Parameters such as IC<sub>50</sub>, cytotoxic effects, duration of treatment, the selective index and the action mechanism of each compound were essential to determine their effectiveness against the parasite, compared with the drug Bdz. One of them was more effective than Bz, and we could observe that the chemical composition of each compound, and the alterations in the chemical structure, can increase or decrease the activity of the compounds against *T. cruzi* intracellular amastigotes. We also observed that cruzain is important to the parasite, since this enzyme is involved in a process that is essential for its development. Cruzain thus represents an important target to be considered in CD therapy, specifically in chronic patients. Due to the limitations of Chagas disease therapy in the chronic phase, more studies reporting the effectiveness of compounds against *T. cruzi* intracellular amastigotes are necessary. In addition, regarding the trypanocidal effects of thiazolidine compounds, we consider that pre-clinical studies, in the future, can be an interesting pathway to evaluate the possibility of use of these compounds in the antichagasic therapy.

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