Identification of a New Amide-Containing Thiazole as a Drug Candidate for Treatment of Chagas’ Disease

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Although the parasitic infection Chagas’ disease was described over 100 years ago, even now there are not suitable drugs. The available drugs nifurtimox and benznidazole have limited efficacies and tolerances, with proven mutagenic effects. Attempting to find appropriate drugs to deal with this problem, here we report on the development and pharmacological characterization of new amide-containing thiazoles. In the present study, we evaluated the in vitro and in vivo effects of new candidates against Trypanosoma cruzi, the etiological agent of Chagas’ disease. The lead amide-containing thiazole derivative had potent in vitro activity, an absence of both in vitro mutagenic and in vivo clastogenic effects, and excellent in vitro selectivity and in vivo tolerance. The compound suppressed parasitemia in mice, modifying the anti-T. cruzi antibodies like the reference drug, benznidazole, and displayed the lowest mortality among the tested drugs. The present evidence suggests that this compound is a promising anti-T. cruzi agent surpassing the lead optimization stage in drug development and leading to a candidate for preclinical study.

C hagas’ disease, or American trypanosomiasis, is the highest-impact parasitic disease in Latin America, despite recent advances in the control of its vectorial and transfusional transmission (1). Mammals can be infected by contaminated feces and urine of the insect vector with Trypanosoma cruzi, transfusion with infected blood, birth canal transmission, or oral transmission by contaminated food (2). Although excellent chemotherapeutic approaches have been described (3–5), nevertheless the pharmacology used to control this parasitic infection remains unsatisfactory. The current specific treatments are based on old and quite nonspecific drugs, benznidazole (Bnz; Radanil; Roche) and nifurtimox (Nfx; Bayer). They are generally well tolerated by children but cause many undesirable side effects in adults and are not effective during the chronic phase of the disease; also, they are mutagenic (6). Thus, there is an urgent need for new therapeutic options.

Previously, we reported a new series of thiazole derivatives which come from a bio-guided design from nitrothiophene 1 (Fig. 1) (7–9). Thiazole 2 (Fig. 1) (10) had a nanomolar 90% effective concentration (EC90) on intracellular T. cruzi amastigotes, excellent selectivity indexes, and an absence of mutagenic effects, displaying high potent activity in the murine model of acute Chagas’ disease. This positioned thiazole 2 as a lead anti-Chagas’-disease compound. However, this series of compounds has some solubility problems, probably due to the high hydrophobic properties. This feature would complicate further drug development. For this reason, we developed new derivatives with improved hydrophilic properties. Thiazole 4 (Fig. 1) was synthesized and shown to be similar in potency to compound 2 against T. cruzi amastigotes (the 50% effective concentration [EC50] of thiazole 4 was 0.72 μM while that for thiazole 2 was lower than 0.25 μM). Additionally, compound 4 proved to have a good preclinical profile, parasite selectivity, an absence of mutagenicity, and biostability and chemical stability. This encourages us to perform the efficacy study in an acute murine model of Chagas’ disease. In addition, an absence of in vivo clastogenic effects was evidenced. As a chemically simple compound, thiazole 4 appears to have features that surpass the lead optimization stage in the Chagas’ disease drug development process.

MATERIALS AND METHODS

Syntheses of new thiazole derivatives (compounds 3 to 11). The designed compounds were synthesized and spectroscopically characterized as described in Table S1 in the supplemental material.

Parasite and mammalian cell drug toxicity. Parasite killing levels caused by the tested compounds were determined as previously described (10–12).

For antiepimastigote studies, the Tulahuen 2 strain, discrete typing unit (DTU) Tc VI (13), growing in an axenic medium (brain heart infusion [BHI]-tryptose), was used. Briefly, compounds were added to the culture (1 × 106 parasites/ml) in different concentrations from a stock solution in dimethyl sulfoxide (DMSO) (DMSO concentration in the culture medium never exceeded 0.4%). The control was run in the presence of antibodies like the reference drug, benznidazole, and displayed the lowest mortality among the tested drugs. The present evidence suggests that this compound is a promising anti-T. cruzi agent surpassing the lead optimization stage in drug development and leading to a candidate for preclinical study.

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of 0.4% DMSO and in the absence of compounds. The percentage of growth inhibition was determined at day 5 after the addition of the compound. The EC50 was defined as the concentration of compound needed to reduce the growth inhibition to 50%. Each antiproliferative experiment was done in duplicate, and each concentration was tested in triplicate. Briefly, for antitrypomastigote studies, the Y strain, DTU Tc II (14), was used. Blood containing 1 × 10^6 trypomastigotes/ml was treated with compound 4 and Bnz (25 μM) for 48 h at 4°C. The percentage of parasite reduction was determined by comparing the treated infected blood with the untreated blood.

For antimastigote studies, the Sylvio X-10 strain, DTU Tc I (15), was used. Briefly, Vero cells, 3 × 10^5 cells/ml in RPMI 1640 medium plus 10% heat-inactivated fetal calf serum (hiFCS), were infected with tissue-derived trypomastigotes at a ratio of parasites to cells of 10:1 for 24 h. The infected cells were treated twice with serial dilutions of each evaluated compound and reference drug (Nfx and Bnz). A second dose of compound or reference drug was added 48 h after the first addition. Control cells were maintained without compound. The incubation times of the drug with the parasites was 72 h. The percentage of infected cells was determined by counting infected and noninfected cells on methanol-fixed and Giemsa-stained slides on 300 cells. Each parasite experiment was tested in duplicate, and each concentration was tested in triplicate.

For mammalian in vitro toxicity, Vero cells were used. Briefly, the compounds dissolved in DMSO were evaluated at serial dilutions of the maximum concentration (2,000 μM) in fresh culture medium. A negative control with DMSO (<0.1%) was included in each experiment. The percent inhibition of cell growth after 72 h of incubation was determined colorimetrically by using thiazole blue (MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and reading the absorbance at 570 nm in a VersaMax Micro microplate reader.

Stability studies. For the pH stability study (16), we utilized the following aqueous solutions: (i) KCl-HCl buffer, pH 1.0; (ii) citrate buffer, pH 6.0; and (iii) borax-HCl buffer, pH 9.0. The stock solution of compound 4 was prepared in DMSO, and the final concentration in the aqueous medium was 1 mM. The solutions were further homogenized and incubated at 37°C for 24 h. After that, thin-layer chromatograms of the ethyl acetate extracts were developed in order to confirm or disprove the presence of decomposition products.

For the determination of liver fraction stability, rat liver microsomal and cytosolic proteins were used. They were prepared according to previously described methodology (17). The protein content of the microsomal and cytosolic fractions was determined by the bicinchoninic acid assay from Sigma, as suggested by the manufacturer. The final concentration in the aqueous medium of thiazole 4 was 400 μM and was prepared from a stock solution in DMSO. The solutions were further homogenized and incubated at 37°C for 1 h. After that, thin-layer chromatography of ethyl acetate extracts was performed in order to confirm or disprove the presence of decomposition products.

**Formulation 1.** Thiazole 4 previously pulverized in a mortar was added to vehicle 1 (V1), composed of saline (80%) and Tween 80 (20%) (vol/vol), and was homogenized by stirring in an ultrasonic chamber (ultrasonic cleaner; Hwashin Instruments; sonic power 405) for 10 min at maximum power. The resulting suspension was stirred gently for 24 h with a horizontal shaker.

**Formulation 2.** Thiazole 4 was dispersed in vehicle 2 (V2) (10%), composed of a surfactant (10%), containing Eumulgin HRE 40 (polyoxyl-40 hydrogenated castor oil), sodium oleate, and soya phosphatidylcholine (8:6:3), and an oil phase (10%) containing cholesterol and phosphate buffer (pH 7.4) (80%). For the preparation of the formulation thiazole 4, previously pulverized in a mortar, cholesterol, Eumulgin HRE 40, and phosphatidylcholine were dissolved in chloroform and the solvent was evaporated under vacuum to dryness. In parallel, sodium oleate was dissolved in phosphate buffer and left in an orbital shaker for 12 h at room temperature. The former was then added to the evaporated residue, and the mixture was homogenized and placed in an ultrasonic bath at full power for 30 min.

**Mutagenicity assay.** For the mutagenicity assay (18–22), the method of direct incubation in the plate was performed. Culture of *Salmonella enterica* serovar Typhimurium TA98, TA100, TA102, TA1535, and TA1537 strains in the agar minimum glucose medium-agar solution, 50× Vogel-Bonner E, and 40% glucose solution was used. First, the direct toxicity of the compounds against the bacteria was assayed. DMSO solutions of the studied compounds at five consecutive dilutions (1/3) were assayed in triplicate. The highest dose without toxic effects was used as the starting one for the Ames test. The influence of the metabolic activation was tested by adding 500 μl of the S9 fraction of mouse liver treated with Aroclor, obtained from Moltox, Inc. (Annapolis, MD, USA). Positive controls of 4-nitro-o-phenylenediamine (20 μg/plate, in the run without S9 activation) and 2-aminofluorene (10 μg/plate, in the case with S9 activation) for TA98, 4-nitro-o-phenylenediamine (20 μg/plate, in the run without S9 activation) and 2-aminofluorene (10 μg/plate, in the run with S9 activation) for TA100, or sodium azide (2 μg/plate, in the run without S9 activation) and 2-aminomethanthe (2 μg/plate, in the run with S9 activation) for TA102, TA1535, and TA1537 and a negative control of DMSO were run in parallel. The revertant number was counted manually. The sample was considered mutagenic when the number of revertant colonies was at least twice the negative control for at least two consecutive dose levels.

**In vivo micronucleus test.** For the *in vivo* micronucleus test (23), approximately 3-month-old CD-1 male mice were housed in polycarbonate cages at an ambient temperature of 25°C and a photoperiod of 12 h throughout the study. Thiazole 4 and vehicle were orally administered two times, days 1 and 2, to groups of five mice at doses of 200 mg/kg of body weight (BW) or 200 μl. Mice were sacrificed 24 h after the last administration, and the bone marrow was prepared for evaluation with slight modifications of the method reported by Schmid (23). At least two slides of the cell suspension per animal were made. The air-dried slides were stained with Giemsa stain (5% in phosphate buffer, pH 7.4). Slides were then examined at ×1,000 magnification. Small round or oval bodies, the size of which ranged from about 1/5 to 1/20 of the diameter of a polychromatic erythrocyte (PCE), were counted as micronuclei. A total of 1,000 PCEs were scored per animal by the same observer for determining the frequencies of micronucleated polychromatic erythrocytes (MNPCe). Cyclophosphamide, 50 mg/kg, administered intraperitoneally (i.p.) 24 h before mouse sacrifice, was used as a positive control. For statistical analysis, the homogeneity of variances of data was tested by the analysis of variance (ANOVA) test (P < 0.05) using EpiInfo (3.5.1) software.

**Acute oral toxicity.** The *in vivo* 50% lethal dose (LD50) for thiazole 4 was determined according to the guidelines of the Organization for Economic Cooperation and Development (OECD) (31). Briefly, healthy young adult male B6D2F1 mice (30 days old, 25 to 30 g) were used in this
Initially, thiazole 4 dissolved in vehicle V2 was administered at 2,000 mg/kg, by orogastric cannula, to one animal. The animal was fasted, maintained, and observed for 14 days according to the OECD guidelines. If the mice survived for the first 48 h, another animal received the same dose. If this repeated, a third animal was dosed with 2,000 mg/kg. In vivo anti-\textit{T. cruzi} studies (acute model). For the acute model (10, 11), BALB/c male mice (30 days old, 25 to 30 g) bred under specific-pathogen-free conditions were infected by intraperitoneal injection with $1 \times 10^3$ blood trypomastigotes of the Y strain. The mice were divided into three groups. One group of animals, $n = 8$, was used as a control (treated orally with the vehicle V1 or V2), and two groups of animals, $n = 8$ each, were treated with compound 4 and Bnz, respectively. Initial parasitemias were counted 5 days postinfection (week 1), and the treatment was begun the following day (6th day). Bnz (at 50 mg/kg body weight [BW]/day, for 14 days) or thiazole 4 (at 50, 100, or 200 mg/kg BW/day, for 14 days) was administered orally, using vehicle V1 or V2. Parasitemias in the control and treated mice were determined once a week after the first administration, for 60 days after beginning of treatment, in tail vein blood. Additionally, the mortality rate was recorded.

\textbf{IgG antibody detection.} For IgG antibody detection (10), all the sera obtained after centrifugation of the blood that were extracted from infected mice were tested twice by enzyme-linked immunosorbent assay (ELISA) at 30 and 60 days after the start of treatment. An in-house ELISA kit (Chagas’ disease test; Instituto de Investigaciones en Ciencias de la Salud [IICS], Asunción, Paraguay) was used according to the procedure recommended by the manufacturer (IICS Production Department, Asunción, Paraguay). The optical density values were obtained in an ELISA plate reader (Titerek Unistan I). The Wilcoxon test was used in order to compare the levels of anti-\textit{T. cruzi} antibodies between experimental groups.

\textbf{Histopathology.} At the end of the experiments, samples of the tissues of the heart, skeletal muscle, intestine, and urinary bladder were taken and stored in 10% formaldehyde, followed by dehydration in alcohol solutions and xylol and embedment in paraffin. Each organ was embedded.

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{EC}_{50} (\mu M) & \textbf{EC}_{90} (\mu M) & \textbf{SI}_V \textsuperscript{b} \\
\hline
4 & 0.72 ± 0.08 & 40.2 ± 3.4 & 433 \\
Bnz & 3.3 ± 0.3 & - & 300 \\
Nfx & 0.45 ± 0.01 & 2.4 ± 0.2 & 200 \\
\hline
\end{tabular}
\caption{Biological characterization of compound 4 against intracellular amastigotes of Sylvio X-10 strain infecting Vero cells\textsuperscript{a}}
\textsuperscript{a} Each compound concentration was evaluated in quadruplicate for 72 h.
\textsuperscript{b} SI$_V$, selectivity index, EC$_{50,\text{Vero cells}}$/EC$_{50,\text{T. cruzi}}$ (amastigotes, Sylvio X-10). 
\textsuperscript{c} —, not studied.
\end{table}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{Parasitemias of mice treated with different doses of thiazole 4 (50 mg/kg BW/day, 0.103 mmol/kg BW/day, black triangles; 100 mg/kg BW/day, 0.205 mmol/kg BW/day, gray triangles; or 200 mg/kg BW/day, 0.410 mmol/kg BW/day, white triangles), mice treated with 50 mg/kg BW/day (0.192 mmol/kg BW/day) of Bnz (black squares), and control group treated with V2 (white circles). Highlighted regions correspond to the period of treatment (between days 6 and 19 postinfection). (Inset) Enlarged region showing the behavior of compound 4 at the highest studied doses. The parasites per ml were the average of eight mice, and the standard deviations were $\pm 10\%$. The parasitemias of compound 4- and Bnz-treated mice were significantly different from the control (V2) ($P < 0.05$, Student $t$ test). Errors were omitted to avoid confusion.}
\end{figure}
separately in paraffin and cut in a microtome set at 6 μm. Slides were stained with hematoxylin–eosin and observed under a Zeiss microscope at ×10, ×40, and ×100 magnifications. Photographs were taken at all of these magnifications with an Olympus X-785 digital camera coupled to the microscope.

The experimental protocols with animals were evaluated and supervised by the local Ethics Committee of the Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, and the research adhered to the Principles of Laboratory Animal Care (24).

RESULTS

Design, syntheses, and antiparasitic evaluation of new thiazole derivatives. Using thiazole 2 (Fig. 1) as the template, a new generation of derivatives with modified hydrophilicity was prepared (derivatives 3 to 8; see Table S1 in the supplemental material) with an effort to include water-soluble moieties, i.e., carboxylic acid, amide, hydrazide, and hydrophilic heterocycles. The best in vitro anti- \textit{T. cruzi} compounds were selected from the activities observed in the epimastigote forms of the parasite, comparing the EC_{50} values (see Table S1). The most hydrophilic compounds, thiazoles 3 and 5 to 8, were inactive against this form of the parasite, while thiazole 4, with one of the highest lipophilicities of this series, displayed the best antiparasitic activity. Thiazole 4 possesses in its chemical structure a substituent, 4-(3-phenyl-2-propenyl)piperazinyl, which was previously identified by us to be responsible for the anti- \textit{T. cruzi} activity, i.e., compound 11 (see Table S1) (8, 25). For this reason, we developed compound 9 (see Table S1), which was very active against epimastigotes, having also high lipophilicity. Consequently, thiazoles 4 and 9 were as potent against epimastigotes as the parent compound 2 and as the reference drugs Bnz and Nfx.

Thiazole 4 was active against \textit{T. cruzi} trypomastigotes and prevented the intracellular growth of amastigotes. Thiazole 4 was tested in vitro against the bloodstream trypomastigote form of \textit{T. cruzi}, the Y strain. The compound, added to infected blood with trypanosomes at a concentration of 25 μM and kept at 4°C for 48 h, produced a 40% reduction of parasite cell density, in comparison to that of the control (no drug added to the medium). Bnz, used as the trypanosomicidal reference drug, produced a 90% reduction of parasite cell density under the same conditions. Additionally, compound 4 was active against amastigotes of the \textit{Sylvio} X-10 strain (Table 1; see also Table S2 in the supplemental material). This compound was able to eliminate intracellular amastigotes infecting Vero cells after 72 h of treatment. To explore the selectivity of thiazole 4 against \textit{T. cruzi}, we evaluated the in vitro nonspecific mammal cytotoxicity, using the Vero line of monkey kidney epithelial cells (Table 1; see also Table S2). Thiazole 4 was equipotent to Nfx, against intracellular amastigotes, and 4-fold more active than Bnz; additionally, it was more selective than the reference drugs (best selectivity index). Thiazole 4 had the best inhibitory growth effect (best EC_{50}) compared with analogue 9 or the compounds 10 and 11 (see chemical structures in Table S1). Compound 11 was equipotent to Bnz (similar EC_{50}) with the best EC_{90} value of all the studied compounds.

Genotoxicity studies. In order to progress in the lead optimization discovery stage, the different potential toxic effects of the best-developed compounds were analyzed. In this sense, in vitro mutagenicity was analyzed using the method of direct incubation in a plate of \textit{Salmonella} Typhimurium cultures (18, 19). In contrast to the reference drugs, Bnz and Nfx, all the new compounds were not mutagenic against the TA98 strain of \textit{S. Typhimurium} (see Table S3 in the supplemental material).

To complete the OECD recommendations, thiazole 4 was evaluated against \textit{S. Typhimurium} TA100, TA102, TA1535, and TA1537 strains without and with metabolic activation (see Table S4 in the supplemental material). The compound fulfilled the OECD requirements, demonstrating its absence of mutagenic effects.

The absence of mutagenic effects of thiazole 4 encouraged us to perform further studies related to biosafety; consequently, in vivo oral genotoxicity and acute toxicity were analyzed.

In the first assay, thiazole 4, orally administered at 200 mg/kg body weight, displayed the same level of micronucleated polychromatic erythrocytes (MNPCes) as did the vehicle alone while the reference genotoxic agent, cyclophosphamide, intraperitoneally administered at 50 mg/kg body weight, produced 10-fold more MNPCes than the vehicle (see Table S5 in the supplemental material).

The oral acute toxicity was determined following the up-and-
down procedure described by the OECD. We found that the LD₅₀ was higher than 2,000 mg/kg BW for thiazole 4 (see Table S6 in the supplemental material).

Additional preclinical studies of thiazole 4. Thiazole 4, containing an amide moiety, could be hydrolysable. We analyzed the stability of compound 4 under simulated physiological conditions, using aqueous solution at different pHs (1.0, 6.0, and 9.0). Additionally, we evaluated the stability of thiazole 4 in the hepatic rat cytosolic and microsomal fractions (see Table S7 in the supplemental material). The compound was stable at the end of the assay, for 24 h in aqueous solutions with pHs 1.0, 6.0, and 9.0, and in liver metabolic fractions for 1 h at 37°C.

On the other hand, for the in vivo studies thiazole 4 was initially suspended in a mixture of saline-Tween 80 (4:1, vol/vol) as vehicle (V1) in order to employ an aqueous-solution-like formulation. The use of different doses in this aqueous vehicle was limited by its low suspension ability. Additionally, the biological results were very erratic. Thus, studies to improve the vehicle system to adequately dispose the compound were undertaken. To this purpose, we selected microemulsion as a vehicle (25). This microemulsion is a mixing of water and oil stabilized by a surfactant system, which is thermodynamically stable and, depending on composition, exhibits a wide range of nanostructures. Consequently, the second vehicle (V2) was a microemulsion composed of cholesterol (10%) as the oil phase, the surfactant (soya phosphatidylcholine–sodium olate–polyoxyl-40 hydrogenated castor oil [3:6:8, 10%]), and phosphate buffer, pH 7.4 (80%), as the aqueous phase. This vehicle is a homogeneous and stable suspension for at least 1 month at 4°C and more than 20 days at 30°C (see Fig. S1 in the supplemental material).

Thiazole 4 was effective in the murine model of acute T. cruzi infection. Mice were treated orally with compound 4, Bnz, or vehicle (V1 or V2) for 14 days beginning 6 days postinfection. Under our experimental conditions, vehicle-treated mice demonstrated high levels of parasitemia, with parasitemia peaks around day 27 postinfection.

Parasitemias, anti-T. cruzi IgG antibody levels, and animal mortalities were significantly reduced in animals treated with thiazole 4 suspended in V2 at the three studied doses, 50, 100, and 200 mg/kg BW (Fig. 2 and 3A and B). When thiazole 4 was suspended in V1, at 50 mg/kg BW, the suppression of the parasitemia was lower than in vehicle V2 (see Fig. S2 in the supplemental material). At the highest studied dose, 200 mg/kg BW, thiazole 4 was not toxic when administered daily in healthy animals but was not well tolerated when administered daily for 14 days to infected...
animals. However, it showed improvement in the effect on parasitemia and antibody depletion. The positive effect of thiazole 4 on infected animals was clearly observed, taking into account changes in the animal flexibility, mobility, food intake, and grooming behavior (see Fig. S3 in the supplemental material).

**Histopathology.** Tissue sections were taken at the end of the acute experiment, day 60 after beginning treatment. No significant alterations in the liver, urinary bladder, or intestinal tissues were found. The heart tissue of the mice that received V2 showed clear differences in histopathology from the thiazole 4- and Bnz-treated mice. Whereas in the untreated infected animals the mononuclear inflammatory infiltrates were pronounced in the pericardium and between the myocardial fibers (Fig. 4C), in the infected animals treated with thiazole 4 or Bnz clear diminutions of these infiltrates were observed (Fig. 4A and B).

**DISCUSSION**

Bnz and Nfx are the only anti-*T. cruzi* drugs available on the market for human use, although they cause very significant undesirable toxic effects (26, 27).

Rational design allowed planning the preparation of thiazole 4 (Fig. 1; see also Table S1 in the supplemental material) that, according to Lipinski’s rule of five (see Table S8 in the supplemental material), would be orally administered with better bioavailability than the parent compound, thiazole 2 (Fig. 1) (10). Thiazole 4 is *in vivo* 1.5- to 4.5-fold more active than the parent compound 2 when the parasitemia loads at the maximums are compared (Fig. 5A and B) (10). Additionally, thiazole 4, unlike Bnz and Nfx (see Table S3) (6, 28, 29), was not mutagenic in the Ames test against the five strains recommended by the OECD, was not genotoxic *in vivo* at the assayed dose, and possessed a high LD$_{50}$.

We demonstrate the efficacy of thiazole 4 against *T. cruzi* infection. This compound was shown to be effective against epimastigotes (see Table S1 in the supplemental material), trypomastigotes, and intracellular amastigotes (Table 1). *In vivo*, thiazole 4 was able to reduce parasite loads in blood, being at least as effective as Bnz (Fig. 2). Thiazole 4, in doses about half of that and near that of Bnz (at 50 and 100 mg/kg BW, respectively), exhibited a particular biological profile. The second maximum parasitemia peak that appeared in the untreated animals at day 40 was abolished (Fig. 2). At the maximum dose, 200 mg/kg BW, thiazole 4, like Bnz at 50 mg/kg BW, shifted the parasitemia maximum 21 days with respect to the untreated animals (inset, Fig. 2). These data could show that thiazole 4 affected *in vivo* trypomastigotes, depleting the bloodstream and the amastigote forms. This effect was corroborated by the absence or the shifting of the second parasitemia peak.

The level of IgG antibodies could be one of the most reliable parameters to determine the cure of Chagas’ disease, since antibodies remain circulating in peripheral blood in response to even minimal or undetectable infection (30). The mice treated with thiazole 4 displayed low serological values 60 days after the beginning of the treatment (Fig. 3A). Although for some animals these titers were slightly higher than those for Bnz, there were not statistically significant differences between them.

Amastigote nests were not found in the histological sections analyzed in any of the group of animals analyzed. However, mice that received V2 displayed advanced signs of infection (lymphoplasmocytic inflammatory infiltrates) in heart tissue that were diminished in treated mice. Heart tissue sections displayed the lowest degree of damage in the thiazole 4-treated group, while in the Bnz-treated group the damage was greater (Fig. 4).

Furthermore, the adequate stability of thiazole 4, under physiological conditions, and its higher solubility in the developed microemulsion V2 facilitate its oral administration to mice.

In conclusion, we reported here a promising chemotherapeutic approach against *T. cruzi*, with thiazole 4 having a curative effect on experimental Chagas’ disease in the acute phase of a murine model of the infection. In this model, thiazole 4 and Bnz administered at the same doses led to comparable survival and cure levels. Therefore, thiazole 4 emerged as a promising candidate for further tests as an anti-*T. cruzi* agent. Future work with this promising new anti-*T. cruzi* agent will involve tests in the chronic phase of the disease and use of other treatment protocols or drug combinations.
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