PONTIFICIA UNIVERSIDAD CATÓLICA DEL ECUADOR

FACULTAD DE CIENCIAS EXACTAS Y NATURALES

CARRERA DE MICROBIOLOGÍA

Activity of bispirazoles against *Trypanosoma cruzi*

Disertación previa a la obtención del título de Licenciada en Microbiología

Ambar Nicol Galarza Jarrín

Quito, 2023

Evaluation of the activity of bispirazoles against recombinant *Trypanosoma cruzi* strains expressing ß-galactosidase

Disertación previa a la obtención del título de Licenciada en Microbiología

Ambar Nicol Galarza Jarrín

Quito, 2023

Yo Jaime Costales Cordero PhD, certifico que la disertación de Microbiología de la estudiante Ambar Nicol Galarza Jarrín ha sido concluida de conformidad con las normas establecidas; por lo tanto, puede ser presentada para la calificación.

Dr. Jaime Costales Cordero
DIRECTOR DE LA DISERTACIÓN
Quito, 16 de junio del 2023

Para mis padres, hermana y abuelitos, por estar y apoyarme siempre.

AGRADECIMIENTOS

Al Centro de Investigación para la Salud en América Latina (CISeAL) de la Pontificia Universidad Católica del Ecuador, por permitirme realizar mi trabajo de disertación en sus instalaciones y ayudarme con el financiamiento requerido para el mismo.

A Jaime Costales Cordero, PhD, por estar siempre pendiente de mi trabajo y por su ayuda incondicional, tanto en este trabajo como en mi formación académica.

A mi familia que fue un pilar fundamental, especialmente a mis padres, hermana y abuelitos que han estado pendientes de mí en cada momento y me han impulsado siempre a cumplir mis metas.

A mis amigos incondicionales Melanie, Daniela, Victoria Paúl y Alex, que siempre confiaron en mí y estuvieron pendientes de cada paso que iba dando.

A los scouts por la confianza que siempre han tenido en mí, especialmente al grupo 5 Muchachos de Mafeking por el soporte que me han brindado para mejorar en diferentes ámbitos de mi vida.

A los profesores de la Carrera de Microbiología, por el conocimiento impartido y el constante apoyo, especialmente a Martín Marcial, PhD, Mtr. Elenita Granda y Anita Villacís, PhD.

A Michelle del Salto, por los conocimientos impartidos que me ayudaron tanto en el ámbito profesional como personal, y porque a pesar de la distancia constantemente está pendiente y a Mateo, Sebastián y Josué por su apoyo.

Tabla de contenido

Lista de figuras

Lista de tablas

Evaluation of the activity of bispirazoles against a recombinant *Trypanosoma cruzi* strains expressing ß-galactosidase

Ambar Nicol Galarza Jarrín[1] and Jaime A. Costales [1*]

[1] Center for Research on Health in Latin America, Escuela de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador, San Pedro, 170170, Ecuador

AG: angalarza@puce.edu.ec

JAC: jacostales@puce.edu.ec

*Correspondence: jacostalesc@puce.edu.ec

Abstract

Background: Nifurtimox and benznidazole are the only drugs currently approved to treat Chagas disease, caused *Trypanosoma cruzi*. However, they display unsatisfactory efficacy and induce undesirable side effects. Therefore, identifying new compounds with specific activity against *T. cruzi* is of great interest.

Methods. The *in vitro* activity of twenty-five bispirazoles was evaluated against intracellular amastigotes of the recombinant Tulahuen ß-gal strain of *T. cruzi*. Their cytotocicity against mammalian cells was evaluated via resazurin reduction assays. BNZ was used as a reference drug.

Results: In the initial two repetitions of the screen, one bispirazole (2X) presented specific *in vitro* activity against *T. cruzi*, with an IC_{50} value of 3,416 µM and no measurable cytotoxicity at concentrations up 100 µM. BNZ, employed as reference drug, displayed a IC_{50} of 4,23 µM and a CC_{50} up to 100 µM. Compound 2X´s activity was not reproducible in subsequent replicates.

Conclusions: One evaluated compound was selectively toxic against intracellular *T. cruzi* amastigotes *in vitro* and did not show cytotoxic activity against mammalian cells. Its IC_{50} value is comparable to those obtained for BNZ, the reference drug. Further studies are warranted to confirm the anti-*T. cruzi* activity of compound 2X.

Key words: Chagas disease, cytotoxicity, bispirazole, parasitological treatment, colorimetric assay, *in vitro*.

1. Introduction

Chagas disease (CD), also known as American trypanosomiasis, is a neglected tropical infectious disease. This chronic systemic parasitic disease, caused by the protozoan parasite *Trypanosoma cruzi*, is transmitted mainly by hematophagous vectors (family Triatominae, subfamily Reduviidae), commonly known as kissing bugs or triatomine bugs [7]. CD is considered endemic in 21 countries in Latin America, and currently affects approximately 6 million people [14]. According to the Pan-American Health Organization (PAHO), 30 000 new cases are registered annually, among which there are 12 000 deaths and 8 600 are newborns infected during gestation [12].

The main transmission route is vectorial. Other infection routes are transfusion of contaminated blood units, transplantation of contaminated organs, oral transmission and congenital transmission [8].

T. cruzi is a kinetoplastid hemoflagellate protozoan capable of infecting multiple hosts, including humans [22]. The parasite´s life cycle begins when a triatomine bug feeds on the contaminated blood of an infected mammalian host, which contains trypomastigotes. These differentiate into epimastigotes in the foregut of the triatomine, then move to the hindgut, and transform into metacyclic trypomastigotes that are finally released in the triatomine´s feces [18]. Triatomines bite the host, usually during sleep, to feed on its blood [24]. At the time of the bite, or shortly after, the vector defecates, placing its parasite-containing feces in the vicinity of the bite wound [17]. By scratching or touching their eyes, the bitten person involuntarily inoculates *T. cruzi* into the bite or their mucous membranes. The parasite infects local cells and gains access to the bloodstream from where it spreads and invades different types of nucleated cells through a lysosome-mediated mechanism [4]. Once infection has been established in the host cell cytoplasm, the parasite replicates as non-flagellated amastigotes, which multiply and then revert to trypomastigotes, which in turn exit the host cell, reach the bloodstream and disseminate to new cells.

CD comprises two phases, acute and chronic. The acute phase lasts approximately 4 to 8 weeks post-infection, and can go unnoticed since the symptoms are usually fever, fatigue, headache, loss of appetite, nausea, diarrhea and vomiting, which can be easily confused with other pathologies, and eventually disappear on their own [23]. In the chronic phase, the

parasites remain hidden in reticuloendothelial cells of the spleen, liver, lymph nodes and myocardium [11]. Clinical manifestations appear after several years, with cardiac (cardiomyopathy, heart rhythm disturbances and cardiac arrest) or digestive (megaesophagus, megacolon and difficulties in swallowing or defecating) complications [6].

Currently, there are only two drugs approved for the parasitological treatment of CD: benznidazole (BNZ) and nifurtimox (NFX), both of which are nitroheterocyclic compounds. Factors such as early treatment initiation (during the acute phase), the strain of *T. cruzi*, the patient's age and immune status affect treatment efficacy [21]. However, these drugs are not ideal, require long treatment courses and can induce side effects, such as neurological alterations (disorientation, paresthesias, insomnia, seizures, polyneuritis, dizziness and headache), digestive (nausea, vomiting, anorexia, weight loss and abdominal pain), asthenia, dermatitis, arthralgias, granulocytopenia, thrombocytopenia, and are mutagenic compounds [12].

Fexinidazole (FNX) was a promising drug for CD treatment, with a higher efficiency than BNZ and NFX in preclinical studies, showing high cure rates for different *T. cruzi* strains in animal models [2]. In 2014, in Bolivia, with a cohort comprising forty-seven adult patients suffering chronic CD, a prospective, double-blind, multicenter, randomized, placebo-controlled clinical trial was initiated. Six treatment regimens were tested to determine if their effectiveness and safety. Although FNX was determined to be an effective antiparasitic, because it reduced parasite load to undetectable levels in chronic patients after eight days, the trial was suspended due to safety concerns. One fatal adverse event and twenty-four serious adverse events of neutropenia, hepatic, neuropsychiatric or nervous system abnormalities, such as insomnia, anxiety, depression and neuropathy were reported in the next twenty months [25]. Additional trials with lower FNZ doses were later carried out in Spain; however, they failed to prove parasitological cure [27].

Therefore, there is a pressing need for developing new treatments for Chagas disease. In this context, here, we have tested the *in vitro* anti-*T. cruzi* activity of twenty-five bispirazoles. The synthesis of this compounds and their activity against T. *brucei* and *Leishmaina mexicana* have been previously reported [3]. Some of these compounds displayed activity against these kinetoplastid parasites; therefore, testing them against *T, cruzi* was of interest.

2. Materials and Methods

2.1. Bispirazoles

Twenty-five previously described bispirazoles [3], were tested in this study. Bisirazoles in powder form were kindly provided by Dr. Jorge Heredia Moya, from the Center for Biomedical Research (CENBIO) of Universidad UTE. Compounds were stored at 4°C. Stock solutions were prepared in DMSO and maintained at -20 °C until use.

2.2.Mammalian cell culture

Macaca mulata LLC-MK2 monkey kidney cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1 % penicillin/streptomycin (DMEM 10). Cells were incubated at 37°C with 5 % CO and 98 % relative humidity. Passages were performed weekly.

2.3.Parasite culture

Tulahuen β-gal strain trypomastigotes were produced infecting LLC-MK2 cells monolayers in 75 cm^2 culture flasks. Cells were infected for 48 hours with $1{,}5x10^5$ trypomastigotes in 10ml DMEM supplemented with 2 % fetal bovine serum and 1 % penicillin/streptomycin (DMEM2), under the same incubation conditions used for mammalian cells. After 48 hours, the cell monolayer was washed with sterile PBS and medium was replaced and trypomastigotes collected starting at day 5 post infection, pelleted by centrifugation and washed 2X in sterile PBS. The amount of parasites was determined microscopically, counting with a hemocytometer.

2.4.Evaluation of trypanocidal activity of bispirazoles

LLC-MK2 cells ($2x10^4$/well) were placed in 200 µl of DMEM10 in each well of a 96-well plate and cultured for 24 hours at 37 °C with 5 % CO_2 and 98 % relative humidity. Subsequently, cell monolayers were infected with trypomastigotes of the Tulahuen β-gal

strain of *T. cruzi* at a multiplicity of infection (MOI) 2, in 200 µl of DMEM2 for 24 hours under the same incubation conditions. After infection, the culture medium was removed and cells were washed 4 times with 1X PBS. Finally, ten 2-fold serial dilutions (400 µM to 0.78125 µM concentration range) of each bispirazole in DMEM without phenol red were prepared and each dilution was applied in duplicate wells of a 96 well-plate. Plates were incubated for 72 hours at 37 °C with 5 % CO_2 and 98 % relative humidity. Seventy-two hours post-infection, 25 µl of a solution containing 500 µM chlorophenol red β-D-galactopyranoside (CPRG) and Triton (0,5 %) in PBS, were added to each well. Finally, the plate was incubated for 24 hours at 37 °C with 5 % CO_2 and 98 % relative humidity and the absorbance was read at 490 nm in multimodal GloMax microplate reader (Promega).

2.5. Evaluation of cytotoxicity against mammalian cells

A 96-well plate was seeded with $2x10^4$ LLC-MK2 cells/well, and cells were allowed to grow for 24 hours at 37 °C with 5 %CO_2 and 98 % relative humidity. Subsequently, culture medium was removed and compounds were added in duplicate in 1:2-fold serial dilutions in DMEM10, with concentrations ranging between 400 µM - 0,78125 µM. Plates were incubated for 48 hours, and 10 µl of 3 mM resazurin sodium salt (RZN) in PBS were added per well. Plates were incubated for 24 hours under the same conditions and cell viability was measured by RZN reduction, measuring fluorescence (530-560 nm excitation and 590nm emission) in a multimodal GloMax microplate reader (Promega).

2.6.Giemsa staining of infected mammalian cells treated with the studied bispirazoles

Three sterile circular coverslips per well, were placed in three mini-Petri dishes. In each mini-Petri dish, 2 ml of DMEM10 with $2x10^5$ LLC-MK2 cells were placed and incubated for 24 hours. Culture medium was removed, and cell monolayers were infected MOI 2of *T. cruzi* in 2ml DMEM2, plates were incubated for 24 hours. After infection, culture medium was discarded and cells were washed 4 times with 1X PBS, bispirazoles were added at their respective IC50 concentration against *T. cruzi* and incubated for 72 hours.

Finally, cell monolayers were rinsed in 1X PBS, the coverslips were removed, and cells fixed with 4% paraformaldehyde and stained with Giemsa. Photographs were obtained

under the 100X lens in a BX51 Olympus microscope equipped with a DP72 camera using Cell F imaging software.

2.7.Statistical analysis

The half maximal inhibitory concentration (IC_{50}) was calculated using statistic software GraphPad prism 8,0. IC_{50} and CC_{50} values of each chemical compound were determined using a nonlinear regression with curve fitting [Model: log (inhibitor) vs. response (three parameters)]. A confidence interval of 95% was established. The IC_{50} (inhibitory concentration) and the CC_{50} (cytotoxic concentration) were defined as the chemical concentration that inhibited less than 50% of the parasitic growth.

3. Results

3.1.Activity of studied compounds against T. cruzi

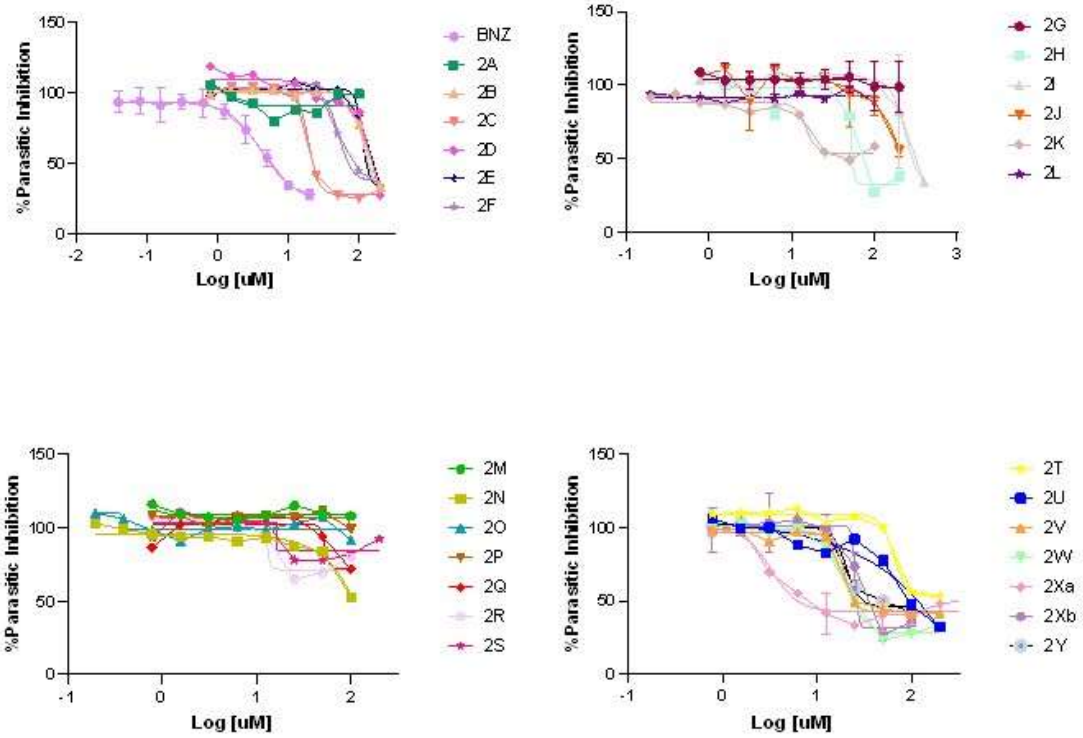
The activity of each chemical compound against *T. cruzi* is shown in Table 1. BNZ was used as reference drug, yielding an IC_{50} of 4.23 µM. Some bispirazoles (2C, 2K, 2V, 2W, 2X, 2Y) were found to affect *in vitro* intracellular amastigote growth, with IC_{50}s below 20 µM. In some cases, compounds were found not to affect the parasite, with IC_{50} greater than 100 or >200 µM (2A, 2B, 2D, 2E, 2F, 2G, 2H, 2I, 2J, 2L, 2M, 2N, 2O, 2P, 2Q, 2R, 2S, 2T), including compounds previously reported to be active against *T. brucei* (2F, 2N, 2Q, 2R, 2W, 2X, 2Y) and *Leishmania mexicana* (2A, 2D, 2I, 2J, 2K, 2N, 2Q, 2R, 2S, 2T, 2V, 2W, 2X). In our study, the best results were obtained for compounds 2X, 2Y and 2K with IC_{50}s of 3,416, 12,35 and 14,82, respectively. The initial two replicates for compound 2X (designated as 2Xa in Table 1) had an IC_{50} of 3,416. For a yet undetermined reason, subsequent replicates of this compound displayed IC_{50}s of 25,48 (2Xb in Table 1).

Table 1. IC_{50} and CC_{50} values of bispirazoles and BNZ against mammalian cells and T. cruzi.

Compound [a]	IC_{50} against *T. cruzi* (± SD) 72h [b]	CC_{50} against mammalian cells 72h [c]	Selectivity index [d]
BNZ	4,23 ± 3,50	>100	>23,64
2A °	>200	N.D.	N.D.
2B	>100	N.D.	N.D.
2C	17,92 ± 9,72	>100	N.D.
2D °	>100	N.D.	N.D.
2E	>200	N.D.	N.D.
2F *	51 ± 4,17	N.D.	N.D.
2G	>200	N.D.	N.D.
2H	57,47 ± 4,26	N.D.	N.D.
2I °	>100	N.D.	N.D.
2J °	54,22 ± 3,59	N.D.	N.D.
2K °	14,82 ± 4,86	>100	N.D.
2L	>200	N.D.	N.D.
2M	>100	N.D.	N.D.
2N °*	>100	N.D.	N.D.
2O	>200	N.D.	N.D.
2P	>200	N.D.	N.D.
2Q °*	>200	N.D.	N.D.
2R °*	>200	N.D.	N.D.
2S	>200	N.D.	N.D.
2T	54,22 ± 3,59	N.D.	N.D.
2U	63,51± 7,82	N.D.	N.D.
2V	18,44 ± 3,12	>200	>10,85
2W °*	20,16 ± 4,47	>200	>9,92
2Xa °*	3,294 ± 8,15	>100	>29,27
2Xb °*	25,48 ± 5,13	>100	>3,92
2Y °*	12,54 ± 1,63	>100	>7,97

[a] Compound showed activity in previous studies against *L. mexicana* ° and/or *T. brucei* * ; [b] IC_{50} against *T. cruzi*; [c] CC_{50} against mammalian cells; [d] selectivity index = CC_{50}/IC_{50}. N.D. = Not done

(a)


%Parasitic Inhibition
Log [uM]
BNZ
2A
2B
2C
2D
2E
2F
2G
2H
2I
2J
2K
2L
2M
2N
2O
2P
2Q
2R
2S
2T
2U
2V
2W
2Xa
2Xb
2Y

(b)

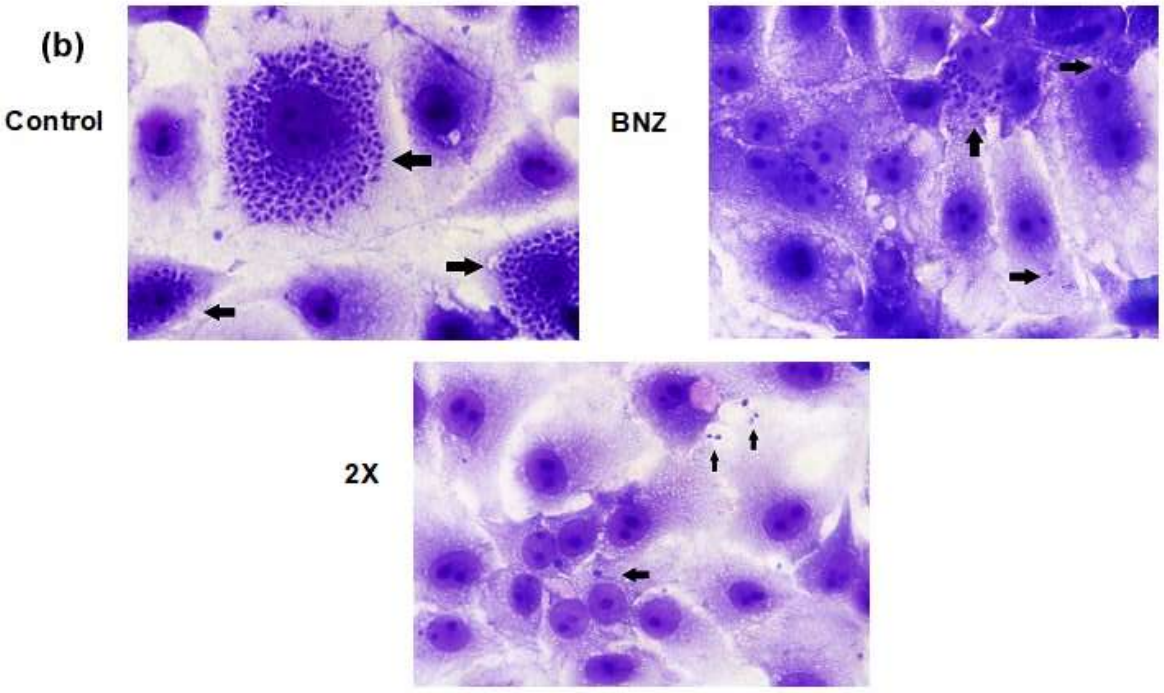

Control
BNZ
2X

Figure 1. Activity of studied compounds against *T. cruzi*. (a) Curves for activity of each studied bispirazole against intracellular amastigotes of Tulahuen ß-gal strain of *T. cruzi*.LL-MK2 cells were infected for 24 hours with trypomastigotes from *T. cruzi* Tulahuen ß-gal strain (MOI 2) and were exposed to each of the 27 compounds in a ten two-fold serial dilutions (400-0,78125 µM concentration range). BNZ was included as a reference drug (20-0,03936 µM concentration range). After 72 hours of exposure to the compounds, antiparasitic activity was evaluated via colorimetric measurement of ß-galactosidase, as indication of amastigote multiplication within infected cells. Curves corresponding compounds are displayed in groups of seven, in alphabetical order, according to the compound designation. Averages and standard deviations from three independent replicates are shown (b) Representative images showing Giemsa-stained infected cells, with the concentration corresponding to the IC50 of each compound: 2X (IC_{50}=[25.5µM]), BNZ (IC_{50}=[4.2µM] and vehicle control.

3.2.Cytotoxicity of studied bispirazoles against mammalian cells

Cytotoxicity was evaluated exclusively for compounds displaying IC50s under 20 µM against *T. cruzi*. The results of the cytotoxicity analysis are displayed in Table 1. BNZ was used as a reference drug, and proved not to affect mammalian cells at the tested concentrations.

Tested chemicals were not cytotoxic, with CC50 against mammalian cells >100 µM (Table 1).

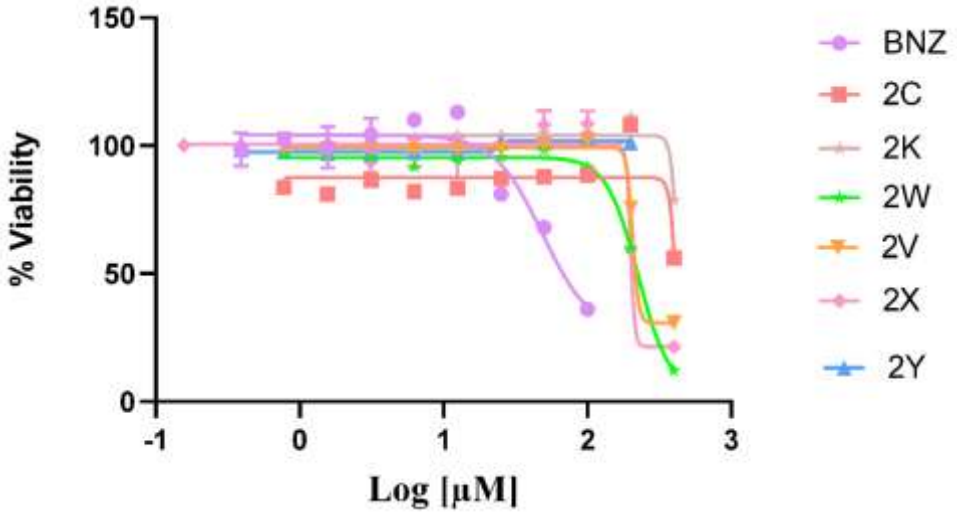


Figure 2. Mammalian cell cytotoxicity of compounds active against *T. cruzi*. Only compounds displaying activity against *T cruzi* at concentrations < 25 µM were analyzed.

LLC-MK2 cells were exposed to ten two-fold serial dilutions (400-0,78125 µM concentration range) of selected compounds. After 72 hours of exposure, cell viability was evaluated with RNZ reduction. Percent viability is shown. BNZ as refence drug (20-0,03936 µM concentration range).

4. Discussion

Currently available drugs for CD treatment are scarce, not effective, toxic and must be used over long periods of time. BNZ and NFX are nitroheterocyclic drugs used to treat CD; however, these drugs have poorly understood pharmacokinetic (PK) and pharmacodynamic (PD) properties. For this reason, they frequently cause serious side effects, like neurological and digestive disorders, asthenia, dermatitis, arthralgias, granulocytopenia, thrombocytopenia, and are mutagenic compounds [6].

BNZ is approved by the FDA for treatment of CD in pediatric patients (ages 2 to 12), with cure rates 60-90% when administered in early stages of the disease. BNZ had antiparasitic activity through the action of free radical intermediates and electrophilic metabolites that eliminate macromolecules such as DNA, lipids and proteins [10].

NFX is effective during acute phase of the infection in children (from 0 to 18 years old) or recently infected patients. NFX metabolization yields nitrile derivatives, which have antiparasitic activity [20]. NFX, is 70% effective in curing CD during the acute phase and 20% in the chronic phase [19]

Bispirazoles are a group of organic compounds characterized by the presence of two pyrazole rings connected by a spacer group. These compounds have diverse pharmacological activities and potential applications in medicine. Bispirazoles exhibit a range of biological properties, including antimicrobial, anti-inflammatory, anticancer, antiviral, insecticide, antifungal, phytotoxic, antibiotic, antifungal, antitumor, hormonal and antiparasitic activities [1]. Previously, a bispirazole panel was tested against *L. mexicana* and *T. brucei brucei*, kinetoplastids closely related to *T. cruzi*. Several compounds displayed antiparasitic activity, resulting in reduced the proliferation of these parasites *in vitro* [3].

Here, we have evaluated the *in vitro* anti-proliferative activity of the same twenty-five bispirazoles against intracellular *T. cruzi* amastigotes of the Tulahuen ß-gal strain. BNZ was used as a positive control, and it displayed an IC_{50} of 4,23 µM and an CC_{50} >100 in our assays. Bispirazole 2X displayed potent activity against intracellular *T. cruzi* amastigotes (IC_{50}= 3,416 µM) and a low cytotoxicity against mammalian cells (CC_{50} >100) in our initial assays. However, in later replicates, the antiparasitic activity of this organic compound was much less intense (IC_{50} of 25,48 µM). The causes for this change in activity remains unclear; however, they could include compound deterioration during storage or variations in hots cell permeability when mammalian cells batches were renewed in the assays. Further studies with newly synthesized batches of compound 2X are warranted. Previously, compound 2X displayed an IC_{50} of 7 µM against *L. mexicana* and an IC_{50} of 1,9 µM against *T. brucei* [3].

Previously, the same 25 bispirazoles were studied against *L. mexicana* and *T. brucei*. Thirteen of them (2A, 2D, 2I, 2J, 2K, 2N, 2Q, 2R, 2S, 2T, 2V, 2W, 2X) showed activity against *L. mexicana*, with ICC_{50}s ranging from 3 to 13 µM. Additionally, seven bispirazoles (2F, 2Q, 2R, 2W, 2X, 2Y), were reported to inhibit growth in *T. brucei*, with IC_{50} values ranging from 0,9 to 10 µM [3]. In our study, three bispirazoles presented antiparasitic activity against *T. cruzi* (2K,2X, 2Y), with IC_{50} values ranging from 3 to 15 µM. Interestingly, compound 2X has shown effect against these three kinetoplastid parasites.

The 25 studied bispirazoles have a pirazol ring in common, which has been a key structure in different drugs with clinical use [3]. Regarding the compounds active against *T. cruzi*, some particular characteristics are: 2K, and 2X are electron receptors, while 2Y has a hydroxyl group. Additionally, 2X has a trifluoromethyl group [3].

Based on our results, further studies to clarify the causes driving the variation in the IC_{50} value of compound 2X in our assays. After testing the efficiency of compound 2X, future studies should include cidality testing, testing against other *T. cruzi* strains, testing in combination with BNZ to gain insights into mode of action, testing of compounds with related structures to determinate which might be even more selectively toxic to *T. cruzi*.

CD is a disease that generates great concern in health systems due to the complications it causes and the reduced existing treatments; therefore, the search for new compounds that

are effective against *T. cruzi* and that do not generate side effects or harmful effects against host cells is of great importance.

5. Conclusions

We have identified one chemical organic compound (2X), which is potentially selectively toxic against intracellular *T. cruzi* amastigotes *in vitro*. In previous studies, this compound also displayed activity against other kinetoplastids. In our assays, compound 2X presented an IC_{50} value comparable with BNZ, the drug of choice for CD treatment. When tested side by side, compound 2X displayed an IC_{50} lower than other compounds previously reported as active against *T. cruzi* in the literature [26]. Confirmation of the activity of compound 2X and further testing is warranted, including cidality testing, evaluation of structurally related compounds and activity against different *T. cruzi* strains.

Acknowledgements

The authors would like to thank of Universidad Tecnológica del Ecuador (UTE) researchers, especially Dr. Jorge Heredia, as well as Michelle Del Salto, from CISeAL, for their support. Funding for this research was provided by the Pontificia Universidad Católica del Ecuador.

References

1. Aguilar, L.E.; Romero B.J.C.; Heredia M.J. Synthesis of 4-Arylallylidenepyrazolone Derivatives. *MDPI Chemestry*. **2021** 8, 7.
2. Bahia, M.T.; De Andrade, I.M.; Martins, T. A.; Do Nascimento, Á.F.; Diniz, L.F.; Caldas, I.S.; Talvani, A.; Trunz, B.B.; Torreele, E.; y Ribeiro, I.; Fexinidazole: a potential new drug candidate for Chagas disease. *PLoS Negleted Tropical Disesases* **2012** 6, e1870.
3. Barreiro, O.; Quiroga, C.; Muñoz, E.: Rojas, P.; Medeiros, A.; Comini, M.; Heredia, J. Evaluation of the Anti-Leishmania mexicana and -Trypanosoma brucei Activity and Mode of Action of 4,40 -(Arylmethylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ol). *MDPI Biomedicines* **2022,** 10.
4. Bern, C.; Kjos, S.; Yabsly, M.; Montgomery, S. Trypanosoma cruzi and Chagas' Disease in the United States. Clinical Microbiology Reviews **2011** 24,655-681.
5. Cadena, J.; Guamán, L.; Romero, J., Bailon, N.; Murillo, K.; Ortiz, N.; Heredia, J. Synthesis of 4,4′-(arylmethylene)bis(3-methyl-1-phenyl-1H-pyrazol-5-ols) and evaluation of their antioxidant and anticancer activities. BMC Chemistry **2021**, 15.
6. CDC (2022). Enfermedad el Chagas Hoja informativa detallada. Available online: https://www.cdc.gov/parasites/chagas/es/informativa/detallada.html#:~:text=La%20enfermedad%20de%20Chagas%20tiene,primeros%20meses%20de%20la%20infecci%C3%B3n.
7. Gonzales, N.; Morocoima, A.; Martínez, C.; Carrasco, J. Infección por Trypanosoma cruzi y polimorfismo del Citocromo B del ADN

mitocondrial en Triatoma maculata de Anzoategui y Portuguesa, Venezuela. Boletín de Malariología y Salud Ambiental **2010,** 50.

8. Lidani, C.; Andrade, F.; Bavia, L.; Damasceno, F.; Beltrame, M.; Messias, M.; Sandri, T. Chagas Disease: From Discovery to a Worldwide Health Problem. Frontiers in Public Health **2019**, 7.
9. Lourenço, A.M.; Faccini, C.C.; Costa, C.A.J.; Mendes, G.B.; Fragata F.A.A. Evaluation of in vitro anti-Trypanosoma cruzi activity of medications benznidazole, amiodarone hydrochloride, and their combination. Revista da Sociedade Brasileira de Medicina Tropical **2018**, 51, 52–56.
10. Mazzeti, A.L.; Capelari O.P.; Bahia, M.T.; y Mosqueira, V.C.F. Review on Experimental Treatment Strategies Against Trypanosoma cruzi. Journal of Experimental Pharmacology **2021**, 13, 409–432.
11. Molina, I.; Salvador, F.: Sánchez, A. Actualización en enfermedad de Chagas. Enfermedades Infecciosas y Microbiología Clínica **2016**, 43, 132-138.
12. Murillo, G. Enfermedad de Chagas (tripanosomiasis americana). Medicina Interna México **2018**, 34, 959-970.
13. MSP (2021). Subsistema de vigilancia sive- alerta enfermedades transmitidas por vectores ecuador, se 01- 2021. Available online: https://www.salud.gob.ec/wp-content/uploads/2021/01/Vectores-SE-01.pdf
14. Nunes, M.; Dones, W.; Morillo, C.; Encina, J.; Ribero, A.; Chagas Disease: an overview of clinical and epidemiological aspects. Journal of the American College of Cardiology **2013**, 62, 767-776.

15. OMS (2020). La enfermedad de Chagas (tripanosomiasis americana). *Datos y cifras.* Available online: https://www.who.int/es/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis)
16. OPS (2021). Enfermedad de Chagas. Available online: https://www.paho.org/es/temas/enfermedad-chagas
17. Palmezano, J.; Plazas, L.; Rivera, K.; Rueda, V. Enfermedad de chagas: realidad de una patología frecuente en Santander, Colombia. Revista de los estudiantes de medicina de la universidad industrial de Santander **2014**, 28, 81-90.
18. Pereira, A.; Pérez, M. Tripanosomosis. Enfermedad de Chagas y enfermedad del sueño. Offarm **2003**, 22, 104-111.
19. Perez, J.; Crespillo, C.; Bosch, P,; Molina, I. Trypanocidal treatment of Chagas disease. Enfermedades Infeccionas y Microbiología Clínica **2021**, 39, 458-470.
20. Rojo, G.; Castilloa, C.; Duasoa, J.; Liempia, A.; Droguetta, D.; Galantib, N.; Mayac, J.D.; López M.R.; Kemmerling, U. (2014). Toxic and therapeutic effects of Nifurtimox and Benznidazol on Trypanosoma cruzi ex vivo infection of human placental chorionic villi explants. Acta tropica **2014**, 132, 112-118.
21. Salazar, P.; Bucio, M.; Cabrera, M.; De Alba, M.; Castillo, D.; Zenteno, E.; Rojo, J.; Fernandez, S.; Perera, M. (2016). Enfermedad de Chagas en México. Revista de la Facultad de Medicina de la UNAM **2016**, 59, 6-16.
22. Tanowitz, H.; Kirchhoff, L.; Simon, D.; Morris, S.; Weiss, L.; Wittner, M.; (1992). Chagas' disease. Clinical Microbiology Reviews **1999**, 5, 400-419.
23. Teixeira, A.; Nitz, N.; Guimaro, M.; Gomes, C.; Santos, C.; Chagas disease. Postgraduate Medical Journal **2006**, 82(974) 788-798.

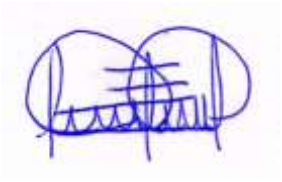
24. Toso, A.; Vial, F.; Galanti, N. Transmisión de la enfermedad de Chagas por vía oral. Revista Médica Chile **2011**.

25. Torrico, F.; Gascón, J.; Ortiz, L.; Pinto, J.; Rojas, G.; Palacios, A.; Barreira, F.; Blum, B.; Schijman, A. G.; Vaillant, M.; Strub W.N.; Pinazo, M.J.; Bilbe, G.; Ribeiro, I. A Phase 2, Randomized, Multicenter, Placebo-Controlled, Proof-of-Concept Trial of Oral Fexinidazole in Adults with Chronic Indeterminate Chagas Disease. Clinical Infectious Diseases **2022**, 76, 1187-1194.

26. Vela, A.; Coral A.M.; Sereno, D.; Costales, J.A.; Barnabé, C.; Brenière, S.F. In vitro susceptibility of Trypanosoma cruzi discrete typing units (DTUs) to benznidazole: A systematic review and meta-analysis. PLoS neglected tropical diseases, **2021**, 15.

27. Watson, J.A.; Strub W.N.; Tarral, A.; Ribeiro, I.; Tarning, J.; y White, N.J. Pharmacokinetic-Pharmacodynamic Assessment of the Hepatic and Bone Marrow Toxicities of the New Trypanoside Fexinidazole. Antimicrobial agents and chemotherapy **2019**, 63(4), e02515-18.

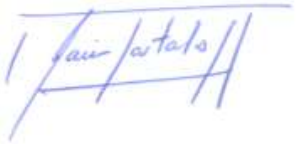
Firma de la estudiante y fecha:



Ambar Nicol Galarza Jarrín

Quito, 16 de julio del 2023

Firma del director de disertación y fecha:



PhD. Jaime Costales Cordero

Quito, 16 de julio del 2023

Firma de la coordinadora de la carrera y fecha:

MSc Sonia Estrella

Quito, 16 de julio del 2023