

2021-08-01

## In The Search For Novel Treatments For Chagas' Disease Using Cutting-Edge Imaging Technology

Karsten Dieter Amezcua Winter  
*University of Texas at El Paso*

Follow this and additional works at: [https://scholarworks.utep.edu/open\\_etd](https://scholarworks.utep.edu/open_etd)



Part of the [Biology Commons](#), [Molecular Biology Commons](#), and the [Parasitology Commons](#)

---

### Recommended Citation

Amezcua Winter, Karsten Dieter, "In The Search For Novel Treatments For Chagas' Disease Using Cutting-Edge Imaging Technology" (2021). *Open Access Theses & Dissertations*. 3214.  
[https://scholarworks.utep.edu/open\\_etd/3214](https://scholarworks.utep.edu/open_etd/3214)

This is brought to you for free and open access by ScholarWorks@UTEP. It has been accepted for inclusion in Open Access Theses & Dissertations by an authorized administrator of ScholarWorks@UTEP. For more information, please contact [lweber@utep.edu](mailto:lweber@utep.edu).

IN THE SEARCH FOR NOVEL TREATMENTS FOR CHAGAS' DISEASE USING  
CUTTING-EDGE IMAGING TECHNOLOGY

KARSTEN DIETER AMEZCUA WINTER

Master's Program in Biological Sciences

APPROVED:

---

Rosa A. Maldonado, D.Sc., Chair

---

Siddhartha Das, Ph.D.

---

Delfina Dominguez, Ph.D.

---

Anita Quintana, Ph.D.

---

Stephen L. Crites, Jr., Ph.D.  
Dean of the Graduate School

Copyright ©

by

Karsten Dieter Amezcua Winter

2020

## **Dedication**

To my parents, grandparents and supporting friends.

IN THE SEARCH FOR NOVEL TREATMENTS FOR CHAGAS' DISEASE USING  
CUTTING-EDGE IMAGING TECHNOLOGY

by

KARSTEN DIETER AMEZCUA WINTER, B.S.

THESIS

Presented to the Faculty of the Graduate School of

The University of Texas at El Paso

in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE

Department of Biological Sciences

THE UNIVERSITY OF TEXAS AT EL PASO

August 2021

## **Acknowledgements**

I want to thank my great laboratory members Itzel Tejeda, Caresse Torres, and specially to Felipe Rodriguez who trained me and guided me since day one and still does without a doubt. I want to thank Dr. Almeida and his laboratory members to help me all these years. Specially a warm hug and appreciation to Dr. Susana Portillo and Dr. Nasim Rodriguez who guided me and helped me in every way possible. I would like to thank Dr. Varela and Denisse Gutierrez who helped me with the use of the In-Cell analysis.

Most importantly I want to thank Dr. Rosa Maldonado who put her hopes in me. She has been the most supporting human being and role model in science to me. If it was not for Dr. Maldonado I would not have gotten so far as a scientist and as the person I am today. She is one of the main reasons I will be joining the D.D.S program at The University of Maryland at Baltimore 25'.

## Abstract

Chagas disease (ChD) is caused by *Trypanosoma cruzi* (*T. cruzi*), an intracellular protozoan parasite. ChD has a global mortality of 15,000 annual deaths, and approximately, 8-10 million people are infected. There is growing concern in the United States as autochthonous cases of ChD have been reported in the southern region. The two available treatments are only partially effective and highly toxic. N-aroyle derivatives and  $\alpha$ ,  $\beta$ -unsaturated ketones have been previously tested against *Leishmania*, a closely related parasite, demonstrating selective toxicity towards the microorganism. The objective of this study is to evaluate a drug library consisting of 21  $\alpha$ ,  $\beta$ -unsaturated ketones and 15 N-aroyle derivatives as anti-trypanosomal treatments. We hypothesized there will be at least one effective candidate from the drug libraries against *T. cruzi*. High-throughput screening was used to evaluate this. Epimastigote forms of *T. cruzi* CL Brenner-*luc* were used to assess the anti-parasitic activity of the compounds through a luciferase viability assay. Six compounds showed low toxicity to Human bone osteosarcoma epithelial cells (U2OS) and Rhesus monkey kidney epithelial cells (LLC-MK2). Six of  $\alpha$ ,  $\beta$ -unsaturated ketones and three N-aroyle compounds showed good anti-trypanosomal activity with an EC<sub>50</sub> ranging from 0.19 nM to 1.00  $\mu$ M and a selectivity index (SI) ranging from 33 to 526. Our future directions include a) to determine the compounds activity against amastigotes (intracellular form of the parasite) by High-Content Imaging (HCI), b) tested in the murine model of Chagas disease and c) to explore the possible mode of action of the lead(s) compounds.

## Table of Contents

Acknowledgements .....	v
Abstract .....	vi
Table of Contents .....	vii
List of Tables .....	viii
List of Figures .....	ix
Chapter 1: General Information .....	1
1.1 - Trypanosoma <i>cruzi</i> Background .....	1
1.1 - $\alpha$ , $\beta$ -unsaturated ketones and N-aroyl derivatives .....	2
Chapter 2: Therapeutic Effect Analysis of $\alpha$ , $\beta$ -unsaturated ketones and N-aroyl derivatives Against Trypanosoma <i>cruzi</i> .....	6
References: .....	29
Vita.....	33



**List of Tables**

Table 1:..... 13

Table 2:..... 24

Table 3:..... 25

## List of Figures

Figure 1 .....	3
Figure 2 .....	4
Figure 3 .....	5
Figure 4 .....	16
Figure 5 .....	18
Figure 6 .....	20
Figure 7 .....	22
Figure 8 .....	28

## Chapter 1: General Information

### 1.1 Chagas Disease Importance

Chagas disease (ChD) is caused by the infection of the protozoan parasite *Trypanosoma cruzi*. It is an endemic disease in Latin America [1]. The prevalence of this disease is more notable in rural communities. It is estimated that 6 to 7 million people are infected worldwide [2]. Currently, more than 300,000 patients in the United States are evaluated to be chronically infected with Chagas Disease [4-5]. Most of the focus of infection in the U.S. is in southern states as shown in Figure 1. The main route of transmission is by triatomine (known as “kissing bug” in the United States) vector, as well as blood transfusion, organ transplants, and contaminated food or beverages. [4, 11]. In recent decades patients have been found positive in the United States and Europe due to globalization and the prominence of these vectors in those areas. *T. cruzi* infection in Texas (Figure 1 and 2) [11].

Chagas disease infection consists of two phases, the acute phase and the chronic phase [5]. The acute phase consisting from the time of the infection up to weeks or months. A few patients develop symptoms at the acute phase such as, “chagoma” (inflammation at the infection area), fever, nausea, between other general symptoms [5-7]. Early detection at the acute phase can influence the faith of the patient. Patient’s early treatment can help predict a satisfactory outcome. At the end of the acute phase, symptomatic patients stop showing any symptoms. Consequently, infection switches into the chronic phase [10]. The chronic phase lasting life-long without treatment [5]. Approximately 20-30% of patients suffering with chronic stage will develop abnormal organ outgrowth causing heart failure (cardiomyopathy) and/or gastrointestinal problems (megacolon and/or megaesophagus) [6, 16].

## 1.2 *Trypanosoma cruzi* Life Cycle

The replicative stage (non-infective), known as epimastigotes *T. cruzi* are found in the midgut of the triatomine [11]. Thereafter, the parasite is transported to the hindgut where it transforms to the infective form, metacyclic trypomastigotes *T. cruzi*. When the triatomine takes a blood meal from the host, it deposits infected feces containing metacyclic trypomastigotes, simultaneously. Metacyclic trypomastigotes parasites can be introduced to any mucosal membrane (eye, nose or mouth) or through a bite wound if the feces are deposited close enough. In such event, parasites can invade the host cells. Within the cell, the metacyclic trypomastigote will transform into intracellular amastigotes. The intracellular amastigotes will reproduce by binary fission and convert into intracellular trypomastigotes causing the cell to burst. Subsequently, the trypomastigotes will infect adjacent cells and/or ingested through a blood meal uptake of another triatomine. When the parasite infects the triatomine, it will be converted into epimastigote *T. cruzi* in the midgut. After the epimastigotes migrate to the hindgut undergoing metacyclogenesis transforming metacyclic trypomastigote, repeating its life cycle (Figure 3) [10].

## 1.3 Current Treatments

Nifurtimox and benznidazole are the only market drugs available and both have FDA approval. The compounds can be used together or separate, depending on the response of the patient. They are most effective when the patient is on the acute phase [16, 17, 18]. However, there is only an approximate 15% of effectivity when the patient is on the chronic stage [17]. On the other hand, treatment using these compounds is long lasting up to 60 days and are highly toxic. Therefore, we are in a search for a novel treatment against *T. cruzi* infection in the chronic phase [8, 10, 18].

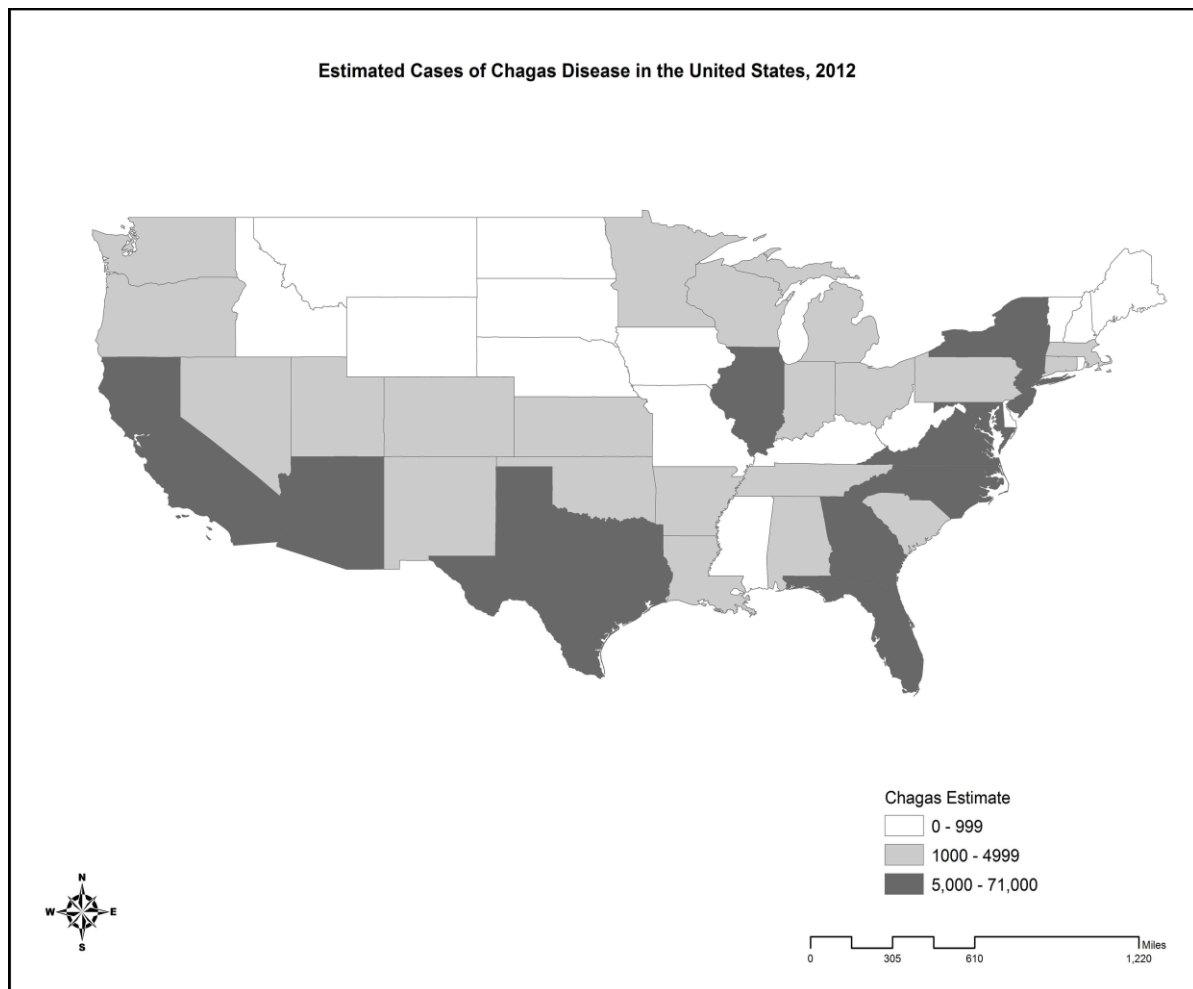


Figure 1. Estimation of Chagas Disease Cases in the United States, 2012. [6].

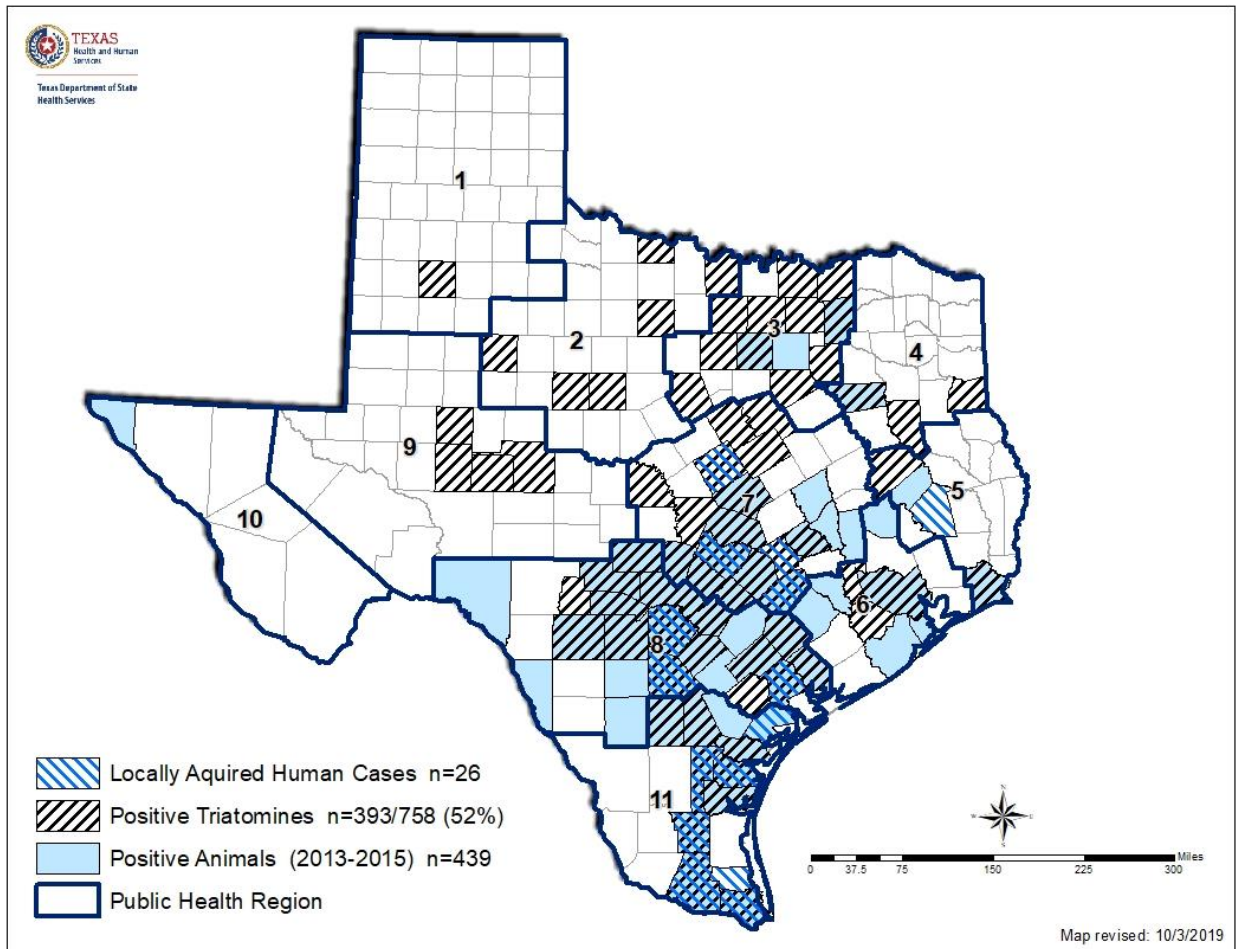


Figure 2. Demographic distribution of *Trypanosoma cruzi* infection in Texas (2013-2018)

[7].

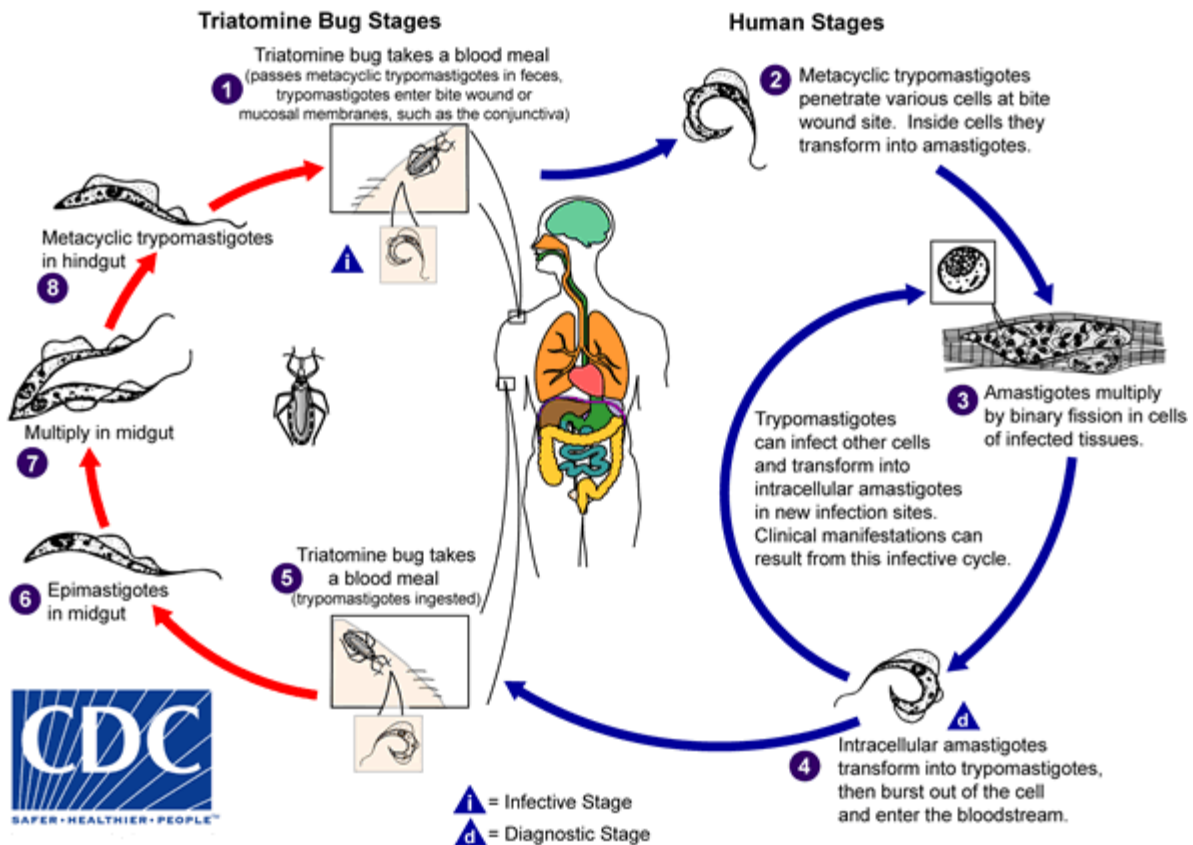


Figure 3. Life cycle of *Trypanosoma cruzi*.

[https://www.cdc.gov/parasites/images/chagas/AmerTryp\\_LifeCycle.gif](https://www.cdc.gov/parasites/images/chagas/AmerTryp_LifeCycle.gif)

Figure 3. Life cycle of *Trypanosoma cruzi*

## Chapter 2: Therapeutic Effect Analysis of $\alpha$ , $\beta$ -unsaturated ketones and N-aroyl derivatives

### 2.1 $\alpha$ , $\beta$ -unsaturated Ketones and N-aroyl Derivatives

Previous studies performed by our laboratory have shown that the organic compounds,  $\alpha$ ,  $\beta$ -unsaturated ketones and N-aroyl derivatives, have a potential anti-trypanosomal activity to be a potential treatment for Chagas disease [9]. Trypanosomatids carry a thiol-dependent metabolism which is used as a detoxification defense mechanism [16]. The organic compounds,  $\alpha$ ,  $\beta$ -unsaturated ketones (enols) and N-aroyl derivatives drugs have shown potential interaction with cellular thiols [14]. The unsaturated ketone alkylate, the N-acetyl-L-cysteine and other biomolecules that contain a thiol group and the phenolic hydroxy group behave as a free oxygen radical scavenger [15, 22]. These types of compounds have previously been published in our lab against the protozoan parasite *Leishmania major* [23, 24]. *Trypanosoma cruzi* and *Leishmania major* belong to the Trypanosomatidae family [9]. Both species contain a thiol-dependent detoxification defense mechanism using a redox pathway [12, 16, 21]. This relatedness indicate that the activity of the compounds will have good anti-parasitic effects.

### 2.2 Hypothesis

We hypothesize that the new generation of  $\alpha$ ,  $\beta$ -unsaturated ketones and N-aroyl derivatives will have improved antiparasitic activity against *Trypanosoma cruzi*.

**SPECIFIC AIM 1** – To determine the potential toxicity of  $\alpha$ ,  $\beta$ -unsaturated ketones and N-aroyl derivatives against mammal cells using an *in vitro* toxicity assay. The cell lines used will be Human Bone Osteosarcoma Epithelial Cells (U2OS) and Rhesus monkey kidney epithelial cells



(LLC-MK2) at different concentrations of the  $\alpha$ ,  $\beta$ -unsaturated ketones and N-aroyl derivatives compounds.

**SPECIFIC AIM 2** – To evaluate the anti-trypanosomal activity of the  $\alpha$ ,  $\beta$ -unsaturated ketones and N-aroyl derivatives. The drug screening will be accomplished by a bioluminescent assay using *T. cruzi* CL Brenner-*luc*, which overexpresses luciferase. Several concentrations of the new generation of  $\alpha$ ,  $\beta$ -unsaturated ketones and N-aroyl derivatives will be used to treat the parasites.

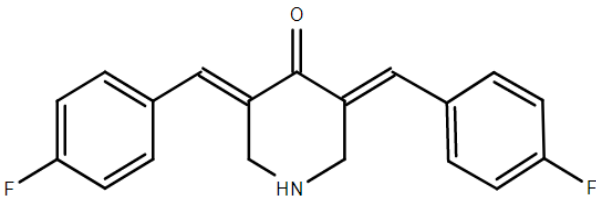
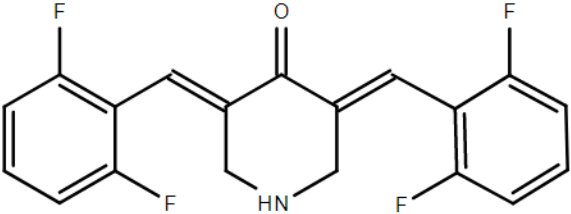
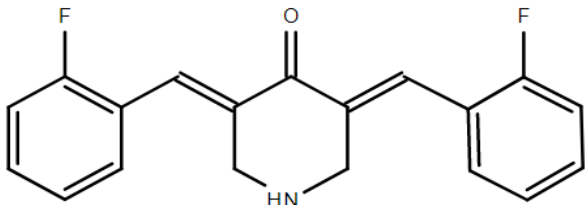
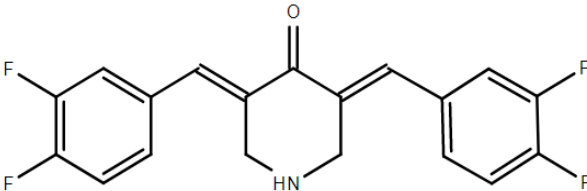
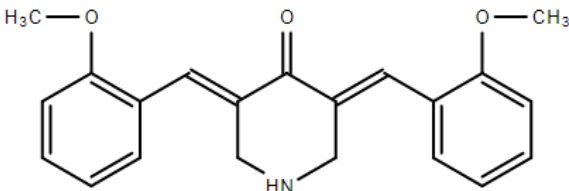
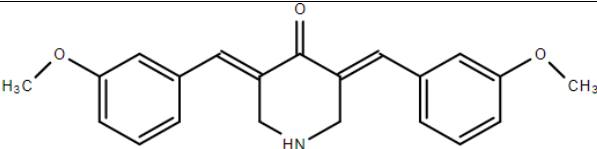
## **2.3 Materials and Methods**

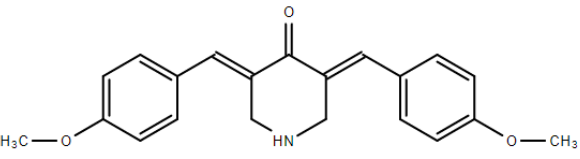
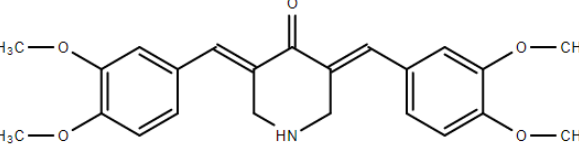
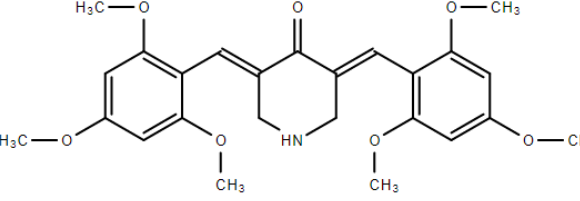
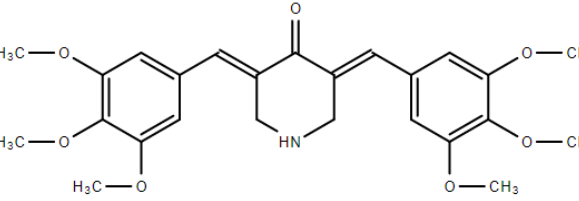
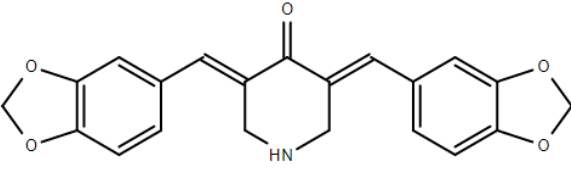
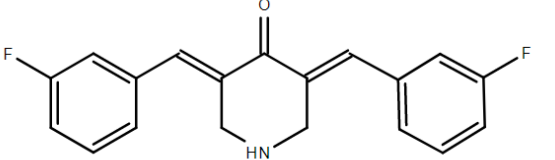
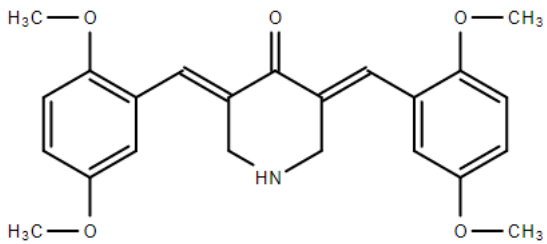
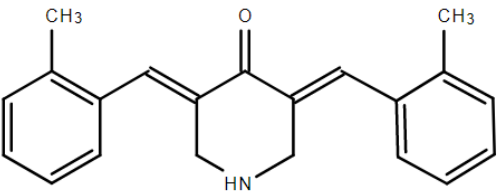
### **2.3.1 Cellular Strains and Compounds Tested**

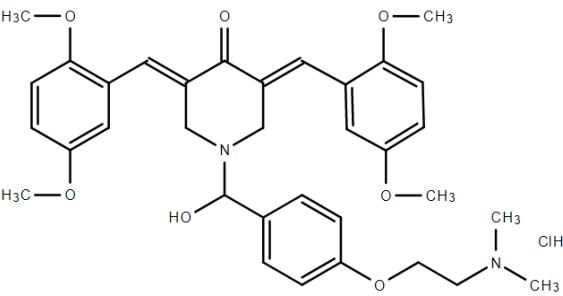
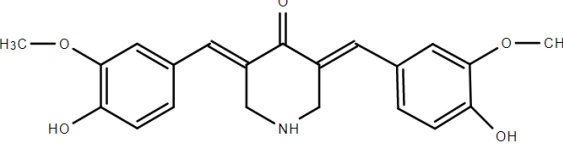
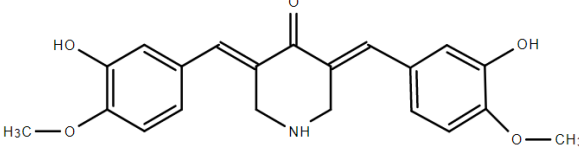
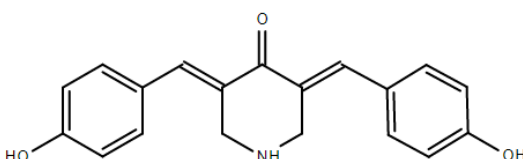
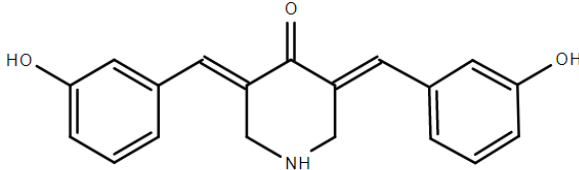
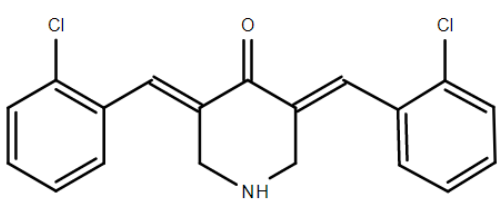
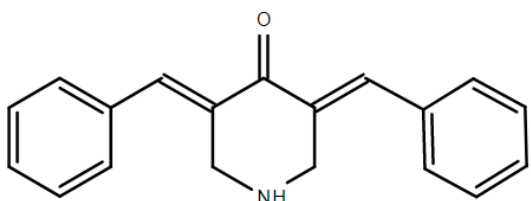
The parasite strain used was *Trypanosoma cruzi* CL Brener-*luc*, a transgenic mutant overexpressing luciferase, the Human Bone Osteosarcoma Epithelial Cells (U2OS) and Rhesus monkey kidney epithelial cells (LLC-MK2).

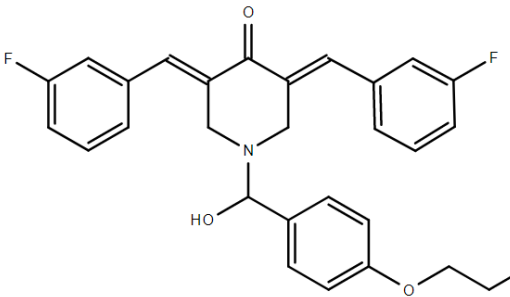
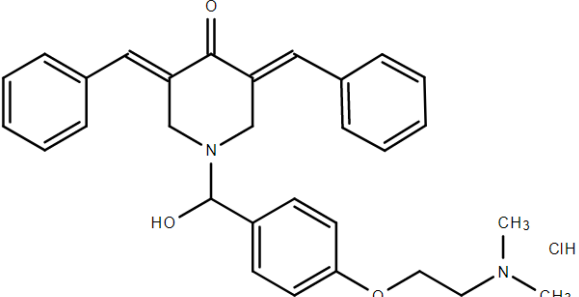
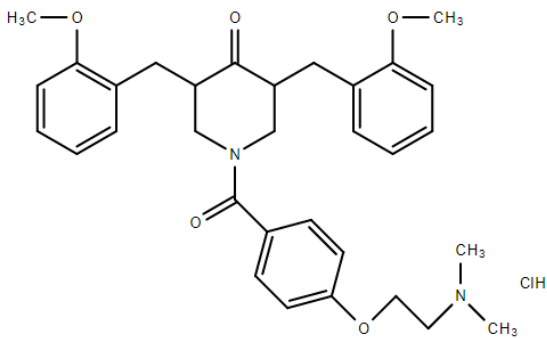
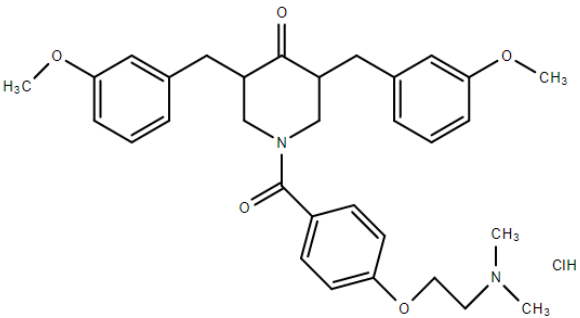
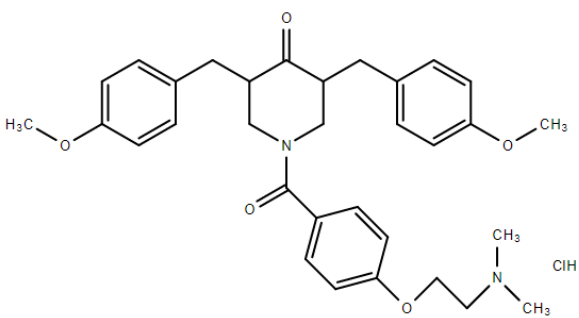
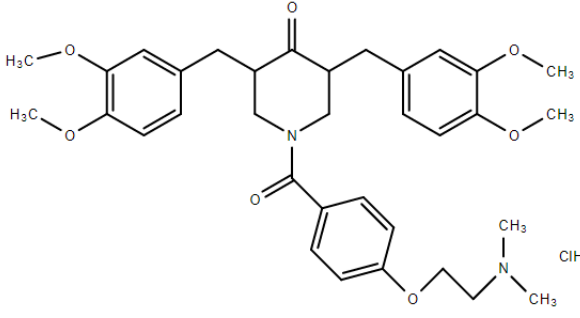
Two sets of compounds were tested, 21  $\alpha$ ,  $\beta$ -unsaturated ketones and 15 N-aroyl derivatives. Compounds were provided by our collaborator Dr. Jonathan Dimmock from The University of Saskatchewan, Canada.

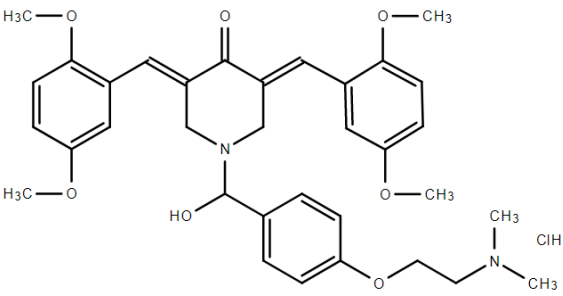
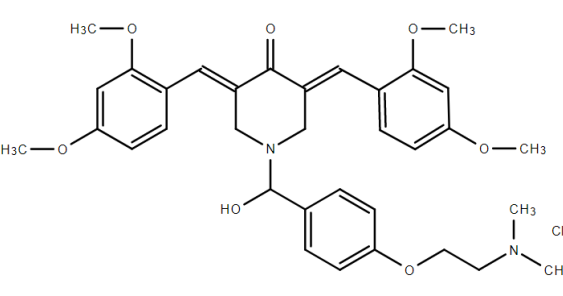
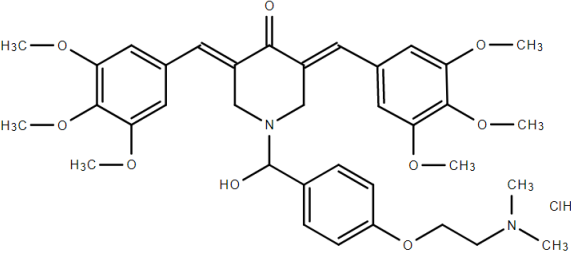
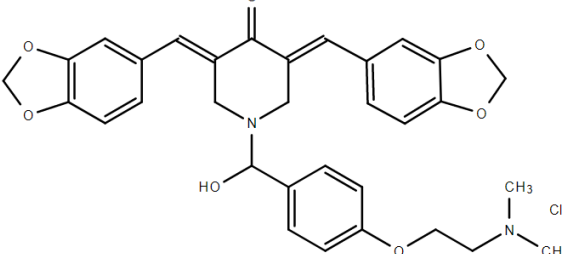
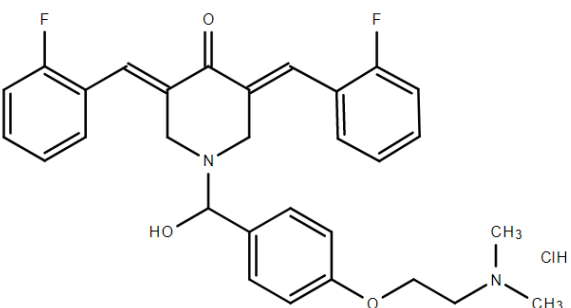
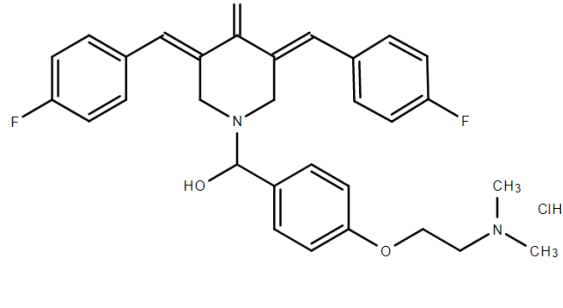
Table 1. The compound structures were drawn using the “Draw structure” tool available at ChemSpider by the Royal Society of Chemistry

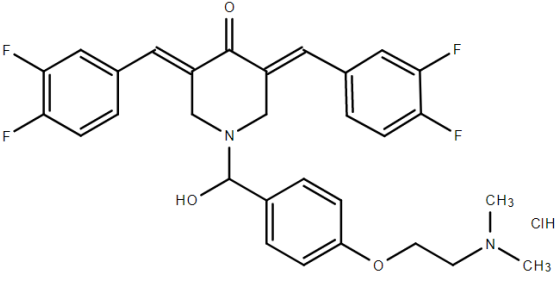
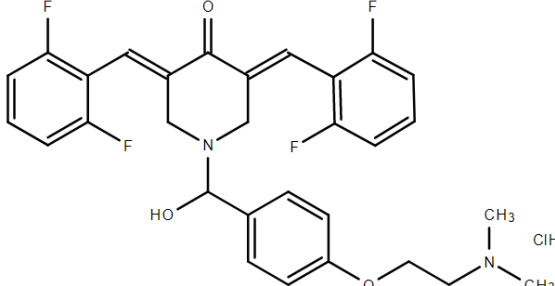
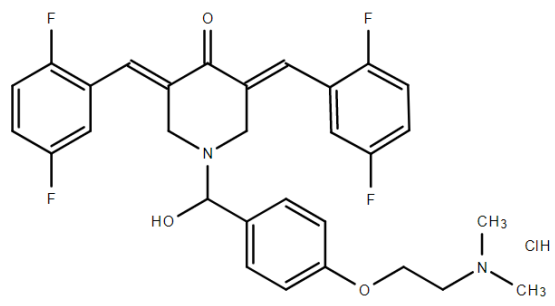
$\alpha$ , $\beta$ -unsaturated ketones	
1833	2446
	
2443	2553
	
2453	2548
	
1834	2423

	
<p>2550</p> 	<p>2551</p> 
<p>2552</p> 	<p>2444</p> 
<p>2549</p> 	<p>2554</p> 
<p>2062</p>	<p>2555</p>

	
2556	2557
	
2558	2448
	
1831	
	

N-aryl derivatives	
2567	2067
	
2572	2559
	
2560	2561
	
2562	2563

	
<p>2564</p>	<p>2565</p>
	
<p>2566</p>	<p>2568</p>
	
<p>2569</p>	<p>2570</p>

	
<p>2571</p>	
	

### 2.3.2 Epimastigote *T. cruzi* Culture

Epimastigote *T. cruzi* CL Brenner-*luc* was cultured in LIT (Liver Infusion Tryptose, 10% inactivated FBS) media with G418 antibiotic [8].

### 2.3.3 Mammalian Cell Culture

Human bone osteosarcoma epithelial cells U2OS (ATCC # HTB-96) and Rhesus monkey kidney epithelial cells LLC-MK2 (ATCC # CCL-7) were cultured in DMEM (Dulbecco's Modified Eagle Medium) with 10% iFBS (heat-inactivated Fetal Bovine Serum) and 1% antibiotics ampicillin (10,000 U/mL) and streptomycin (10,000  $\mu$ /mL) in 0.9% sodium chloride.

### 2.3.4 *In vitro* Epimastigote *Trypanosoma cruzi* Luciferase Assay

The survival of the parasite *T. cruzi* CL Brenner-*luc* was obtained by the direct measure of bioluminescence. This procedure was performed three independent times in quadruplicates.

*T. cruzi* culture was transferred into a 50 mL conical tube and centrifuged at 3000 rpm for 5 min. The supernatant was discarded and the pellet was kept. The pellet was resuspended on 1 mL of LIT supplemented with 200  $\mu$ M G418. The suspension was transferred to a 1 mL microcentrifuge tube. The parasites were counted using a hemacytometer to obtain a final concentration of  $1 \times 10^6$  parasite per well.

The parasites were added in a 96-well transparent plate with transparent flat bottom with the exception of the blank control (media only), followed by the addition of the compounds diluted in media. In a concentration range from 1.00  $\mu$ M to 8 nM (stock dilution in DMSO). The plate was incubated at 37°C for 72 hrs. After incubation, 35  $\mu$ L of Dual-Glo® Luciferase Assay System was



added. The plates were analyzed using the Thermo Luminoskan Ascent Microplate Reader and the data obtained was normalized. Data normalization was adjusted using each plate's controls.

### **2.3.5 *In vitro* U2OS and LLC-MK2 Mammalian Cell Line Toxicity**

The media was removed from the culture flask and the cells washed twice with 5 mL of warm PBS (phosphate-buffered saline) into the flask. Thereafter, trypsin was added and incubated at 37°C for 5 min. After incubation, 8 mL of DMEM supplemented with 10% inactivated FBS (Fetal Bovine Serum) and 5 mL of penicillin and streptomycin. The suspension is then transferred to a 50 mL conical tube, kept on ice, to then be counted using a hemacytometer.

The cells were added to the 96-well Falcon black/ clear bottom plate to incubate 37°C for 4 hrs. for cells seeding. The controls used were 1% DMSO (positive) and Benznidazole at a final concentration of 800 µM (negative). After incubation the drugs were added in concentration range from 66 µM to 8.25 µM (U2OS) and 100.0 µM to 1.5 µM (LLC-MK2). Follow, the plate was incubated at 37°C 5% CO<sub>2</sub> for 72 hrs. After incubation, 2% of Hoechst and 2% of PI (propidium iodine) was added to each well for staining and incubated for 1 hr. at 37°C. The plate was analyzed using the In-Cell Analyzer.

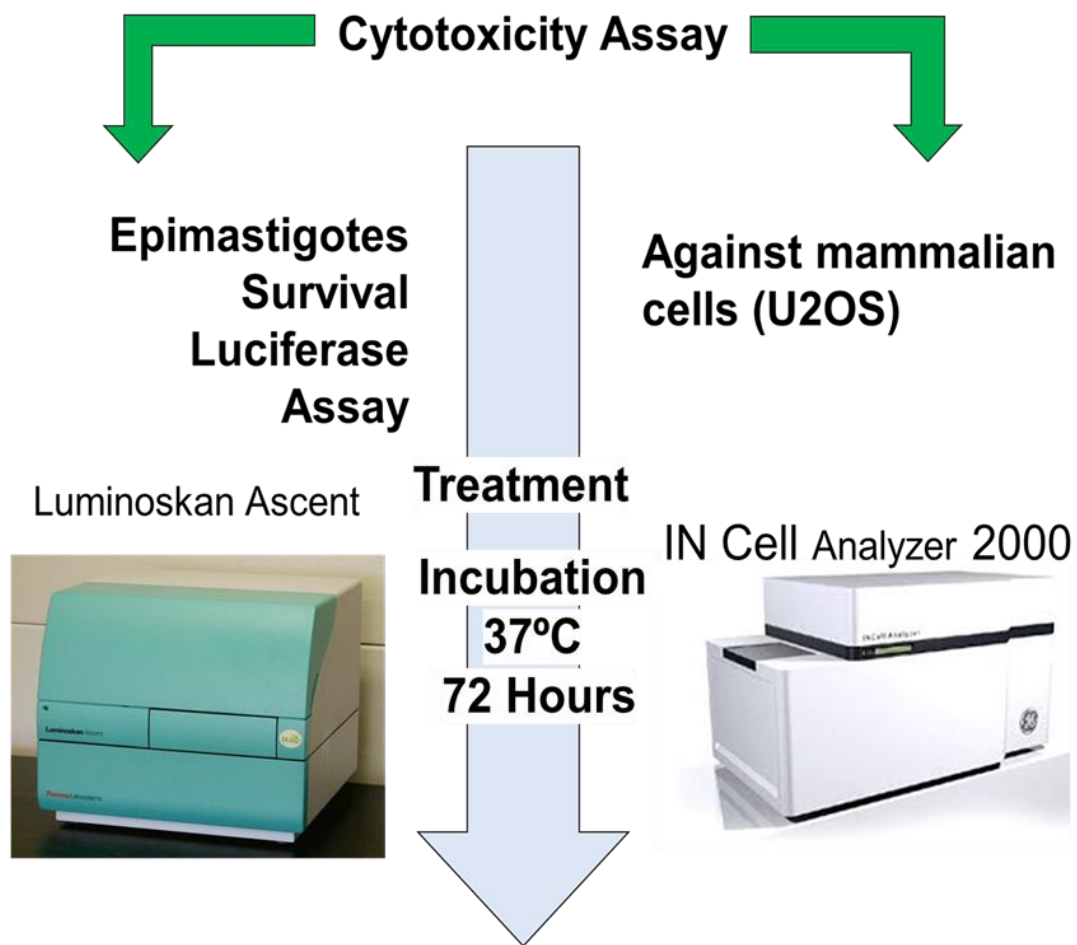


Figure 4. Epimastigote *T. cruzi* Cl Brenner-*luc* and U2OS cell line toxicity flow chart.

## 2.4 Results.

### 2.4.1 Cytotoxicity Analysis of N-aroyle Derivatives and $\alpha$ , $\beta$ -unsaturated Ketones

#### Treatments Against *T. cruzi* CL Brenner-*luc in vitro*

The agents were pre-screened to observe their anti-parasitic effectivity against epimastigote *T. cruzi* CL Brenner-*luc in vitro*. The highest concentration tested was 1.00  $\mu$ M, followed by 0.50  $\mu$ M and 0.25  $\mu$ M. The parasites were treated with the compounds and incubated for 72 hrs. at 37°C.

Many compounds were discarded at the first trial since they showed partial to non-antiparasitic effects. In figure 5, six compounds that showed antiparasitic effects are shown. The compounds NC2443, NC2067, NC2507, NC1833, NC2553, and NC2446 showed an EC<sub>50</sub> below the concentration of 0.50  $\mu$ M. The compounds NC2067 and NC1833 reached the EC<sub>50</sub> at the around 18  $\mu$ M, NC2446 and NC2567 at 0.50  $\mu$ M and NC2443 and NC2553 did not reach their EC<sub>50</sub>. The calculated z-score was 0.8689 with a p-value of 0.0440 with alpha 0.05 portraying significance among treatments.

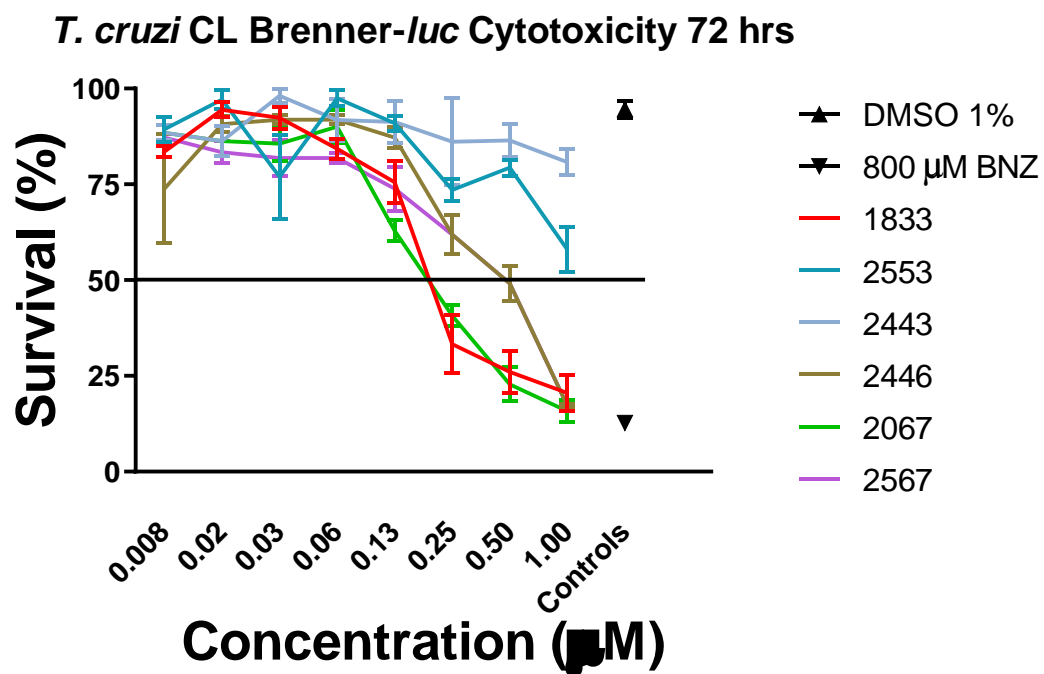


Figure 5. Cytotoxicity assay for  $\alpha$ ,  $\beta$ -unsaturated ketones treating *T. cruzi*. Assay yield significant effect (P-value = 0.0440  $\alpha=0.05$ )

#### **2.4.2 Toxicity Analysis Against U2OS and LLC-MK2 Mammalian Cell Lines Using N-aroyl Derivatives and $\alpha$ , $\beta$ -unsaturated Ketones Treatment *in vitro***

The compounds that showed anti-trypanosomal activity were further tested against U2OS (human bone osteosarcoma epithelial cells) and LLC-MK2 (Rhesus monkey kidney epithelial cells) cell lines, independently. A concentration of  $10^4$  cells were added to each well. After cell seeding, the treatment was added and incubated for 72 hrs. After incubation, the cells were analyzed using a live/dead assay with staining using Hoechst and PI dyes. The procedure allowed us to differentiate and determine the live cells percentage.

The compounds NC2553 and NC1833 showed low toxicity against U2OS cell line with survival of 80% at the highest concentration of 66  $\mu$ M. Compounds NC2443, NC2446, NC2067 and NC2567 showed an approximate  $EC_{50}$  of 45  $\mu$ M. The controls used were untreated acting as our positive control, 1% DMSO as our vehicle control and 1% hydrogen peroxide as our negative control. Our choice of 1% peroxide was used to obtain cell death baseline. Compounds that showed low toxicity are strong candidates for *in vivo* trials. The calculated z-score was 0.9096, this is a reflection of an accurate assay as it is less than one standard deviation from the mean.

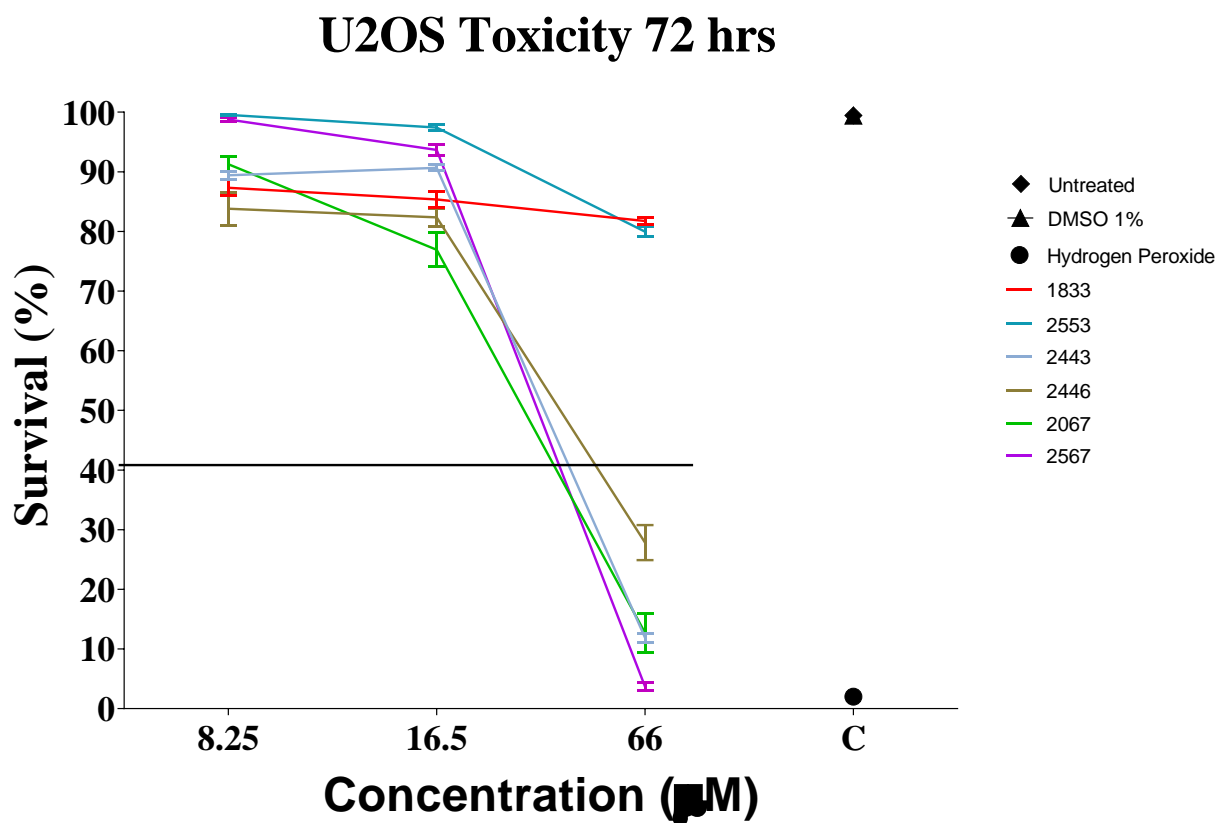


Figure 6. Cytotoxicity Assay Against U2OS Cell Line at three different concentrations of  $\alpha$ ,  $\beta$ -unsaturated ketones. The highest concentration of the compound was 66  $\mu$ M, diluted two times.

Assay yield a highly significant effect (P-value<0.0001,  $\alpha$ =0.05)

The compound NC1833 showed the lowest toxicity against LLC-MK2 cells with a survival over 50% at its highest concentration of 100  $\mu$ M, followed by NC 2446 with an EC<sub>50</sub> of 75  $\mu$ M. The compounds NC2553, NC2443, NC2067, and NC2567 had an EC<sub>50</sub> ranging from 12-37  $\mu$ M shown in Table 1. The positive control was untreated cells, followed by 1% DMSO as the vehicle control. 800  $\mu$ M Benznidazole was used instead of 1% hydrogen peroxide; we wanted to compare our compound toxicity against mammalian cells to the current approved treatment. The survival shown for 800  $\mu$ M Benznidazole was lower than 50%. The calculated z-score was 0.7736, measurement of accuracy of this assay.

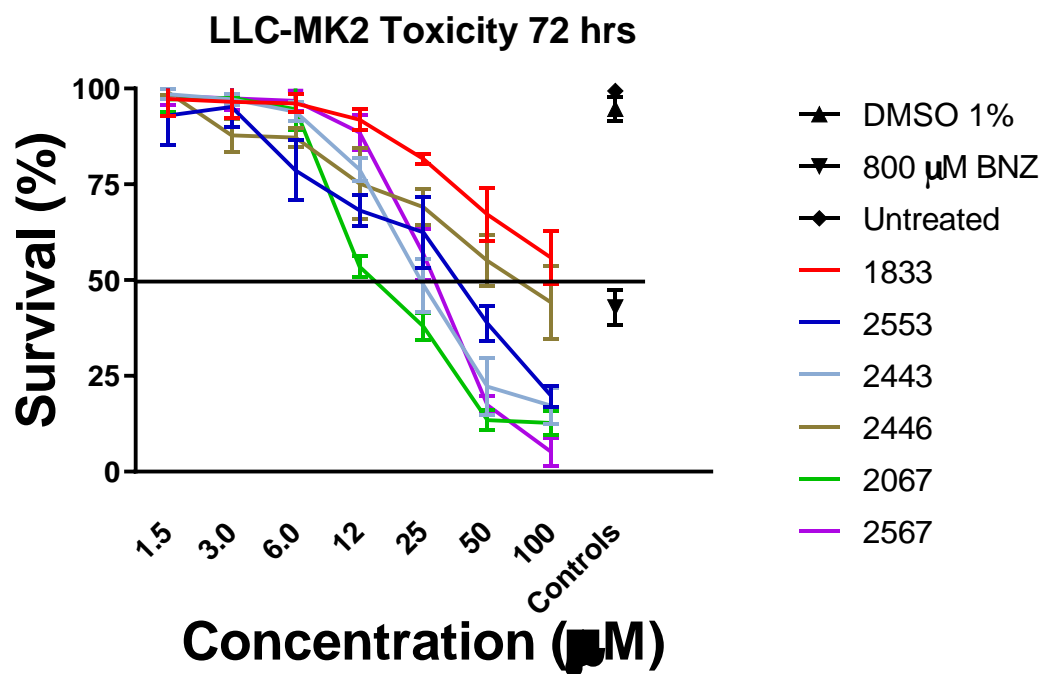


Figure 7. Cytotoxicity Assay Against LLC-MK2 Cell Line at seven different concentrations of  $\alpha$ ,  $\beta$ -unsaturated ketones. The highest concentration of the compound was 100  $\mu$ M, serial diluted six times. Assay yield highly significant values of treatments (P-value<0.0001,  $\alpha$ =0.05)



### 2.4.3 Calculation of Selective Index (SI)

The selective index was calculated by dividing the EC<sub>50</sub> of the compound against *T. cruzi* over the EC<sub>50</sub> of compound causing toxicity against the mammalian cell lines. The SI allowed us to the window range of toxicity of the compounds against epimastigote *T. cruzi* CL Brenner-*luc* and U2OS human cell line *in vitro*. The selective index (SI) was calculated by dividing the EC<sub>50</sub> of the compound against *T. cruzi* over the EC<sub>50</sub> of compound causing toxicity against the mammalian cell line.

The compound with the highest selective index is NC1833 towards U2OS at 347 and LLC-MK2 at 526. This compound offered the highest selectivity against *T. cruzi* while showing low toxicity against both mammalian cells. The compound that followed was NC2567 with an SI of 237 with U2OS cell line and 195 with LLC-MK2. The third most selective compound was NC2446 with an SI with U2OS of 90 and 200 for LLC-MK2. While the compounds NC2553, NC2443 and NC2067 showed an SI range from 33-72.

Table 2. Calculated EC<sub>50</sub> table of cytotoxicity assay against epimastigote *T. cruzi* and toxicity assays against U2OS and LLC-MK2 cell lines, independently

Drug	Epimastigotes (μM)	U2OS (μM)	Selective Index	LLC-LMK2 (μM)	Selective Index
2553	1	66	66	37	37
2443	1	42	66	37	37
2446	0.5	45	90	100	200
2067	0.25	8.25	33	18	72
2567	0.19	45	237	37	195
1833	0.19	66	347	100	526

Table 3. Two-way ANOVA analysis of drug concentration and toxicity among treatments. The quality of the assays was calculated by z-score and two-way ANOVA,  $\alpha=0.05$ . Z-score test and ANOVA were performed based on GraphPad article “Calculating a Z-factor to assess the quality of a screening assay” and GraphPad group comparison analysis. This helped us determine the accuracy and significance of the experiments.

<b>Two-way ANOVA</b>	Ordinary				
Alpha	0.05				
<b>Source of Variation</b>	<b>% of total variation</b>	<b>P value</b>	<b>P value summary</b>	<b>Significant?</b>	
Interaction	22.46	<0.0001	****	Yes	
Row Factor	62.46	<0.0001	****	Yes	
Column Factor	14.31	<0.0001	****	Yes	
<b>ANOVA table</b>	<b>SS</b>	<b>DF</b>	<b>MS</b>	<b>F (DFn, DFd)</b>	<b>P value</b>
Interaction	16478	10	1648	F (10, 54) = 158.4	P<0.0001
Row Factor	45817	2	22908	F (2, 54) = 2201	P<0.0001
Column Factor	10495	5	2099	F (5, 54) = 201.7	P<0.0001
Residual	561.9	54	10.41		
<b>Data summary</b>					
Number of columns (Column Factor)	6				
Number of rows (Row Factor)	3				
Number of values	72				

## 2.5 Conclusion and Future Directions

The discovery of a novel treatment for Chagas disease is necessary since millions of patients in indigenous areas in Central and South America suffer from this morbid disease. Migration of the illness in recent years will also affect more communities around the globe, including the United States. An estimate of 8 million people is presumed to be infected making the development of a treatment of utmost importance. The treatments available are not easily accessible to low socioeconomic populations in rural areas. Additionally, the current treatments are highly toxic and poorly effective in the chronic stage. Previous research of these compounds against closely related parasite, *Leishmania major*, were proven effective on *in vitro* models. Thus, these novel compounds provide promising anti-parasitic effects against *T. cruzi* with low toxic effects against mammalian cells *in vitro*.

This research indicates that N-aroyl derivatives and  $\alpha$ ,  $\beta$ -unsaturated ketones drugs are potential drug candidates against *T. cruzi*. The z-scores of all three assays were between 0.5 and 1.0, according to the GraphPad article these values classify our assay as excellent. Two-way ANOVA was performed with independent variables of drug concentration and survival. It showed a significance level of  $p < 0.0001$  for LMK-2 and U2OS cell lines and  $p = 0.0440$  for *T. cruzi*, which proves significant within our confidence interval of  $\alpha = 0.05$ . Therefore, we fail to reject our hypothesis and treatments can move to next stage of experimentation.

The SI of 6 the total compounds, ranged from 33 to 526. Our leading compound NC1833 showed high anti-parasitic effects against *T. cruzi* CL Brenner-*luc* at the concentration of 19 nM and 80% cell survival against U2OS mammalian cell line at the concentration of 66  $\mu$ M, (SI of 347) and more than 50% survival against LLC-MK2 cell line (SI of 526). High selective indexes are indicative of a larger therapeutic window when tested in a murine model. The results obtained

create a baseline of the effectivity against trypomastigotes and the existing toxicity to mammal cells. Experiments were performed in triplicates and were proven to be replicable as they were repeated at least three different times with similar results. Error bars are shown in all graphs to portray this.

Future directions for this project are testing its anti-proliferative effects to further discriminate compounds and select the most effective ones. Proliferation assay will be performed as follows: Cell lines U2OS and LLC-MK2, will be infected with trypomastigote *T. cruzi* CL Brenner-*luc*. After infection the cells will be treated with our leading compounds independently. The cells will then be incubated at 37 °C for 48 hrs. After incubation, the cells will be fixed using 4% paraformaldehyde. Once fixed, they will be stained using the Alexa Fluor 488 green and DAPI. Image acquisition and analysis will be performed using Biotek's Cytation 5 Cell Imaging Multi-Mode Reader.

The *in vitro* cytotoxicity and the proliferation assays will allow us to determine if these compounds are good candidates to be tested in a murine model. It is necessary to understand if these novel treatments can stop the intracellular amastigotes proliferation within the cell. These promising results fulfill our specific aims and indicate that N-aroyle derivatives and  $\alpha$ ,  $\beta$ -unsaturated ketones drugs are potential candidates for treatment against *T. cruzi* as we fail to reject our hypothesis.

This array of compounds has shown the potential to become novel treatments against Chagas Disease. This is highly important due to the lack of treatments available. The compounds have shown to have lower toxicity levels against mammalian cells and good antiparasitic effects compared to the Benznidazole. This project creates a background for future research using N-aroyle derivatives and  $\alpha$ ,  $\beta$ -unsaturated ketones to treat Chagas Disease.

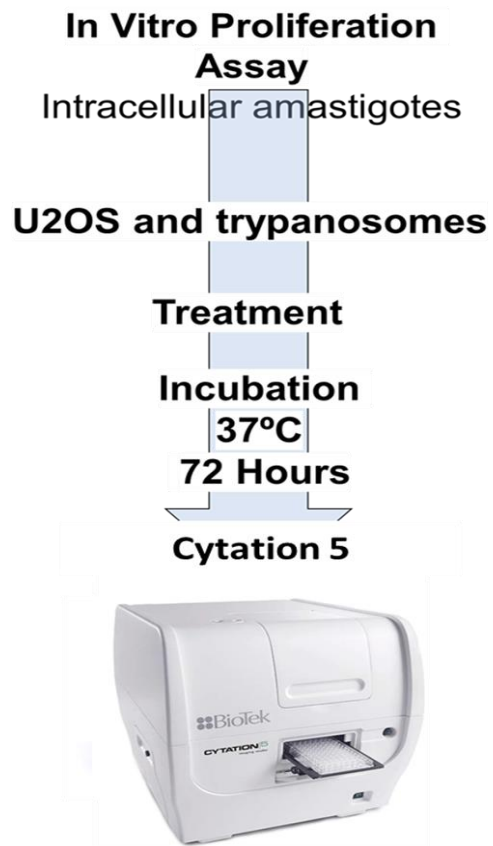


Figure 8. Intracellular amastigote *T. cruzi* CL Brenner-*luc* proliferation assay flow chart

## References

- [1] E. Chatelain, “Chagas disease research and development: Is there light at the end of the tunnel?,” *Comput. Struct. Biotechnol. J.*, vol. 15, pp. 98–103, Jan. 2017, doi: 10.1016/j.csbj.2016.12.002.
- [2] “Chagas disease (American trypanosomiasis).” <https://www.who.int/westernpacific/health-topics/chagas-disease> (accessed Aug. 29, 2020).
- [3] B. L. Herwaldt et al., “Characteristics of Patients for Whom Benznidazole Was Released Through the CDC-Sponsored Investigational New Drug Program for Treatment of Chagas Disease — United States, 2011–2018,” *Morb. Mortal. Wkly. Rep.*, vol. 67, no. 29, pp. 803–805, Jul. 2018, doi: 10.15585/mmwr.mm6729a3.
- [4] J. Bermudez, C. Davies, A. Simonazzi, J. Pablo Real, and S. Palma, “Current drug therapy and pharmaceutical challenges for Chagas disease,” *Acta Trop.*, vol. 156, pp. 1–16, Apr. 2016, doi: 10.1016/j.actatropica.2015.12.017.
- [5] P. J. Hotez et al., “Chagas Disease: ‘The New HIV/AIDS of the Americas,’” *PLoS Negl. Trop. Dis.*, vol. 6, no. 5, p. e1498, May 2012, doi: 10.1371/journal.pntd.0001498.
- [6] J. Manne-Goehler, C. A. Umeh, S. P. Montgomery, and V. J. Wirtz, “Estimating the Burden of Chagas Disease in the United States,” *PLoS Negl. Trop. Dis.*, vol. 10, no. 11, p. e0005033, Nov. 2016, doi: 10.1371/journal.pntd.0005033.
- [7] “Chagas | Data.” <https://www.dshs.state.tx.us/DCU/disease/chagas/Chagas-Disease-Data.aspx> (accessed Sep. 06, 2020).
- [8] A. C. C. Silva, M. C. A. Brelaz-de-Castro, A. C. L. Leite, V. R. A. Pereira, and M. Z. Hernandez, “Chagas Disease Treatment and Rational Drug Discovery: A Challenge That Remains,” *Front. Pharmacol.*, vol. 10, 2019, doi: 10.3389/fphar.2019.00873.

- [9] Vasquez, M. A., Iniguez, E., Das, U., Beverley, S. M., Herrera, L. J., Dimmock, J. R., & Maldonado, R. A. (2015). Evaluation of  $\alpha$ ,  $\beta$ -Unsaturated Ketones as Antileishmanial Agents. *Antimicrobial Agents and Chemotherapy*, 59(6), 3598-3601. doi:10.1128/aac.04056-14
- [10] Herwaldt, Barbara L., Cindy P. Dougherty, Christopher K. Allen, Julian P. Jolly, Megan N. Brown, Patricia Yu, and Yon Yu. “Characteristics of Patients for Whom Benznidazole Was Released Through the CDC-Sponsored Investigational New Drug Program for Treatment of Chagas Disease — United States, 2011–2018.” *Morbidity and Mortality Weekly Report* 67, no. 29 (July 27, 2018): 803–5.
- [11] Bern, Caryn, Louisa A. Messenger, Jeffrey D. Whitman, and James H. Maguire. “Chagas Disease in the United States: A Public Health Approach.” *Clinical Microbiology Reviews* 33, no. 1 (December 18, 2019).
- [12] Khare, Shilpi, Advait S. Nagle, Agnes Biggart, Yin H. Lai, Fang Liang, Lauren C. Davis, S. Whitney Barnes, et al. “Proteasome Inhibition for Treatment of Leishmaniasis, Chagas Disease and Sleeping Sickness.” *Nature* 537, no. 7619 (September 2016): 229–33.
- [13] Mutus, Bulent, Jerome D. Wagner, Christopher J. Talpas, Jonathan R. Dimmock, Oludotun A. Phillips, and R. Stephen Reid. “1-p-Chlorophenyl-4,4-Dimethyl-5-Diethylamino-1-Penten-3-One Hydrobromide, a Sulfhydryl-Specific Compound Which Reacts Irreversibly with Protein Thiols but Reversibly with Small Molecular Weight Thiols.” *Analytical Biochemistry* 177, no. 2 (March 1, 1989): 237–43.
- [14] Das, Swagatika, Umashankar Das, Armando Varela-Ramírez, Carolina Lema, Renato J. Aguilera, Jan Balzarini, Erik De Clercq, Stephen G. Dimmock, Dennis K. J. Gorecki, and Jonathan R. Dimmock. “Bis[3,5-Bis(Benzylidene)-4-Oxo-1-Piperidinyl]Amides: A Novel Class of Potent Cytotoxins.” *ChemMedChem* 6, no. 10 (October 4, 2011): 1892–99.



- [15] Couto, Narciso, Jennifer Wood, and Jill Barber. “The Role of Glutathione Reductase and Related Enzymes on Cellular Redox Homeostasis Network.” *Free Radical Biology and Medicine* 95 (June 1, 2016): 27–42.
- [16] Salassa, Betiana Nebaí, and Patricia Silvia Romano. “Autophagy: A Necessary Process during the *Trypanosoma Cruzi* Life-Cycle.” *Virulence* 10, no. S11 (May 28, 2019): 460–69.
- [17] Egui, Adriana, M. Carmen Thomas, Ana Fernández-Villegas, Elena Pérez-Antón, Inmaculada Gómez, Bartolomé Carrilero, Ángel del Pozo, et al. “A Parasite Biomarker Set for Evaluating Benznidazole Treatment Efficacy in Patients with Chronic Asymptomatic *Trypanosoma Cruzi* Infection.” *Antimicrobial Agents and Chemotherapy* 63, no. 10 (July 29, 2019): e02436-18, /aac/63/10/AAC.02436-18.atom.
- [18] Revollo, Susana, Bruno Oury, Andrea Vela, Michel Tibayrenc, and Denis Sereno. “In Vitro Benznidazole and Nifurtimox Susceptibility Profile of *Trypanosoma Cruzi* Strains Belonging to Discrete Typing Units TcI, TcII, and TcV.” *Pathogens* 8, no. 4 (December 2019): 197.
- [19] Silva, Ana Catarina Cristovão, Maria Carolina Accioly Brelaz-de-Castro, Ana Cristina Lima Leite, Valéria Rêgo Alves Pereira, and Marcelo Zaldini Hernandes. “Chagas Disease Treatment and Rational Drug Discovery: A Challenge That Remains.” *Frontiers in Pharmacology* 10 (2019).
- [20] Ulrich, Kathrin, Caroline Finkenzeller, Sabine Merker, Federico Rojas, Keith Matthews, Thomas Ruppert, and R. Luise Krauth-Siegel. “Stress-Induced Protein S-Glutathionylation and S-Trypanothionylation in African Trypanosomes—A Quantitative Redox

Proteome and Thiol Analysis.” *Antioxidants & Redox Signaling* 27, no. 9 (February 15, 2017): 517–33.

[21] Costa, Kelli Monteiro da, Raphael C. Valente, Eduardo J. Salustiano, Luciana B. Gentile, Leonardo Freire-de-Lima, Lucia Mendonça-Previato, and José O. Previato. “Functional Characterization of ABCC Proteins from *Trypanosoma Cruzi* and Their Involvement with Thiol Transport.” *Frontiers in Microbiology* 9 (2018).

[22] Ulrich, Kathrin, and Ursula Jakob. “The Role of Thiols in Antioxidant Systems.” *Free Radical Biology and Medicine, Early Life on Earth and Oxidative Stress*, 140 (August 20, 2019): 14–27.

[23] Paiva, Claudia N., Emiliano Medei, and Marcelo T. Bozza. “ROS and *Trypanosoma Cruzi*: Fuel to Infection, Poison to the Heart.” *PLOS Pathogens* 14, no. 4 (April 19, 2018): e1006928.

[24] Costa, Kelli Monteiro da, Eduardo J. Salustiano, Raphael do Carmo Valente, Leonardo Freire-de-Lima, Lucia Mendonça-Previato, and José Osvaldo Previato. “Thiol Efflux Mediated by an ABCC-like Transporter Participates for *Trypanosoma Cruzi* Adaptation to Environmental and Chemotherapeutic Stresses.” Preprint. *Microbiology*, March 29, 2020. <https://doi.org/10.1101/2020.03.26.009753>.

## **Vita**

Karsten Amezcua was born in Jalisco, Mexico in 1994. He is the oldest of three sons. He migrated to El Paso, Texas in 2011. He obtained his Bachelor's in Science in Biochemistry on 2018 at The University of Texas at El Paso. Afterwards, he was accepted in the Masters of Science in Biological Sciences on Spring 2020 under the mentorship of Dr. Rosa Maldonado at the laboratory of molecular and anti-parasitic therapies. He had presented at distinctive national and state scientific conferences where he has been recognized at the highest levels.

Karsten Dieter Amezcua Winter

Permanent Address: 1809 Dean Jones, El Paso, Texas, 79936.

Email address: [karsten\\_dieter@hotmail.com](mailto:karsten_dieter@hotmail.com)