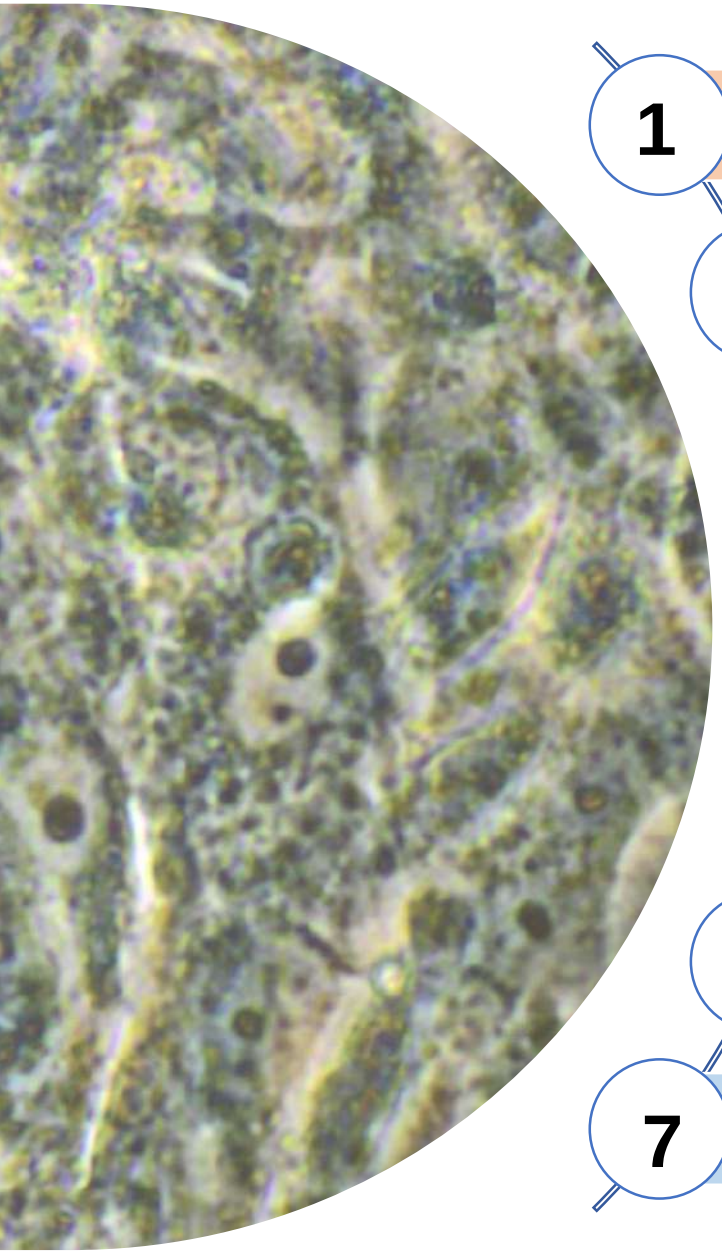
# Identification of anti-parasitic compounds against *Trypanosoma cruzi*, the causal agent of Chagas disease, through the evaluation of diverse chemical collections

Nieves Martínez Peinado

Thesis directors: Dr. Julio Alonso Padilla and Prof. Joaquim Gascón
Tutor: Jordi Vila Estapé

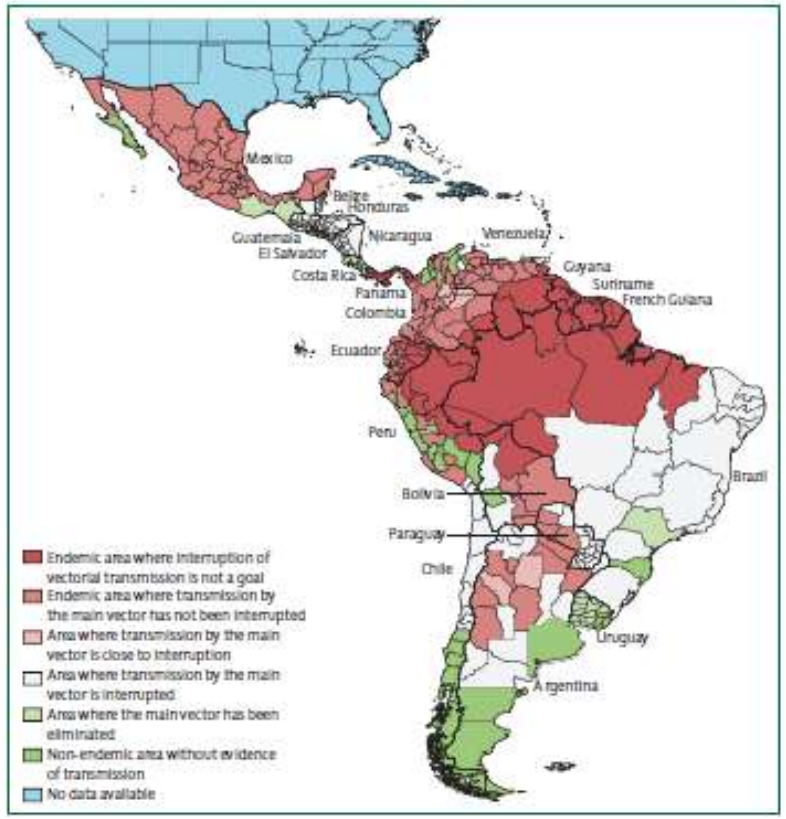


# 1. INTRODUCTION

- Is a neglected tropical disease (NTD) caused by the Kinetoplastid protozoan parasite *Trypanosoma cruzi* (*T.cruzi*).

## EPIDEMIOLOGY

- ~7 million people are affected by the disease, mainly in Latin America where is endemic in 21 countries.
- Spread to non-endemic areas → global health problem.




(Perez-Molina J.A. et al, Lancet 2018)

## TRANSMISION

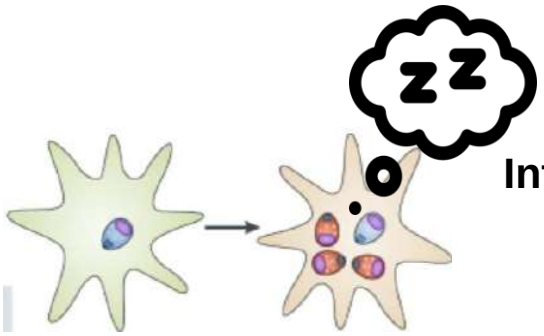
- Main route: triatomine insects (*family Reduvidae*).
- Blood transfusion, organ transplants, vertical transmission.

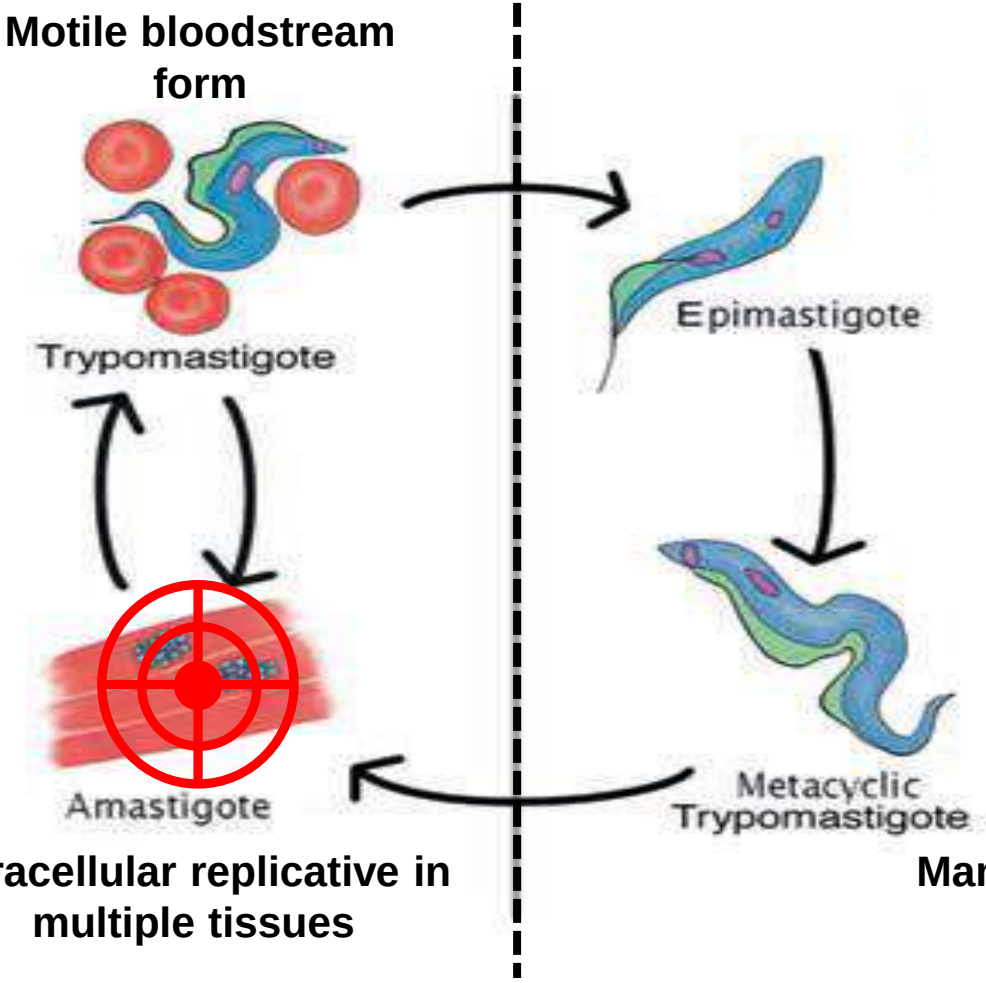
# 1. INTRODUCTION

## *TRYPANOSOMA CRUZI* LIFE CYCLE

**Mammalian host stage**

Multiple hosts
(>100 species)
Multiple tissues






**Intracellular replicative in multiple tissues**

**Mammalian first infective motile form**

**Replicative in insect gut**

**Triatomine insect vector stage**

Multiple vectors

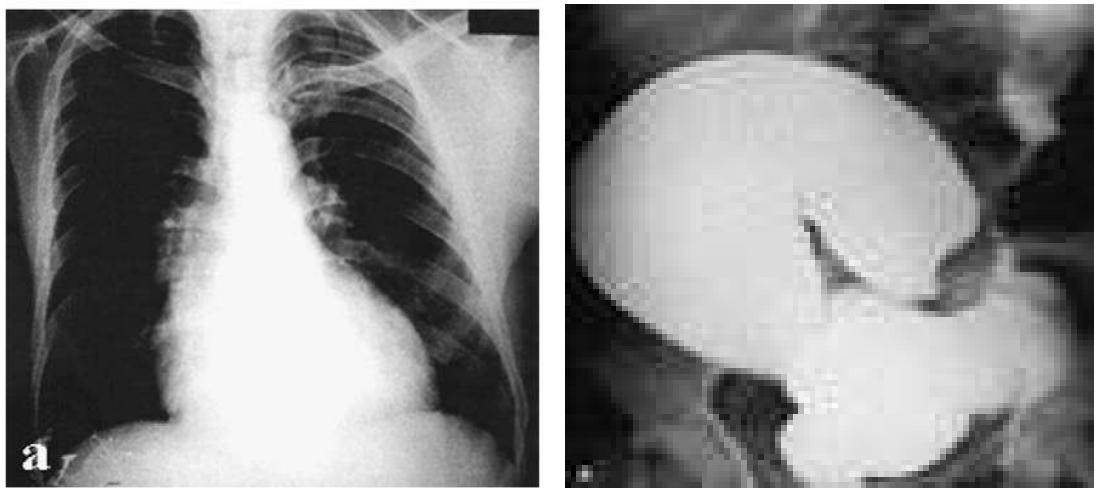
(Atwood et al., Science 2005)

# 1. INTRODUCTION

## CLINICAL MANIFESTATIONS

Chagas disease may progress in two clinical phases:

- Acute
- Chronic
  - Indeterminate
  - 30-40% infected: cardiac and/or digestive damage

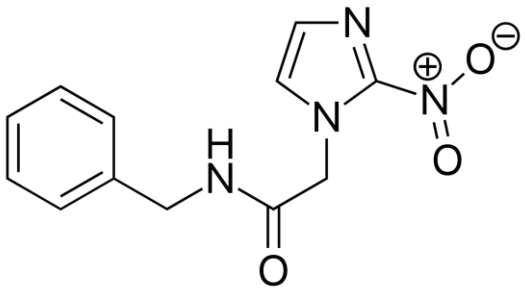



(Coura J.R. et al, Acta Tropica 2010)

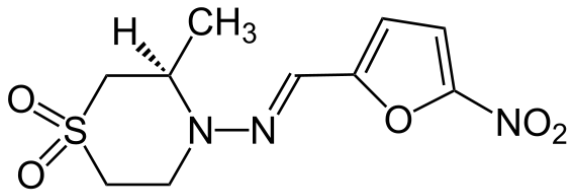
## TREATMENT

- Only two drugs available: Benznidazole (Bnz) and Nifurtimox (Nfx).
- Good efficacy in acute phase but diminished as the disease progress.
- High toxicity and frequent adverse events.

Bnz



Nfx



**URGENT NEED OF NEW ANTI CHAGASIC DRUGS FOR CRHONIC PHASE!**

# 1. INTRODUCTION

## DRUG DISCOVERY

gsk

### New Compound Sets Identified from High Throughput Phenotypic Screening Against Three Kinetoplastid Parasites: An Open Resource

Imanol Peña[1], M. Pilar Manzano[2], Juan Cantizani[2], Albane Kessler[2], Julio Alonso-Padilla[3], Ana I. Bardera[1], Emilio Alvarez[1], Gonzalo Colmenarejo[1], Ignacio Cotillo[2], Irene Roquero[1], Francisco de Dios-Anton[1], Vanessa Barroso[1], Ana Rodriguez[3], David W. Gray[4], Miguel Navarro[5], Vinod Kumar[6], Alexander Sherstnev[7], David H. Drewry[8], James R. Brown[6], Jose M. Fiandor[2] & J. Julio Martin[1]

(Peña I et al., Scientific Reports 2015)

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

### Randomized Trial of Posaconazole and Benznidazole for Chronic Chagas' Disease

Israel Molina, M.D., Jordi Gómez i Prat, M.D., Fernando Salvador, M.D., Begoña Treviño, M.D., Elena Sulleiro, M.D., Núria Serre, M.D., Diana Pou, M.D., Sílvia Roure, M.D., Juan Cabezos, M.D., Lluís Valerio, Ph.D., Albert Blanco-Grau, M.D., Adrián Sánchez-Montalvá, M.D., Xavier Vidal, Ph.D., and Albert Pahissa, Ph.D.

### Proteasome inhibition for treatment of leishmaniasis, Chagas disease and sleeping sickness

Shilpi Khare[1]*, Advait S. Nagle[1]*, Agnes Biggart[1], Yin H. Lai[1], Fang Liang[1], Lauren C. Davis[1], S. Whitney Barnes[1], Casey J. N. Mathison[1], Elmarie Myburgh[2,3], Mu-Yun Gao[1], J. Robert Gillespie[4], Xianzhong Liu[1], Jocelyn L. Tan[1], Monique Stinson[1], Ianne C. Rivera[1], Jaime Ballard[1], Vince Yeh[1], Todd Groessl[1], Glenn Federe[1], Hazel X. Y. Koh[5], John D. Venable[1], Badry Bursulaya[1], Michael Shapiro[1], Pranab K. Mishra[1], Glen Spraggon[1], Ansgar Brock[1], Jeremy C. Mottram[2,3], Frederick S. Buckner[4], Srinivasa P. S. Rao[5], Ben G. Wen[1], John R. Walker[1], Tove Tuntland[1], Valentina Molteni[1], Richard J. Glynne[1] & Frantisek Supek[1]

NOVARTIS

(Khare S, Nature 2016)

### Spontaneous dormancy protects *Trypanosoma cruzi* during extended drug exposure

**Fernando J Sánchez-Valdéz[1†‡], Angel Padilla[1,2†], Wei Wang[1], Dylan Orr[1], Rick L Tarleton[1,2*]**

[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, United States; [2]Department of Cellular Biology, University of Georgia, Athens, United States

# 1. INTRODUCTION

**Academia**

**Basic research**

Host-pathogen interactions

Mechanisms of pathogenesis

Computational screenings to identify conserved essential proteins

**Potential targets**

**Target-based screening**

**Phenotypic assays**

1 **Primary screening**

- HTS anti-*T. cruzi* assays
- Cytotoxicity on host cells (Vero cells, 3T3, L6)

2 **Secondary assays**

- Cytotoxicity on human cells (HepG2 cells)
- Intracellular amastigote assay
- Representative panel of *T. cruzi* strains
- Trypomastigote assay
- Rate of kill
- Wash out assays
- CYP51 depriorization assay

**+ Throughput -**

- Activity and cytotoxicity comparable to existing treatments (variable progression criteria)
- • Rapid acting drugs
  • Activity against persister forms

**Public Private**

**Potential hits**

3

- • Docking analysis/
  • Target devonvolution
  • In silico ADMET properties
- • Experimental validation of mechanism of action

**Potential leads**

**Lead optimization**

Improve *in vitro* potency, physchem properties, DMPK, and safety profile

**Preclinical evaluation**

4 Animal models

- • Rapid screening *in vivo*
  • Acute
  • Chronic

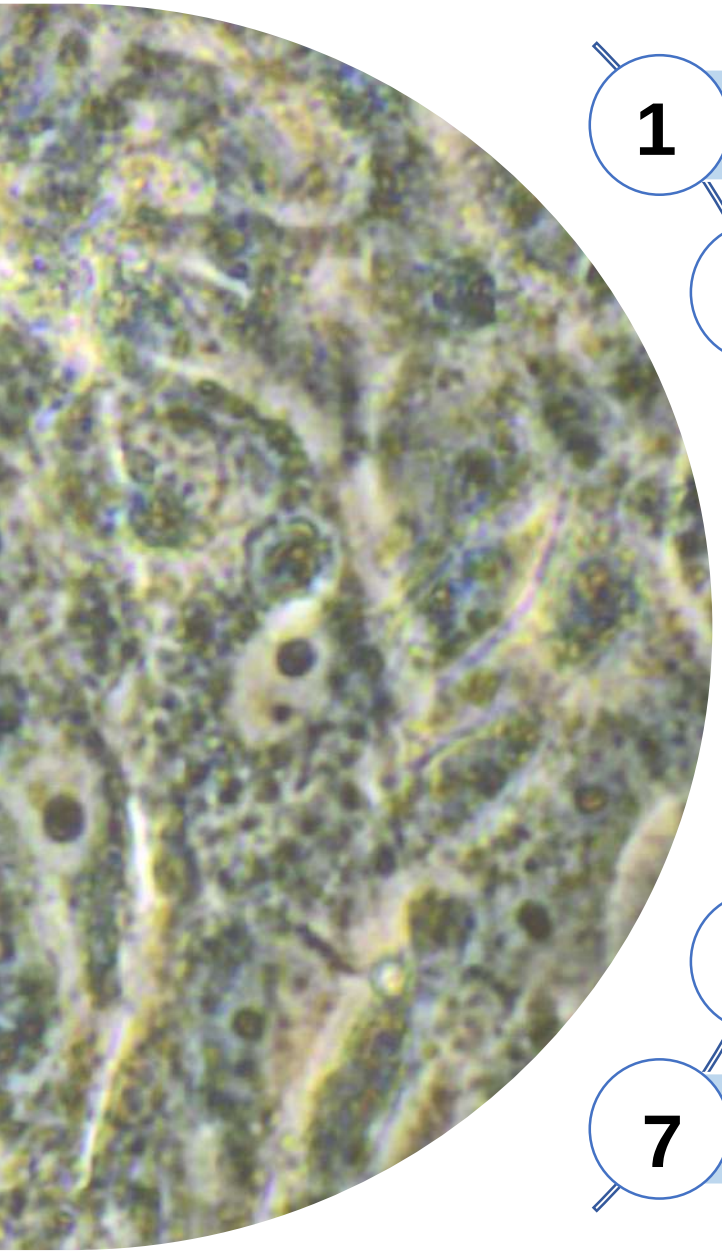
PK/PD analysis

Translatable capacity to achieve parasite clearance or improve disease progression

**Clinical candidate**

**Industry**

Adapted from Gabaldón-Figueira et al., Exp Opin 2023



1. Introduction
2. Hypothesis
3. Objectives
4. Methods
5. Results and discussion
6. Limitations
7. Conclusions

## 2. HYPOTHESIS

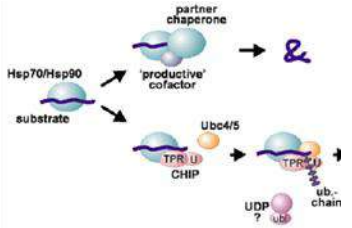
The exploration of the structural diversity and biological properties from different chemical collections obtained through collaborations will allow to preclinically prioritize chemical entities for the treatment of Chagas disease.

### Amaryllidaceae plants

- Natural compounds are a valuable source of active biological substances
- Unique alkaloid constituents

### Licensed drugs

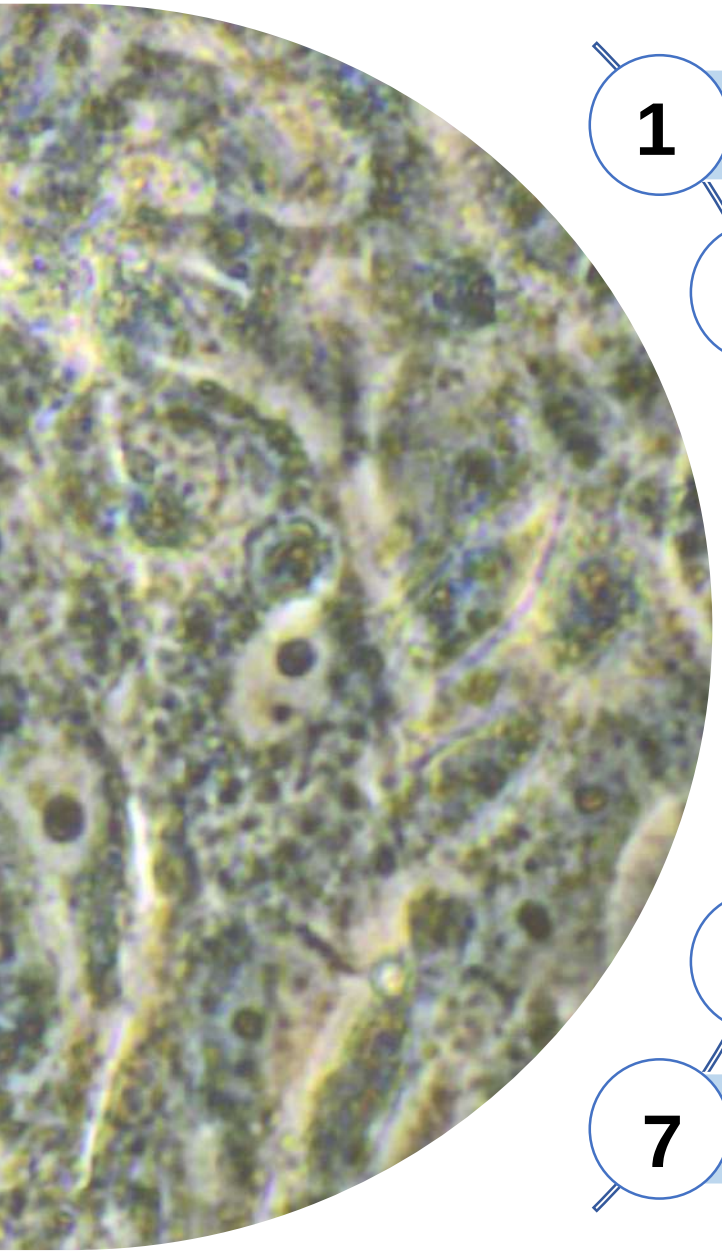
- A fast-track and low-cost strategy
- Pharmacological characteristics and safety profiles
- Posaconazole and E1224




### Metabolism modifier compounds

- Metabolic coupling of intracelular pathogens with host cells is essential for successful colonization of the host
- Potential anti-parasitic treatments

### *In silico* target identification



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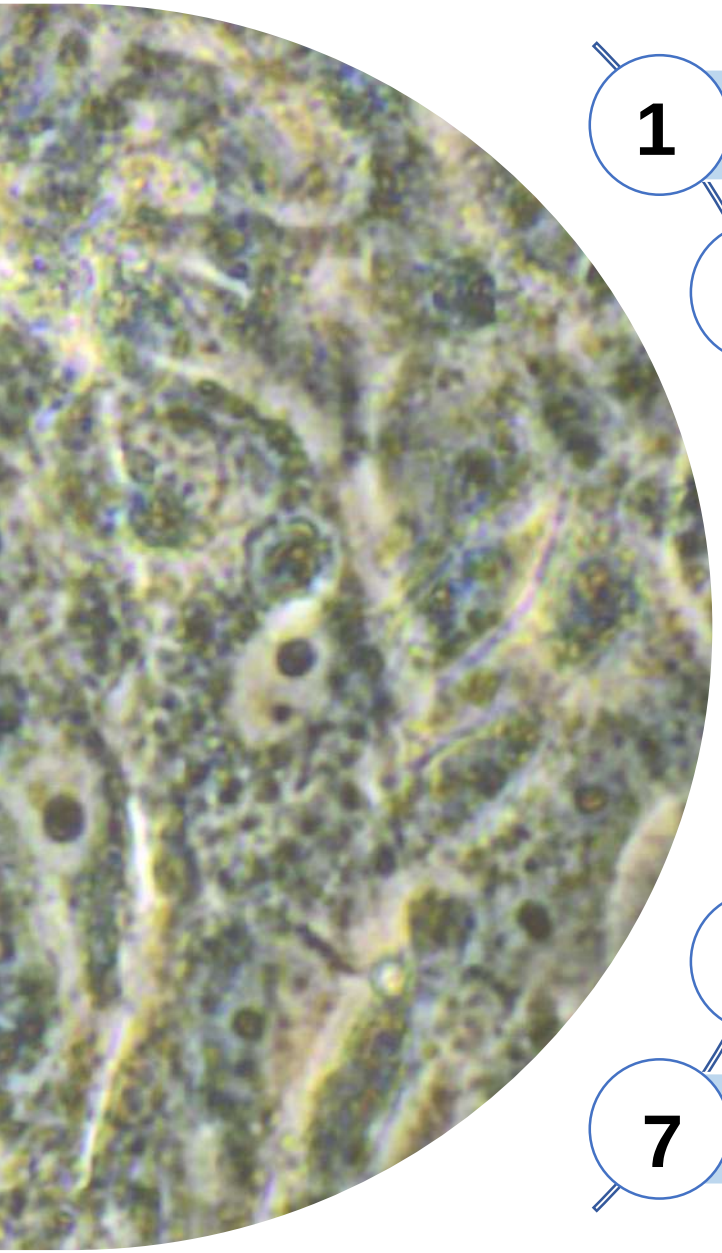
## 3. OBJECTIVES

The **main objective of this work is the identification of compounds or drugs with potent and specific activity against the parasite *T. cruzi*** among different chemical collections.

- Specific Objective 1: development of a statistically robust and reproducible *in vitro* screening cascade to identify compounds specifically acting against *T. cruzi*.
- Specific Objective 2: identification of Amaryllidaceae plant extracts or alkaloids isolated from them with specific anti-*T. cruzi* activity.
- Specific Objective 3: evaluation of the anti-*T. cruzi* activity of a collection of licensed drugs through *in vitro* and *in vivo* experiments.
- Specific Objective 4: exploration of the capacity to modulate or inhibit *T. cruzi* growth of a collection of metabolism modifier compounds.
- Specific Objective 5: deciphering *T. cruzi* molecular targets and mechanisms of action of hit compounds using *in silico* molecular docking studies and the AlphaFold protein database.



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# 4. METHODS

## Amaryllidaceae plants

Prof. Jaume Bastida

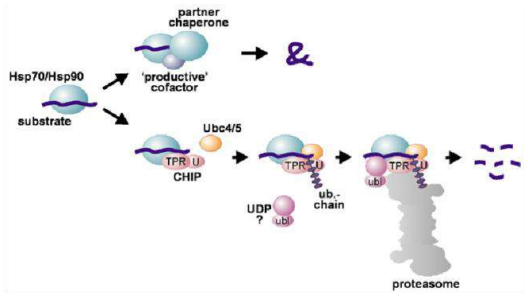
Dra. Gabriela Feresin

## Drugs for repurposing

Prof. Joaquim Gascón

Dr. Juan Bustamante

## Metabolism modifier compounds




Dra. Alhelí Rodríguez-Cortes

**X compounds**

Anti-*T. cruzi* assay on Vero cells → $IC_{50} < X \times$ BNZ $IC_{50}$

**X compounds**

Vero cell toxicity assay → SI > X

**X compounds**

HepG2 cell toxicity assay → SI > X

**X compounds**

Anti-amastigote assay → SI > X

**X compounds**

*In silico* model

**X compounds**

*In vivo* model

**X compounds**

# 4. METHODS

**X compounds**

**Anti-*T. cruzi* assay on Vero cells** ↓ IC50 < X x BNZ IC50

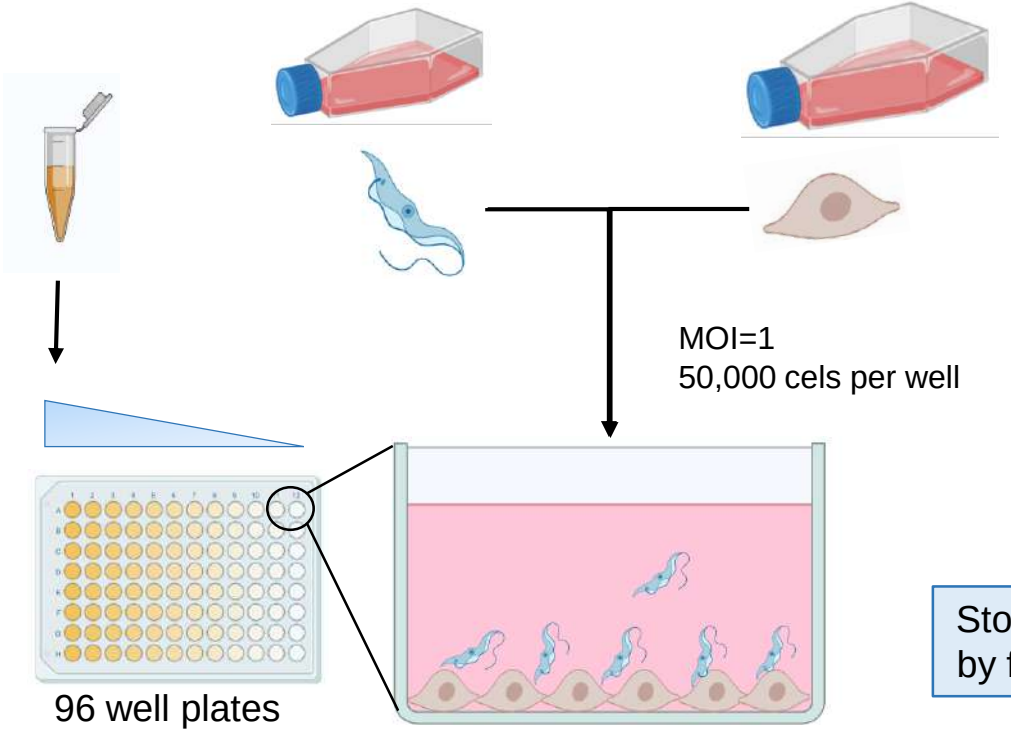
**X compounds**

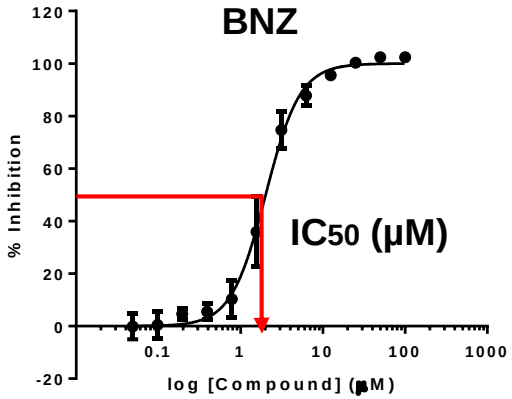
## Anti-*T. cruzi* assay on Vero cells

*T. cruzi* Tulahuen expressing β-galactosidase reporter gene

(Fred Buckner, University of Washington, US).

Vero cells




**Colored substrate (CPRG)**

Principle of action: enzymatic activity increase indicates *T.cruzi* replication.

**Day 0-1** → **Day 1** → **Day 5** → **Day 6**

Stop assay by freezing

Incubation 37º C 5% CO2

(Bettiol et al., PLoS NTD 2009)

# 4. METHODS

**X compounds**

Anti-*T. cruzi* assay on Vero cells

↓ IC50 < X x BNZ IC50

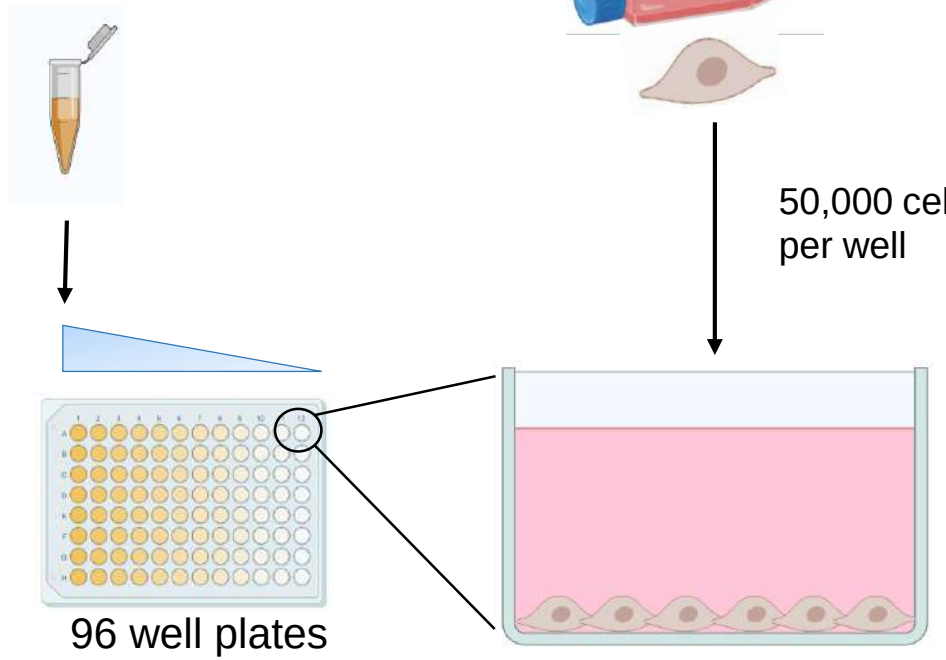
**X compounds**

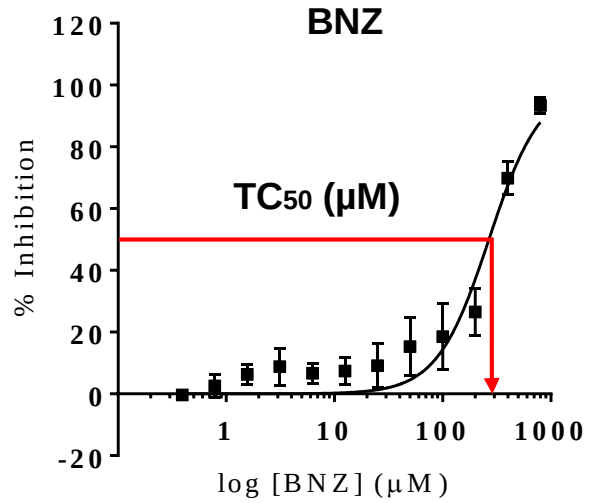
**Vero cell toxicity assay**

↓ SI > X

**X compounds**

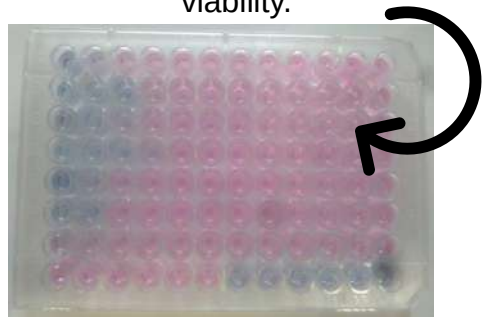
## Vero cell toxicity assay




**Fluorescent susbtrate (AlamarBlue)**

Principle of action: use of reducing power of living cells to cuantify viability.



# 4. METHODS

**X compounds**

Anti-*T. cruzi* assay on Vero cells

↓ IC50 < X x BNZ IC50

**X compounds**

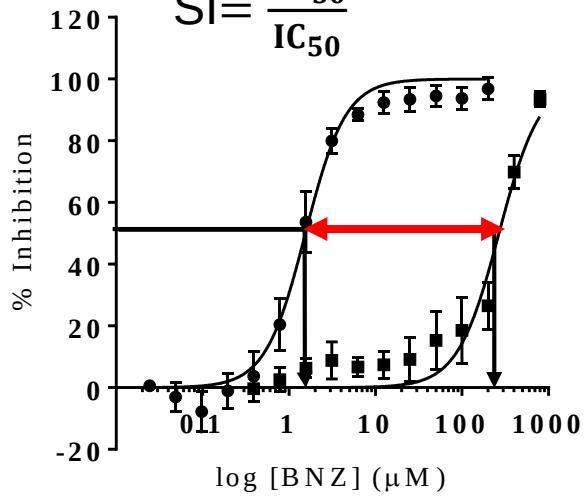
**Vero cell toxicity assay**

↓ SI > X

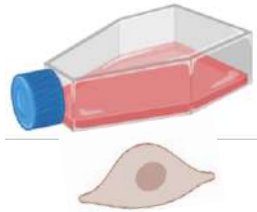
**X compounds**

## Vero cell toxicity assay

$$SI = \frac{TC_{50}}{IC_{50}}$$




Vero cells



50,000 cels per well

**Fluorescent susbtrate (AlamarBlue)**

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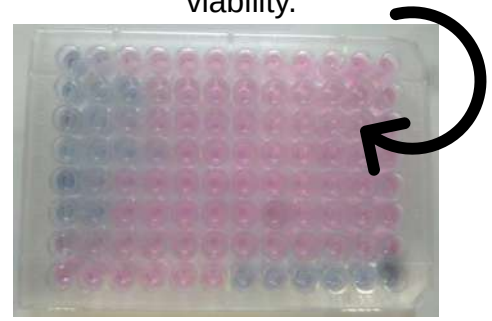
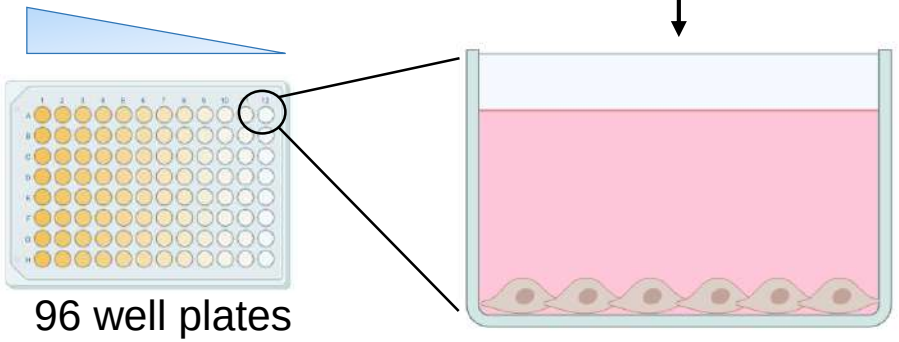




96 well plates

**Day 0-1** → **Day 1** → **Day 5**

Incubation 37º C
5% CO2

# 4. METHODS

**X compounds**

Anti-*T. cruzi* assay on Vero cells

↓ IC50 < X x BNZ IC50

**X compounds**

Vero cell toxicity assay

↓ SI > X

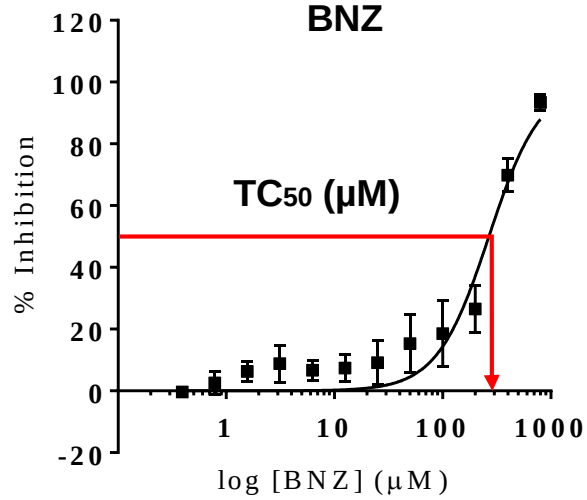
**X compounds**

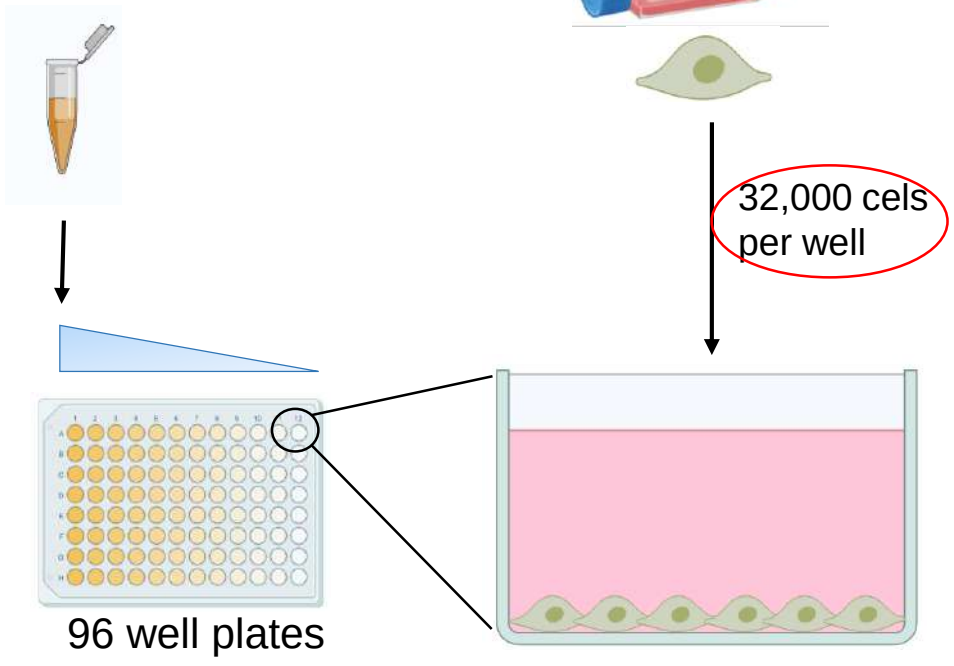
**HepG2 cell toxicity assay**

↓ SI > X

**X compounds**

## HepG2 cell toxicity assay

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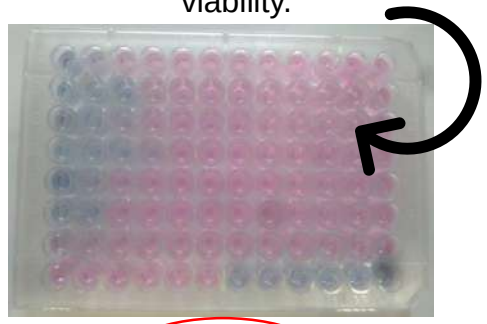

**Day 0-1** → **Day 1** → **Day 3**

Incubation 37º C
5% CO2

# 4. METHODS

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Anti-*T. cruzi* assay on Vero cells ↓ IC50 < X x BNZ IC50

**X compounds**

Vero cell toxicity assay ↓ SI > X

**X compounds**

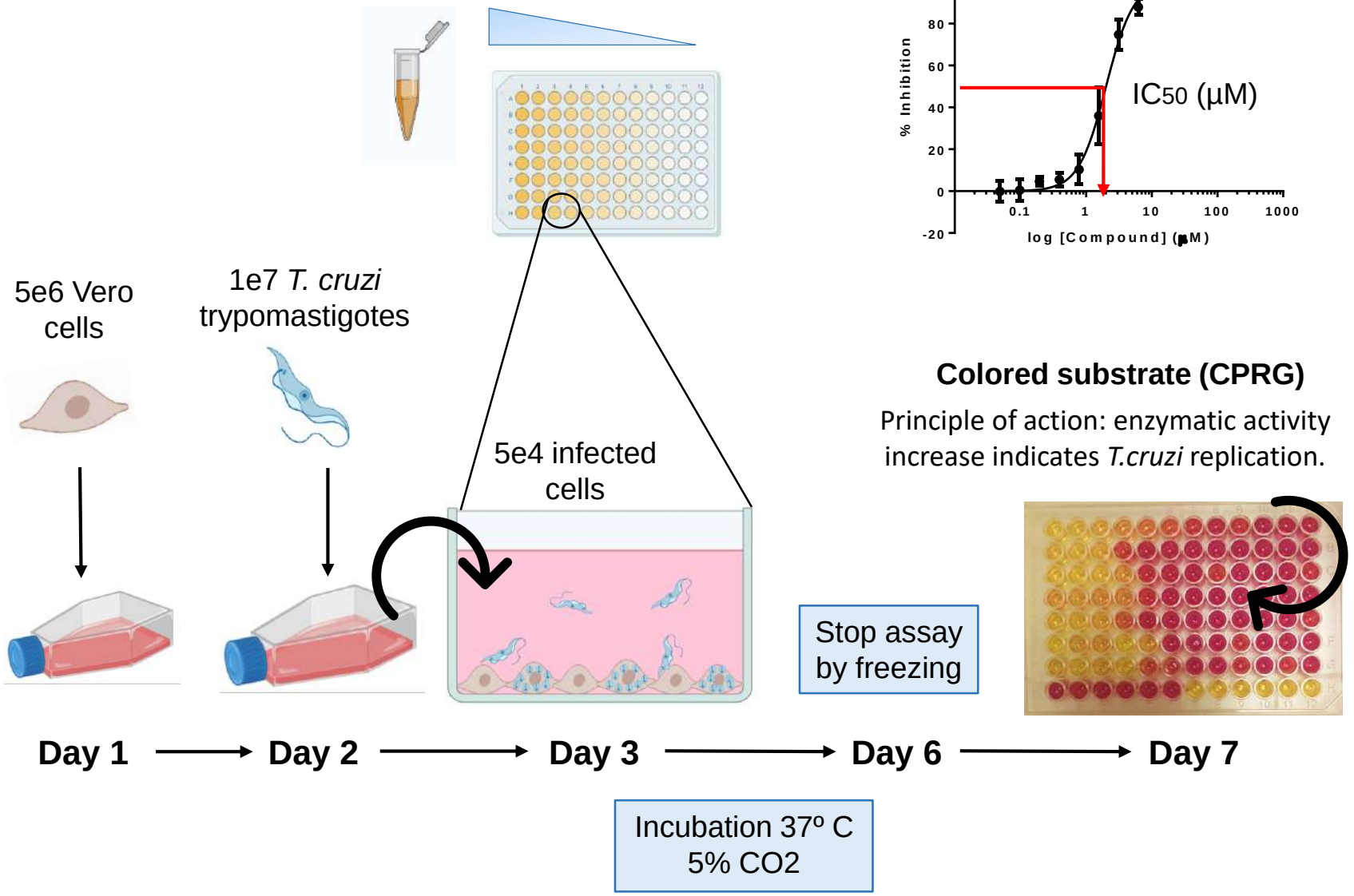
HepG2 cell toxicity assay ↓ SI > X

**X compounds**

**Anti-amastigote assay** ↓ SI > X

**X compounds**

## Anti-amastigote assay

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**X compounds**

Vero cell toxicity assay

↓ SI > X

**X compounds**

HepG2 cell toxicity assay

↓ SI > X

**X compounds**

Anti-amastigote assay

↓ SI > X

**X compounds**

NYU ***In vivo* model**

↓

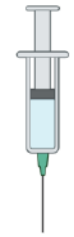
**X compounds**

## *In vivo assays*

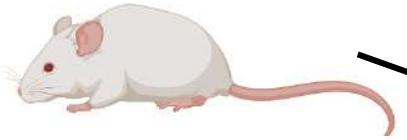
NYU

Dra. Ana Rodriguez

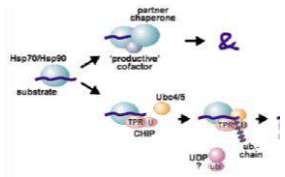
*T. cruzi* Brazil strain (DTU I) expressing firefly luciferase



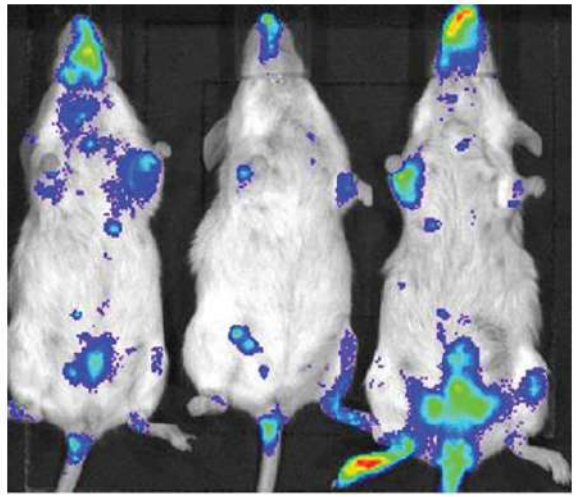
Acute *in vivo* model



Balb/c mice



Chronic *in vivo* model



# 4. METHODS

**X compounds**

Anti-*T. cruzi* assay on Vero cells → IC50 < X x BNZ IC50

**X compounds**

Vero cell toxicity assay → SI > X

**X compounds**

HepG2 cell toxicity assay → SI > X

**X compounds**

Anti-amastigote assay → SI > X

**X compounds**

NYU *In vivo* model

**X compounds**

***In silico* model**

**X compounds**

## *In silico* target identification

Ros-Lucas et al., 2022

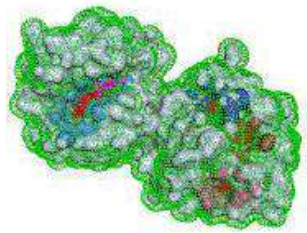
10,596 genes
All Tc Brenner
Esmeraldo genes

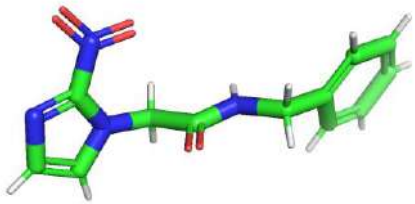
AlphaFold

2,298 proteins

P2Rank

4,351 pockets of
2,298 proteins



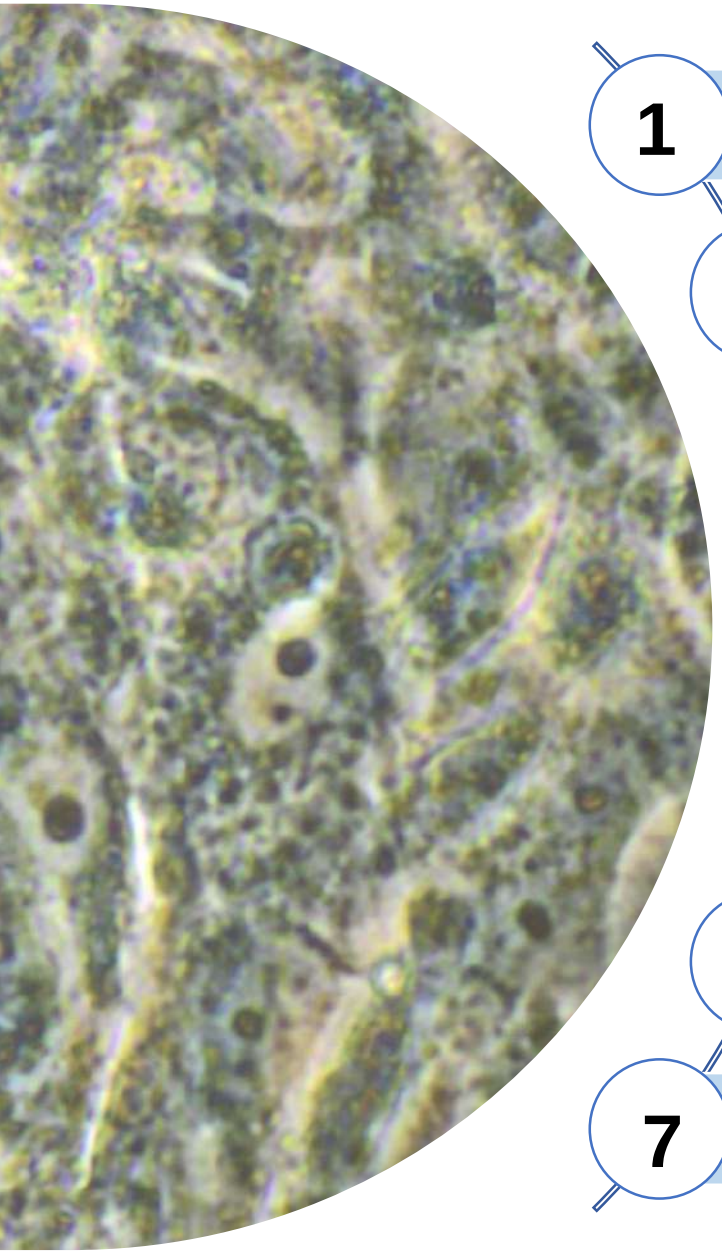


PDBQT files
Calculate radius of gyration

AutoDock Vina

| | L1 | L2 | L3 | Ln |
|---|---|---|---|---|
| R1 | -8.4 | -5.2 | -8.5 | -8.3 |
| R2 | -7.2 | -6.3 | -7.9 | -6.3 |
| R3 | -6.4 | -8.7 | -8.1 | -7.2 |
| R4 | -7.3 | -6.5 | -8.3 | -6.5 |
| Rn | -7.4 | -7.6 | -8.3 | -7.2 |

**Select top 3% best receptors**



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- Chapter I. Amaryllidaceae plants
- Chapter II. Drug repurposing
- Chapter III. Metabolism modifier compounds
- Chapter IV. *In silico* target identification

# 5. RESULTS AND DISCUSSION. CHAPTER I

Prof. Jaume Bastida

UNIVERSITAT DE BARCELONA

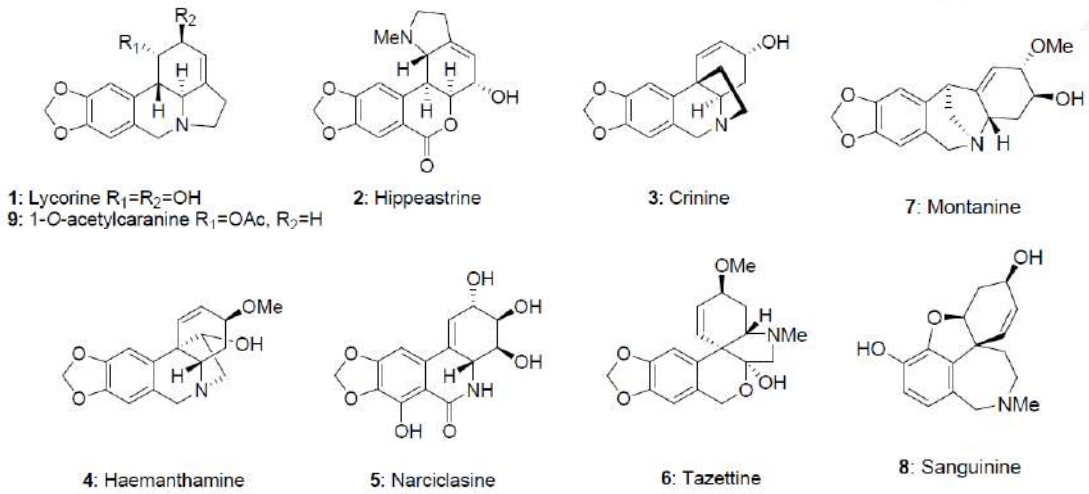
Martinez-Peinado *et al. Parasites Vectors* (2020) 13:299
https://doi.org/10.1186/s13071-020-04171-6

Parasites & Vectors

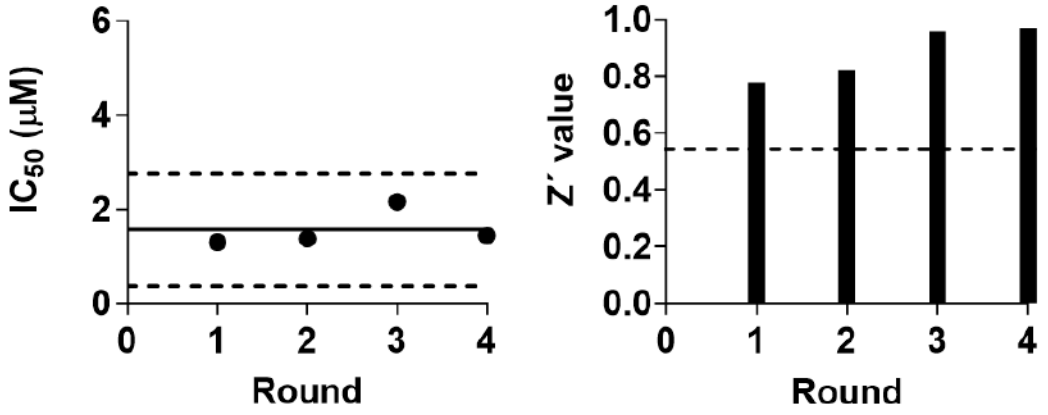
RESEARCH Open Access

## Amaryllidaceae alkaloids with anti-*Trypanosoma cruzi* activity

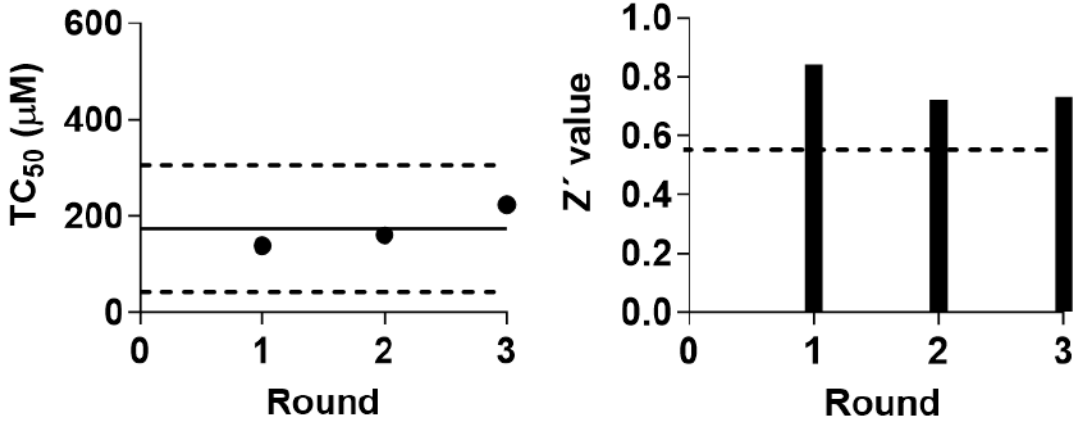
Nieves Martinez-Peinado[1], Nuria Cortes-Serra[1], Laura Torras-Claveria[2], Maria-Jesus Pinazo[1], Joaquim Gascon[1], Jaume Bastida[2] and Julio Alonso-Padilla[1*]




## Anti-*T. cruzi* assay




## Vero cell toxicity assay




*Z´* parameter to assess the reproducibility and quality (0.5-1)

# 5. RESULTS AND DISCUSSION. CHAPTER I



Martinez-Peinado *et al. Parasites Vectors* (2020) 13:299
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Parasites & Vectors

RESEARCH Open Access

## Amaryllidaceae alkaloids with anti-*Trypanosoma cruzi* activity

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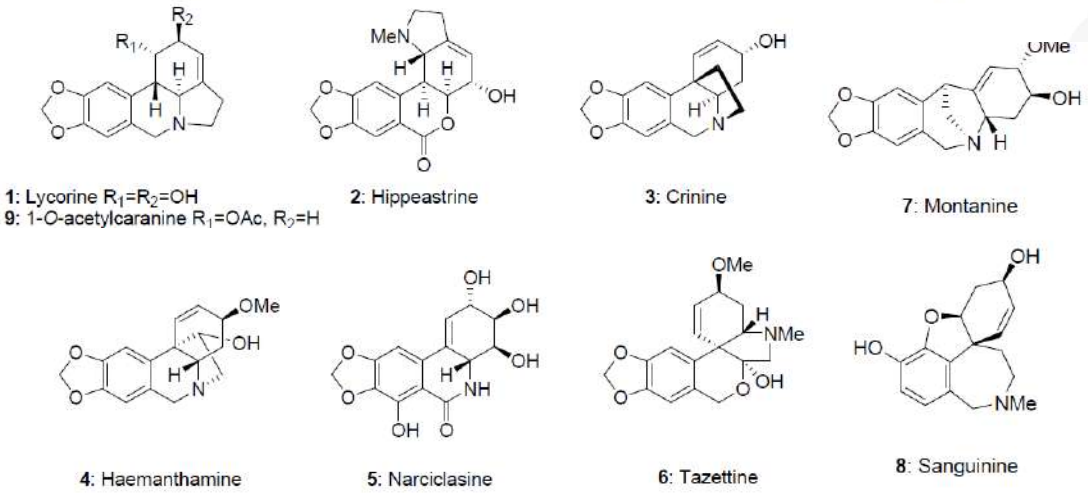
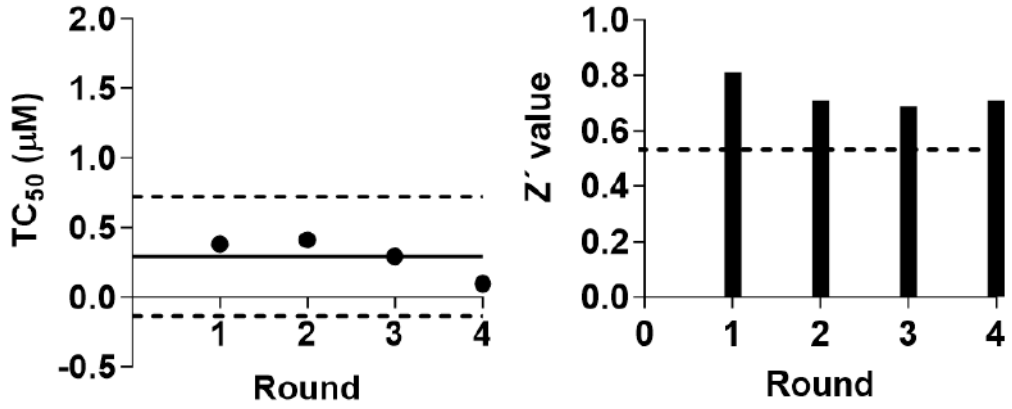
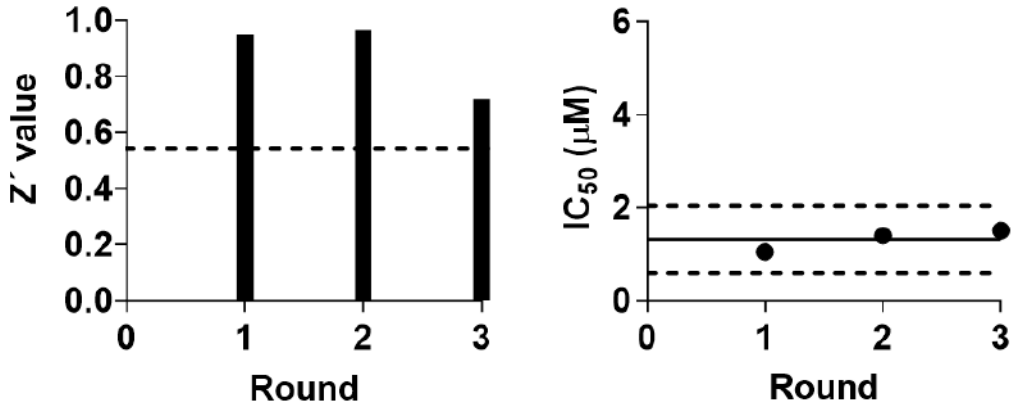



## HepG2 cell toxicity assay




## Anti-amastigote assay




*Z´* parameter to assess the reproducibility and quality (0.5-1)

# 5. RESULTS AND DISCUSSION. CHAPTER I

**9 alkaloids**

Anti-*T. cruzi* assay on Vero cells → $IC_{50}$ < 5x BNZ

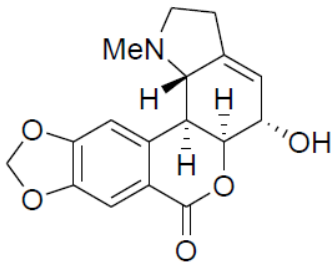
**5 alkaloids**

Vero cell toxicity assay
HepG2 cell toxicity assay → SI > 10

**Hippeastrine**

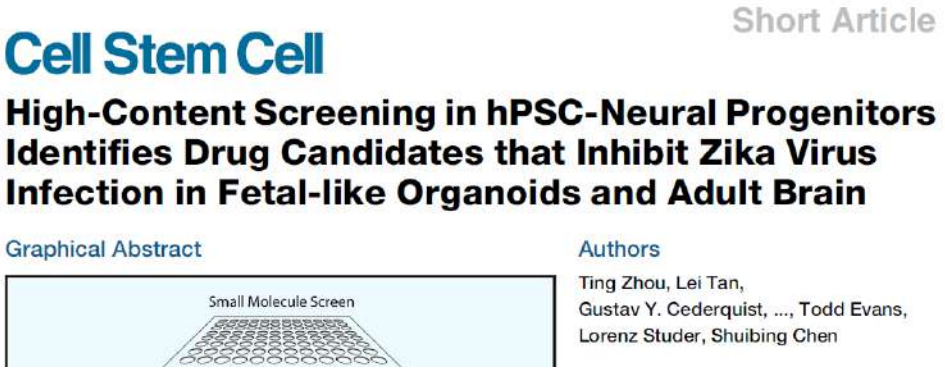
Anti-amastigote assay → SI > 10

**Hippeastrine**




Hippeastrine

| Alkaloid | $IC_{50}$ (μM) | $TC_{50}^{a}$ (μM) | $SI^{a}$ | $TC_{50}^{b}$ (μM) | $SI^{b}$ | $IC_{50}$ (μM) | $SI^{a}$ | $SI^{b}$ |
|---|---|---|---|---|---|---|---|---|
| **BNZ** | 1.56 | 173.4 | 111.2 | 168.76 | 108.2 | 1.20 | 144.5 | 140.6 |
| **Lycorine** | 0.70 | 5.21 | 7.5 | 21.87 | 31.2 | | | |
| **Hippeastrine#** | 3.63 | 45.99 | 12.7 | 128.10 | 35.2 | 3.31 | 13.8 | 38.7 |
| **Haemanthamine** | 1.59 | 11.52 | 7.3 | 42.48 | 26.7 | | | |
| **Narciclasine** | 0.49 | 0.66 | 1.3 | 2.73 | 5.5 | | | |
| **Montanine** | 1.99 | 5.04 | 2.5 | 46.10 | 23.1 | | | |



Short Article

Cell Stem Cell

**High-Content Screening in hPSC-Neural Progenitors Identifies Drug Candidates that Inhibit Zika Virus Infection in Fetal-like Organoids and Adult Brain**

Graphical Abstract

Small Molecule Screen

Authors

Ting Zhou, Lei Tan, Gustav Y. Cederquist, ..., Todd Evans, Lorenz Studer, Shuibing Chen

# 5. RESULTS AND DISCUSSION. CHAPTER I

**9 alkaloids**

Anti-*T. cruzi* assay on Vero cells → $IC_{50}$ < 5x BNZ $IC_{50}$

**5 alkaloids**

Vero cell toxicity assay
HepG2 cell toxicity assay → SI > 10

**Hippeastrine**

Anti-amastigote assay → SI > 10

**Hippeastrine**

Contents lists available at ScienceDirect

Phytomedicine

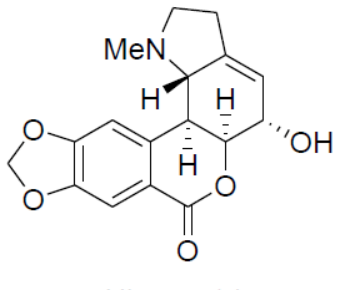
journal homepage: www.elsevier.com/locate/phymed

Original Article

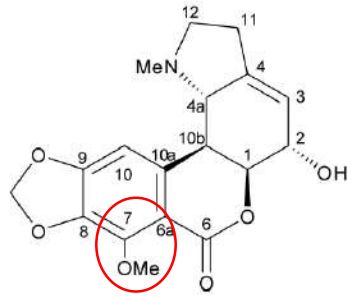
## Candimine from *Hippeastrum escoipense* (Amaryllidaceae): Anti-*Trypanosoma cruzi* activity and synergistic effect with benznidazole

Javier E. Ortiz [a,b,1], Mauricio Piñeiro [a,b,1], Nieves Martinez-Peinado [c,d], Patricia Barrera [e], Miguel Sosa [e], Jaume Bastida [d], Julio Alonso-Padilla [c,f,§], Gabriela E. Feresin [a,b,§,*]

[a] Instituto de Biotecnología, Facultad de Ingeniería, Universidad Nacional de San Juan, Av. Libertador General San Martin, 1109 O San Juan, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), CCT CONICET San Juan, Argentina
[c] Barcelona Institute for Global Health (ISGlobal), Hospital Clinic-University of Barcelona, 08036 Barcelona, Spain
[d] Departament de Biologia, Sanitat i Medi Ambient, Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, 08028 Barcelona, Spain
[e] Facultad de Ciencias Médicas, Instituto de Histología y Embriología "Dr. Mario H. Burgos", Universidad Nacional de Cuyo-CONICET, CC 56 (5500) Mendoza, Argentina
[f] CIBER de Enfermedades Infecciosas, Instituto de Salud Carlos III (CIBERINFEC, ISCIII), Madrid, Spain




**Hippeastrine**
$IC_{50}$=3.63, SI=12.7
$IC_{50(amastigote)}$=3.31, SI=13.8




**Candimine**
$IC_{50}$=2.49, SI=102.57
$IC_{50(amastigote)}$=1.60, SI=159.63

# 5. RESULTS AND DISCUSSION. CHAPTER I

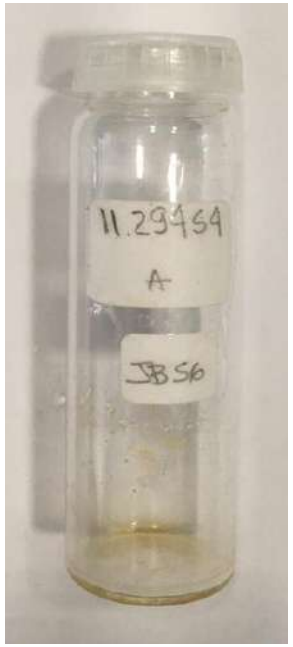
Martínez-Peinado *et al. Parasites Vectors* (2021) 14:337
https://doi.org/10.1186/s13071-021-04837-9

Parasites & Vectors

RESEARCH Open Access

## Amaryllidaceae plants: a potential natural resource for the treatment of Chagas disease

Nieves Martínez-Peinado[1], Nuria Cortes-Serra[1], Luciana R. Tallini[2,3], Maria-Jesus Pinazo[1], Joaquim Gascon[1], Jaume Bastida[2*] and Julio Alonso-Padilla[1*]




Prof. Jaume Bastida

UNIVERSITAT DE BARCELONA

**79 extracts**

Anti-*T. cruzi* assay on Vero cells → $IC_{50}$ > 30x BNZ $IC_{50}$

**35 extracts**

Vero cell toxicity assay → SI > 20

**7 extracts**

HepG2 cell toxicity assay → SI > 20

**6 extracts**

Anti-amastigote assay → SI > 10

**2 extracts**

# 5. RESULTS AND DISCUSSION. CHAPTER I

Martínez-Peinado *et al. Parasites Vectors* (2021) 14:337
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Parasites & Vectors

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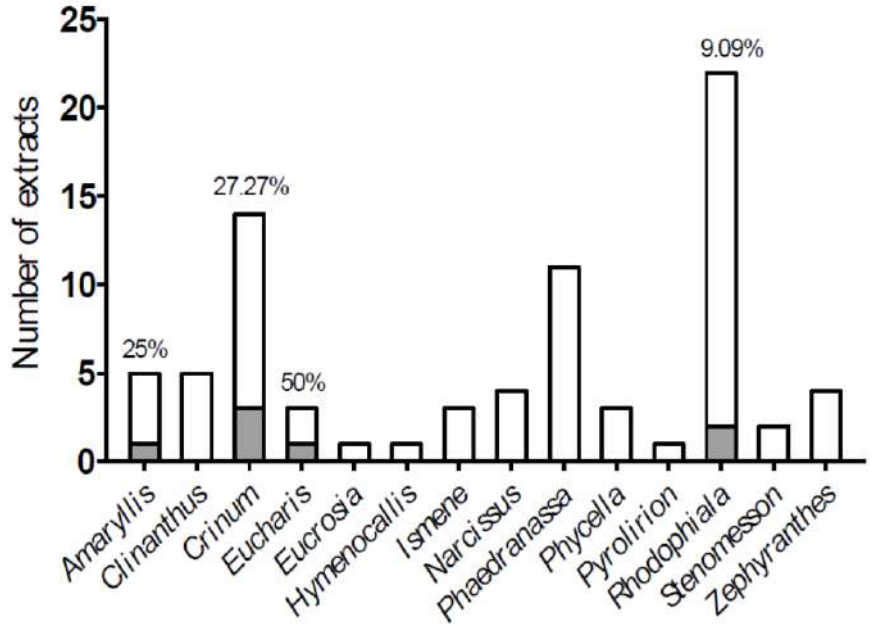



Distribution of anti-*T. cruzi* selective extracts per plant genus

**79 extracts**

Anti-*T. cruzi* assay on Vero cells → $IC_{50}$ > 30x BNZ $IC_{50}$

**35 extracts**

Vero cell toxicity assay → SI > 20

**7 extracts**

HepG2 cell toxicity assay → SI > 20

**6 extracts**

Anti-amastigote assay → SI > 10

**2 extracts**

# 5. RESULTS AND DISCUSSION. CHAPTER I

| | | | | Vero cells assay | | | HepG2 cell assay | Anti-amastigote assay | |
|---|---|---|---|---|---|---|---|---|---|
| **Extract number** | **Plant species of origin** | **Country of collection** | **Part of the plant*** | **$IC_{50}$ (ppm)** | **$TC_{50}$ (ppm)** | **SI** | **$TC_{50}$ (ppm)** | **$IC_{50}$ (ppm)** | **SI** |
| **BNZ** | - | | | 0.40 | 69.60 | 174 | 51.47 | 0.53 | 131.4 |
| **51** | *Amaryllis belladonna* | Chile | B | 1.65 | 41.97 | 25.4 | 128.2 | 37.29 | 1.12 |
| **81** | *Crinum amabile* | Venezuela | B | 5.42 | 211.5 | 38.9 | 266.9 | 25.86 | 8.2 |
| **93** | *Crinum amabile* | Ecuador | B | 2.21 | 60.69 | 27.5 | 111.3 | 20.57 | 2.9 |
| **56** | *Crinum erubescens* | Bolivia | B | 9.50 | 234.7 | 24.7 | 678.3 | 11.10 | 21.1 |
| **101** | *Eucharis formosa* | Ecuador | B | 9.71 | 346.7 | 35.7 | 778.9 | 26.93 | 12.9 |
| **23** | *Rhodophiala andicola* | Chile | B | 6.20 | 134.9 | 21.8 | 77.37 | - | - |
| **24** | *Rhodophiala andicola* | Chile | AP | 6.13 | 228.4 | 37.3 | 188.1 | 10.18 | 22.4 |

# 5. RESULTS AND DISCUSSION. CHAPTER I

Phytomedicine 101 (2022) 154126

Contents lists available at ScienceDirect

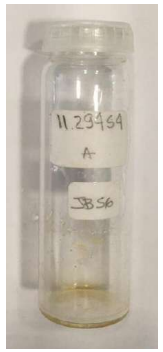
Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

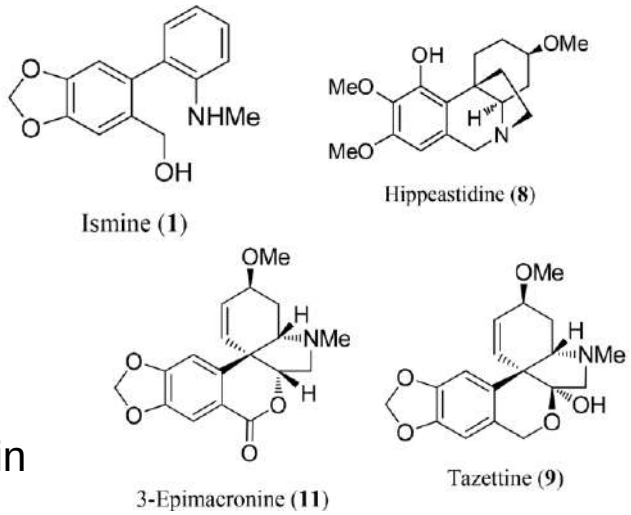
Original Article

## Anti-*Trypanosoma cruzi* activity of alkaloids isolated from *Habranthus brachyandrus* (Amaryllidaceae) from Argentina

Nieves Martinez-Peinado [a,b,1], Javier E. Ortiz [c,d,1], Nuria Cortes-Serra [a,b], Maria Jesus Pinazo [a,b], Joaquim Gascon [a,b], Alejandro Tapia [c], German Roitman [e], Jaume Bastida [f], Gabriela E. Feresin [c,d,*], Julio Alonso-Padilla [a,b,**]



GC-MS spectra, NMR
Cromatographic techniques




Prof. Jaume Bastida  Dr. Gabriela Feresin

**AE-HBr extract 3 alkaloids**

Anti-*T. cruzi* assay on Vero cells

$IC_{50}$ > 30x BNZ $IC_{50}$

**AE-HBr extract ismine**

Vero cell toxicity assay

SI > 20

**AE-HBr extract ismine**

HepG2 cell toxicity assay

SI > 20

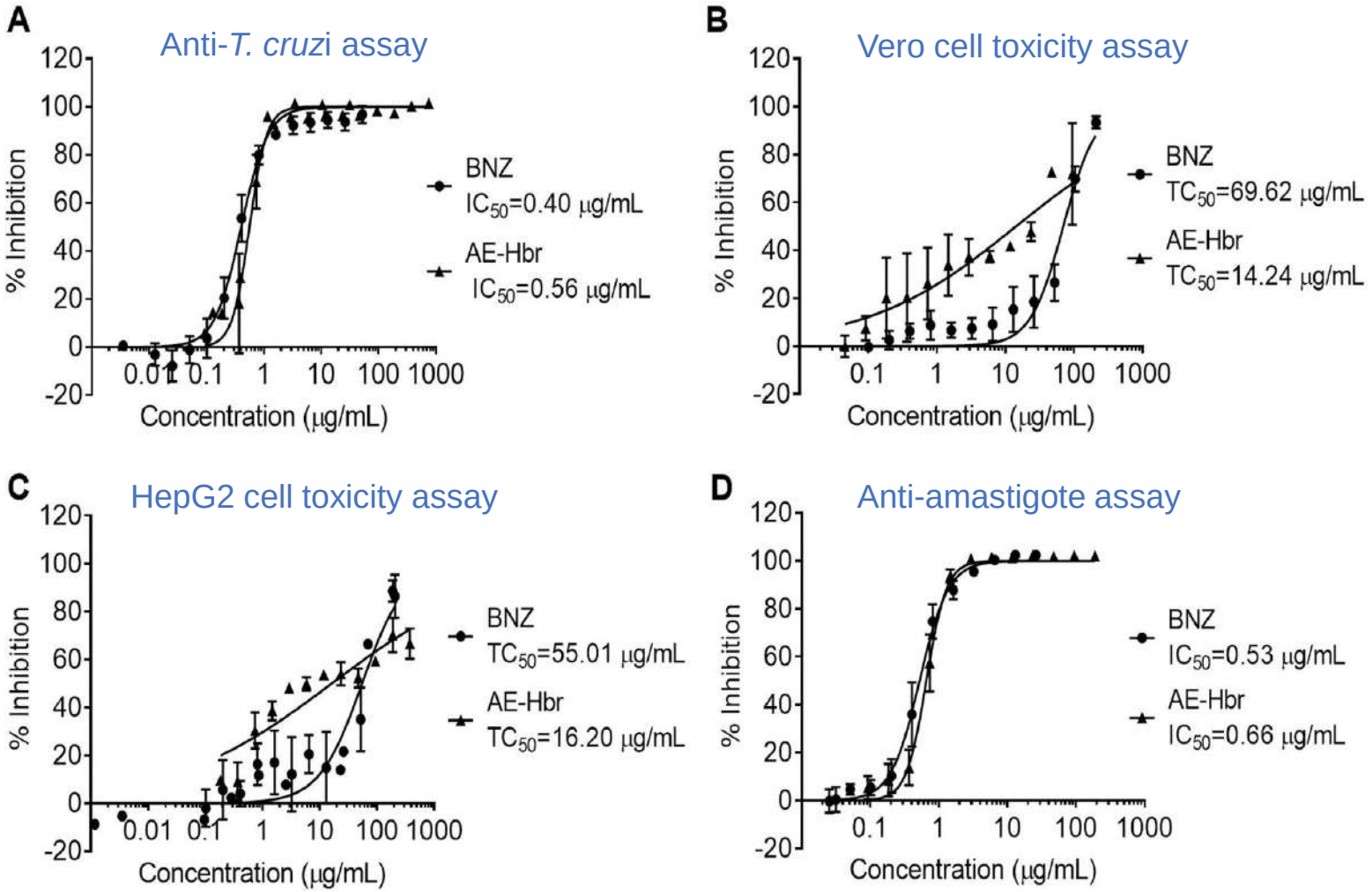
**AE-HBr extract ismine**

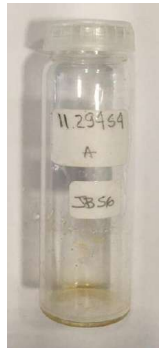
Anti-amastigote assay

SI > 10

**AE-Hbr extract**

# 5. RESULTS AND DISCUSSION. CHAPTER I



GC-MS spectra, NMR
Cromatographic techniques

**X compounds**

Anti-*T. cruzi* assay on Vero cells → $IC_{50} < X \times BNZ\ IC_{50}$

**X compounds**

Vero cell toxicity assay → SI > X

**X compounds**

HepG2 cell toxicity assay → SI > X

**X compounds**

Anti-amastigote assay → SI > X

**X compounds**

Alkaloids responsable of AE-Hbr extract activity?

# 5. RESULTS AND DISCUSSION. CHAPTER II

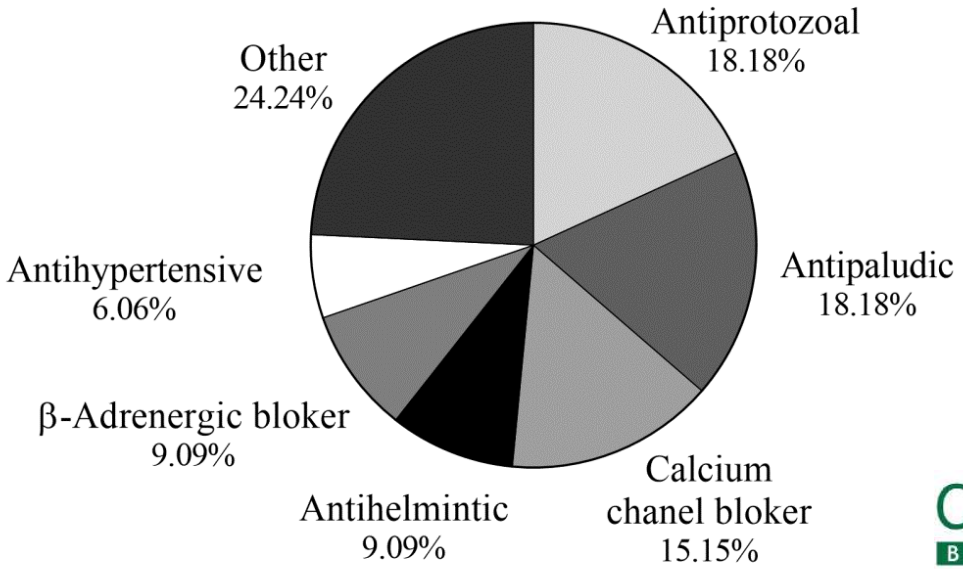
microorganisms

MDPI

*Article*

## Identification of *Trypanosoma cruzi* Growth Inhibitors with Activity In Vivo within a Collection of Licensed Drugs

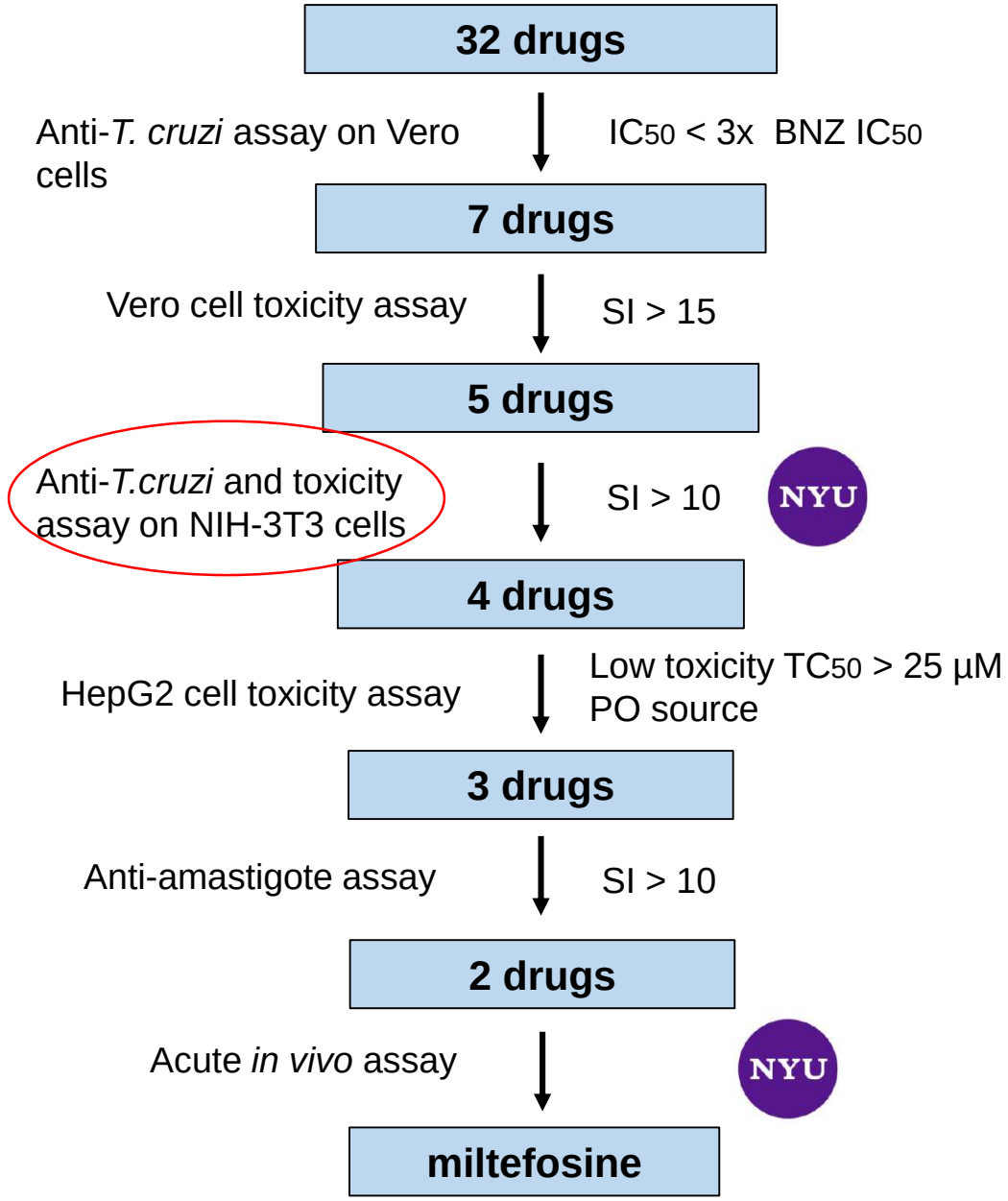
Nieves Martinez-Peinado [1,†], Nuria Cortes-Serra [1,†], Julian Sherman [2], Ana Rodriguez [2], Juan M. Bustamante [3], Joaquim Gascon [1], Maria-Jesus Pinazo [1,*] and Julio Alonso-Padilla [1,*]




CLÍNIC BARCELONA Hospital Universitari

UNIVERSITY OF GEORGIA

Dr. Juan Bustamante
Prof. Joaquim Gascón

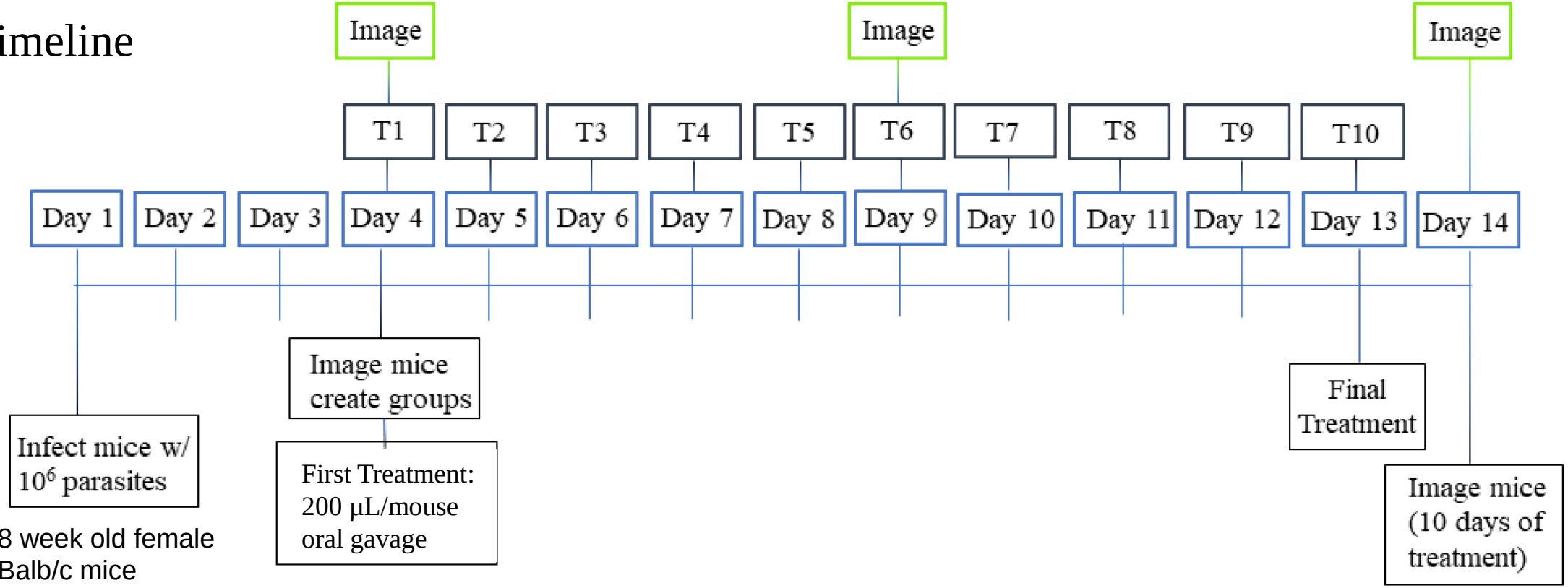


| | Vero cells assays | | | NIH-3T3 cells assays* | | | HepG2 cells assays | Anti-amastigote assay | |
|---|---|---|---|---|---|---|---|---|---|
| **Drug** | **$IC_{50}$ (µM)** | **$TC_{50}$ (µM)** | **SI** | **$IC_{50}$ (µM)** | **$TC_{50}$ (µM)** | **SI** | **$TC_{50}$ (µM)** | **$IC_{50}$ (µM)** | **SI** |
| **BNZ** | 1.93 | 242.2 | 125.5 | - | - | - | 229.8 | 2.66 | 91.1 |
| **Atovaquone – proguanil** | 1.26 | 27.13 | 21.5 | 1.32 | 50 | >50 | 34.36 | 1.85 | 14.7 |
| **Miltefosine** | 0.018 | 78.99 | 4,388.3 | 0.037 | 1.95 | 52.7 | 51.28 | 1.25 | 63.2 |
| **Lidocaine#** | 0.016 | 0.23 | 14.4 | | | | | | |
| **Nifedipine** | 0.19 | 1.967 | 10.4 | | | | | | |
| **Pentamidine** | 1.01 | 78.96 | 78.2 | 0.13 | 5.9 | 45.4 | 39.4 | | |
| **Piperaquine tetraphosphate - dihydroartemisinin** | 3.95 | 75.27 | 19.1 | 4.05 | 27.33 | 6.8 | | | |
| **Verapamil** | 3.44 | 197.4 | 57.4 | 0.21 | 5.72 | 27.2 | 170.5 | 122.5 | 1.6 |

# values expressed as drug % (v/v).

# Acute *in vivo* model

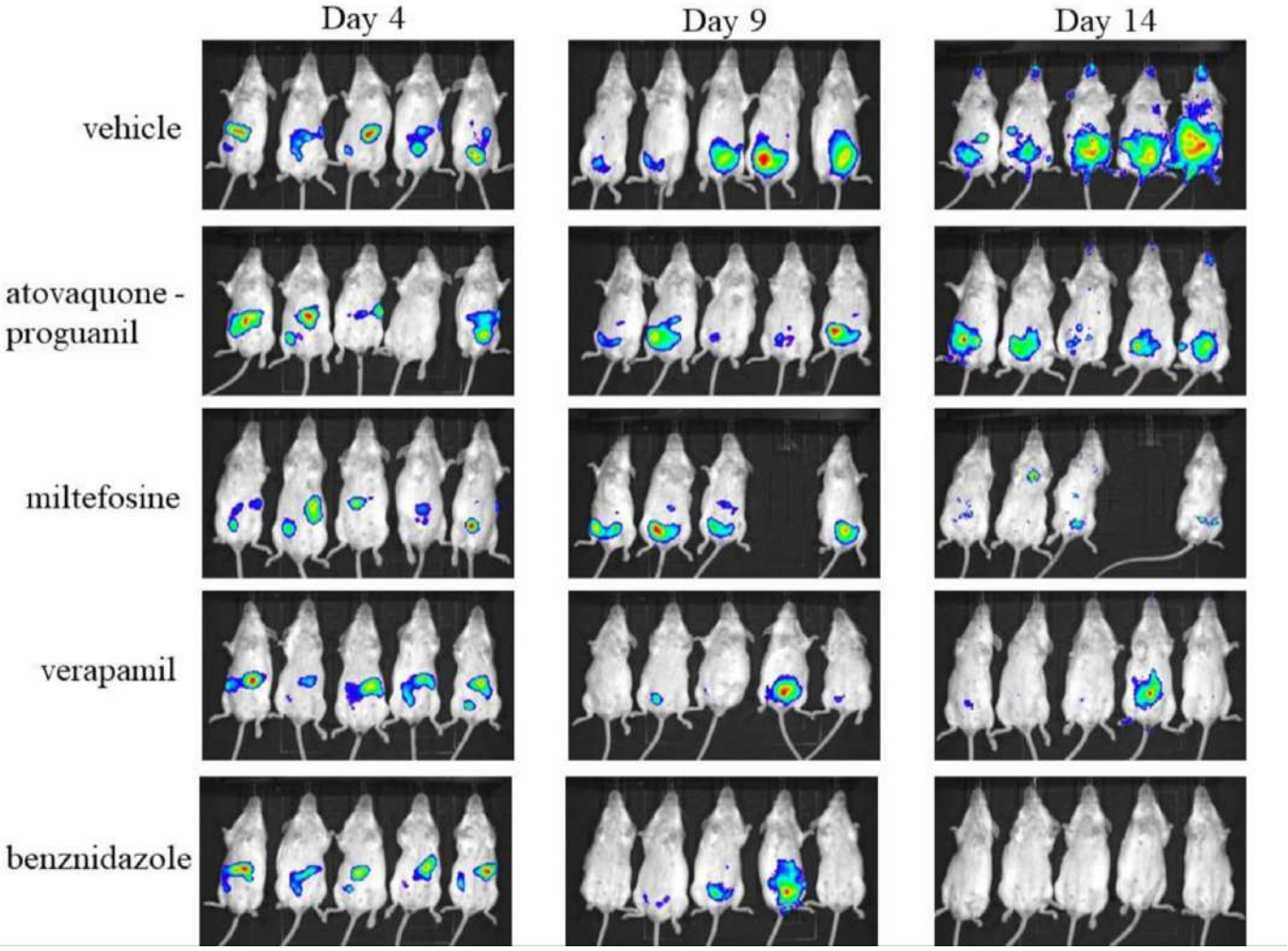
Timeline




8 week old female Balb/c mice

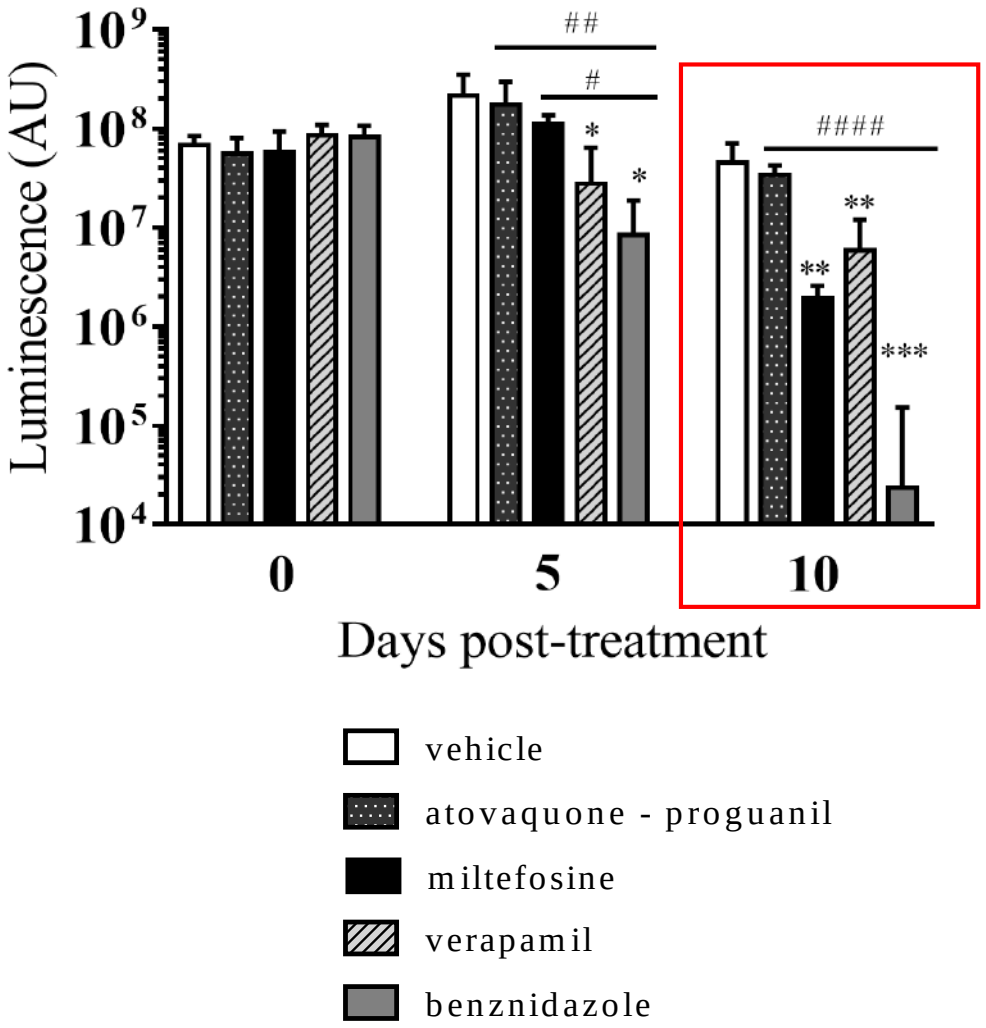
BNZ, miltefosine and autovaquone-proguanil: 30 mg/kg/day
verapamil: 5 mg/kg/day

# Acute *in vivo* model




*T. cruzi* Luc Brazil (DTU I)

*significate differences with vehicle
# significative differences with BNZ



# 5. RESULTS AND DISCUSSION. CHAPTER II

frontiers | Frontiers in Cellular and Infection Microbiology

ORIGINAL RESEARCH
published: 05 July 2022
doi: 10.3389/fcimb.2022.855119

## Miltefosine and Benznidazole Combination Improve Anti-*Trypanosoma cruzi In Vitro* and *In Vivo* Efficacy

*Julián Ernesto Nicolás Gulin[1,2], Margarita María Catalina Bisio[1,3], Daniela Rocco[1], Jaime Altcheh[1], María Elisa Solana[4,5] and Facundo García-Bournissen[1,6]**

OPEN ACCESS

Analysis

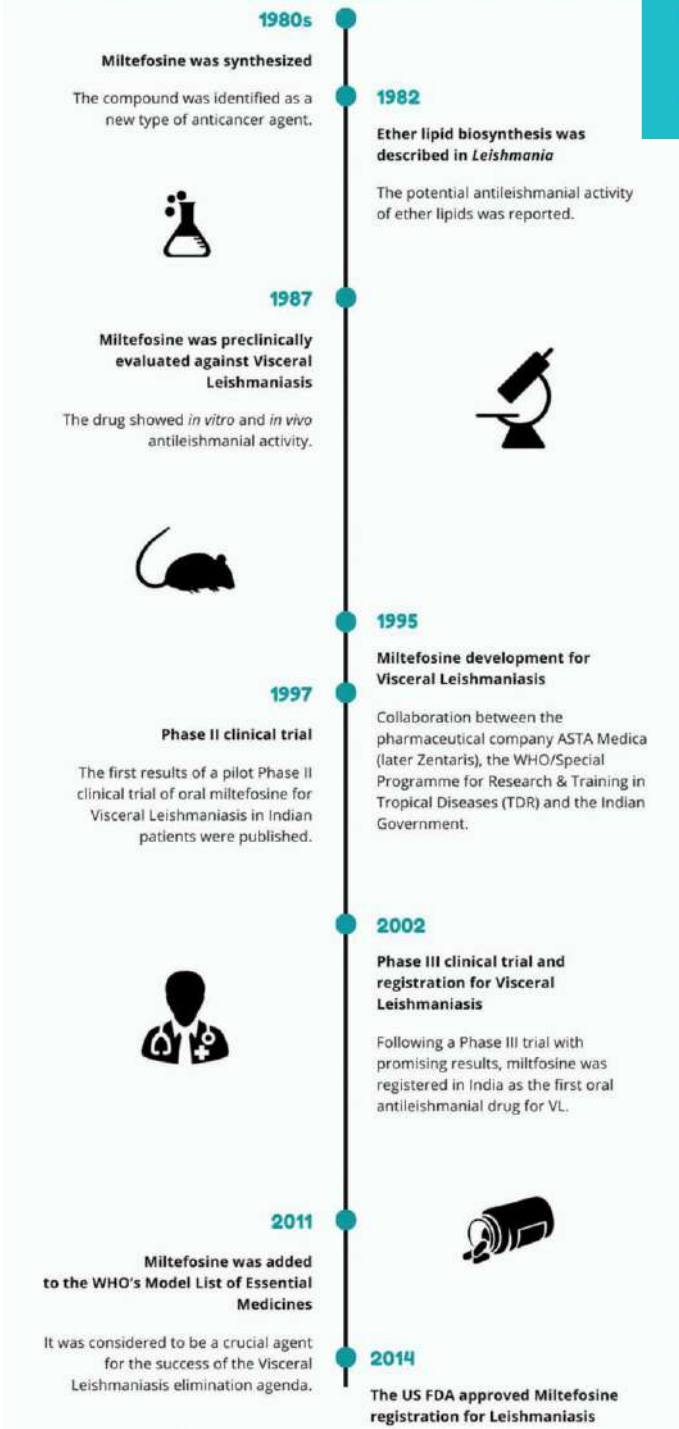
BMJ Global Health

## Why miltefosine—a life-saving drug for leishmaniasis—is unavailable to people who need it the most

REVIEW

## Miltefosine in the treatment of leishmaniasis: Clinical evidence for informed clinical risk management

**MILTEFOSINE**
**DISCOVERY & DEVELOPMENT**



# 5. RESULTS AND DISCUSSION. CHAPTER III

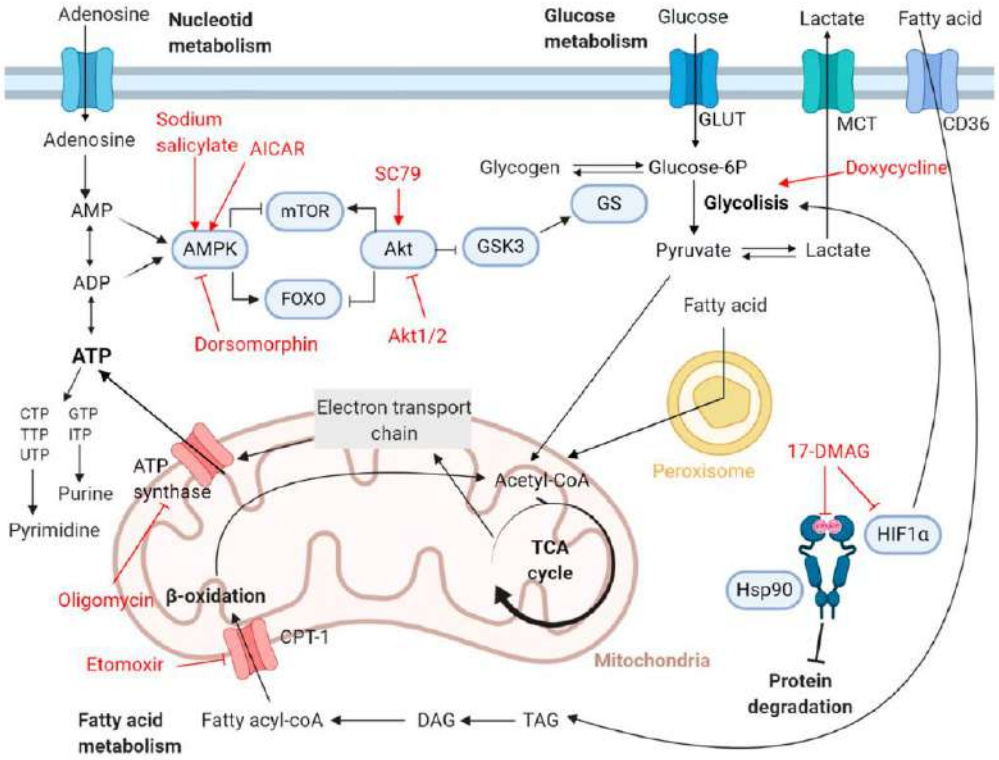
International Journal of *Molecular Sciences*

MDPI

*Article*

## Anti-*Trypanosoma cruzi* Activity of Metabolism Modifier Compounds

Nieves Martinez-Peinado [1], Clara Martori [2], Nuria Cortes-Serra [1], Julian Sherman [3], Ana Rodriguez [3], Joaquim Gascon [1], Jordi Alberola [2], Maria-Jesus Pinazo [1], Alheli Rodriguez-Cortes [2,*] and Julio Alonso-Padilla [1,*]




UAB Universitat Autònoma de Barcelona

Dra. Alhelí Rodríguez-Cortes

**9 compounds**

Anti-*T. cruzi* assay on Vero cells → $IC_{50}$ < 5 x BNZ $IC_{50}$

**3 compounds**

**AlamarBlue**
**Crystal violet**
Vero cell toxicity assay → SI > 10

**17-DMAG**

Anti-amastigote assay → SI > 10

**17-DMAG**

*In silico* model

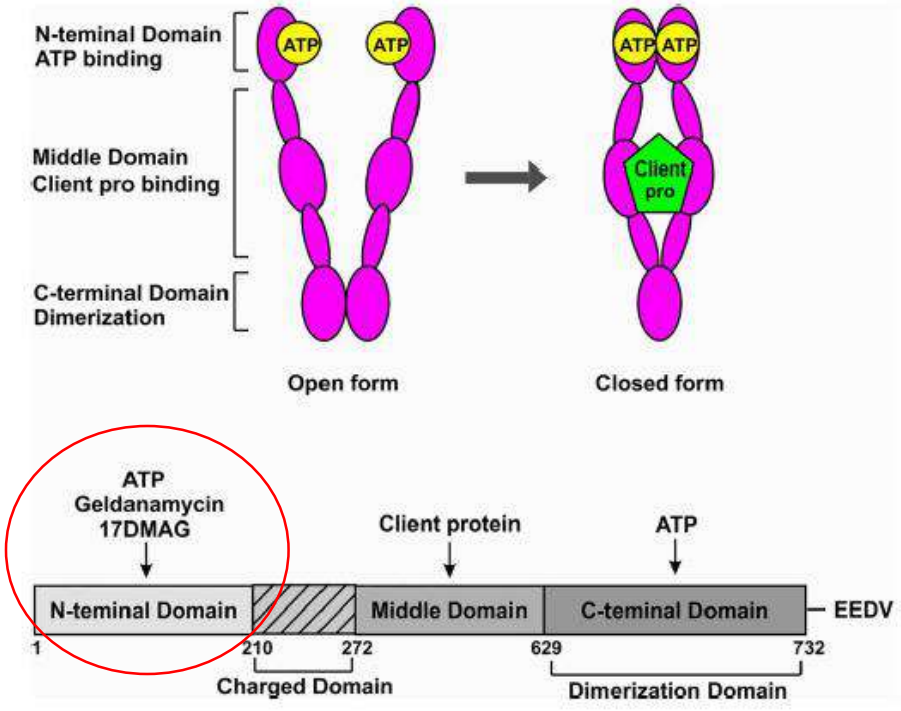
**17-DMAG**

Chronic *in vivo* model

**-**

# 5. RESULTS AND DISCUSSION. CHAPTER III

| | | Vero cell assays Alamar Blue | | Crystal violet | | Anti-amastigote assay | |
|---|---|---|---|---|---|---|---|
| **Compound** | **$IC_{50}$ (µM)** | **$TC_{50}$ (µM)** | **SI** | **$TC_{50}$ (µM)** | **SI** | **$IC_{50}$ (µM)** | **SI** |
| **BNZ** | 1.63 | 243.8 | 149.6 | 140.2 | 86 | 2.02 | 120.69 |
| **17-DMAG** | 0.017 | 6.2 | 366.5 | 2.97 | 174.7 | 0.17 | 36.5 |




(Mellyart et al., BiomedPharma 2018)

SCIENTIFIC REPORTS
natureresearch

OPEN

## A docking-based structural analysis of geldanamycin-derived inhibitor binding to human or *Leishmania* Hsp90

Received: 20 June 2018
Accepted: 13 September 2019
Published online: 14 October 2019

Luana Carneiro Palma[1], Luiz Felipe Gomes Rebello Ferreira[2], Antonio Luis de Oliveira Almeida Petersen[1], Beatriz Rocha Simões Dias[1], Juliana Perrone Bezerra de Menezes[1], Diogo Rodrigo de Magalhães Moreira[3], Marcelo Zaldini Hernandes[2] & Patricia Sampaio Tavares Veras[1]

OPEN ACCESS Freely available online
PLOS | NEGLECTED TROPICAL DISEASES

## Exploring the *Trypanosoma brucei* Hsp83 Potential as a Target for Structure Guided Drug Design

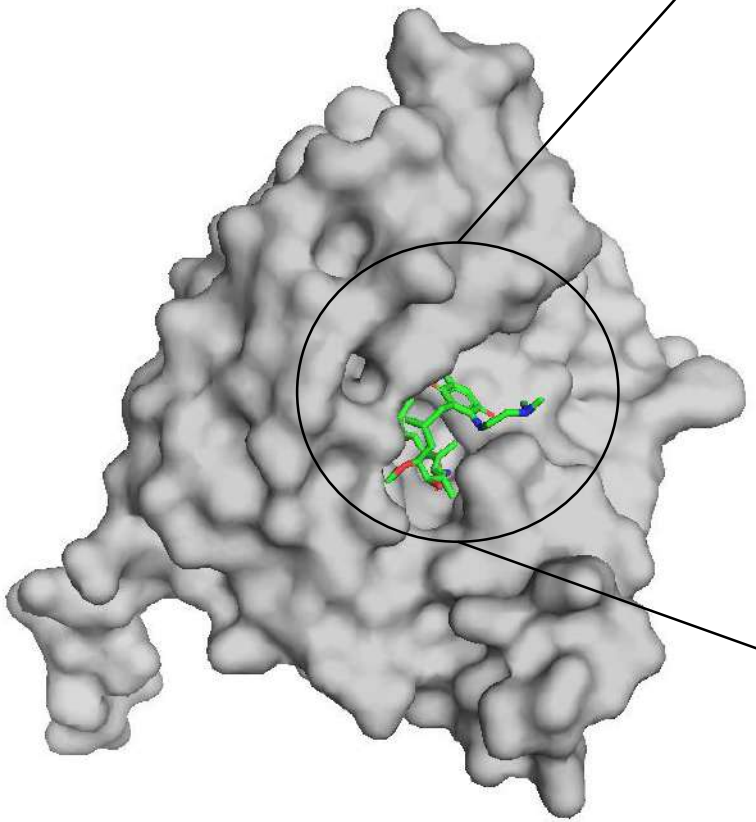
Juan Carlos Pizarro[1,2]*, Tanya Hills[1], Guillermo Senisterra[1], Amy K. Wernimont[1], Claire Mackenzie[3], Neil R. Norcross[3], Michael A. J. Ferguson[3], Paul G. Wyatt[3], Ian H. Gilbert[3], Raymond Hui[1]

1 The Structural Genomics Consortium (SGC), University of Toronto, Toronto, Ontario, Canada, 2 Department of Tropical Medicine, School of Public Health and Tropical Medicine, Tulane University, New Orleans, Louisiana, United States of America, 3 Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee, Scotland, United Kingdom

## *In silico* study:

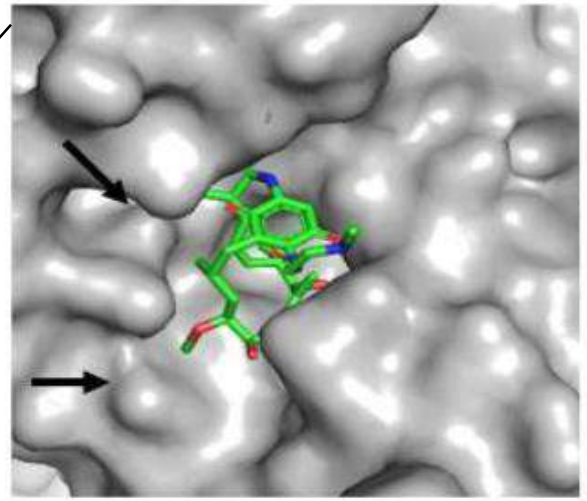
### Site-directed mutagenesis

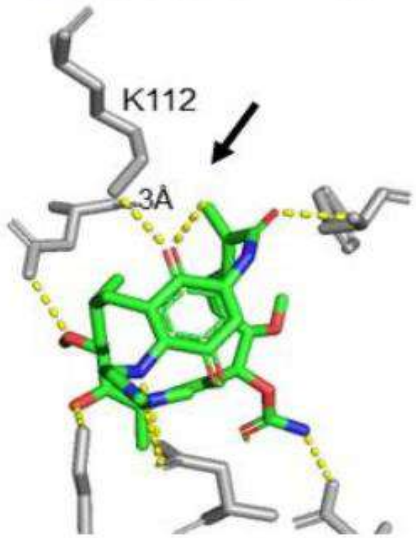
73% secuence identity between human Hsp90 N-terminal and *T. cruzi* Hsp83 N-terminal



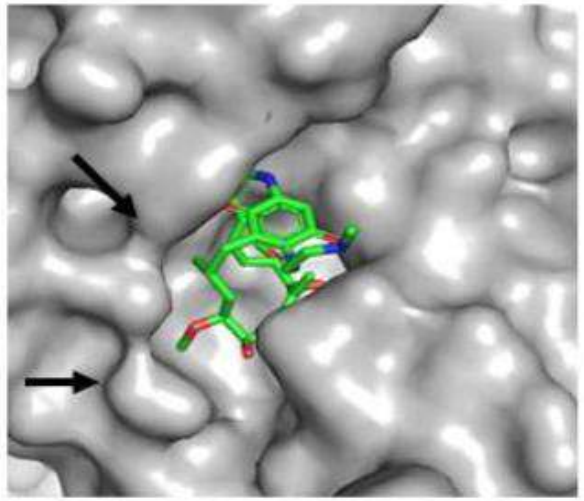
(PDB: 1OSF)

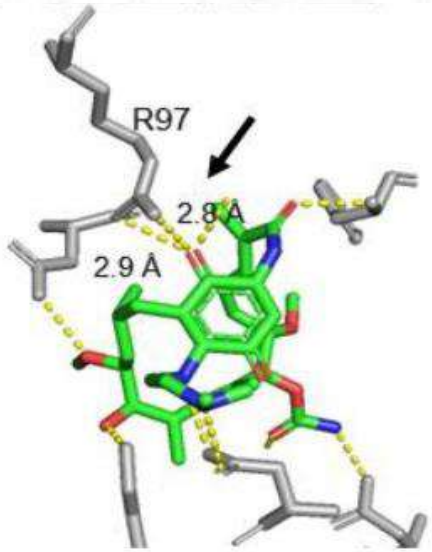
**Human Hsp90**






***T. cruzi* Hsp83**

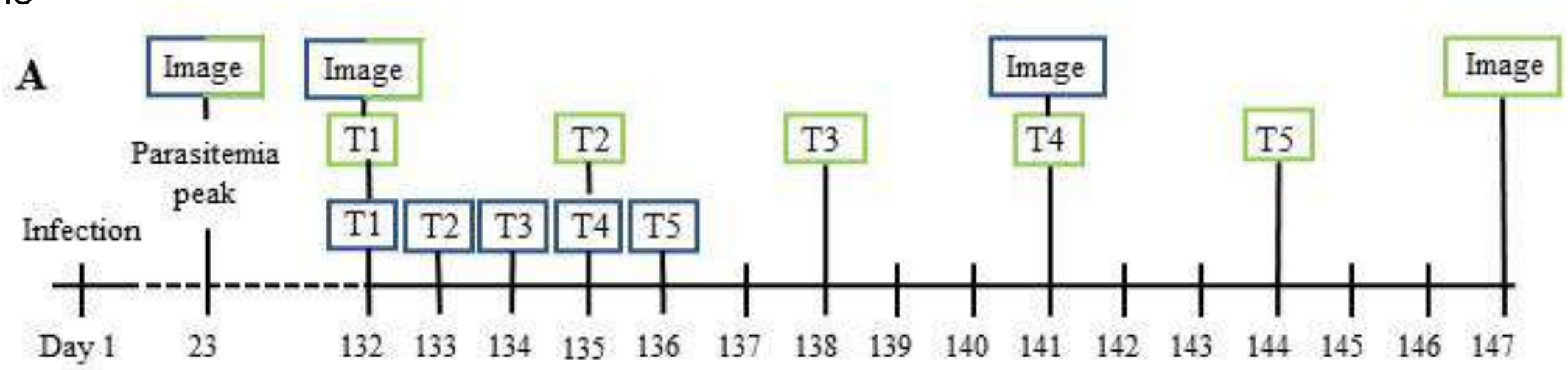





(PyMOL Molecular Graphics System)

# Chronic *in vivo* model

Timeline




BNZ intraperitoneal administration: 30 mg/kg/day
17-DMAG intraperitoneal administration: 30 mg/kg/day

MAJOR ARTICLE

Potent Antitrypanosomal Activities of Heat Shock Protein 90 Inhibitors In Vitro and In Vivo

Kirsten J. Meyer[1] and Theresa A. Shapiro[1,2]
[1]Department of Pharmacology and Molecular Sciences and [2]Division of Clinical Pharmacology, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland

OPEN ACCESS Freely available online
PLOS | NEGLECTED TROPICAL DISEASES

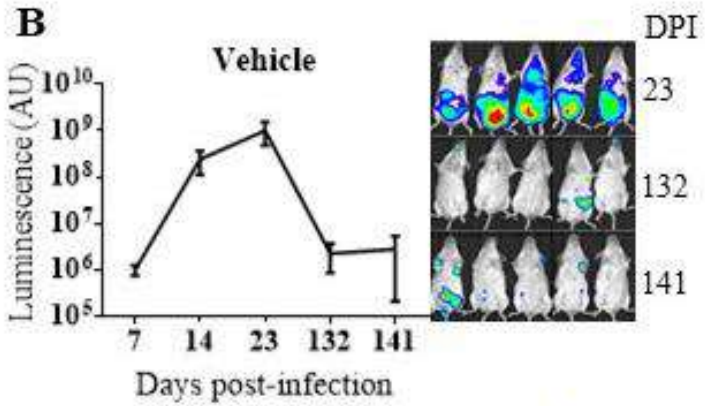
Chemotherapeutic Potential of 17-AAG against Cutaneous Leishmaniasis Caused by *Leishmania (Viannia) braziliensis*

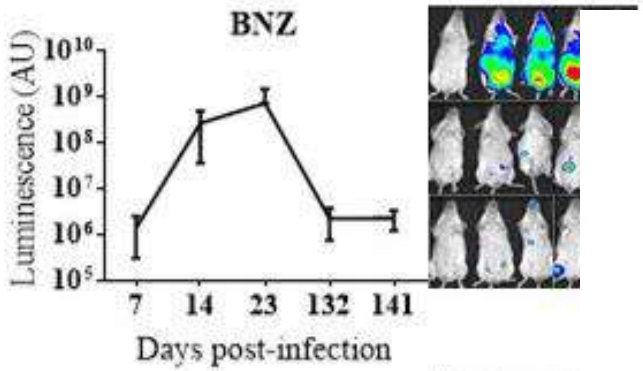
Diego M. Santos[1¤], Antonio L. O. A. Petersen[1], Fabiana S. Celes[1], Valeria M. Borges[1,2], Patricia S. T. Veras[1], Camila I. de Oliveira[1,2*]

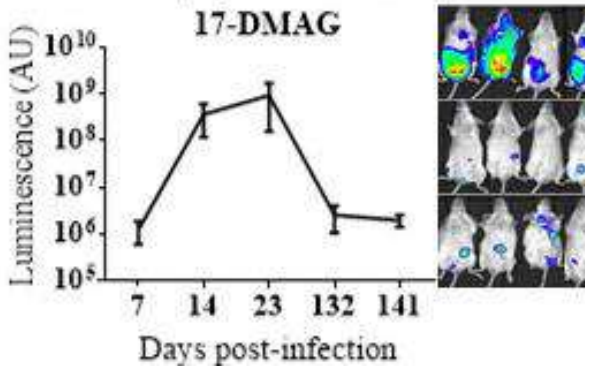
# Chronic *in vivo* model

BNZ and 17-DMAG:
30 mg/kg/day

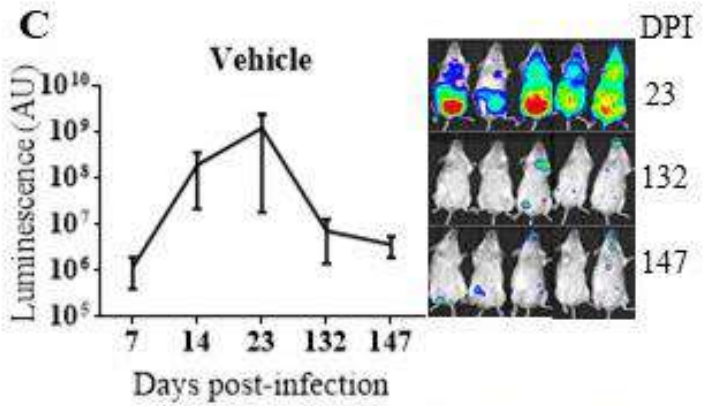
**Daily treated**

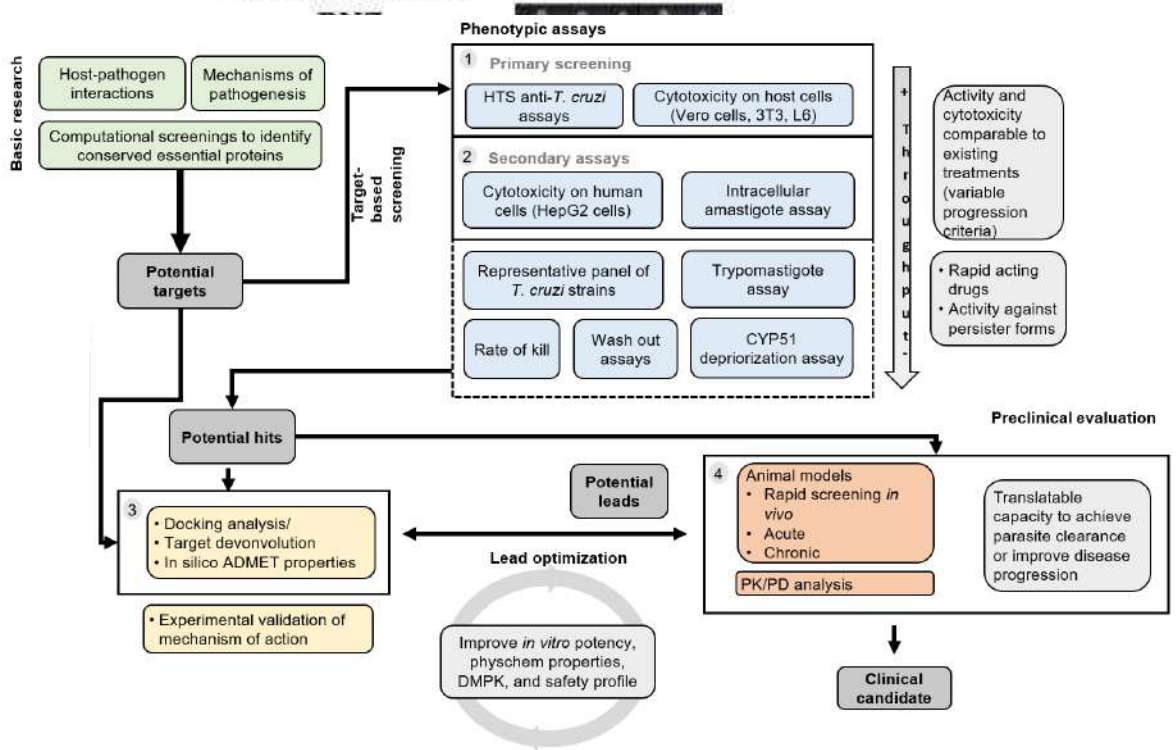


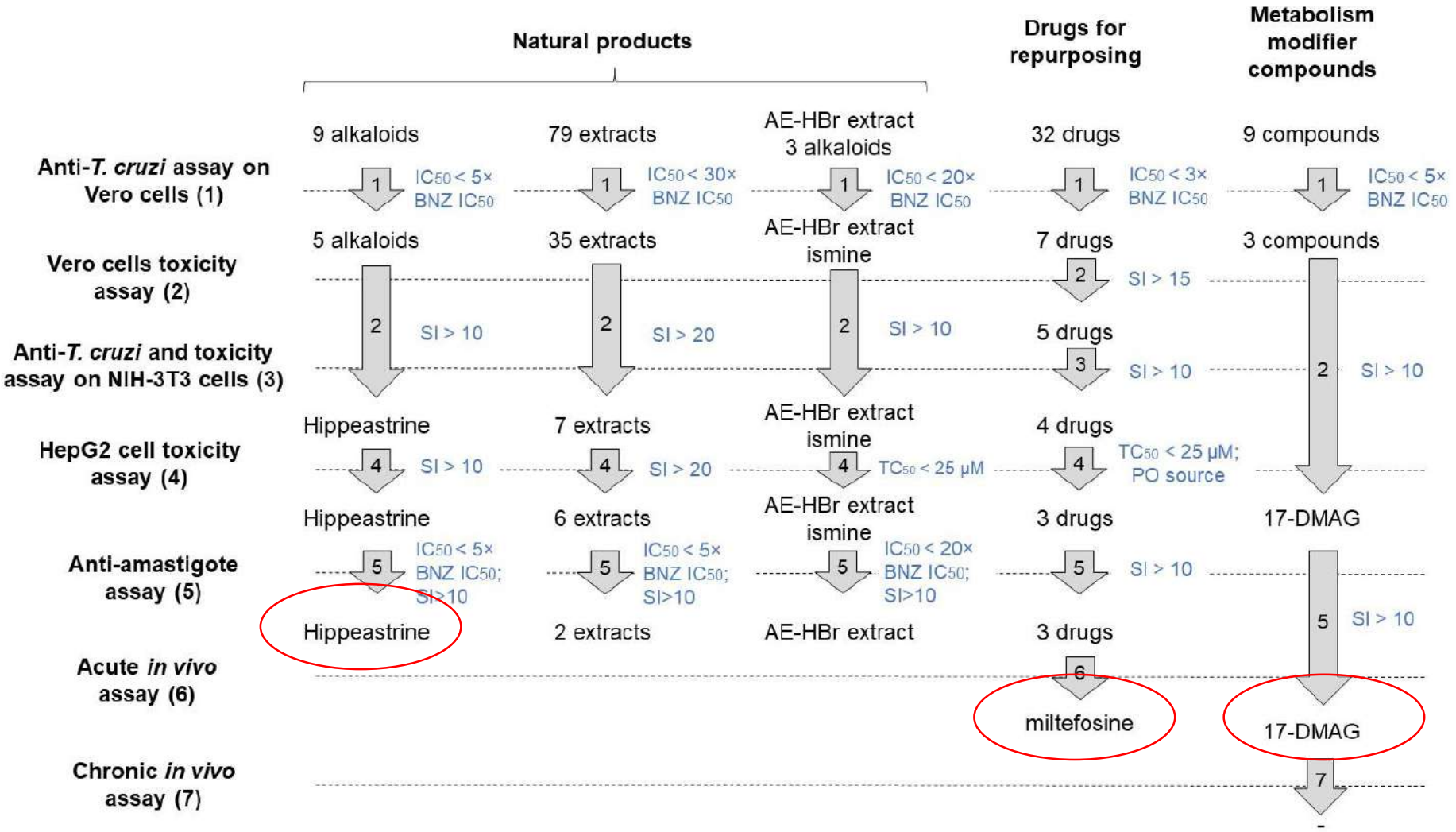




**Intermitently treated**



# 5. RESULTS AND DISCUSSION

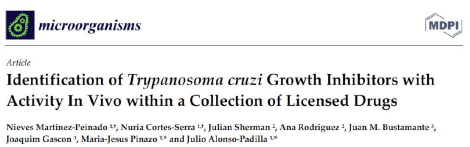




Parasites & Vectors

RESEARCH Open Access

Amaryllidaceae alkaloids with anti-*Trypanosoma cruzi* activity


*microorganisms*

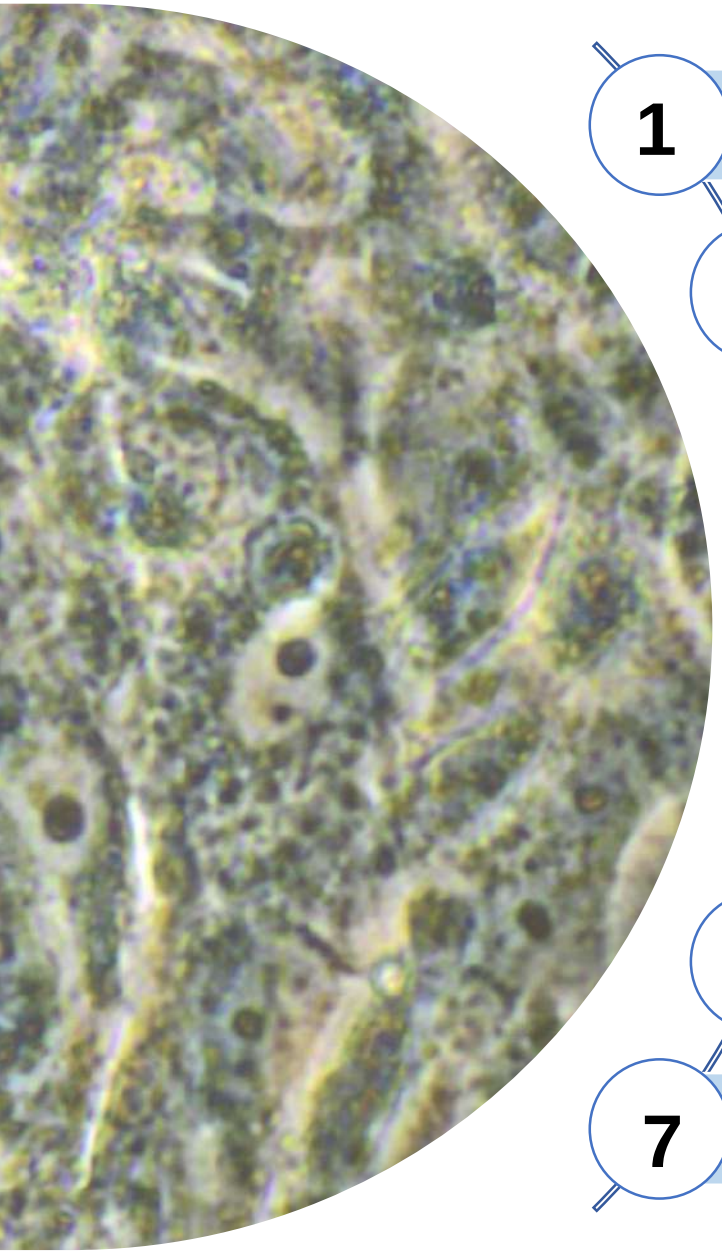
Article

Identification of *Trypanosoma cruzi* Growth Inhibitors with Activity In Vivo within a Collection of Licensed Drugs


International Journal of *Molecular Sciences*

Article

Anti-*Trypanosoma cruzi* Activity of Metabolism Modifier Compounds



1. Introduction
2. Hypothesis
3. Objectives
4. Methods
5. Results and discussion
6. Limitations
7. Conclusions

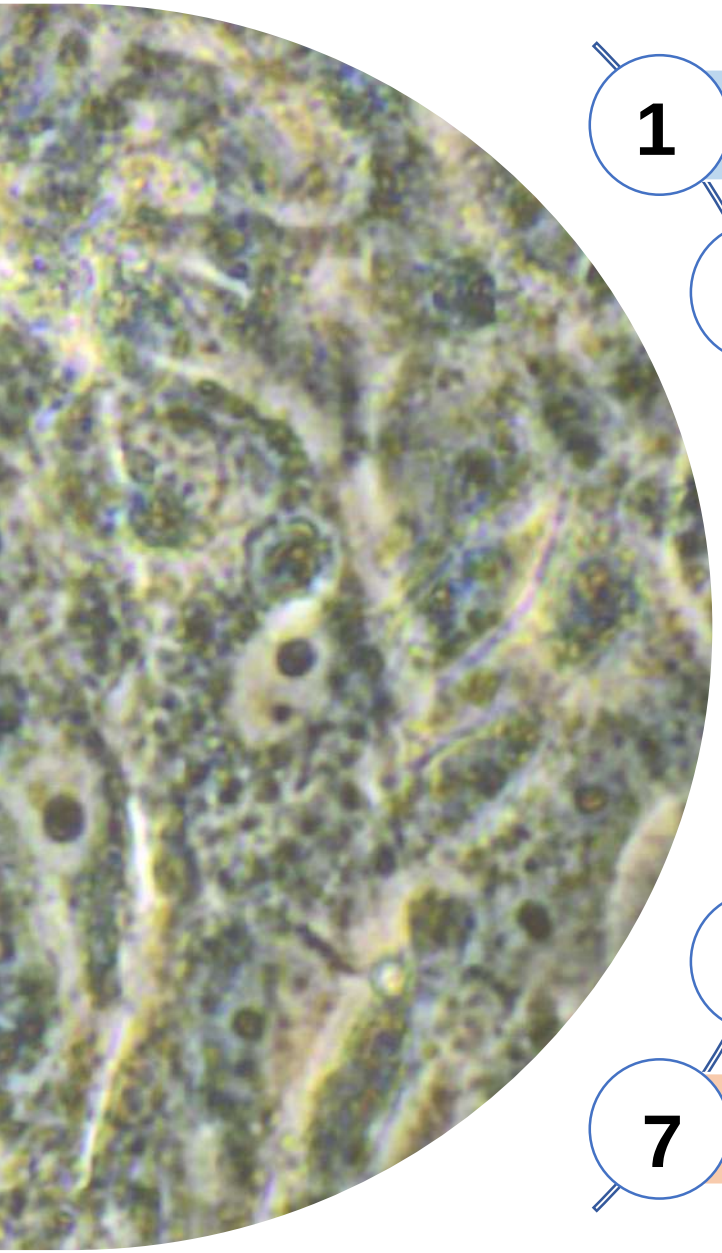
## 6. LIMITATIONS

- Chemical collections:
  a. Unknown alkaloid profile of some of the extracts.

- Our *in vitro* screening cascade would benefit from extra secondary assays:
  a. Evaluation against a panel of diverse *T. cruzi* strains and host cells.
  b. Wash-out.
  c. Rate of kill.
  d. CYP51 depriorization.

- *In vivo* assays*:*
  a. Lack of resources to continue studying crhonically infected mice for a longer period of time.

- *In silico* study:
  a. AlphaFold models vs crystalized proteins.
  b. Receptor rigid molecular docking.



1. Introduction
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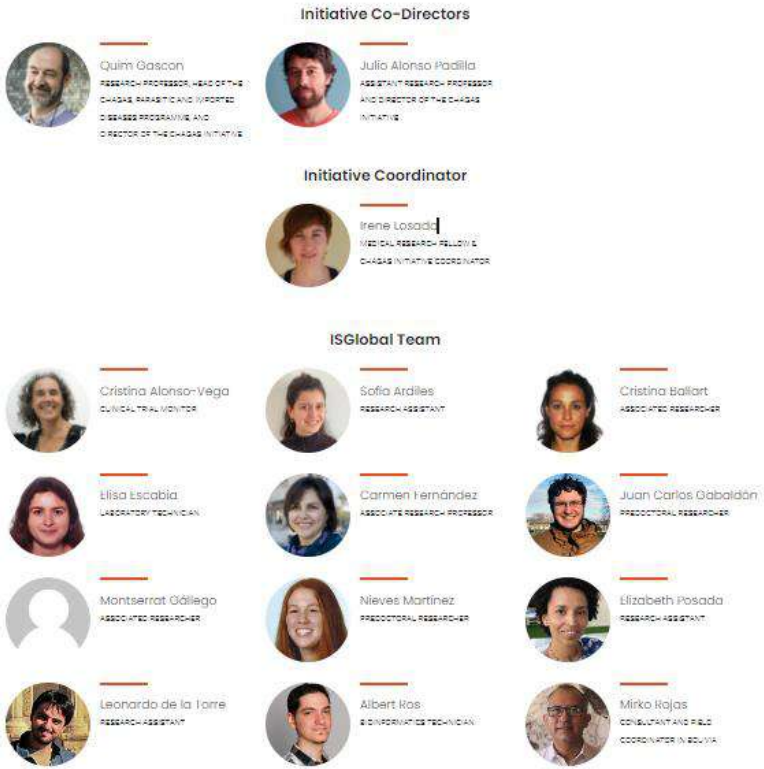
## 7. CONCLUSIONS

1. The screening cascade established as part of this thesis encompasses *in vitro*, *in silico* and *in vivo* assays that allow the identification of compounds/drugs with specific activity against *T. cruzi*.
2. Amaryllidaceae plants are a source of biological active alkaloids with anti-*T. cruzi* properties.
3. *C. erubescens*, *R. andicola* and *H. brachyandrus* extracts were active against *T. cruzi* and deserve further exploration to elucidate the alkaloid or alkaloids responsible of such anti-parasitic activity.
4. The alkaloids hippeastrine and ismine were found to be active against the parasite forms infecting mammalian cells and showed low toxicity to Vero and HepG2 cells. However, ismine lacks activity against the replicative amastigote forms.
5. Miltefosine performance *in vitro* and *in vivo* would encourage further investigating its use against *T. cruzi*.
6. The metabolism modifier compound 17-DMAG showed the highest *in vitro* potency against the parasite among all tested compounds, but failed to work in a mouse model of chronic *T. cruzi* infection.
7. Our *in silico* target identification pipeline has allowed us to identify potential molecular targets and hypothesize on the compounds´ MOA, although experimental validation would be needed.
8. In summary, we have found compounds with selective anti-*T. cruzi* activity. Although some of them deserve further attention, none has worked *in vivo* as good as the current anti-*T. cruzi* standard drug: benznidazole.

# Muchas gracias!

Chagas Initiative




Prof. Jaume Bastida

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Dr. Juan Bustamante

Dra. Alhelí Rodríguez-Cortes