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In vitro experimentation of newly synthesised compounds against *Trypanosoma cruzi*

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In vitro experimentation of newly synthesised compounds against *Trypanosoma cruzi*

Compounds First screening

In vitro activity against epimastigotes

- $IC_{50} < 20\ \mu M$
- $SI > 10$

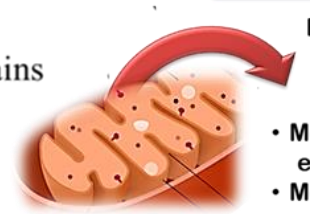
Hit compounds Second screening

In vitro activity against trypomastigotes

- Trypanocidal activity > BZN (preferably $IC_{50} < 10\ \mu M$)
- $SI > 10$ (preferably > 100)
- Max. Activity > 90%
- Potency against different *T. cruzi* strains (including BZN-resistant strains)
- Fast-acting and cidal MoA

Lead compounds

Mechanism of Action



Abstract:
Chagas disease is a chronic, systemic and parasitic disease caused by *Trypanosoma cruzi*. It is endemic in 21 American countries and, according to World Health Organisation, 8 million people are infected, causing 12,000 deaths and 56,000 new cases each year. Chagas disease is naturally transmited by haematophagous insects of the subfamily Triatominae, but there are other ways of transmission such as blood transfusions, organ transplantation, congenital transmission and food contamination.
There is currently no vaccine or effective treatment for the disease. The current treatment is based on chemotherapy with two obsolete nitroheterocyclic compounds: Benznidazole and Nifurtimox. These drugs are not effective in the chronic phase, where most cases are diagnosed, and they are highly toxic. In the present work, three families of newly synthesised compounds have been evaluated as potential anti-*T. cruzi* candidates with better properties than the current ones.
The biological experimentation is divided into two phases: 1) *in vitro* screening phase where the activity of the compounds against different morphological forms of the parasite is evaluated; and 2) mode of action determination phase where selected compounds are subjected to study in order to know their trypanocidal mechanism.
To conclude, the selected compounds are potential candidates for *in vivo* assays and could be the starting point for the development of new antichagas agents.

Keywords: Alternative treatment; Anti-chagas agents; Chagas disease; *Trypanosoma cruzi*

Introduction

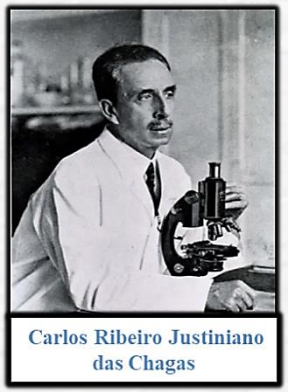
Chagas Disease & *Trypanosoma cruzi*

6-8 million infected people

70-100 million people at risk of infection

28 thousand new cases/year

14-50 thousand deaths/year



Carlos Ribeiro Justiniano das Chagas

World Health Organization

- Parasitic, systemic, chronic and life-threatening illness.

- Caused by tropical infection with the triatomine-transmitted protozoan parasite *Trypanosoma cruzi.*

- Primary transmission by triatomine defecation. Secondary transmission: blood transfusions, congenital route…

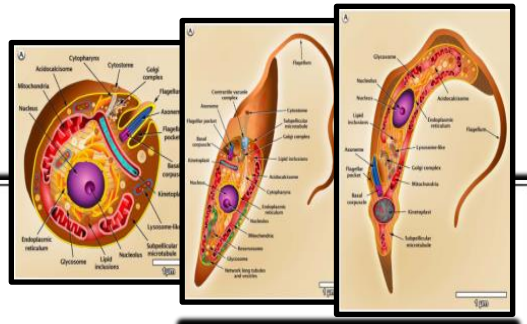
Introduction

WORLD HEALTH PROBLEM

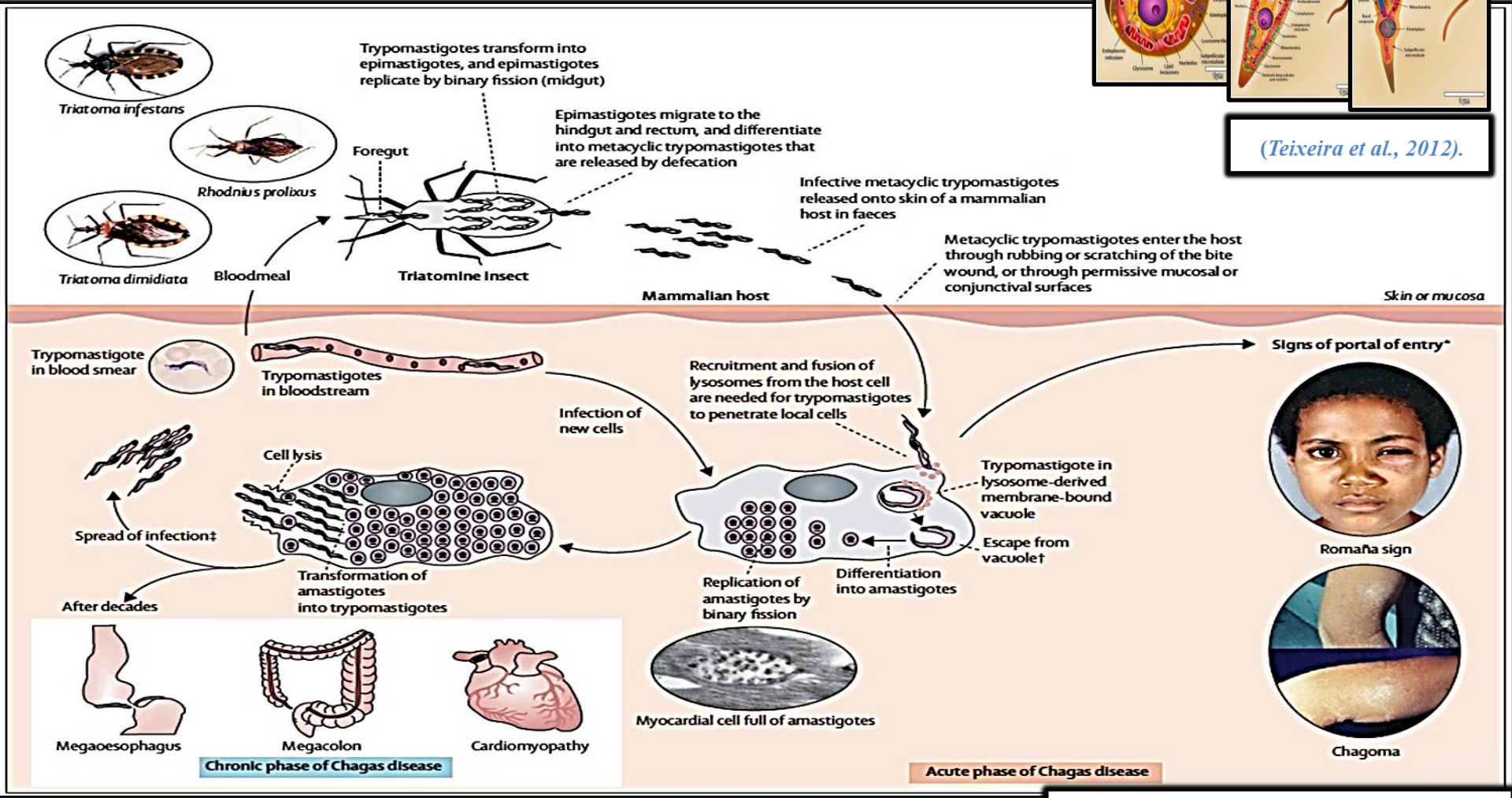
Lidani KCF et al., 2019

Introduction

Morphology and life cycle



(Teixeira et al., 2012).

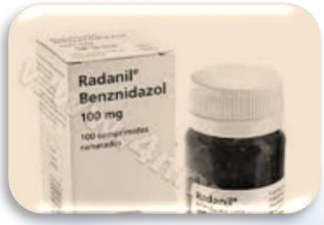


Lyfe cicle of T. cruzi (Rassi et al., 2010).

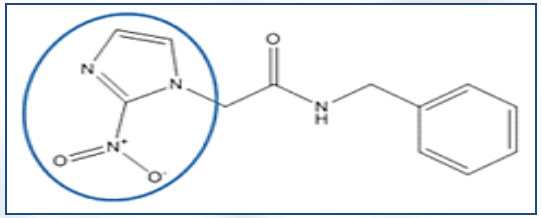
Introduction

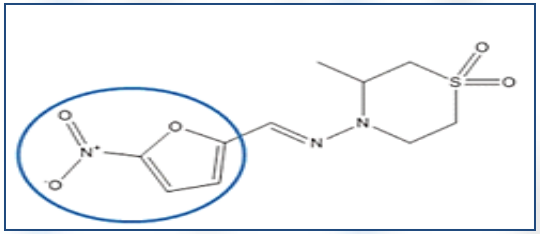
Treatment

No effective vaccine

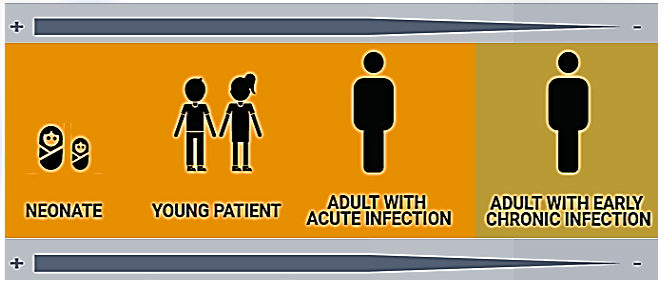


Benznidazol & Nifurtimox





- **Low efficacy in the chronic phase (< 20% cure rate).**
- **Limited efficacy in the acute phase (60-80% cure rate).**
- **Contraindicated during pregnancy and in patients with renal or hepatic insufficiency.**
- **Toxic side-effects.**
- **Long treatment periods.**
- **High cost.**



Treatment efficacy after infection *ISGlobal*

Introduction

Objectives

1. **Determination of the trypanocidal activity** of different chemical compounds belonging to chemical families whose previous members have shown good results against trypanosomatids.

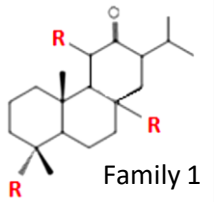
2. **Comparison of the results with the reference drug BZN**, in order to know the advantages and disadvantages of the compounds tested against it.

3. **Elucidation of the possible mechanism of action of potential compounds.**

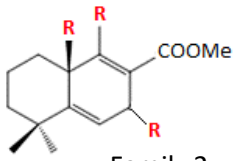
4. **Selection of the compounds with the best results** for further experimentation, mainly *in vivo* studies.

Results and discussion

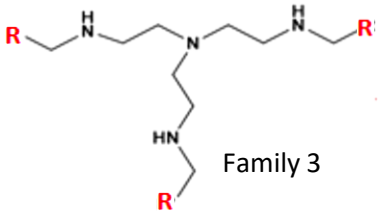
Origin	Compound's family
Department of Organic Chemistry Faculty of Science (University of Granada)	Family 1 (13 compounds) Family 2 (15 compounds)
Department of Inorganic Chemistry Institute of Molecular Science (University of Valencia)	Family 3 (10 compounds)



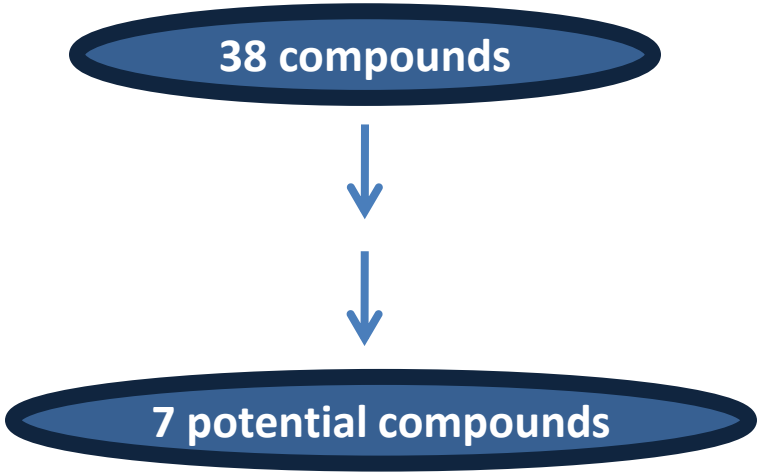
Family 1



Family 2



Family 3



Results and discussion

Activity and toxicity

Potential compounds

IC50 ≤ 10 µM SI > 10

Activity of benznidazole and compounds against forms of *Trypanosoma cruzi*, and toxicity on mammalian Vero cells.

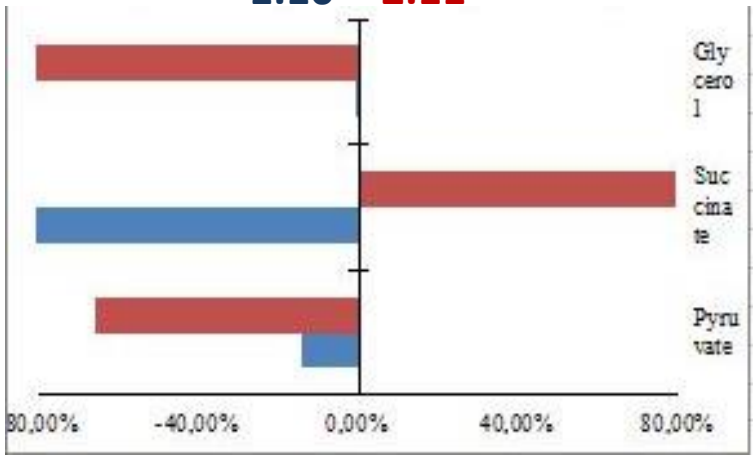
Compound	Activity IC50 (µM)		Toxicity IC50 Vero Cells (µM)	Selectivity index (SI)	
	Epimastigote	Trypomastigote		Epimastigote	Trypomastigote
1.10	1.2	0.3	13.4	11.6	44.6
2.11	1.4	0.8	52.7	37.9	65.9
2.13	4.1	7.8	77.8	18.9	10
3.2	3.9	6.0	846.8	217.1	141.7
3.3	6.3	5.2	1121.9	178.1	216.5
3.6	9.9	8.0	373.4	37.7	46.6
3.10	6.3	2.7	858.2	136.2	317.9
BNZ	15.8	6.2	13.6	0.9	2.2

The compounds with the best activity profile are listed in this table.
The value is the mean of three separate determinations. BZN, benznidazole (reference drug)

Results and discussion

Alterations in metabolic excretion

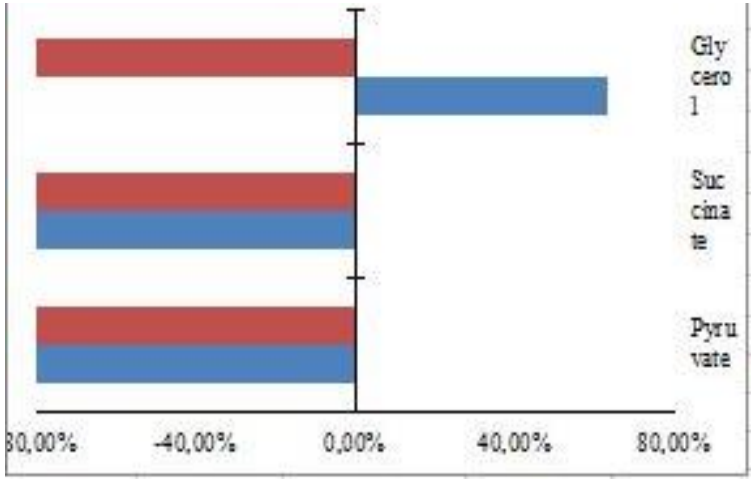
1.10 – 2.11



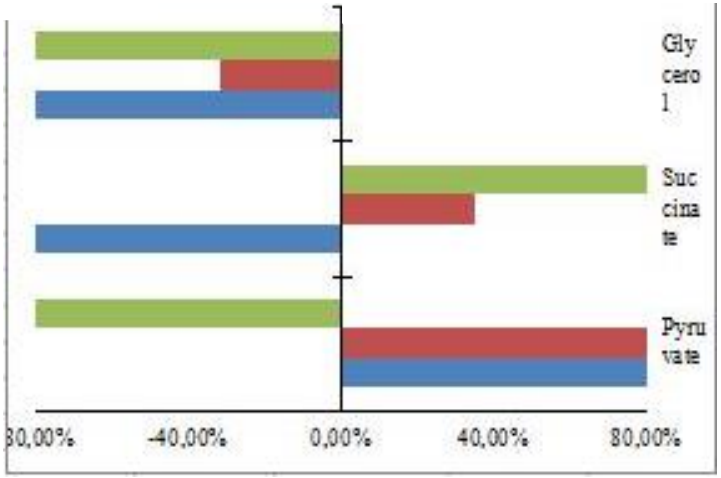
Variation of catabolites excreted by epimastigotes of *Trypanosoma cruzi* exposed to 21 at IC_{25} concentrations in comparison to control parasites. The value is the mean of three separate determinations ± standard deviation. * Significant differences between control and treated parasites for α = 0.05.

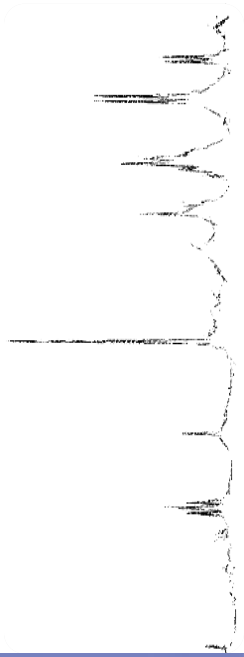
- **<10 % → Not significant differences**
- **> 10, <25 % → Slight differences**
- **>25 % → Significant differences**

2.13 – 3.2



3.3 – 3.6 – 3.10





Results and discussion

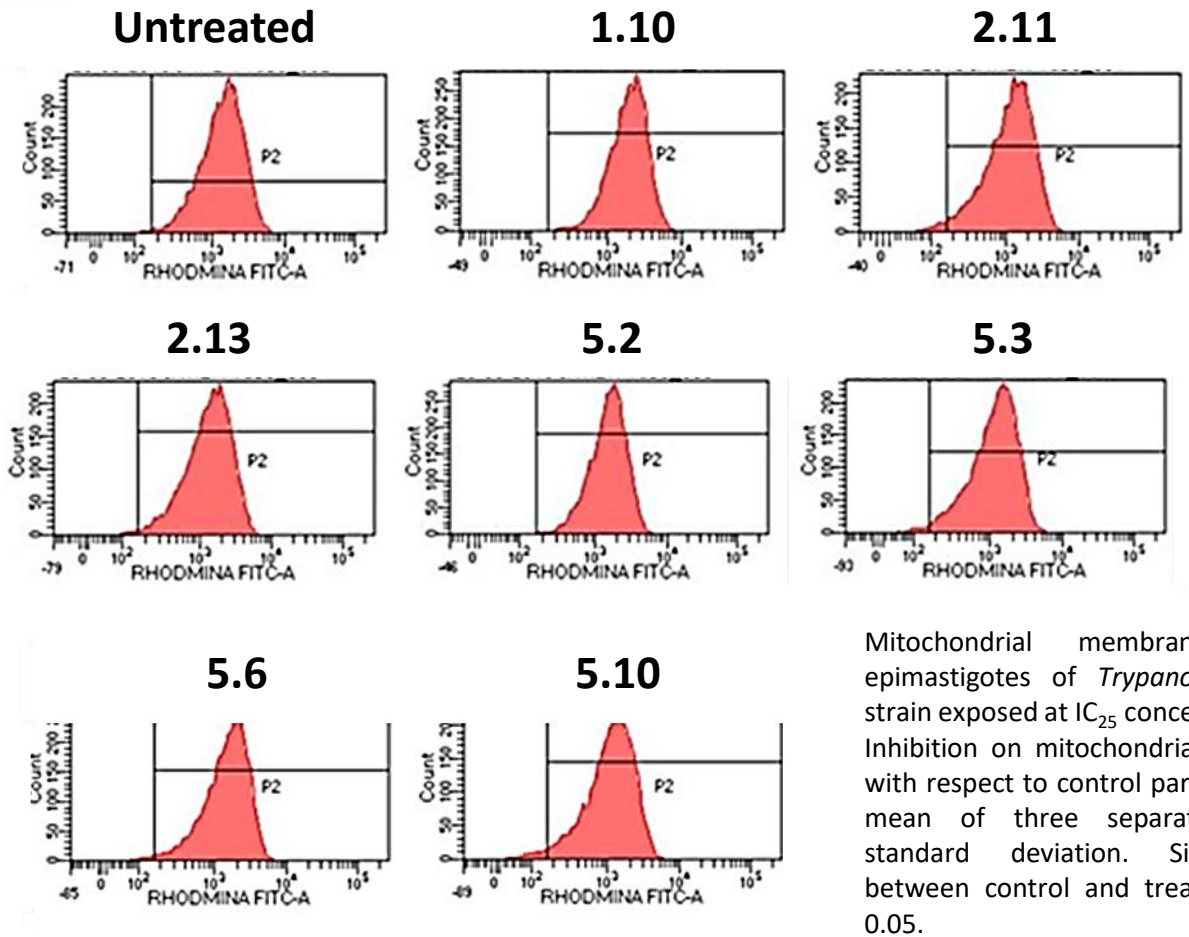
Alterations in metabolic excretion

	Glycerol	Succinate	Pyruvate
1.10	-1,0%	-167,0%	-14,0%
2.11	-164,3%	+148,9%	-66,0%
2.13	+62,8%	-252,0%	-88,2%
3.2	-271,9%	-154,0%	-213,9%
3.3	-260,7%	-218,2%	+206,4%
3.6	-31,7%	+34,6%	+766.5%
3.10	-133,3%	+796,5%	-163,6%

- <10 % → Not significant differences
- > 10, <25 % → Slight differences
- >25 % → Significant differences

Results and discussion

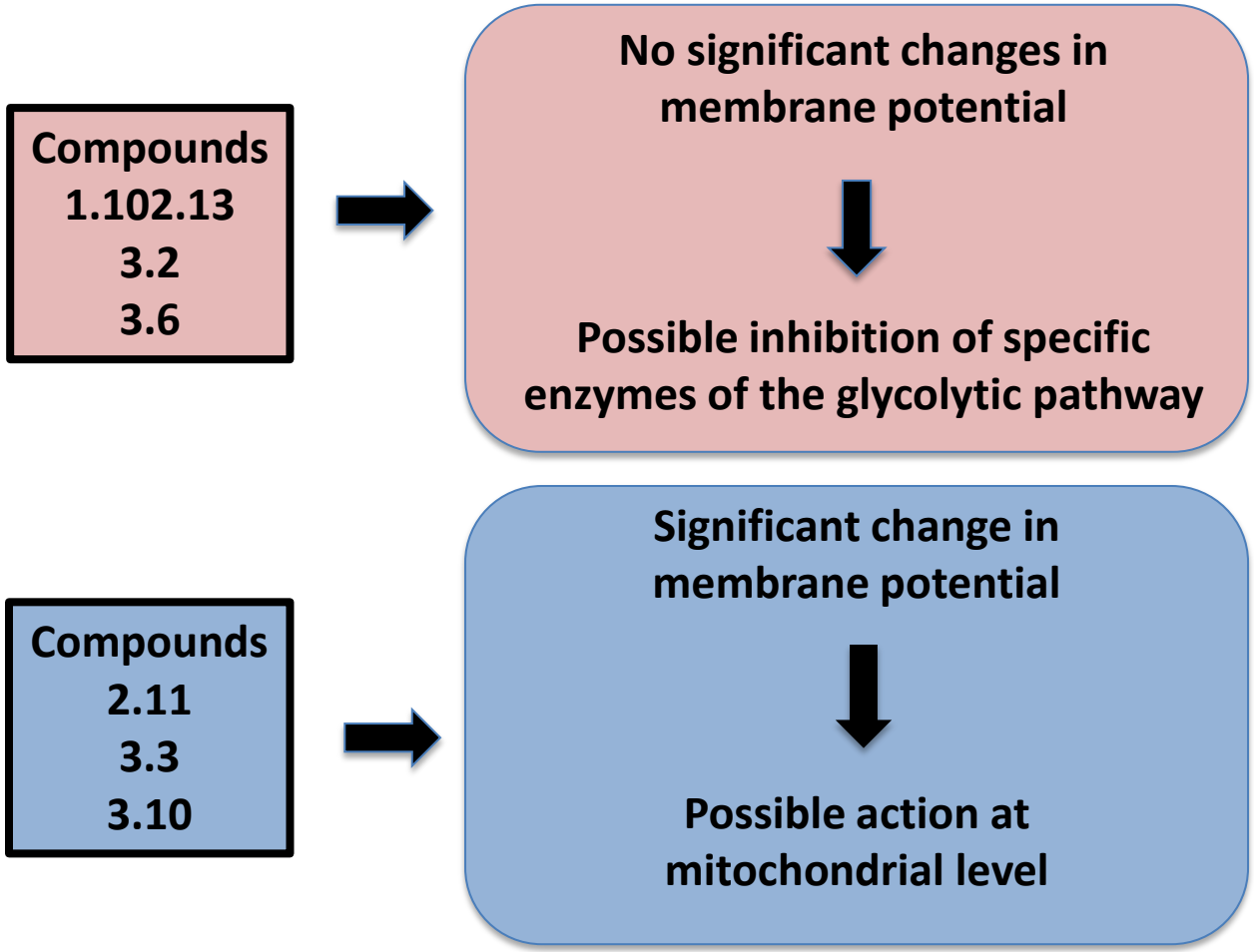
Effect on mitochondrial membrane potential and nucleic acid levels



Mitochondrial membrane potential from epimastigotes of *Trypanosoma cruzi* Arequipa strain exposed at IC_{25} concentrations:
Inhibition on mitochondrial membrane potential with respect to control parasites. The value is the mean of three separate determinations ± standard deviation. Significant differences between control and treated parasites for α = 0.05.

Results and discussion

Effect on mitochondrial membrane potential and nucleic acid levels



Conclusions

1. From the 46 tested compounds, **7 of them** (**1.10, 2.11, 2.13, 3.2, 3.3, 3.6, and 3.10**) are considered **potential antichagasic agents** due to their low toxicity and high antichagasic activity.

2. The selected compounds show SI in trypomastigote forms at least 5 times higher than the SI of the reference drug BNZ, even improving it 145 times for compound 3.10.

3. The selected compounds present a mechanism of action on **energy metabolism**, with significant changes in the excretion of all the metabolites studied.

4. In addition, compounds 2.11, 3.3 and 3.10 caused a **significant inhibition of the mitochondrial membrane potential**, which could be the final cause of their trypanocidal activity, causing a rapid and severe **energy collapse**.

5. The next step of this work will be the evaluation of the *in vitro* trypanocidal activity against amastigote forms and subsequent *in vivo* study in order to overcome a new barrier towards the goal of an **effective alternative treatment for clinical use**.

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