3rd International Electronic Conference on Medicinal Chemistry

1-30 November 2017

chaired by Dr. Jean Jacques Vanden Eynde

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Arylidene ketones with Potent Trypanosomicidal Activity that Causes Late Apoptosis/Necrosis Like Nifurtimox

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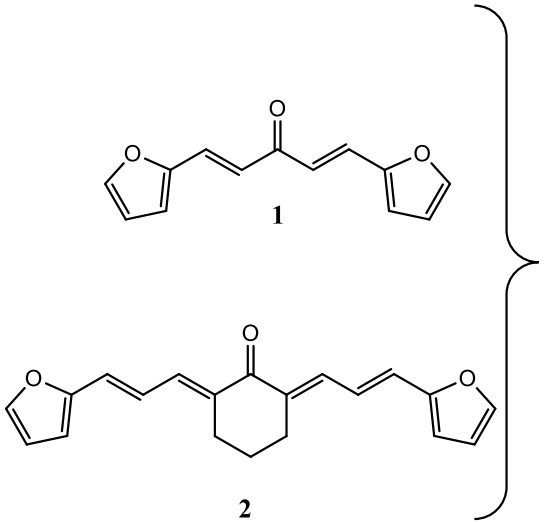
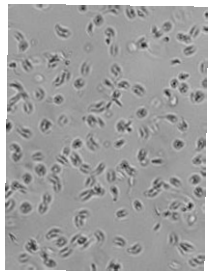
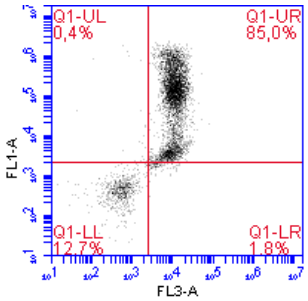
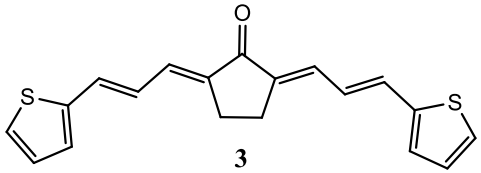
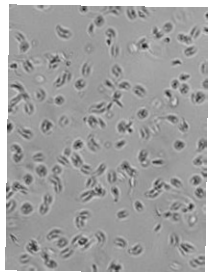


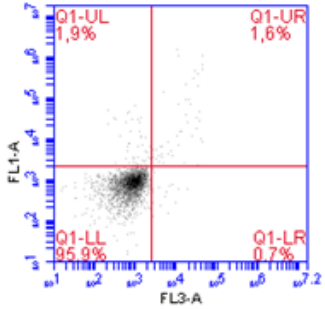


Late Apoptosis/Necrosis









Abstract

Chagas disease is caused by the parasite *Trypanosoma cruzi (T. cruzi) and* it remains the major parasitic disease in Latin America. The chemotherapy employed to control the parasitic infection is based on two drugs: Nifurtimox (Nfx) and Benznidazole (Bnz), requiring long-term treatment that can give rise to severe side effects. They are not active against all *T. cruzi* strains, exhibit low efficiency in long-term chronic infections, and are mutagenic. The search of new drugs is an urgent need.

In this work, we used three symmetrical diarylidene ketones containing thiophene and furane, **1**, **2** and **3**. These molecules showed good to excellent trypanosomicidal activity and selectivity to the parasite, affected cruzipain, a proteolytic enzyme of the parasite, and the glycolytic enzyme, triosephosphate isomerase of *T. cruzi* (TcTIM) without affecting human´s TIM and showing effectiveness in protecting infected mice and without toxic effects *in vivo*. Arylidene ketones **1** and **2** causes after 24 h late apoptosis/necrosis at a concentration of 20 times the value of its IC_{50} (approximately 80% of late apoptosis/necrosis) as Nfx. It should be studied what happens with compound **3** since no death is observed by apoptosis or necrosis at a dose of 20 times the value of their IC_{50} as **Bnz**.

Keywords: *Trypanosoma cruzi*, arylidene ketones, Apoptosis/Necrosis

Chagas disease

- Chagas disease remains the major parasite disease in Latin America
- Migration of infected people has spread the disease to non-endemic areas, presenting a new worldwide challenge.
- Approximately 6-8 million of people is infected and more than 70 million is at risk of getting the disease.

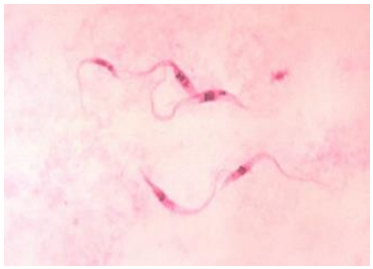
http://www.dndi.org/diseases/chagas.html

Salvatella R., Gonzalez, J. Reservorios animales de *T. cruzi* en Uruguay. *Rev. Med. Uruguay* **1986**, *2*:101.

Neghme A. Hipotesis acerca de la evolucion de la tripanosomiasis americana. *Parasitología al Día* **1982**, *6*:23.

Usinger R., Wygodzinsky P., Ryckrnan R. The biosystematics of Triatominie. *Ann. Rev. Entomol.* **1966**, *2*:309 .

Agent that causes the illness



T. cruzi hematosidual monoflagelate protozoan

It is transmitted principally through insect dejections of the order *Triatomea, Triatomea infestans* known as "vinchuca“ in Argentina, Chile, Paraguay and Uruguay.

Other vias of transmissions are: transfusions of contaminated blood, infected organ transplant, mother– child transmission, ingestion of contaminated food

Salvatella R., Gonzalez, J. Reservorios animales de *T. cruzi* en Uruguay. *Rev. Med. Uruguay* **1986**, *2*:101 .

Neghme A. Hipotesis acerca de la evolucion de la tripanosomiasis americana. *Parasitología al Día* **1982**, *6*:23.

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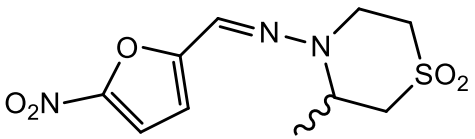
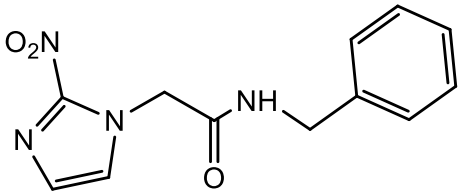
Current pharmacotherapy



Nifurtimox



Benznidazole

Both drugs are:

- Toxic
- They are not good in eliminating the amastigote form of the parasite
- Mutagenic

Cerecetto, H., González, M. Chemotherapy of Chagas disease: status and new developments. *Curr. Trop. Med. Chem.* **2002**, *2:* 1187.

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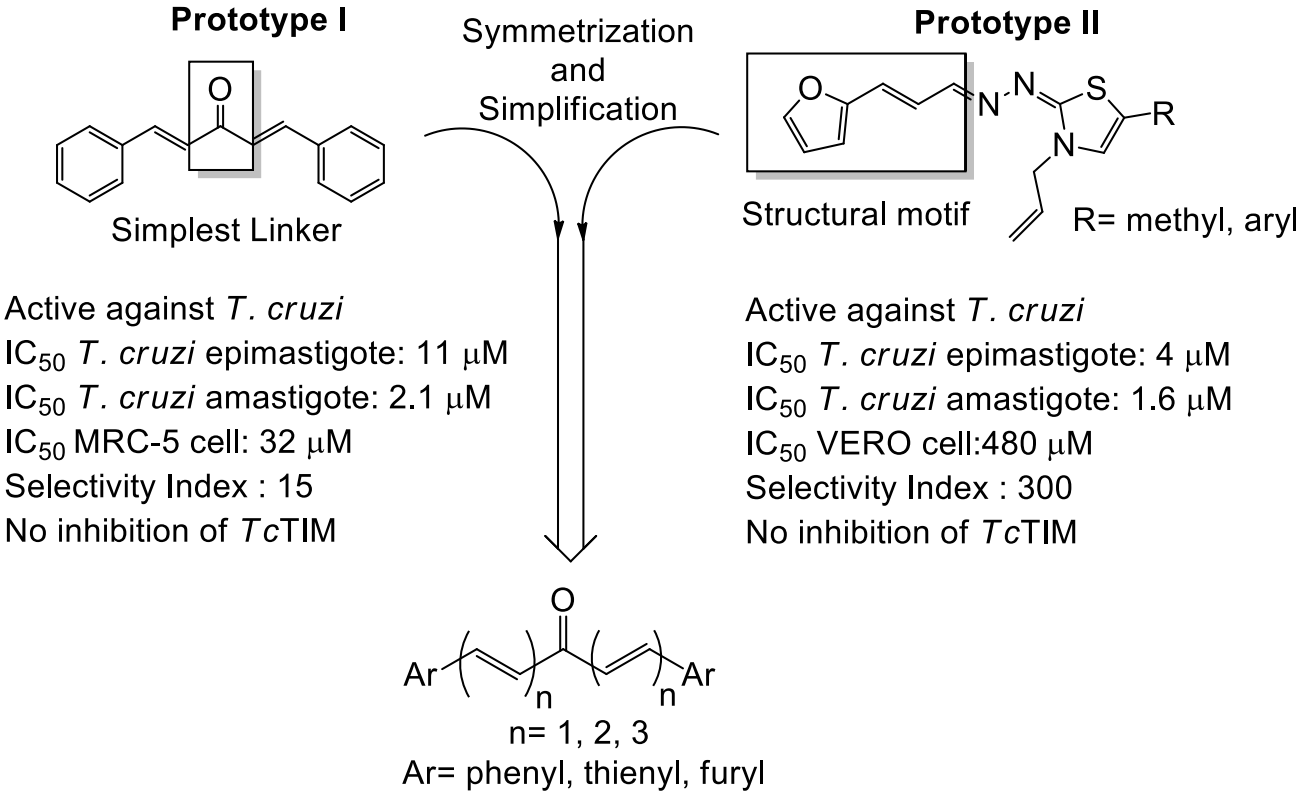
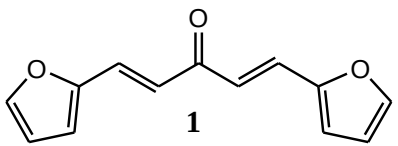
Background of the present work

Trypanosomicidal structures used for the design of new and simpler symmetric diarylidene ketone from dibenzalketone (**Prototype I**) and furylthiazolidines (**Prototype II**).



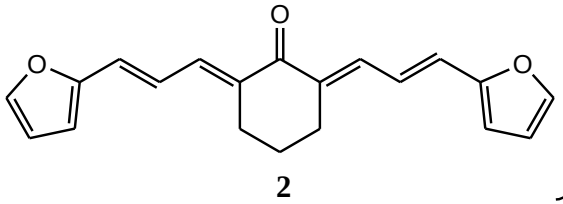
Aguilera, E., Varela J., Birriel, E., Serna E., Yaluff G., Vera de Bilbao ,N., Aguirre-López, B., Cabrera, N., Díaz Mazariegos, S., Truena de Gómez-Puyou, M. Gómez-Puyou, A., Perez-Montfort, R., Minini, L., Merlino, A., Cerecetto, H., González, M., Alvarez, G. *ChemMedChem*, **2016**, *11*, 1328-1338.

Arylidene ketones more active and selective



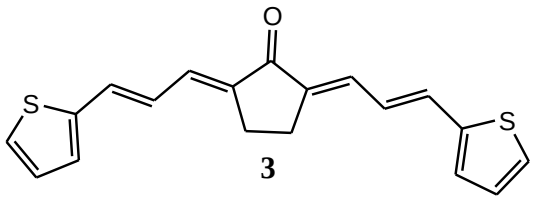
1

IC_{50} *T. cruzi* epimastigote: (5±0,7)μM
IC_{50} J774.1: (60±3)μM
Selectivity Index: 12
IC_{50}TcTIM: (3,0±0,7)μM
hTIM: No active
IC_{50}Cruzipain:100μM



2

IC_{50} *T. cruzi* epimastigote: (0,6±0,2)μM
IC_{50} J774.1: (10±2)μM
Selectivity Index: 17
IC_{50}TcTIM: (0,086±0,007)μM
hTIM: No active
IC_{50}Cruzipain (37,0±0,1)μM



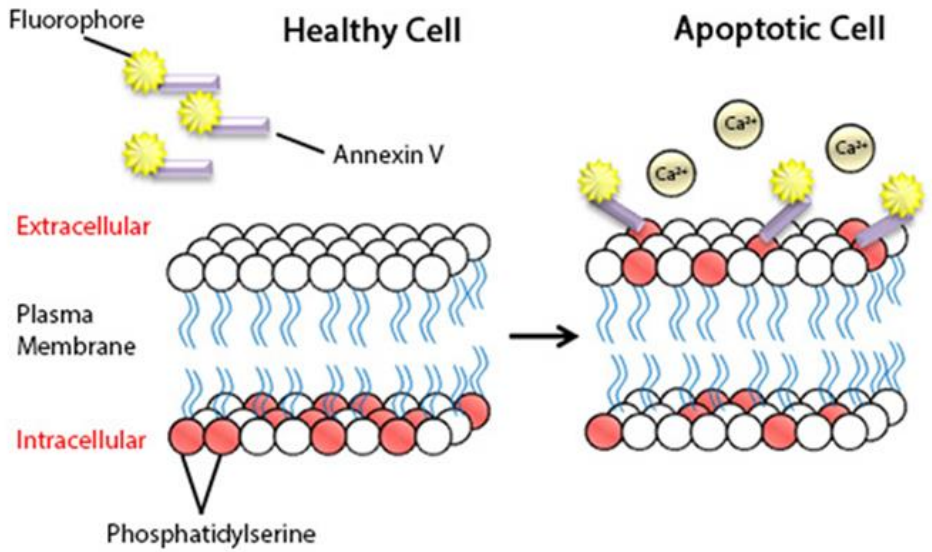
3

IC_{50} *T. cruzi* epimastigote: (0,04±0,01)μM
IC_{50} J774.1: (15±4)μM
Selectivity Index: 375
IC_{50}TcTIM: (4,7±1,1)μM
hTIM: No active
IC_{50}Cruzipain (42±2)μM

Aguilera, E., Varela J., Birriel, E., Serna E., Yaluff G., Vera de Bilbao ,N., Aguirre-López, B., Cabrera, N., Díaz Mazariegos, S., Truena de Gómez-Puyou, M. Gómez-Puyou, A., Perez-Montfort, R., Minini, L., Merlino, A., Cerecetto, H., González, M., Alvarez, G. *ChemMedChem*, **2016**, *11*, 1328-1338.

Apoptosis/ Necrosis

Annexin V acts by binding to phosphatidylserine exposed in apoptotic cells. Propidium iodide is used to identify necrotic cells as it binds to the DNA or RNA of cells as the integrity of the membrane disappears. For Chagas disease or any disease, it is preferable that the parasite dies by apoptosis than by necrosis because it is less invasive and cause less inflammation events.



Vermes, I., Haanen, C., Steffens-Nakken, H., Reutelingsperger, C. *J Immunol Methods*. **1995**, *184*, 39-51.
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Cornelissen, M., Philippe, J., De Sitter, S., De Ridder, L. *Apoptosis*. **2002**, *7*, 41-47.
Fried, J., Perez, A. G., Clarkson, B. D. *J Cell Biol*. **1976**, *71*, 172-181.

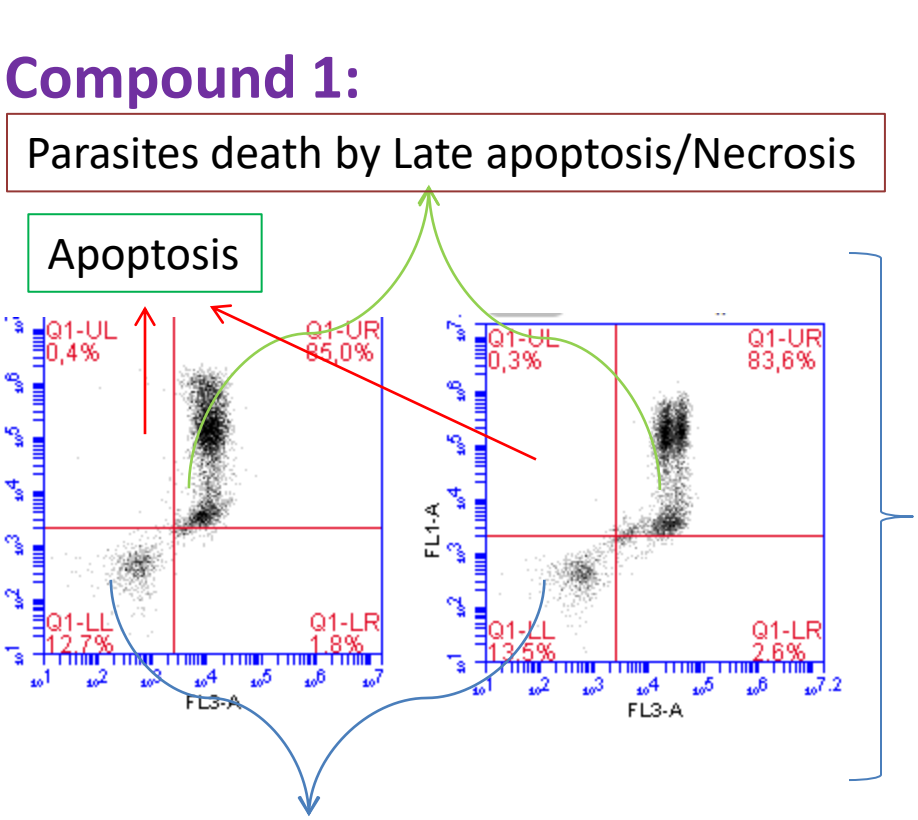
Materials and methods

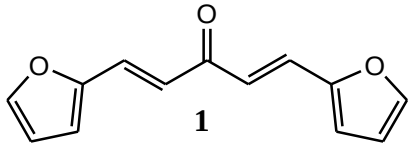
- $1x10^6$ of *T. cruzi* strain Tulahuen 2 were incubated with the compounds to be evaluated on a 12-well plate.
- The compounds were incubated at 28 ° C for 24 h. Compound **1** at a concentration of 100 µM; **2** at a concentration of 12µ; **3** at a concentration of 0,8 µM (20X IC_{50}). **Nfx** and **Bnz** were as control reference drugs, at concentrations 160µM and 140µM respectively (20X IC_{50}). Untreated parasites were incubated with the solvent (in this case dimethylsulfoxide) in a concentration that never exceeding 1%.
- Finally the Cell death mechanism was analyzed using the Dead Cell Apoptosis Kit (Thermo Fisher Scientific) following the manufacturer's instructions. Briefly, untreated and drug treated parasites were harvested by centrifugation, washed with 1X PBS and incubated for 15 min at room temperature (RT) with 5 mg/mL Alexa Fluor® 488 annexin V (AV) and 10 mg/mL propidium iodide (PI) diluted in annexin-V binding buffer containing Ca2+. Cells were immediately analyzed on a flow cytometer. Dual-parameter flow cytometric analysis was performed on an Accuri C6 (BD Bioscience) flow cytometer, using a 533/30 nm signal detector (FL1-H) for AV and a 670 nm long pass PI emission signal detector (FL3-H). Fluorescence intensity was acquired for 10,000 events and the data were analyzed using BD CSampler software (BD Bioscience).

Results and discussion

All graphs shown correspond to the evaluation of compounds at 20X IC_{50}

Compound 1:





Compound **1** produced more than 80% of Late Apoptosis/Necrosis (all the results were done by duplicate) at 24 h post incubation.

Interestingly, we did not observed at 24 h post incubation with 10 or 20 X IC_{50} this effect (these high doses were used based in their IC_{50} were obtained at 5 days according to the assay used in our research group).

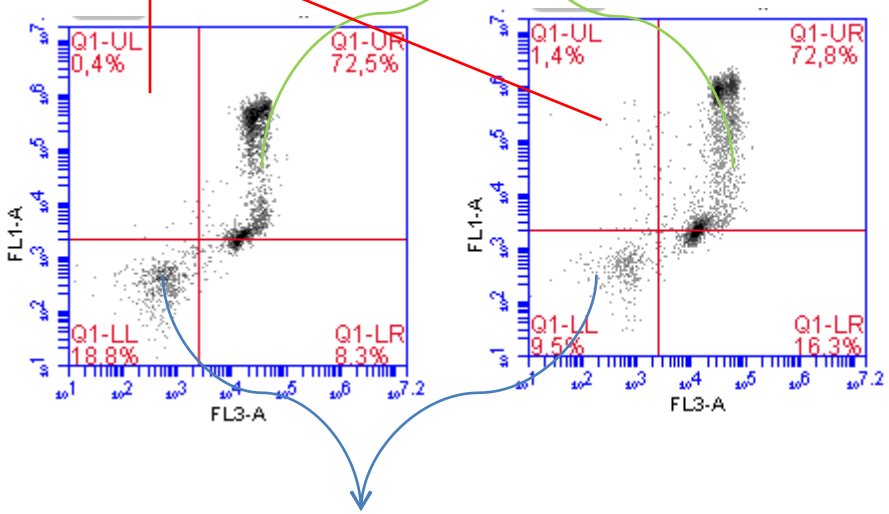
Apoptosis was not significantly observed

Aguilera, E., Varela J., Birriel, E., Serna E., Yaluff G., Vera de Bilbao ,N., Aguirre-López, B., Cabrera, N., Díaz Mazariegos, S., Truena de Gómez-Puyou, M. Gómez-Puyou, A., Perez-Montfort, R., Minini, L., Merlino, A., Cerecetto, H., González, M., Alvarez, G. *ChemMedChem*, **2016**, *11*, 1328-1338.

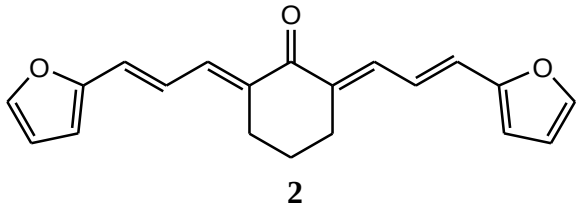
Compound 2:

Parasites death by Late apoptosis/Necrosis

Apoptosis



Parasites that did not die by apoptosis or necrosis

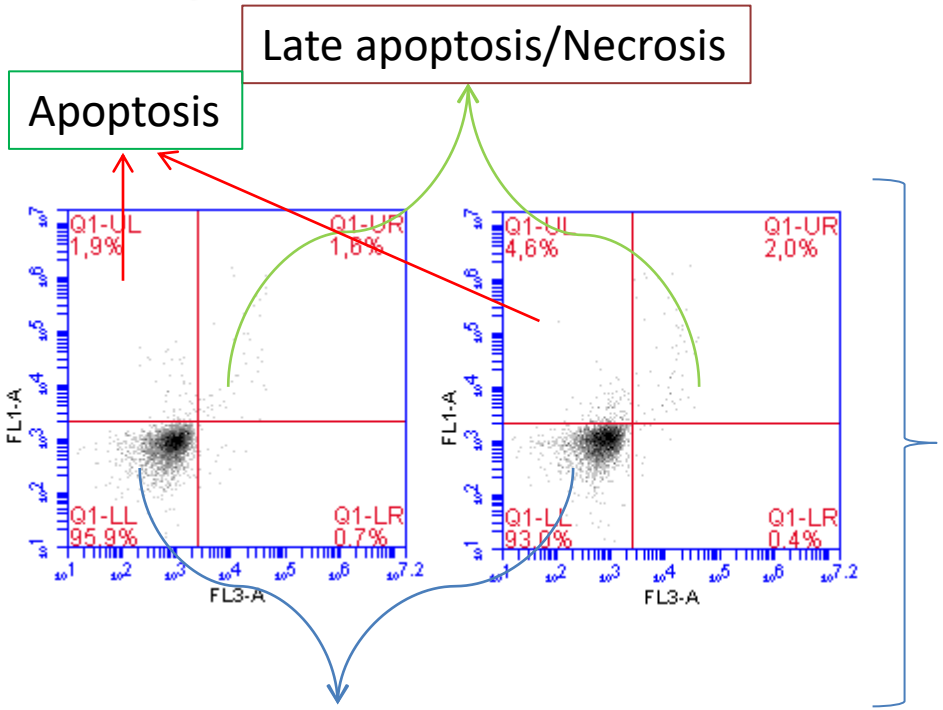


2

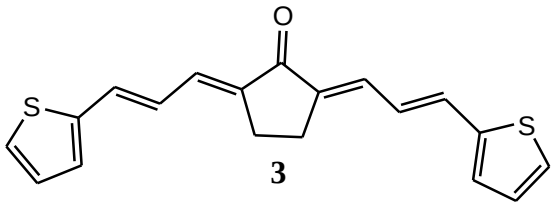
Compound **2** produced more than 70 % of Late Apoptosis/Necrosis at 24 h post incubation. It was evaluated at 20X IC_{50}.

Apoptosis was not significantly observed

Compound 3:



Parasites that did not die by apoptosis or necrosis



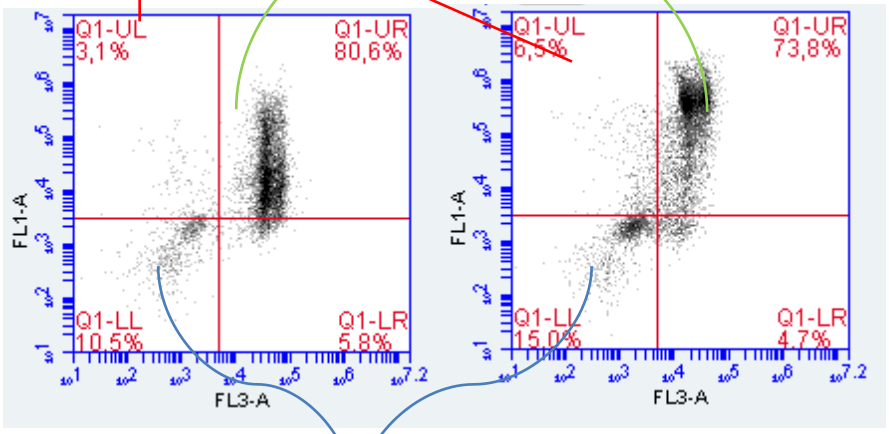
Compound **3** did at 20X IC_{50} not produced Apoptosis nor Necrosis at 24 h post incubation.

The cells were alive or suffer different types of cellular death like autophagia in this condition.

Nifurtimox (Nfx):

Parasites death by Late apoptosis/Necrosis

Apoptosis

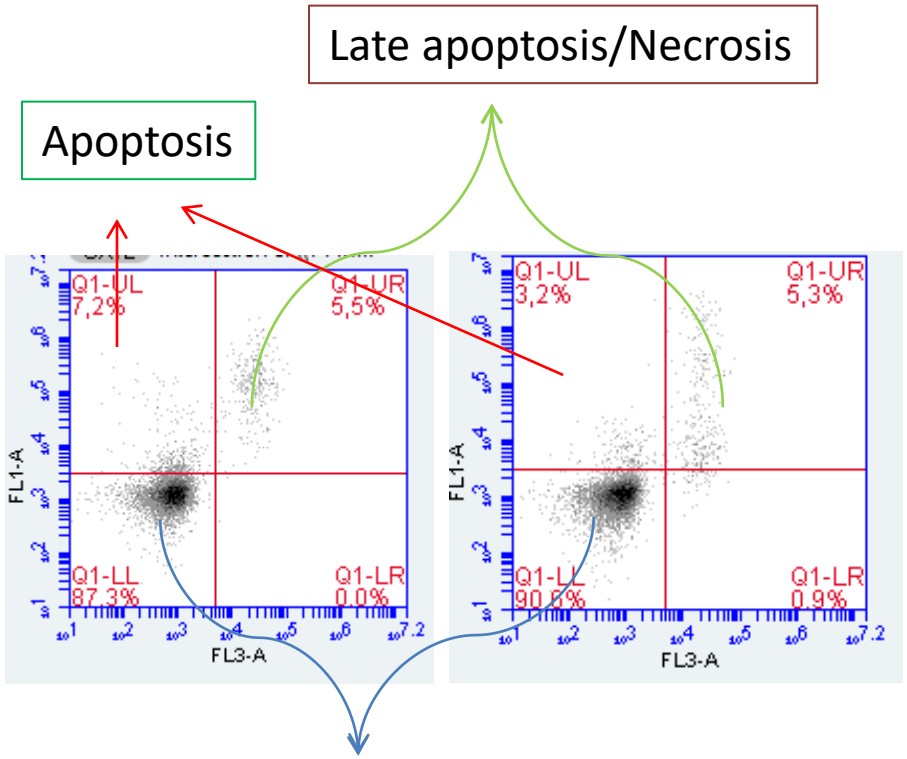


Parasites that did not die by apoptosis or necrosis

Nfx produced more than 75 % of Late Apoptosis/Necrosis at 24 h post incubation evaluated at a concentration of 20X IC_{50}. The same effect on Necrosis of **NFx** was observed previously using TUNNEL microscopy and ^{1}H RMN

Benitez, D., Pezaroglo, H., Martínez, V., Casanova, G., Cabrera, G., Galanti, N., González, M., Cerecetto, H. Para*sitology*. **2012**, 139(4),506-515

Benznidazole (Bnz):

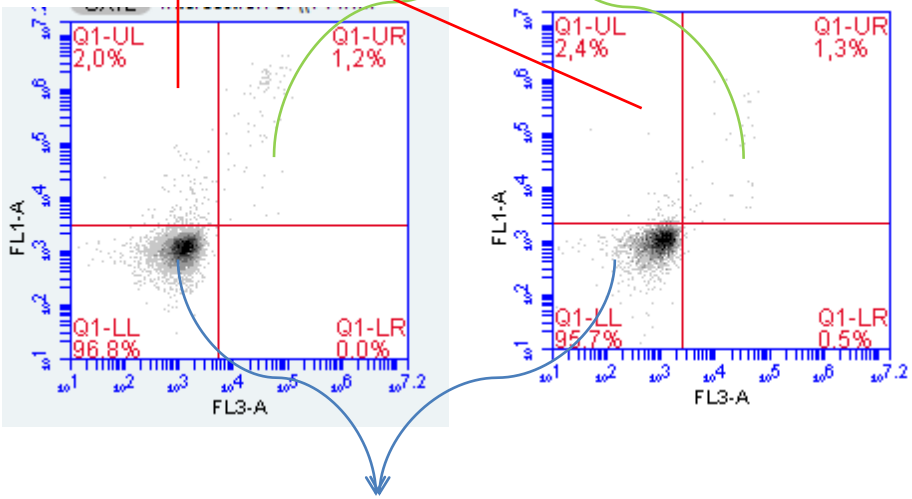


Bnz did not produced Apoptosis nor Necrosis at 24 h post incubation evaluated at a concentration of 20X IC_{50}. The cells were alive or suffer different type of cellular death like autophagia in this condition

Control (untreated parasites):

Late apoptosis/Necrosis

Apoptosis



As we expected in untreated Parasites nor Apoptosis or Necrosis was observed.

Parasites that did not die by apoptosis or necrosis

Conclusions

- Comparing compounds **1** and **2** (the two aryldene ketones containing furyl) with **Nfx**, we observed that they presented a similar behavior. Both causes Late Apoptosis/Necrosis in the parasite.
- Moreover compound **1** at 20X IC_{50} causes more death at the parasite than the reference drug (**Nfx**).
- We must study what happened with compound **3** (the arylidene ketone containing a thiophenyl) and **Bnz.**
- Probably a process of autophagy it happening on compound **3** and **Bnz.**
- We are performing different studies to know which process could be ongoing with compound **3** and **Bnz**.

Acknowledgments

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PEDECIBA

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