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GROWTH INHIBITORY EFFECT OF JUVENILE HORMONE ANALOGUES ON EPIMASTIGOTES OF *Trypanosoma cruzi*

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Abstract: Several compounds, structurally related to the insect growth regulator Fenoxycarb, exhibited interesting inhibition action to control proliferation of *Trypanosoma cruzi*, the parasite responsible for Chagas' disease. Some of these drugs were shown to be potent growth inhibitors of this parasite. All of these drugs had previously presented juvenoid activity on several non-related bug species such as *Tenebrio molitor*, *Galleria mellonella*, *Dysdercus cingulatos*, and *Pyrrhocoris apterus*.

Introduction

American trypanosomiasis (Chagas' disease) is a major health problem in Central and South America. The number of infected people has been estimated close to 20 million individuals, from which 2 to 3 million people develop the characteristic clinical symptoms of the chronic stage of Chagas' disease, and 45,000 of them die every year.[1-3] The etiologic agent for this illness is the protozoan *Trypanosoma cruzi* which is transmitted in rural areas by Reduviid insects like *Rhodnius* and *Triatoma* and by transfusion of infected blood even where Chagas' disease is not endemic.[4,5]

The parasite presents a complex life cycle that involves interactions between mammals and Reduviid bugs: it replicates within the crop and midgut of Chagas' disease vectors. Then, it is released with the excrements as non dividing metacyclic trypomastigotes which invade the mammalian host through the wounds produced by the blood sucking activity and it replicates as intracellular amastigotes. This form is liberated as non dividing bloodstream trypomastigotes that invade other tissues.[6]

Two drugs are currently in use to control this illness, Nifurtimox and Benznidazole, present significant drawbacks such as severe side effects, long term treatments and lack of specificity against all stages of this disease.[7-10] Moreover, as Chagas' disease may be transmitted by blood transfusion, it is crucial

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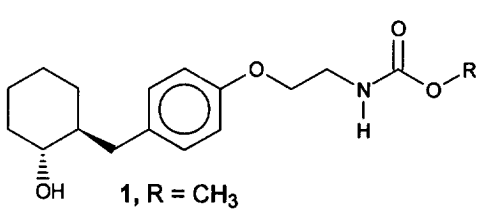
to eradicate the parasite from blood blanks. The only chemotherapeutic agent employed for this aim is the dye Crystal Violet[11] that proved to be carcinogenic in mammals[12] bringing some doubts about its safety. For the mentioned reasons, there is a demanding need for new, safe and efficacious drugs to be use in order to control Chagas' disease.[13]

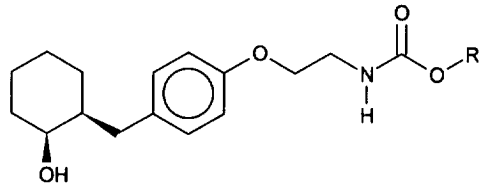
Results and Discussion

We have reported that compounds structurally related to the insect growth regulator Fenoxycarb (*N*-{2-[(4-phenoxyphenoxy)ethyl]} ethyl carbamate)[14] present a surprising dual action as juvenile hormone analogues[15] against Chagas' disease vector *Triatoma infestans*[16,17] and as growth inhibitors of *T. cruzi*.[18-23] This work had been motivated bearing in mind that juvenilized Chagas' disease vector were less susceptible to infection with *T. cruzi* than non treated vectors.[24]

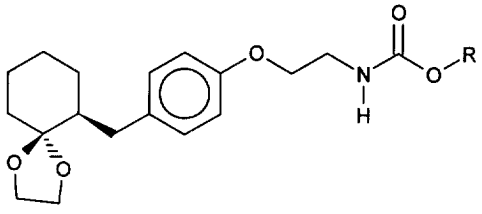
Figure 1. Chemical Structures of Juvenoids with Trypanostatic Activity



1, R = CH_3
2, R = CH_2CH_3
3, R = $CH_2CH_2CH_3$
4, R = $CH(CH_3)_2$
5, R = CH_2CCH



6, R = $CH_2CH_2CH_3$



7, R = $CH_2CH_2CH_3$
8, R = $CH(CH_3)_2$

In the present study, we wish to inform the trypanostatic activity exhibited by compounds **1-8** which had previously presented high activity as juvenoids on several non-related insect species such as *Tenebrio molitor*, *Galleria melonela*, *Dysdercus cingulatus*, and *Pyrrhocoris apterus*.[25] This work was encouraged by the trypanostatic action presented by several drugs on *T. cruzi* which formerly behaved as juvenoids on *T. infestans*. Therefore, it was very interesting to assay potent juvenile hormone analogues on *T. cruzi* proliferation in order to know if it exits a correlation between juvenoid action and inhibition of *T. cruzi* growth. We selected compounds **1-8** because they are very active drugs and they present attractive

modifications on Fenoxycarb framework: (a) the terminal phenoxy group has been replaced by different cyclohexylmethyl groups lacking its aromaticity; (b) the carbamate functionality was kept but several *O*-alkyl susbtituents were chosen. These compounds were prepared according to published procedures.[25]

Among the tested drugs, *O*-*n*-propyl and *O*-ethyl derivatives were very active drugs favoring the former ones. The relative stereochemistry between the alcohol group and the main chain did not have any significant influence on biological activity. The oxidation state of the saturated terminal six membered ring did not improve the biological action. Contrary, ketal derivatives also showed modest inhibition values. Therefore, compounds **2**, **3** and **6** were active compounds as inhibitors of *T. cruzi* growth (epimastigotes) with IC_{50} values of 67.3, 37.3 and 45.7 μg/mL, respectively. Compound **5** was moderately active, at a concentration of 50 μg/mL presented an inhibition value of 25%, while drugs **4** and **8** presented at concentrations of 100 μg/mL also modest activity values with inhibition percents close to 25%. The rest of the assayed drugs exhibited vanishing action as growth inhibitors of *T. cruzi*. In addition, no changes in the normal morphology of the parasites were observed during culturing.

These results are very encouraging because, in this case, not only there is cellular activity in compounds that mimic juvenile hormone action on Chagas' disease vector but also trypanostatic action is observed with juvenile hormone analogues on bug pests non-related among them and, remarkably, non related to *T. infestans*. Moreover, although the inhibitory action on *T. cruzi* by juvenoids is moderate, it suggests a biological explanation in which the presence of juvenile hormones in the midgut of Chagas' disease vector impairs *T. cruzi* (epimastigotes) multiplication. It is worthy to notice that this compound proved to be certainly not toxic on mammals.[25]

Biological Assays

Trypanosoma cruzi epimastigotes (Y strain) were grown in 20 mL screw-cap tubes at 28 °C in a liquid medium containing brain-heart infusion (37 g/L), hemin chlorohydrate (20 mg/L) (dissolved in 50% triethanolamine) and 10% newborn calf serum. The initial inoculum contained 2-3 x 10^6 cells/mL (as determined by counting in a Neubauer chamber) in a final volume of 1 mL. The

Table. Biological Activity of Juvenoids on the Epimastigote Forms of *Trypanosoma cruzi*.

Drug	IC_{50} (μg/mL)	Drug	IC_{50} (μg/mL)
1	> 200	**2**	62.3 ± 3.4
3	37.3 ± 1.6	**4**	194 ± 24.0
5	> 100	**6**	45.7 ± 1.6
7	> 200	**8**	145 ± 12.0
Ketoconazole	1.7 ± 0.2		

concentration of cells was determined by measuring the absorbance of the culture medium containing parasites at 600 nm against a blank with culture medium alone. Each drug was tested at four different concentrations (1, 10, 50 and 100 μg/mL) each one in quadruplicate. Drugs were dissolved in ethanol. The parasites were cultured in the presence of the compounds for 5 days. A control without drug was done with each group that was tested. The maximum amount of solvent used (1% ethanol) did not have any significant effect on the epimastigotes growth. Ketoconazole,[26] a well known trypanocidal drug, was used as positive control. The values of IC_{50} were estimated by linear and polynomial regression. The results are presented in the Table.

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