Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 22, pp. 2783-2786, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00517-3

BIOLOGICAL EVALUATION OF TWO POTENT INHIBITORS OF *Trypanosoma cruzi* EPIMASTIGOTES AGAINST THE INTRACELLULAR FORM OF THE PARASITE

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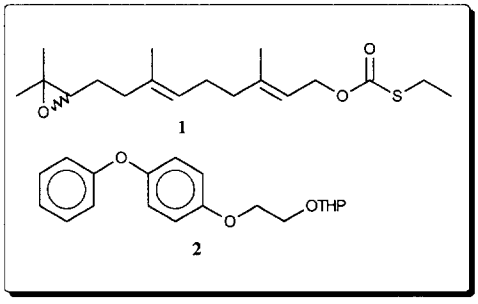
Abstract: (±)-*S*-Ethyl-[3,7,11]-trimethyl-10,11-epoxy-2E,6E-dien-1-yl]-thiolcarbonate (**1**) and 4-phenoxyphenoxy ethyl tetrahydropyranyl ether (**2**) were shown to be very active antireplicative agents against intracellular *Trypanosoma cruzi* amastigotes in in vitro assays. Both of them had previously presented high biological activity as growth inhibitors of the epimastigote form of the parasite.

Introduction

Chagas' disease or American trypanosomiasis is a serious health problem that affects millions of people in Central and South America.[1] The causative agent for this disease is the hemoflagellate protozoan *Trypanosoma cruzi*, which is transmitted in rural areas to humans and other mammals by Reduviid bugs such as *Rhodnius* and *Triatoma* species and in large urban centers by transfusion of infected blood.

The parasite occurs in three main morphological forms: the epimastigotes that are the dividing forms that replicate within the crop and midgut of Chagas' disease vectors, the nondividing highly infective metacyclic and bloodstream trypomastigotes, and the clinically more relevant form amastigotes, which are found within the cells in host tissues.[2]



Despite the public health problem of Chagas' disease, no new drugs are available for its chemotherapy. The two drugs presently in use, Nifurtimox (4-([5-nitrofurfurylidene]-amino)-3-methylthiomorpholine-1,1-

dioxide) and Benznidazole (*N*-benzyl-2-nitro-1-imidazoleacet amide), cause severe side effects and lack efficacy against the chronic stage of the disease.[3-6] In addition, due to the risk that the parasite may be transmitted by transfusions of contaminated blood, it is quite desirable to eliminate this parasite in blood to be transfused. At the present time, the drug use for blood sterilization is a dye discovered to be effective for this purpose many years ago (Gentian Violet,[7] which suffers from limitations due to its safety).[6]

Results and Discussion

In this paper, we wish to inform the biological activity of (±)-*S*-ethyl-[3,7,11]-trimethyl-10,11-epoxy-2E,6E-dien-1-yl]-thiolcarbonate (**1**) and 4-phenoxyphenoxy ethyl tetrahydropyranyl ether (**2**), the former is structurally related to insect juvenile hormones and the latter to the insect growth regulator Fenoxycarb (*N*-ethyl 2[4-phenoxyphenoxyethyl] carbamate),[8] which is a juvenile hormone of insects mimic. These drugs were selected for being the most representative members of both families of compounds that had previously exhibited vigorous action to control proliferation of the epimastigote forms of *T. cruzi*.[9-11] It is very interesting to point out that both families of drugs were primarily designed as juvenile hormone analogues for Chagas' disease vector, *Triatoma infestans*[12,13] and became antitrypanostatic agents bearing in mind that juvenilized *T. infestants* were seven times less susceptible to natural infection with this parasite.[14] Compound **1** was straightforwardly prepared by acylation of *trans,trans*-farnesol with ClC(O)SEt/py followed by selective epoxydation at the terminal double bond[15] and so was compound **2** by coupling of 4-phenoxyphenol with the tetrahydropyranyl derivative of bromoethanol by a modified Williamson procedure.[16] There is strong evidence to believe that these compounds act at the sterol biosynthetic pathway.[17,18] Although this pathway differs in *T.cruzi* and mammalian cells,[19] we can not rule out any potential toxic side effect in mammalian cells. In addition, both of these drugs present small to vanishing biological activity against trypomastigotes.[20]

Experiments were performed to determine the activities of compounds **1** and **2** against *T. cruzi*-infected L_6E_9 myoblasts. When either compound **1** or **2** was used, there was a significant dose-dependent reduction in the percentage of myoblasts containing intracellular amastigotes and in the number of parasites per 100 myoblasts, this effect being more noticeable after 48 h in the presence of either drug. At 40 μg/mL compound **1** practically eliminated all parasites, while compound **2** reduced the numbers to less than 10% of the controls. Compound **1** was used at higher concentrations than compound **2** because it was ineffective at lower concentrations. The results are presented in the table. At the concentrations used the compounds were not toxic to the host cells as determined by their normal morphology and no direct effects of the drugs were detected on the parasites incubated alone for as long as 1 h under similar conditions.

Biological Assays

The method for tissue culture testing was adapted from Barr et al.[21] To determine the activity of the compounds against *T. cruzi*-infected L_6E_9 myoblasts, confluent cell myoblast cell monolayers were prepared as described before by Moreno et al.[22] on 0.9 x 0.9 cm coverslips in tissue culture chambers (3 coverslips per treatment). Myoblasts were trypsinized and counted in a Neubauer hemacytometer. The same amount of cells were inoculated in each chamber. The monolayers were washed three times with phosphate buffered saline (PBS) at 37 °C after 4 h. Some monolayers were exposed to a suspension of tissue culture-derived trypomastigotes according to Moreno et al.[22] in DMEM-10% FBS (1 mL/chamber). The final concentration of trypomastigotes was calculated based on a ratio of 4:1 parasites to L_6E_9 cells. The parasites were allowed to internalize within the myoblast for 24 h. At this time, a set *T. cruzi*-infected cultures was fixed and stained with Giemsa and was designated as the 24-h control culture. The media from the remaining slides was removed and fresh DMEM-10% FBS alone (control) or containing compounds **1** or **2** were added to the cultures. After a further 24 h of incubation at 37 °C, a set of *T. cruzi*-infected cultures (untreated control and drug-treated) was fixed and stained with Giemsa. Media were removed from other cultures and again, fresh DMEM-10% FBS alone (control) or containing compounds **1** or **2** were added to the cultures. Cultures were incubated (37 °C) for a further 24 h after which they were fixed and stained with Giemsa. Infection was assessed by the percentage of myoblast with intracellular parasites and by the number of parasites present in 100 myoblasts. A minimum of 200 cells were screened in each culture.

Table. Growth inhibition of Intracellular *T. cruzi* by 1 and 2

Compd. 1	% Myoblast with Parasites	Parasites No. per 100 Myoblasts	Compd. 2	% Myoblast with Parasites	Parasites No. per 100 Myoblasts
None (24 h)	21.25 ± 6.25	46.5 ± 12.5	None (24 h)	21.25 ± 6.25	46.5 ± 12.5
None (48 h)	16.25 ± 1.75	118 ± 9.0	None (48 h)	24.50 ± 1.25	107 ± 0.5
30 (μg/mL)	23.25 ± 4.75	77.75 ± 17.75	5 (μg/mL)	22.5 ± 1.0	90.25 ± 5.25
40 (μg/mL)	10.25 ± 2.75	26.25 ± 10.75	10 (μg/mL)	21.25 ± 0.25	75.75 ± 0.25
None (72 h)	20.5 ± 2.5	694.5 ± 166.5	None (72 h)	18.25 ± 0.75	536.75 ± 15.25
30 (μg/mL)	9.75 ± 1.75	27 ± 8.5	5 (μg/mL)	22.0 ± 0.5	113.75 ± 3.25
40 (μg/mL)	2.75 ± 0.25	5.5 ± 2.5	10 (μg/mL)	12.5 ± 1	44.75 ± 8.75

Acknowledgments: The authors would like to thank the National Research Council of Argentina (CONICET) and the University of Buenos Aires for partial financial support and UMYMFOR for spectra and NIH grant AI23259 to R. D.

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19. By the time this manuscript was submitted an interesting paper was published where the authors report cure on experimental Chagas' disease employing a bis-triazole derivative ((R)-2-(2,4-difluorophenyl)-1-(3-[(Z)-4-(2,2,3,3-tetrafluoropropoxy)styryl]-1,2,4-triazol-1-yl)-propan-2-ol). The target of this compound is also the sterol biosynthetic pathway. Urbina, J. A.; Payares, G.; Molina, J.; Sanoja, C.; Liendo, A.; Lazardi, K.; Piras, M. M.; Piras, R.; Perez, N.; Wincker, P.; Ryley, J. F. *Science* **1996**, *273*, 969.
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(*Received in USA* 24 *June* 1996; *accepted* 21 *October* 1996)