Biological Activities of 7-Epiclusianone

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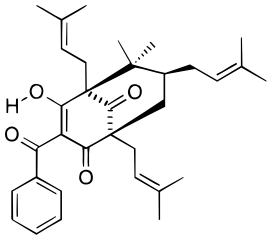
7-Epiclusianone, isolated from *Rheedia gardneriana*, was tested in several biological assays. It was active *in vitro* against trypomastigotes of *Trypanosoma cruzi* but inactive *in vivo* in experimentally infected mice. It was also active against *Artemia salina*, but inactive against the fungus *Cladosporium sphaerospermum* and the snail *Biomphalaria glabrata*.

The polyprenylated benzoquinone 7-epiclusianone (**1**) was isolated from the hexane extract of the pericarp of the *Rheedia gardneriana* Miers ex Planch. & Triana, a small tree of the Clusiaceae family.[1] Previous work with this compound disclosed its activity against the phytobacteria *Clavibacter michiganense* subsp. *michiganense* (MIC = 4 μg/mL) and the enterobacteria *Listeria monocytogenes* and *Staphylococcus aureus* (IC_{100} = 80 μg/mL).[2] These results stimulated us to further evaluate this substance in other biological models, including pathogenic (*Trypanosoma cruzi*) and nonpathogenic organisms (*Biomphalaria glabrata*, *Artemia salina,* and *Cladosporium sphaerospermum*).



7-epiclusianone

Chagas' disease, caused by the flagellate protozoan *Trypanosoma cruzi*, affects 18 million people in Latin America and is responsible for the death of 45 000 patients every year.[3] The treatment relies on only two available drugs, nifurtimox and benznidazole, which are relatively efficient in the acute phase of the disease, but almost ineffective in the chronic phase.[4] Nowadays, one of the most important mechanisms of Chagas' disease transmission in many countries is by blood transfusion.[5] In highly endemic areas it is strongly recommended to use chemoprophylatic measures such as the addition of gentian violet to clear trypomastigotes from blood banked for transfusion.[6] Although effective, this triphenylmethane dye is not well accepted because of such undesirable effects as coloring the skin and possible mutagenicity.[7] Thus, new drugs to prevent or treat Chagas' disease are urgently needed, and, in this regard, the title compound was evaluated in vitro and *in vivo* for its effects against *T. cruzi*.

Table 1. *In Vitro* Activity of 7-Epiclusianone and the Control Drug Gentian Violet on Trypomastigote Forms of *Trypanosoma cruzi* Present in Blood of Experimentally Infected Mice

	concentration (μg/mL)	trypanocidal activity (% ± S.D.)[a]
drug		
7-epiclusianone	500	92 ± 1.0
	250	38 ± 3.7
	125	17 ± 1.2
controls		
vehicle		0
gentian violet	7.5	47 ± 1.7
DMSO	2.5%	2 ± 4.8

[a] The experiments were run in duplicate and repeated twice. The activity is expressed as percent reduction of the parasite number in infected murine blood ± standard deviation (S.D.) as compared with the control without drug.

Intestinal schistosomiasis is another endemic disease that affects millions of people in tropical countries and is caused by the helminth *Schistosoma mansoni*.[3] In Brazil, the most important intermediate host of this parasite is the snail *Biomphalaria glabrata*. The population control of this snail is considered an important complementary measure to chemotherapy to interrupt the transmission of the disease. In this regard, the development of cheap plant-derived molluscicides that are accessible to poor communities was recommended by WHO.[8] The bioassay using *B. glabrata* has been described as an important tool to find new molluscicides and was used to evaluate 7-epiclusianone.

This quinone was also tested in the brine shrimp (*Artemia salina* Leach.) lethality test and bioautographic assays with the fungus *Cladosporium sphaerospermum*. The former has been used as a surrogate assay to detect antitumor or cytotoxic activity[9] or as a pre-screen system to indicate trypanocidal activity against blood forms of *T. cruzi*.[10] *C. sphaerospermum* has been used to detect antifungal activity of plant extracts or pure compounds.[11]

As shown in Table 1, 7-epiclusianone was active *in vitro* against the trypomastigote forms of *T. cruzi* present in murine blood (LC_{50} = 260 μg/mL, 518 μM). This assay mimics the conditions found in blood banks and is intended to find candidates for chemoprophylatic agents. These compounds should be able to kill all parasites eventually present in donated blood in highly endemic regions, thus avoiding transmission of Chagas' disease via blood transfusion. 7-Epiclusianone presented a LC_{50} about 29 times higher than that of gentian violet (LC_{50} = 7.5 μg/mL, 18

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μM), the reference chemoprophylatic drug. In view of this *in vitro* activity, 7-epiclusianone was also tested in vivo in mice infected with the Y strain of *T. cruzi*. Two different treatment schedules were used: (A) single oral dose of 500 mg/kg of body weight in the peak of parasitaemia and (B) four daily 100 mg/kg of body weight doses starting on the day after infection. Both experiments included control groups with animals untreated and treated with benznidazole and with the vehicle. In the rapid treatment (A), a single, high dose of drug was administered at the peak of parasitaemia, and the parasites were assessed 6 h later.[12] This assay is aimed at evaluating the activity of the drug on the circulating bloodstream trypomastigotes. In the short-term treatment (B), a lower dosage is administered over 4 consecutive days, starting the day after infection. The drug activity is then evaluated on days 6−9, based on its capacity to reduce the parasitaemia peak. This treatment evaluates simultaneously the activity of the drug against the nonproliferative, blood-circulating (trypomastigotes) and the intracellular, proliferative forms of the parasite (amastigotes).

In experiment A, the group treated with the reference drug (benznidazole) showed no parasites in blood 6 h after drug administration, while the group treated with 7-epiclusianone showed the same level of parasitaemia found in control groups treated with vehicle or untreated (data not shown). The failure to control the infection was also observed in the short-term treatment (B), where the onset of the disease was not altered by 7-epiclusianone administration when compared with the untreated groups. The group treated with benznidazole (100 mg/kg/day) over 4 days presented no parasitaemia peak. The *in vivo* inefficacy of the tested drug could be due to several reasons, including poor absorption and/or rapid metabolism.

In view of the lack of activity of the 7-epiclusianone in the mouse model of Chagas'disease, this compound *per se* shows no promise as a chemotherapeutic agent for this disease. Nevertheless, as this is the first report on the significant *in vitro* trypanocidal activity of a substance belonging to this structural class, our results indicate that related natural or synthetic compounds should be evaluated in *T. cruzi* models in an attempt to find more active derivatives that could be used as chemoprophylatic agents for Chagas' disease.

According to McLaughlin, compounds with $LC_{50} = 1000$ ppm in the brine shrimp lethality assay are considered active and potentially cytotoxic against tumor cell lines.[9] 7-Epiclusianone disclosed reasonably strong toxicity in this assay ($LC_{50} = 25$ ppm, 49.7 μM), indicating that it may be an interesting lead compound for the development of new anticancer agents. Investigations in this direction are planned for the near future. 7-Epiclusianone was active against both *A. salina* and *T. cruzi* trypomastigotes, reinforcing the feasibility of using the toxicity assay with *A. salina* as a pre-screen system to detect extracts active against *T. cruzi*, as proposed in a previous paper.[10] Finally, the lack of activity against *C. sphaerospermum* and its inability to kill *B. glabrata* at 20 ppm suggest that this compound is a poor candidate either as a fungicide or as a plant-derived molluscicide.

Experimental Section

***In Vitro* Assay with Trypomastigote Forms of *T. cruzi*.** Blood infected with trypomastigotes of *T. cruzi* Y strain was obtained by retro-orbital bleeding of experimentally infected male Swiss albino mice and diluted with normal murine blood to 2×10^6 trypomastigotes/mL. Stock solutions of the pure compound at 20, 10, and 5 mg/mL in dimethyl sulfoxide (DMSO) were prepared, and 5 μL of each solution was added to 195 μL of infected blood in a 96-well microtiter plate, attaining final concentrations of 500, 250, and 125 μg/mL, respectively. Negative and positive controls containing either 2.5% DMSO or gentian violet at its IC_{50} (7.5 μg/mL) were run in parallel. After 24 h at 4 °C, the number of parasites was determined by placing 5 μL of the tested blood on a glass plate, covering with a 22 × 22 mm coverslip, and counting the parasites in 50 fields at 400× magnification. Each experiment was performed in duplicate and repeated twice. The results were expressed as mean ± standard deviation (S. D.) of the percentage reduction of parasitaemia compared to the control with DMSO alone. At 2.5% DMSO does not interfere with parasite survival.

***In Vivo* Assay with Mice Experimentally Infected with *T. cruzi*.** The drug control—benznidazole: *N*-benzyl-2-nitro-1-imidazolacetamide (Rochagan—Roche)—was dissolved in distilled H_2O. 7-Epiclusianone was initially dissolved in DMSO, followed by the addition of 3 volumes of 2% aqueous sodium methylcellulose to a final concentration of 10 and 50 mg/mL. Both drugs were given to mice by oral route through gavage.

(A) Rapid treatment: Blood of mice infected with the *T. cruzi* Y strain, isolated from an acute human case of Chagas' disease,[13] was collected from the orbital venous sinus (0.2−0.5 mL), diluted in 3.8% sodium citrate (3:1 v/v), and inoculated intraperitoneally (ip) in normal mice. Groups of 20 male Swiss albino mice 3−4 weeks old and weighing 18−20 g were inoculated ip with 5×10^4 trypomastigotes/mouse. Seven days after inoculation, at the peak of parasitaemia, the animals with nearly the same parasitaemia levels, were divided in groups of 3 mice: (a) untreated, (b) treated with DMSO-methylcellulose, (c) treated with benznidazole (500 mg/kg) or (d) treated with 7-epiclusianone (500 mg/kg). The parasitaemia was determined according to Filardi and Brener.[12] Reduction in parasitaemia was determined 6 h after treatment, comparing treated with untreated animals.

(B) Short-term treatment: Mice were inoculated intraperitoneally with 5×10^4 trypomastigotes and divided in groups of 5 individuals: (a) untreated, (b) treated with DMSO−methylcellulose, (c) treated with benznidazole (100 mg/mouse/day), or (d) treated with 7-epiclusianone (100 mg/mouse/day). Mice were treated over 4 consecutive days, starting the day after infection. The parasitaemia was monitored from day 6 to day 9 after infection in fresh blood collected from the mice's tails. The number of parasites was estimated as above.[12] Mortality rate was expressed as the percentage of accumulated deaths within 20 days after the infection.

Brine Shrimp Lethality Assay. An established protocol was employed.[9] Briefly, the drug was assayed at 40, 4, and 0.4 ppm, using 10 second-instar larvae of the brine shrimp (*Artemia salina*, Leach) in triplicate. After 24 h contact at room temperature, the number of surviving organisms was recorded and the LC_{50} calculated using the Probit Statistical Analysis Program. Controls with thymol ($LC_{50} = 10$ ppm) and without drug were made.

Bioautographic Assay with the Fungus *C. sphaerospermum*. The bioautographic assay described by Homans and Fuchs [11] was adopted, employing the *C. sphaerospermum* fungus.

***Biomphalaria glabrata* Lethality Assay.** The assay was run according to Alves and coworkers.[14]

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