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# Leishmanicidal activity of two canthin-6-one alkaloids, two major constituents of *Zanthoxylum chiloperone* var. *angustifolium*

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**Abstract**

The crude alkaloidal extract of *Zanthoxylum chiloperone* stem bark exhibited in vitro activity against various strains of *Leishmania* ssp. at 100 μg/ml. Two active major constituents were isolated and identified as canthin-6-one and 5-methoxycanthin-6-one. The effect of these compounds was also tested in an in vivo assay using BALB/c mice infected with *Leishmania amazonensis*. The mice were treated for 5 weeks postinfection with these alkaloids by oral (14 days) or intralesional route (4 days) at 10 mg/kg daily. The reference drug, *N*-methylglucamine antimonate was administered by subcutaneous injections at 100 mg/kg for 10 days. Intralesional administration of canthin-6-one reduced the parasite burden but not significantly when it was compared with the untreated group, while the reference drug reduced by 91% the parasite loads in the lesion. 

*Keywords:* *Zanthoxylum chiloperone*; Rutaceae; Canthin-6-one; 5-Methoxycanthin-6-one; BALB/c mice; *Leishmania amazonensis*

## 1. Introduction

The leishmaniases are infectious diseases caused by different species of *Leishmania* protozoa and are a globally widespread group of parasitic diseases. These infections are initiated by inoculation of *Leishmania* species into the skin during sand fly bites. In Paraguay, cutaneous and mucocutaneous leishmaniases are caused by several species of *Leishmania* species, *Leishmania amazonensis* and *L braziliensis*. In general, the reported cases (100–500) (OPS, 1996) of *Leishmania* infections in Paraguay are a modest estimation and would substantially increase if active surveillance were implemented throughout the country. In the endemic regions of Paraguay situated at East of Paraguay river, the classic treatments (pentavalent antimonials or amphotericin B) are too expensive or unavailable to the population affected by cutaneous leishmaniasis. The Instituto de Investigaciones en Ciencias de la Salud (IICS) in Paraguay and the French Institute of Research for the Development (IRD) have initiated and developed original investigations of alternative compounds for the treatment of leishmaniasis.

In the course of screening for antileishmanicidal compounds from Paraguayan plants, we have found that the alkaloidal crude extract of the stem bark of *Zanthoxylum chiloperone* var. *angustifolia* (Engl.) Chodat & Hassler (Rutaceae) displayed activity in vitro at 100 μg/ml against three strains of promastigote forms of *Leishmania* species, *L. braziliensis*, *L. amazonensis* and *L. donovani*. *Z. chiloperone* var. *angustifolium* is a tree growing in the Eastern departments of Paraguay known as 'tembetary hu'. In Paraguay, the decoction of the root bark of *Z. chiloperone* var. *angustifolium* is used as antimalaric (Milliken, 1997), emmenagogue and antirheumatic.

In this paper, we report the isolation of two canthin-6-one alkaloids and their in vivo leishmanicidal activity in BALB/c mice infected with *L. amazonensis*.

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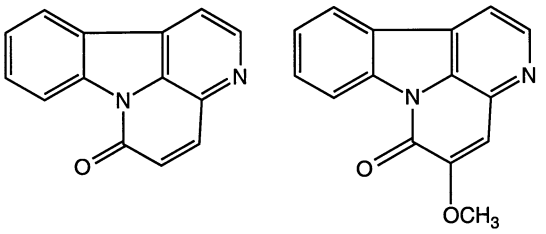


Fig. 1. Chemical structure of canthin-6-one alkaloids.

## 2. Materials and methods

### 2.1. Plant material

Canthin-6-one and 5-methoxycanthin-6-one were isolated from the stem bark of *Z. chiloperone* var. *angustifolium* (Engl.) collected in Paraguay, near Piribebuy, Dept. Cordillera by A. Fournet and isolated by C. Thouvenel and A. Fournet. A voucher specimen (AF 917) has been deposited in the Herbarium of Chemical Sciences Faculty, Asunción, Paraguay (FCQ).

### 2.2. Phytochemical analysis

The active compounds were isolated by fractionation and purification monitored by bioassays as described (Fournet et al., 1994). Briefly, dry and powdered stem bark (1.9 kg) of *Z. chiloperone* was extracted in a Soxhlet extractor with $CH_2Cl_2$. The extract obtained (44 g) was chromatographed on a Si gel flash column and eluted with $CH_2Cl_2$/EtOAc (2:8) mixture to obtain eight fractions. The first fraction was found to contain a single compound crystallizing in acetone (3.2 g), this compound was identified as canthin-6-one by nuclear magnetic resonance spectroscopy (H/C-NMR) with its physical data (Chan et al., 1986). In the sixth fraction, a second compound (150 mg) was obtained and identified as 5-methoxycanthin-6-one 2 (Mitscher et al., 1972). The chemical structures of these compounds are shown in Fig. 1.

*N*-methylglucamine antimonate (Glucantime®) with a pentavalent antimony ($Sb^v$) content 28% by weight was purchased from Aventis, Paris, France and was used as a reference drug.

### 2.3. Animals

Female and male BALB/c mice were supplied by JICA, La Plata, Argentina and bred at the Instituto de Investigaciones en Ciencias de la Salud (IICS), Asunción, Paraguay. Golden hamsters (*Mesocritus auratus*) were used to maintain the parasites.

*L. amazonensis* MHOM/IFLA/BR/67/PH8 was used and maintained by passage every 8 weeks in hamsters. BALB/c ($n = 10$) were inoculated in the right hind footpad with $1 \times 10^6$ amastigotes obtained from donor hamsters. The parasites were delivered in 100 μl of phosphate buffered saline (PBS). Disease progression was monitored by the measurement of lesion diameters weekly for 8 weeks.

### 2.4. Drug treatment

The treatments were initiated 5 weeks after inoculation when the infection was well established, and lesions were obvious. Two days before administration of drug, the mice were randomly divided into groups of ten. *N*-methylglucamine antimonate was dissolved in 50 μl of PBS and administered to BALB/c mice in regimens of 28 mg of $Sb^v$ per kg of body weight daily for 10 days by subcutaneous route or by five intralesional injections in the infected footpad. Canthin-6-one or 5-methoxycanthin-6-one were administered by oral or intralesional route at a dose level of 10 mg/kg body weight and were made up in 50 μl PBS and 5 μl of polysorbate (Tween 80, OSI, France). The canthin-6-one alkaloids were given once daily by oral route for 15 days, or four times by intralesional injections at intervals of 5 days into the infected footpad. The untreated group received daily 50 μl of PBS and 5 μl of Tween 80.

### 2.5. Effect of treatment

The animals were sacrificed 1 week after cessation of treatment to assess parasitological loads in the infected footpad. Briefly, the mice were killed and the lesions of the infected footpad were excised, weighed and homogenized in a tissue glass grinder and then homogenized in 5 ml of RPMI 1640 (Gibco, France) tissue culture medium supplemented with 10% fetal calf serum, 1 ml of glutamine (GIBCO, France) (29.4 mg/l), penicillin (100 U/ml) and streptomycin (100 μg/ml). Smears were prepared from the infected lesions, fixed with absolute methanol, and stained with Giemsa (Prolabo), and amastigotes were counted with an inverted microscope at a magnification of (400) (Buffet et al., 1995; Hill et al., 1983). Five hundred cell nuclei from each animal were examined under oil immersion.

A corrected parasite suppression index was calculated by the following formula:

$$\frac{\text{Mean parasites(or weight)treated mice} \times 100}{\text{Mean parasites(or weight)untreated mice}} - 100$$

### 2.6. Statistical analysis

The data were expressed as means ± standard deviation (S.D.). Comparisons of parasite suppression in

Table 1
In vitro activity of *Z. chiloperone* crude extracts, canthin-6-one and 5-methoxy canthin-6-one towards three strains of promastigote forms of *Leishmania* spp

| Extracts and compound | *L. braziliensis* | *L. amazonensis* | *L. donovani* |
|---|---|---|---|
| *N*-methylglucamine antimonate[a] | > 100 | > 100 | > 100 |
| Pentamidine[a] | 5 | 5 | 5 |
| Alkaloidal extract ($CH_2Cl_2$) | 100 | 100 | 100 |
| Methanolic extract | > 100 | > 100 | > 100 |
| Canthin-6-one | 100 | 100 | 100 |
| 5-methoxycanthin-6-one | 100 | 100 | 100 |

[a] Reference drug.

infected footpad of the untreated and drug-treated groups were done by analysis of variance (ANOVA) and by Student's *t*-test. Data were considered statistically significant at $P < 0.05$.

## 3. Results and discussion

At 100 μg/ml, the alkaloidal extract prepared from the stem bark of *Z. chiloperone* inhibited the growth of three strains of *Leishmania* sp. (see Table 1). This extract was fractionated on a silica gel column to yield two active fractions at 100 μg/ml against the *Leishmania* strains. The EI-MS, $^{1}$H- and $^{13}$C-NMR data of these two alkaloids showed that they were canthin-6-one and 5-methoxy-canthin-6-one, previously isolated from *Z. elephantiasis* (Mitscher et al., 1972), *Brucea antidysentrica* (Simaroubaceae) (Fukamyia et al., 1987), *Eurycoma harmandiana* (Simaroubaceae) (Kanchanapoom et al., 2001), *Peganum nigellastrum* (Zygophyllaceae) (Ma et al., 2000) and *Ailanthus altissima* (Simaroubaceae) (Ohmoto et al., 1976). The effects of treatments with Glucantime®, canthin-6-one or 5-methoxycanthin-6-one by subcutaneous, oral or intralesional routes during the course of infection of BALB/c mice infected with *L. amazonensis* are presented Table 2. The subcutaneous treatment with antimonial drug at 28 mg/kg of $Sb^{v}$ for 10 days reduced the lesion weight by 28.5% and parasite burden in lesion by 90.9% ($P < 0.05$) versus the untreated mice. The intralesional treatment with canthin-6-one was efficient but not significantly; in this condition the lesion weight decreased by 15.0% and the parasite load by 77.6% when compared with the group of untreated mice (see Table 2). This specific treatment produced a slight inflammation of footpad, but generally the treatments with canthin-6-one did not cause any obvious toxicity in the mice. On the other hand, we have observed an increase of the weight lesion when the mice were treated orally with canthin-6-one or with 5-methoxycanthin-6-one by 139.6 and 27.7%, respectively, and an increasing of the parasite loads (171.4 and 68.4%). None of the tested compounds showed a significant leishmanicidal, but the intralesional administration of canthin-6-one displayed an interesting leishmanicidal effect. The effectiveness of this compound and its low toxicity, its lethal dose killing 50% of mice ($LD_{50}$) was evaluated to be upward of 400 mg/kg via the intraperitoneal route, would merit further studies for a topic treatment of the cutaneous leishmaniasis. However, to our knowledge this is the first report of antileishmanicidal activity of canthin-6-one alkaloids but their activity have been previously reported against *Plasmodium falciparum* (Chan et al., 1986; Kardono et al., 1991).

Table 2
Inhibitory effect of treatment with *N*-methyl-glucamine antimonate by subcutaneous route and canthin-6-one and 5-methoxycanthin-6-one by the intralesional or oral route on *L. amazonensis* infected BALB/c mice

| Drug (dosage) | Route of administration | Lesion wt (g) (mean ± S.D.)[a] | Suppression of lesion weight (%) | Suppression of parasite load in lesion | Mean parasites quantitation in lesion |
|---|---|---|---|---|---|
| None (control) | | 0.039 ± 0.009 | – | – | $2.2 \times 10^{7}$ |
| Meglumine antimonate 28 mg $Sb^{v}$ × 10 | Subcutaneous | 0.028 ± 0.010 | −28.5 | −90.9* | $2.0 \times 10^{6}$ |
| Canthin-6-one 10 mg × 15 | Oral | 0.092 ± 0.094 | +139.6 | +171.4 | $6.0 \times 10^{7}$ |
| Canthin-6-one 10 mg × 4 | Intralesional | 0.033 ± 0.023 | −15.0 | −77.6 | $5.0 \times 10^{6}$ |
| 5-methoxy canthin-6-one 10 mg × 15 | Oral | 0.086 ± 0.092 | +27.7 | +68.4 | $3.8 \times 10^{7}$ |
| 5-methoxy canthin-6-one 10 mg × 4 | Intralesional | 0.049 ± 0.043 | +124.2 | −21.6 | $1.7 \times 10^{7}$ |

*, $P < 0.05$ by ANOVA and Student's *t*-test (treated mice vs. control).
[a] Values represent the means ± standard deviation, ten mice per group.

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