

Antiprotozoal Activity of Quinoline Alkaloids Isolated from *Galipea longiflora*, a Bolivian Plant Used as a Treatment for Cutaneous Leishmaniasis

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The stem bark of *Galipea longiflora* is used by the Chimane Indians in Bolivia for the treatment of cutaneous leishmaniasis produced by *Leishmania braziliensis*. Petroleum ether and chloroform extracts of stem, root bark and leaves were found active *in vitro* against *Leishmania* spp and *Trypanosoma cruzi* at 100 µg/mL. The activity guided fractionation of the extracts by chromatography afforded 12 active compounds identified as 2-substituted quinoline alkaloids. BALB/c mice were infected with *Leishmania amazonensis* (strain PH8 or H-142) and treated 24 h after infection with the major alkaloids from the crude alkaloidal extract; 2-phenylquinoline and 2-*n*-pentylquinoline. 2-phenylquinoline was as potent as Glucantime (Rhône-Poulenc) against the strain H-142, but less active than the reference drug against the virulent strain PH8 of *L. amazonensis*. 2-*n*-pentylquinoline did not exhibit any activity. Assays of single local treatments on the rear footpad infection, 2 weeks after the parasitic inoculation, indicated an effect for 2-phenylquinoline by reducing the severity of lesion. However, this activity was found to be slightly lower than that obtained using Glucantime.

Keywords: alkaloids; quinoline; *Galipea longiflora*; Rutaceae; *Leishmania* spp., *Trypanosoma cruzi*.

INTRODUCTION

Cutaneous and mucosal leishmaniasis, caused by the protozoan *Leishmania braziliensis*, are common infections in the subtropical areas of the Department of La Paz (Yungas and Alto-Beni) and the foothills of the Andes (Department of Beni) where the Chimane Indians live. Cutaneous leishmaniasis is known as *espundia* by the natives of this region of Bolivia called *Oriente*. The infection is classically treated with pentavalent antimony (Glucantime, Rhône-Poulenc) for cutaneous leishmaniasis or with amphotericin B for mucosal leishmaniasis. These drugs are administered parenterally to patients in hospitals which are often far from their colonies. These treatments are also too expensive or unavailable for the population suffering from *espundia*. The dispersion of cutaneous leishmaniasis is accentuated in subandean tropical regions by the influx of people descending from higher areas. The use of medicinal plants is commonplace, especially among the Chimane Indians, a group that inhabits the gallery forest and sandbars of the Rio Maniqui and its

tributary streams. We have collected and studied many medicinal plants (Fournet, 1991) and most notably a tree called *evanta*, used by the Chimane Indians for the treatment of cutaneous leishmaniasis and botanically identified as *Galipea longiflora* Krause (Rutaceae). The stem bark, in the form of a poultice, is applied on the cutaneous leishmaniasis until complete cicatrization of the wound. In a preliminary screening, the crude alkaloidal extracts of the stem bark, root bark and leaves of *Galipea longiflora* displayed activity *in vitro* at a concentration of 100 µg/mL against three strains of promastigote forms of *Leishmania* species, *L. braziliensis*, *L. amazonensis* and *L. donovani*, and three strains of epimastigote forms of *Trypanosoma cruzi* (Tulahuen, C8CL1 and Tehuentepec), another trypanosomatid responsible for Chagas' disease. Activity-directed fractionation and purification of crude alkaloidal extracts in petroleum ether and in chloroform of root bark, stem bark and leaves gave 12 active compounds, identified by their physical and spectral data as 2-aryl and 2-alkyl quinolines alkaloids (Fournet *et al.*, 1989, 1991).

This work describes the *in vitro* antileishmanial and trypanocidal activities of the 12 quinolines alkaloids and the *in vivo* activity of two major alkaloids of *Galipea longiflora*, 2-phenylquinoline and 2-*n*-

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27 MARS 1995

O.R.S.T.O.M. Fonds Documentaire

N° : 41533 ex1

Cote : B

pentylquinoline in BALB/c mice infected with *Leishmania amazonensis* (IFLA/BR/67/PH8) or MHOM/GF/84/CAY-H-142). We sought to verify the effect of the most abundant alkaloid of *Galipea longiflora*, 2-phenylquinoline, when administered directly in the rear footpad of infected BALB/c mice by *Leishmania amazonensis*.

MATERIALS AND METHODS

Isolation and chemistry

General experimental procedures. UV spectra were recorded on a Unicam SP 1800 spectrophotometer. IR spectra were measured in KBr with a Perkin Elmer 257 spectrometer. All ^1H and ^{13}C -NMR spectra were recorded in CDCl_3 (δ ppm) on a Bruker AC 200 P spectrometer operating at 200 and 50 MHz respectively. Eims spectra were obtained at 70 eV on a Nermag R 1010 C mass spectrometer.

Plant material. Stem bark (3.5 kg), root bark (350 g) and leaves (3.5 kg) of *Galipea longiflora* Krause were collected by A. Fournet (A.F. 850) in August 1989, at Fatima de Chimane, in the Department of Beni, altitude 400 m, Bolivia. The plant material was identified by Dr J. A. Kallunki of the New York Botanical Garden. A reference specimen is deposited in the National Herbarium of Bolivia (La Paz).

Extraction and isolation. The isolation procedure was effected by activity-directed fractionation and purification of the crude alkaloidal extracts of root bark, stem bark and leaves. Extraction, partitioning, chromatographic separation and physicochemical data of the 12 alkaloids identified were performed as described by Fournet *et al.* (1989) and Fournet (1991).

Biological assays

Parasites. Cultures of *Leishmania* spp and *Trypanosoma cruzi* were obtained from IBBA (Instituto Boliviano de Biología de Altura, La Paz) and identified by isoenzyme analysis.

Five strains of *Leishmania* were used during these investigations, *L. braziliensis* (MHOM/BR/75/M 2903), *L. amazonensis* (IFLA/BR/67/PH8), *L. amazonensis* (MHOM/GF/84/CAY H-142), *L. donovani* (MHOM/IN/83/HS-70) and *L. donovani* (MHOM/BR/74/M 2682), and five strains of *Trypanosoma cruzi*: SC 43 CL2 (Bolivian strain), C8 CL1 (Brazilian strain), Tulahuen (isolated from *Triatoma infestans* in Brazil), TeCL2 (Brazilian strain) and 1979 CL1 (Bolivian strain).

Promastigote forms of *Leishmania* were grown at 28 °C in USMARU medium (Evans, 1987) containing 10% heat inactivated (56 °C for 30 mn) fetal bovine serum and epimastigote forms of *T. cruzi* in liver infusion tryptose medium (LIT, Bacto) supplemented with 10% fetal calf serum at 28 °C with an inoculum of 10^6 cells per mL. Logarithmically growing promasti-

gotes and epimastigotes were maintained by transferring 10^6 cells per mL. The extracts and the fractions of *Galipea longiflora* were dissolved in a known volume of DMSO (dimethyl sulphoxide) and then in medium, from which aliquots were drawn. Parasites were counted after 48 h of contact with drugs in a haemocytometer and the counts were compared with those of controls grown without drug. The 90% inhibitory concentrations (IC_{90}) were chosen for the comparison of susceptibilities of the strains to drugs tested.

N-methylglucamine antimonate (Glucantime) (Rhône-Poulenc, France) and Pentamidine (May and Baker, England), nifurtimox (Bayer, Germany) and benznidazole (Roche, USA) were used as the baseline drugs against which the efficacy of the quinoline alkaloids of *Galipea longiflora* were compared. Each assay was performed in triplicate.

In vivo studies

The mouse footpad infection was used as a model for these experiments (Avila *et al.*, 1990; Coleman *et al.*, 1989).

Female and male BALB/c mice were supplied by the Charles River Breeding Laboratory and were then bred in IBBA (Bolivia). Mice weighed between 18–20 g and were 8 weeks old when bioassays were initiated.

Leishmania amazonensis IFLA/BR/67/PH8 and MHOM/GF/84/CAY-H-142 were used. BALB/c mice ($n=10$ or $n=8$) were infected subcutaneously in the right rear footpad with 1×10^6 amastigotes obtained from donor hamsters. The parasites were delivered in 200 μL phosphate buffered saline (PBS).

The growth of the lesion was determined weekly by measuring the diameter of both rear feet with a direct reading vernier caliper (Kroelin 1ODI 00T6). The size of lesion in millimetres (Index of Leishmaniasis) was calculated by subtracting the measurements obtained from the uninfected foot from that of the infected foot. Measurements commenced 1 day prior to the inoculation of amastigotes and were continued for 8 weeks. For each experiment, the mean and standard error of the mean (SEM) were calculated.

N-methylglucamine antimonate (Glucantime) with a pentavalent antimony (Sb^v) content of 28% by weight was obtained from the Rhône-Poulenc, France and was used as a reference drug.

Two experiments were conducted. Mice in the first experiment were treated by the subcutaneous route. Glucantime was given at a dose of 56 mg Sb^v per kg per day and quinoline alkaloids (2-phenylquinoline and 2-*n*-pentylquinoline) at 100 mg/kg/day. The quinoline alkaloids were dissolved in 40 μL of polysorbate (Tween 80, Prolabo). Glucantime was dissolved in PBS and untreated mice received PBS and Tween 80. Drug treatment commenced 1 day after the inoculation of amastigotes and was continued once daily for 14 days.

In the second experiment, mice were treated directly on the infected rear footpad with a single treatment 14 days after the inoculation of parasites. For this experiment, the mice were treated with Glucantime at a rate of 112 mg Sb^v per kg per day and with the major quinoline alkaloid of *Galipea longiflora*, 2-phenylquinoline at 200 mg/kg/day.

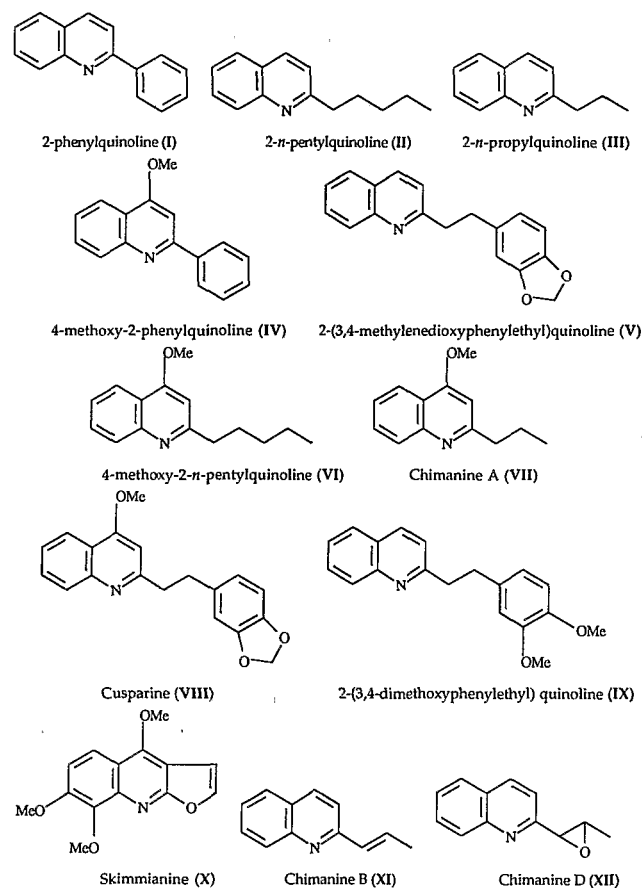


Figure 1. Structures of twelve 2-substituted quinolines isolated from *Galipea longiflora* by activity guided fractionation.

RESULTS AND DISCUSSION

The crude alkaloidal extracts of stem bark, root bark and leaves of *Galipea longiflora* showed an activity on promastigote forms of *Leishmania* ssp. and epimastigote forms of *Trypanosoma cruzi*. The fractionation and purification, monitored by bioassays, led to isolation of 12 active quinoline alkaloids. The new products, the four chimanines (A, B, C and D) were described by Fournet in 1991. The chemical structures of these products are presented in Fig. 1. Chimanine C was not tested against parasites since it was not isolated following the bioassay purification procedure.

The results of the *in vitro* activity of these compounds are shown in Table 1 against five strains of promastigote forms of *Leishmania* species. Three quinoline alkaloids showed activity against all strains of both parasites at 50 µg/mL; 2-*n*-propylquinoline (III), chimanine B (XI) and chimanine D (XII). All these compounds are substituted in position 2 with a propyl chain. We did not notice any different activity against *Leishmania* ssp. and *T. cruzi* or the same type of parasites. All quinoline alkaloids are more potent than Glucantime, but 25–100 times less active than pentamidine against the promastigote forms of *Leishmania* species. It was impossible to confirm *in vitro* activity against the intracellular forms of *Leishmania* either with mouse peritoneal macrophages (Neal and Croft, 1984) or with cell lines U937 (Martinez *et al.*, 1988). This failure seemed to be due to a lack of good technical conditions and effects of altitude (3500 m).

Table 1. Comparison of *in vitro* inhibitory effects (IC₉₀ (µg/mL) on five strains of promastigote forms of *Leishmania* at 48 h after addition of twelve 2-substituted quinolines, pentamidine and N-methylglucamine antimonate

Drug	IC ₉₀ (µg/mL)				
	L.b. ^a (2903)	L.a. ^b (PH 8)	L.a. ^b (H-142)	L.d. ^c (2682)	L.d. ^c (HS70)
2-Phenylquinoline (I)	100	100	100	100	100
2- <i>n</i> -Pentylquinoline (II)	100	100	100	100	100
2- <i>n</i> -Propylquinoline (III)	50	50	50	50	50
4-Methoxy-2-phenylquinoline (IV)	50	50	50	50	50
2-(3,4-Methylenedioxyphenylethyl)quinoline (V)	100	100	100	100	100
4-Methoxy-2- <i>n</i> -pentylquinoline (VI)	100	100	100	100	100
Chimanine A (VII)	100	100	100	100	100
Cusparine (VIII)	100	100	100	100	100
2-(3,4-Dimethoxyphenylethyl)quinoline (IX)	100	100	100	100	100
Skimmianine (X)	100	100	100	100	100
Chimanine B (XI)	25	25	25	25	25
Chimanine D (XII)	25	25	25	25	25
Pentamidine	1	1	1	1	1
N-methylglucamine antimonate	>100	>100	>100	>100	>100

^a *Leishmania braziliensis*,

^b *Leishmania amazonensis*,

^c *Leishmania donovani*

The *in vitro* effects of the quinoline alkaloids, nifurtimox and benznidazole against five strains of epimastigote of *Trypanosoma cruzi* are presented in Table 2. Nine quinoline alkaloids were less active than nifurtimox and benznidazole, and three (2-*n*-propylquinoline (III), chimanine B (XI) and chimanine D (XII) showed a similar activity to the reference drugs.

We chose to determine the *in vivo* activity of two major quinoline alkaloids of the crude alkaloidal extracts of *Galipea longiflora*, in BALB/c mice infected with the strain PH8 or H-142 of *Leishmania amazonensis* but not with the endemic cutaneous strain, *L. braziliensis*. The *in vivo* model of infection with this strain was obtained by coinjection with saliva and parasites isolated from infected sand fly (Samuelson *et al.*, 1991). The major quinoline, 2-phenylquinoline,

Table 2. Comparison of *in vitro* inhibitory effects (IC₉₀ (µg/mL) on five strains of epimastigote forms of *Trypanosoma cruzi* at 48 h after addition of twelve 2-substituted quinolines, benznidazole and nifurtimox

Drug	IC ₉₀ (µg/mL)				
	C8CL1	TeCL2	Tulahuen	1979	CL1 SC43 CL1
2-Phenylquinoline (I)	100	100	100	100	100
2- <i>n</i> -Pentylquinoline (II)	100	100	100	100	100
2- <i>n</i> -Propylquinoline (III)	50	50	50	50	50
4-Methoxy-2-phenylquinoline (IV)	100	100	100	100	100
2-(3,4-Methylenedioxyphenylethyl)quinoline (V)	100	100	100	100	100
4-Methoxy-2- <i>n</i> -pentylquinoline (VI)	100	100	100	100	100
Chimanine A (VII)	100	100	100	100	100
Cusparine (VIII)	100	100	100	100	100
2-(3,4-Dimethoxyphenylethyl)quinoline (IX)	100	100	100	100	100
Skimmianine (X)	100	100	100	100	100
Chimanine B (XI)	25	25	25	25	25
Chimanine D (XII)	50	50	50	50	50
Benznidazole	100	25	50	25	50
Nifurtimox	25	25	25	50	50

composed 67% of the total crude alkaloidal extract of stem bark and 48% of root bark, while 2-*n*-pentylquinoline represents 17% of stem bark. The drugs were daily administered 24 h after parasitic infection at 100 mg/kg for 14 days. The lethal dose killing 50% of mice (LD₅₀) was evaluated to be upward of 400 mg/kg via the intraperitoneal route for each alkaloid. No apparent signs of drug toxicity; weight loss, hair loss or diarrhoea, were observed in any experiment but there was the appearance of a weak inflammatory effect at the site of inoculation. Table 3 shows the results obtained in the first experiment with 2-phenylquinoline (I), 2-*n*-pentylquinoline (II) and Glucantime against the development of lesions in BALB/c mice infected with *Leishmania amazonensis* (PH8) treated with these drugs. After 4 weeks, lesions in mice which had received 2-*n*-pentylquinoline were greater than 0.4 mm with respect to control mice, 1.64 and 1.25 mm respectively. Also mice treated with 2-phenylquinoline (I) had developed smaller lesions (-0.98 mm) than control mice. After 8 weeks, mice treated with 2-phenylquinoline (I), 2-*n*-pentylquinoline (II) and Glucantime had an average lesion size of 5.06, 5.69 and 4.02 mm respectively versus 6.60 mm in the untreated controls.

In the second experiment, the BALB/c mice were infected with the strain H-142 of *Leishmania amazonensis*. The results obtained from this experiment are detailed in Table 4. This strain developed lesions smaller than lesions obtained with the strain PH8 after 8 weeks. During the first week, the lesions increased more rapidly than in the first experiment in all three groups of mice. For the next 7 weeks, the lesions increased more slowly, 0.5 mm in average for each 14 days. During 7 weeks the mice treated with 2-phenylquinoline and with reference drug showed the same development of lesions. Finally, after 8 weeks, mice treated with quinoline alkaloids and mice receiving Glucantime, exhibited identical sizes of lesions, 2.54 and 2.51 mm respectively.

In the third experiment, the BALB/c mice were infected with *Leishmania amazonensis* (PH8) and treated locally with a single dose of 2-phenylquinoline or Glucantime near the site of infection. The results obtained are presented in Table 5. After 6 weeks, the lesion size of mice which received 2-phenylquinoline and the untreated mice did not differ significantly, 2.62 and 2.87 mm respectively. In the last 2 weeks, lesions in control mice increased more rapidly than lesions in

Table 3. Effect of N-methylglucamine antimonate (56 mg of Sb^v per kg per day), 2-phenylquinoline (I) (100 mg/kg/day) and 2-*n*-pentylquinoline (II) (100 mg/kg/day) on the development of *L. amazonensis* PH8 in BALB/c mice (\pm SEM). Treatments were given for 14 days period commencing 1 day after inoculation of *L. amazonensis*

Weeks post-infection	Diameter of lesion ^a			
	Control	Glucantime	2-Phenylquinoline (I)	2- <i>n</i> -Pentylquinoline (II)
2	0.53 (0.14)	0.17 (0.07)	0.67 (0.13)	0.32 (0.06)
4	1.25 (0.16)	0.48 (0.15)	0.97 (0.15)	1.64 (0.15)
6	3.80 (0.41)	2.35 (0.34)	3.52 (0.25)	3.77 (0.22)
8	6.60 (0.43)	4.02 (0.41)	5.06 (0.37)	5.69 (0.35)

^a Average measurement (in mm) for 10 mice and \pm SEM.

Table 4. Effect of N-methylglucamine antimonate (56 mg of Sb^v per kg per day), 2-phenylquinoline (I) (100 mg/kg/day) on the development of *L. amazonensis* H-142 in BALB/c mice (\pm SEM). Treatments were given for 14 days period commencing 1 day after inoculation of *L. amazonensis*

Weeks post-infection	Diameter of lesion ^a		
	Control	Glucantime	2-Phenylquinoline (I)
1	1.07 (0.19)	0.91 (0.17)	0.76 (0.14)
2	1.37 (0.14)	1.32 (0.10)	0.92 (0.15)
4	2.02 (0.22)	1.74 (0.15)	1.54 (0.16)
6	2.85 (0.21)	2.17 (0.24)	2.08 (0.36)
8	3.49 (0.26)	2.51 (0.26)	2.54 (0.21)

^a Average measurement (in mm) for 10 mice and \pm SEM.

mice treated with 2-phenylquinoline or with the reference drug, 2.03, 1.00 and 1.31 mm.

These results explain the antileishmanial activity of stem bark of *Galipea longiflora* Krause, a plant used by the Chimane Indians for the treatment of cutaneous leishmaniasis. The major quinoline alkaloid present in the stem bark and root bark, 2-phenylquinoline (I), did not show a high *in vitro* activity against extracellular forms of *Leishmania* ssp compared with the reference drug, Pentamidine. However, mice infected with *Leishmania amazonensis* (H-142) and treated with 2-phenylquinoline or Glucantime developed an equivalent lesion. This activity of 2-phenylquinoline was not as significant when mice were infected with the strain PH8 of *Leishmania amazonensis*. These results demonstrate the importance of infectivity of strains of *Leishmania amazonensis* which determine different drug responses (Trotter *et al.*, 1980). The other quinoline alkaloid tested, 2-*n*-pentylquinoline did not show any effect when administered in infected BALB/c mice.

In the literature quinolines are reported to have numerous biological activities (Farghaly *et al.*, 1990) molluscicidal (Vieira and Kubo, 1990) as well as antiviral activities (Althaus *et al.*, 1990), and therapeutic applications against malaria (Bryskier and Labro, 1988; Wommarck and Pearson, 1970) and *Pneumocystis carinii* (Queener *et al.*, 1991).

These ethnopharmacological, biological and chemical studies showed that the poultices of stem bark of *Galipea longiflora* are most effective against cutaneous leishmaniasis. This work describes the first example of activity of 2-substituted quinoline against *Leishmania* species.

Table 5. Effect of N-methylglucamine antimonate (112 mg of Sb^v per kg per day), 2-phenylquinoline (I) (200 mg/kg/day) on the development of *L. amazonensis* H-142 in BALB/c mice (\pm SEM). Treatments were given on the infected rear footpad with a single treatment 14 days after the inoculation of *L. amazonensis*

Weeks post-infection	Diameter of lesion ^a		
	Control	Glucantime	2-Phenylquinoline (I)
2	0.35 (0.08)	0.45 (0.05)	0.47 (0.15)
4	1.02 (0.20)	0.53 (0.23)	1.10 (0.18)
6	2.87 (0.29)	1.52 (0.38)	2.62 (0.22)
8	4.90 (0.42)	2.83 (0.58)	3.62 (0.52)

^a Average measurement (in mm) for 8 mice and \pm SEM.

The investigations on the antileishmanial activity of *Galipea longiflora* continue in our laboratory to determine *in vivo* activities of the other quinoline alkaloids isolated towards cutaneous leishmaniasis of the New World (*Leishmania amazonensis* and *L. venezuelensis*) and the parasite of visceral leishmaniasis, *L. donovani*.

Acknowledgements

We express our sincere thanks to Padre Martin Bauer of the Mission Redentorista of Fatima (Department of Beni, Bolivia), to the Chimane Indians who gave essential logistic support and Dr J. A. Kallunki (New York Botanical Garden, NY, USA) for the identification of plant material.

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