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Trypanocidal and Leishmanicidal Properties of Substitution-Containing Chalcones

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Ten chalcones were synthesized and tested as potential leishmanicidal and trypanocidal agents. All tested compounds caused concentration-dependent inhibition of the in vitro growth of *Leishmania braziliensis* and *Trypanosoma cruzi* with no significant toxic effect towards host macrophages. Our results show that the positions of the substituents seem to be critical for their antiprotozoal activities.

Among the kinetoplastid protozoa, which infect invertebrates, mammals, and plants, some species are of particular interest due to their medical importance. These include *Trypanosoma cruzi* (the agent of Chagas' disease), the African trypanosome responsible for sleeping sickness, and several species of *Leishmania*, which cause the various forms of leishmaniasis (9). The World Health Organization has identified Chagas' disease and leishmaniasis as major and increasing public health problems, particularly in Latin America (14, 15, 16, 18). In spite of the socioeconomic importance of these tropical infectious diseases, efforts directed towards the discovery of new drugs and/or vaccines against them are underdeveloped (10, 13). In addition, most of the drugs currently in use (i) were developed several decades ago, (ii) show variable efficacy, (iii) have serious side effects, (iv) are expensive, (v) can require long-term treatment, (vi) may have low activity in immunosuppressed patients, and (vii) present and/or induce resistance in parasites (9, 10, 16). Thus, the need for the development of new, effective, cheap, and safe drugs for the treatment of leishmaniasis and Chagas' disease is very important.

Chalcones, or 1,3-diaryl-2-propen-1-ones, are natural or synthetic compounds belonging to the flavonoid family. Chalcones possess a broad spectrum of biological activities, including antibacterial, anthelmintic, amoebicidal, antiulcer, antiviral, insecticidal, antiprotozoal, anticancer, cytotoxic, and immunosuppressive activities (for reviews, see references 11 and 12). The present study was designed to determine the in vitro leishmanicidal and trypanocidal activities of the 10 substitution-containing chalcones and to investigate the cytotoxic effects of these chalcones on mouse peritoneal macrophages in vitro.

The chalcones used in the present study were synthesized in our laboratory by reaction of the appropriate aryl methyl ketone and aryl aldehyde (in a 1:1 ratio) in the presence of sodium hydroxide and ethanol. The products were then added to cooled diluted acetic acid according to the methodology previously described (8). The synthetic reaction gave substantial yields (55 to 98%) of all the chalcones, and these were characterized by ^{1}H nuclear magnetic resonance and infrared analyses and by microanalysis. The substitution-containing chalcones were dissolved in 0.5% Tween 80 in phosphate-buffered saline to prepare a working solution with a 0.1 M concentration before being passed through 0.22-μm-pore-size Millipore filters. The structures of the chalcones are shown in Table 1.

Cultures of promastigote forms of *Leishmania braziliensis* (strain Lb2904, kindly provided by the Evandro Chagas Institute, Belém, Brazil) and epimastigote forms of *T. cruzi* (strain Y) were grown at 28°C in Schneider's and TC100 media containing 5 and 10% heat-inactivated (56°C for 30 min) fetal bovine serum (FBS), respectively. For the parasite growth inhibition assays, *L. braziliensis* and *T. cruzi* were harvested on days 4 and 5 of the culture, respectively. To assess trypanocidal and leishmanicidal properties, the parasites were washed three times in phosphate-buffered saline by centrifugation at 1,000 × *g* for 10 min at room temperature. The concentration was adjusted to 2×10^6 parasites/ml in TC100 medium plus 10% FBS for *T. cruzi* or in Schneider's medium plus 5% FBS for *L. braziliensis*. One hundred fifty microliters of parasite suspension was added to 96-well plates and incubated at 28°C for 72 h in the presence or absence of the substitution-containing chalcones (3 to 1,000 μM), amphotericin B (10.8 to 1,082.0 nM; used as a control), or benznidazole (10 to 1,000 μM; used as a control). Three to four experiments were carried out in triplicate, and the number of surviving parasites was determined in Neubauer chambers.

The cytotoxic activity of substitution-containing chalcones to mouse peritoneal macrophages was evaluated as previously described (1, 17). To this end, cells were harvested from the peritonea of mice 2 to 3 days after injection of 2 ml of sterile thioglycolate solution (3% [wt/vol] in water). Cytotoxicity (cell viability) was assessed by an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay (17).

The following drugs were used as positive controls in the growth inhibition assays: benznidazole (Rochagan; Roche, São Paulo, Brazil) and amphotericin B (Fungizon; Bristol-Myers Squibb, São Paulo, Brazil).

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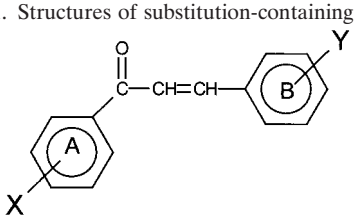
TABLE 1. Structures of substitution-containing chalcones



Chalcone	Ring structure	
	X	Y
C1	4-H	4-H
C2	4-H	4-Cl
C3	4-Cl	4-H
C4	4-Cl	4-Cl
C5	3,4-Cl_2	H
C6	3,4-Cl_2	4-Cl
C7	4-CH_3	4-Cl
C8	4-Br	4-Cl
C9	4-Br	4-H
C10	3,4-Cl_2	4-$N(CH_3)_2$

The data obtained were analyzed by one-way analysis of variance followed by Dunnett's multiple-comparison test. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis of data from individual experiments (GraphPad Software, San Diego, Calif.). The percentages of maximal inhibition (MI) were calculated as follows: MI = {[(number of parasites of vehicle group) − (number of parasites of drug group)]/(number of parasites of vehicle group)} × 100.

The substitution-containing chalcones clearly showed a concentration-dependent inhibitory effect on the in vitro growth of *T. cruzi* epimastigotes. Among the 10 tested chalcones, C1, C2, C3, C8, and C9 demonstrated distinct and potent inhibitory effects on the growth of *T. cruzi*, while the other chalcones inhibited parasite growth at lower but similar levels (Table 2). As observed for *T. cruzi*, substitution-containing chalcones strongly inhibited, in a concentration-dependent manner, the in vitro growth of *L. braziliensis* promastigotes. Chalcones C1 and C2 exhibited the most marked inhibitory effect on the growth of *L. braziliensis*, while the other chalcones (except C3, C5, and C8) inhibited the growth of the parasite to similar extents (Table 2). In a comparison of the mean IC_{50}s, C1 and C2 were about 2- to 13-fold more potent than the other substitution-containing chalcones. However, all chalcones were less potent than the positive control drug, amphotericin B (Table 2). None of the chalcones tested over a concentration range of 10 to 300 μM showed any evidence of cytotoxicity to mouse peritoneal macrophages in vitro as assessed by MTT reduction to formazan. Cytotoxicity to macrophages was observed only at very high chalcone concentrations (≥1,000 μM; results not shown).

The results presented here show that some studied chalcones (Table 1) have a concentration-dependent inhibitory effect on the growth of *L. braziliensis* promastigotes and on *T. cruzi* epimastigotes in vitro. Although leishmanicidal and antimalarial activities have already been reported in the literature for this class of compounds (4, 5, 6; for a review, see reference 12), our results demonstrated that the synthetic chalcones reported in the present study exhibit marked in vitro leishmanicidal and trypanocidal activities. Nevertheless, the mechanisms by which the substitution-containing chalcones showed leishmanicidal and trypanocidal activities were not addressed in this work. Based on the literature, it can be predicted that chalcones could potentially inhibit the activity of fumarate reductase, succinate dehydrogenase, NADH dehydrogenase, or succinate- and NADH-cytochrome *c* reductases in the parasite mitochondria (2, 3, 7, 19, 20). Additional studies are in progress to address this hypothesis. Our results also show that, in vitro, leishmanicidal and trypanocidal concentrations of chalcones showed low cytotoxicity to mouse peritoneal macrophages.

In summary, we show here that some synthesized substitution-containing chalcones, especially C1, exhibited promising leishmanicidal and trypanocidal activities with no evidence of a cytotoxic effect on mouse macrophages. The positions of the substituents seem to be important for the effectiveness of the antiprotozoal activity. C1, which has no substituent groups, revealed both pronounced leishmanicidal and trypanocidal activities.

TABLE 2. Trypanocidal and leishmanicidal activities of substitution-containing chalcones, benznidazole, and amphotericin B on epimastigote and promastigote forms of *T. cruzi* and *L. braziliensis*, respectively

Chalcone or control	Trypanocidal activity		Leishmanicidal activity	
	IC_{50} (μM)[a]	MI (%)	IC_{50} (μM)[a]	MI (%)
C1	24.8 (11.3–54.7)	100	13.7 (9.9–18.9)	100
C2	64.5 (45.9–90.5)	100	21.9 (20.3–23.7)	100
C3	66.7 (47.3–94.1)	94 ± 3	100.5 (76.1–132.8)	100
C4	100.3 (60.0–167.0)	92 ± 5	98.0 (62.6–153.5)	100
C5	82.7 (63.3–108.0)	88 ± 7	182.3 (179.6–185.0)	100
C6	126.4 (73.5–217.4)	93 ± 4	66.7 (43.8–101.6)	100
C7	80.8 (53.3–122.6)	90 ± 6	45.6 (38.1–54.8)	100
C8	65.4 (47.6–89.8)	97 ± 3	129.1 (93.2–178.8)	100
C9	66.6 (44.5–99.8)	100	61.7 (57.7–65.9)	100
C10	89.6 (71.2–112.8)	90 ± 6	57.4 (38.0–86.5)	100
Benznidazole	54.7 (42.8–69.8)	100		
Amphotericin B			0.21 (0.18–0.24)	100

[a] IC_{50} with their respective 95% confidence limits.

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