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Effect of cis-tetrahydro-β-carboline-1(m-nitrophenyl)-3-carbomethoxy on Leishmania amazonensis

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ABSTRACT

Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania,* representing a serious public health problem. The worldwide prevalence is estimated to be 12 million cases, with an incidence of 2 million new cases each year. The available treatments for leishmaniasis exhibit high toxicity and several side effects. Therefore, the development of new antileishmanial drugs has become very important. β-carboline alkaloids are notable because of their wide range of pharmacological properties, including trypanosomatid activity. In the present study, we evaluated the antileishmanial activity of the derivative cis-tetrahydro-β-carboline-1(*m*-nitrophenyl)-3-carbomethoxy (Cis-βCC) against *Leishmania amazonensis.* The alkaloid Cis-βCC inhibited growth of the promastigote and amastigote forms of *L. amazonensis*, with IC_{50} values of 8.7 and 30 μg/mL, respectively. The CC_{50} in a macrophage cell line was 82 μg/mL, demonstrating selectivity for the parasite. Moreover, Cis-βCC reduced the survival index of intracellular amastigotes. Ultrastructural and morphological analysis demonstrated alterations in the number of nuclei and flagella, rounding of the cell body, and alterations in mitochondrial cristae in promastigotes treated with Cis-βCC. These results suggest that Cis-βCC may be useful for the treatment of leishmaniasis, and its activity may be related to alterations in cell division and mitochondrial dysfunction.

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SUBJECT CLASSIFICATION

Parasitic disease, protozoan, treatment

TYPE (METHOD/APPROACH)

Experimental research

1 INTRODUCTION

Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania,* representing a serious public health problem in several developing countries. This disease has a worldwide distribution and has been reported in 88 countries throughout the Americas, Europe, Africa, and Asia. The overall prevalence has been estimated to be 12 million cases, with an incidence of 200,000-400,000 cases of the visceral form and 700,000-1.2 million cases of the cutaneous form reported each year. The population of individuals at risk for the disease is estimated to be 350 million, with 20,000-40,000 deaths attributable to leishmaniasis each year [1, 2]. The parasite presents two evolutive forms in their life cycle: promastigotes (a flagellated form that multiplies in the midgut of the sandfly vector) and amastigotes (obligatory intracellular forms that live inside the vertebrate host's tegument and visceral tissue and invade cells of the mononuclear phagocyte system) [3].

Depending on the parasite species, *Leishmania* infection can cause various disease outcomes in humans, ranging from single self-healing cutaneous lesions to visceral dissemination of the parasite, which may lead to death if not properly treated. Infection with *Leishmania (Leishmania) amazonensis* causes different forms of American cutaneous leishmaniasis, including localized cutaneous leishmaniasis, anergic diffuse cutaneous leishmaniasis, and borderline disseminated cutaneous leishmaniasis, which is a newly recognized intermediate form of the disease [4]. However, this species is a less frequent etiologic agent than the species *Leishmania (Viannia) braziliensis* and *Leishmania (Viannia) guyanensis* because its main sandfly vector, *Lutzomyia flaviscutellata*, presents nocturnal activity and low anthropophily [5-7].

The currently available treatments for leishmaniasis include the pentavalent antimonial compounds amphotericin B desoxycholate and liposomal amphotericin B, but they are expensive, present several side effects, need to be administered intravenously or intramuscularly, and are not entirely effective [8]. The development of new antileishmanial drugs has become very important. β-carboline alkaloids are known for their potent psychoactive and hallucinogenic properties. However, they have also been shown to exert a wide range of pharmacological effects, including anti-trypanosomal activity [9-11] and antileishmanial activity toward parasites of the species *Leishmania infantum* [12]. We recently reported that β-carboline compounds can present activity against *L. amazonensis* [13, 14]. In the present study, we investigated the antileishmanial activity of the derivative cis-tetrahydro-β-carboline-1(*m*-nitrophenyl)-3-carbomethoxy (Cis-βCC) against *L. amazonensis*.

2. MATERIAL AND METHODS

2.1. Chemicals

L-tryptophan (98.5%) was obtained from Vetec. Vanillin (99%) was obtained from Acros Organic. Glacial acetic acid (99.8%) was obtained from Merck. Methanol (99.9%) was obtained from Aldrich. Sulfuric acid (95-98%) was obtained from Dinâmica. Ammonium hydroxide, sodium bicarbonate (99.5%), ethyl acetate, and anhydrous sodium sulfate were obtained from Synth.

2.2. Cis-tetrahydro-β-carboline-1(m-nitrophenyl)-3-carbomethoxy (Cis-βCC) synthesis

The synthesis of Cis-βCC was performed through a condensation reaction of L-tryptophan amino acid (5 mmol) with vanillin (1.1 equivalents). The mixture was submitted to reflux in glacial acetic acid (20 mL) for approximately 2 h. The pH was then adjusted to 5.0 with ammonium hydroxide, and the obtained precipitate was washed with distilled water and filtered with a Buchner filter. The precipitate was dissolved in methanol (10 mL), and 1 mL sulfuric acid was added. The solution was submitted to reflux and agitation for approximately 48 h. After methanol evaporation, the product was neutralized with 10% aqueous sodium bicarbonate solution. The organic phase was extracted with ethyl acetate (3× 10 mL) and dried with anhydrous sodium sulfate. After filtration, the solvent was removed by rotaevaporation.

2.3. Parasites and cells

The promastigote forms of *Leishmania amazonensis* (WHOM/BR/75/Josefa), originally isolated from a human case of diffuse cutaneous leishmaniasis, were cultured at 25°C in Warren's medium (brain-heart infusion plus haemin and folic acid) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco Invitrogen, New York, NY, USA) in a tissue flask. Axenic amastigote cultures were obtained by the *in vitro* transformation of infective promastigotes with a progressive increase in temperature and decrease in pH [15] and cultured at 32°C in Schneider's insect medium (Sigma, St. Louis, MO, USA), pH 4.6, with 20% FBS. J774G8 murine macrophages were maintained in tissue flasks with RPMI 1640

medium (Gibco Invitrogen, New York, NY, USA), pH 7.6, sodium bicarbonate, and L-glutamine and supplemented with 10% FBS at 37°C in a 5% CO_2–air mixture.

2.4. *In vitro* antileishmanial activity against promastigote and axenic amastigote forms

Promastigote forms (10^6 parasites/mL) were incubated in Warren's culture medium (brain-heart infusion plus hemin and folic acid) in the presence or absence of various concentrations of Cis-βCC supplemented with 10% FBS (Gibco Invitrogen, New York, NY, USA) in 24-well culture microplates and incubated at 25°C for 72 h. Axenic amastigote forms (10^6 parasites/mL) were treated with different concentrations of Cis-βCC in 12-well culture microplates and incubated at 32°C for 72 h. The activity of Cis-βCC was evaluated by cell counting using a Neubauer hemocytometer, and the results are expressed as the log number of cells per milliliter after a 72 h incubation period.

2.5. Activity against intracellular amastigotes in infected macrophages

J774G8 mononuclear cells (5×10^5) in 0.5 mL of RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), were placed on coverslips and incubated at 37ºC in a 5% CO_2-air mixture. Non-adherent cells were removed after 24 h by washing with RPMI medium. The macrophage monolayer was infected with promastigote forms (5×10^6 cells/mL) suspended in RPMI medium without FBS and incubated for 90 min in the presence of a 5% CO_2-air mixture. After incubation, the cell monolayer was washed with RPMI 1640 medium to remove non-interiorized parasites. Afterward, the infected macrophages were treated with 10 and 50 μg/mL of Cis-βCC for 24 h. The monolayers were then fixed with Bouin solution and stained with 10% Giemsa. The percentage of macrophages that contained intracellular parasites was analyzed microscopically at 1,000× magnification by examining at least 200 cells.

2.6. Cytotoxicity assay

A suspension of 5×10^5 J774G8 cells in RPMI 1640 medium supplemented with 10% FBS was added to each well in 96-well microplates. The macrophage monolayer was then incubated for 48 h in the presence of increasing concentrations of Cis-βCC. The monolayer was then fixed, washed, and stained using the sulforhodamine method. Control cells without Cis-βCC were included. The macrophage monolayer was fixed with 50 µl/well of 10% trichloroacetic acid at 4°C for 1 h. The well plates were washed with water, and 50 µl sulforhodamine B (0.4% w/v) in 1% acetic acid solution was added to each well. The microplate was then maintained at 4°C for 30 min and washed five times with 1% acetic acid to remove sulforhodamine B. Afterward, 150 µl of 10 mM unbuffered Tris-base solution (Sigma) was added to each well. The absorbance of each individual well was read in a plate reader (BIO-TEK Power Wave XS) at 530 nm. Dose-response curves were plotted. The values are expressed as a percentage of control optical density. The 50% cytotoxicity concentration (CC_{50}) was estimated by regression analysis.

2.7. Morphological and ultrastructural analysis

Morphological analysis was performed using scanning electron microscopy (SEM), and ultrastructural analysis was performed using transmission electron microscopy (TEM). Promastigotes (10^6 cells/mL) treated with 50 and 100 μg/mL Cis-βCC or medium alone for 72 h at 25ºC were washed in PBS and fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). For SEM, after fixation, the cells were placed on a specimen support with poly-L-lysine and washed in cacodylate buffer. The samples were then dehydrated in graded ethanol, critical-point-dried in CO_2, coated with gold, and observed in a Shimadzu SS-550 SEM. For ultrastructural analysis, the parasites were washed with 0.1 M sodium cacodylate buffer and postfixed with osmium tetroxide for 60 min. After dehydration in acetone, the material was incubated in an acetone-epon mixture for 24 h at room temperature and then transferred to pure Epon at 60ºC for 72 h. Ultrathin sections obtained in an ultramicrotome were stained with uranyl acetate and lead citrate and examined in a Zeiss 900 transmission electron microscope.

3 RESULTS AND DISCUSSION

Several disadvantages associated with the current treatments for leishmaniasis have led to the need to study new compounds and strategies to improve the treatment of this disease. We evaluated the antileishmanial activity of the β-carboline compound Cis-βCC against different evolutive forms of *L. amazonensis*.

Cis-βCC inhibited the proliferation of the promastigote and amastigote forms of *L. amazonensis*, presenting IC_{50} values of 8.7 μg/mL and 30 μg/mL, respectively, after 72 h of treatment (Fig. 2). The cytotoxicity of this compound for mammalian cells was evaluated by treating J774G8 macrophages with several concentrations of Cis-βCC. A CC_{50} value of 82 μg/mL was observed (data not shown), which resulted in a selectivity index (SI) of 9.4 and 2.7 for promastigotes and amastigotes of *L. amazonensis*, respectively.

The activity of Cis-βCC against intracellular amastigotes internalized by intraperitoneal macrophages was evaluated. The survival index markedly decreased in treated cultures (30.4% and 14.9% for 10 and 50 μg/mL concentrations, respectively; Fig. 3). We also observed an increase in the number of parasite nuclei, in which 26.3% of the treated promastigotes presented two or more nuclei. In control cells, this rate was of 2%, suggesting that Cis-βCC can interfere with cellular division (Fig. 4.A). Similarly, Monte Neto et al. [16] demonstrated that a lignin, yanyambin, altered nuclei numbers in treated *Leishmania* promastigotes, suggesting that this lignin interferes with the process of cell division, having not only cytotoxic but also cytostatic effects.

The ultrastructural alterations observed in the promastigote forms treated with Cis-βCC were analyzed by SEM and TEM. The SEM analysis showed that the compound induced the rounding of cell bodies and altered flagella, reflected by a

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reduction of the number of flagella, their absence, or multiple flagella (Fig. 5). The increase in the number of nuclei, observed by optical microscopy, was confirmed by TEM (Fig. 4Bb). Consistent with our findings, Pedroso et al. [14] reported that *N*-benzyl-1-(4-methoxy)phenyl-9H-β-carboline-3-carboxamide caused a rounding of the cell body and an increase in the number of flagella in promastigote forms of *L. amazonensis*, suggesting that alterations in cell division occurred. Moreover, Boursereau and Coldham [17] demonstrated that the β-carboline ring system of 1-amino β-carbolines can intercalate in the base pairs of DNA, which could contribute to biological activity, mainly in cancer cells.

The TEM analysis in the present study also verified alterations in mitochondrial cristae, indicating damage of this organelle (Fig. 4Bc). Volpato et al. [13] demonstrated that the β-carboline compound *N*-butyl-1-(4-dimethylamino)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxamide had considerable antileishmanial activity associated with mitochondrial dysfunction, reflected by depolarization of the mitochondrial membrane and an increase in the formation of mitochondrial superoxide anions, followed by an apoptotic process, indicated by a loss of cell membrane integrity.

In conclusion, the present results demonstrated that Cis-βCC may be a potential new drug for the treatment of leishmaniasis, suggesting that its mechanism of action could be related to alterations in cell division and mitochondrial dysfunction.

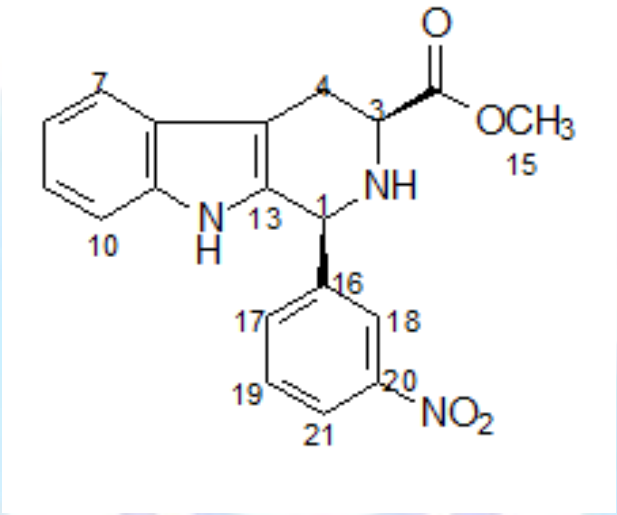

Figure 1. Structure of Cis-tetrahydro-β-carboline-1(*m*-nitrophenyl)-3-carbomethoxy.

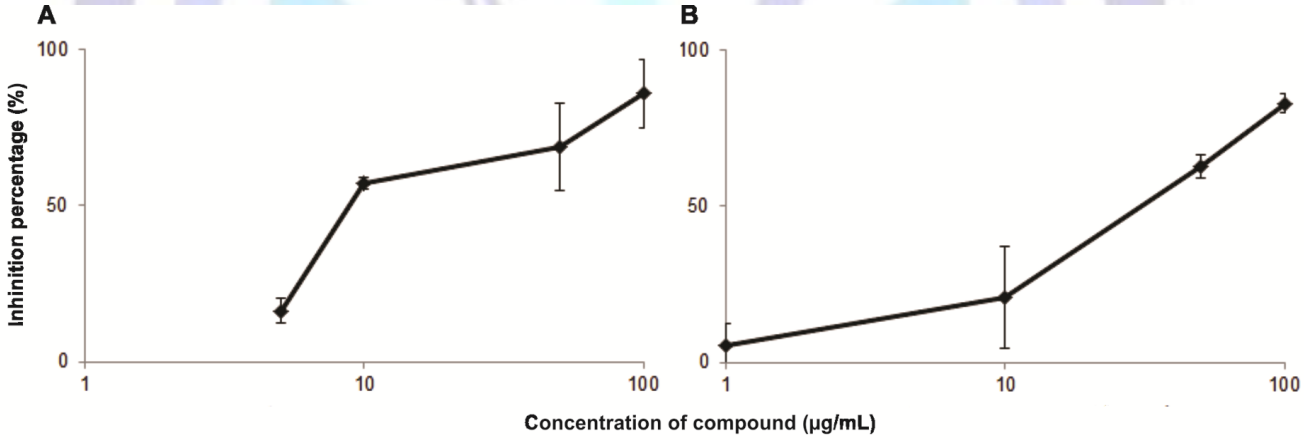

Figure 2. Antileishmanial activity of cis-tetrahydro-β-carboline-1(*m*-nitrophenyl)-3-carbomethoxy against promastigotes (A) and amastigotes (B) of *Leishmania amazonensis* after 72 h of treatment.

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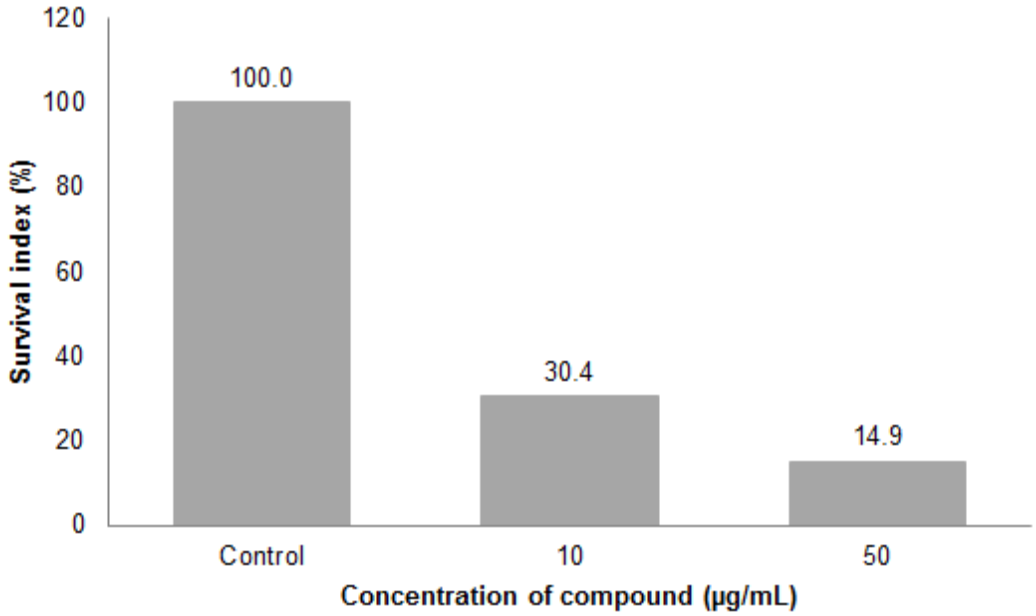

Figure 3. Survival index of amastigote forms internalized in J774G8 macrophages after treatment with cis-tetrahydro-β-carboline-1(*m*-nitrophenyl)-3-carbomethoxy for 24 h.

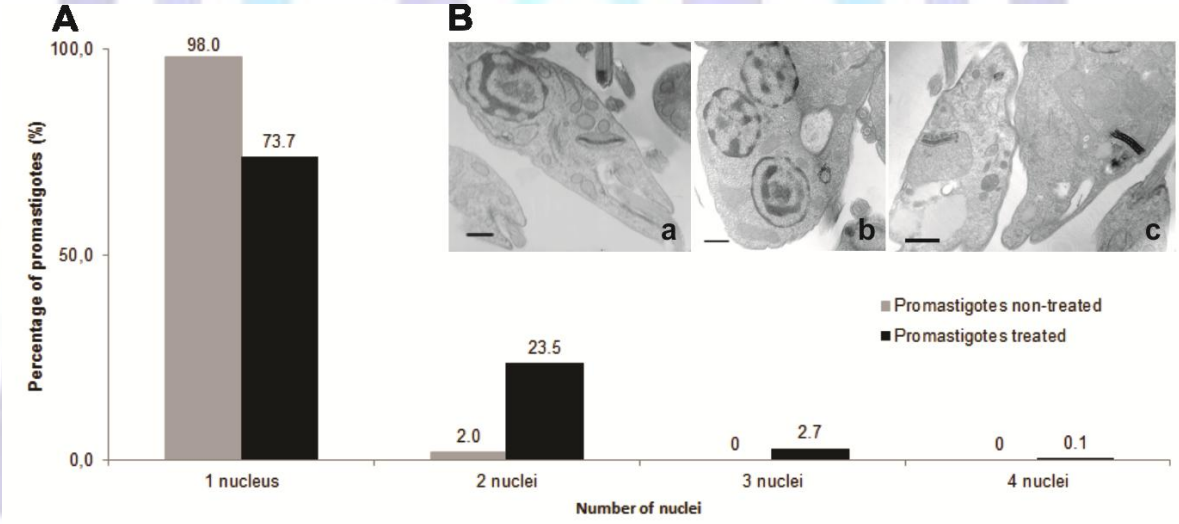

Figure 4. (A) Number of nuclei in promastigote forms of *Leishmania amazonensis* treated or not treated with 15 µg/mL cis-tetrahydro-β-carboline-1(*m*-nitrophenyl)-3-carbomethoxy (Cis-βCC). (B) Ultrastructural effects in promastigote forms treated with 8.7 µg/mL (IC_{50}) of Cis-βCC. (a) Control parasites. (b, c) Cis-βCC-treated parasites. Scale bars = 1 µm.

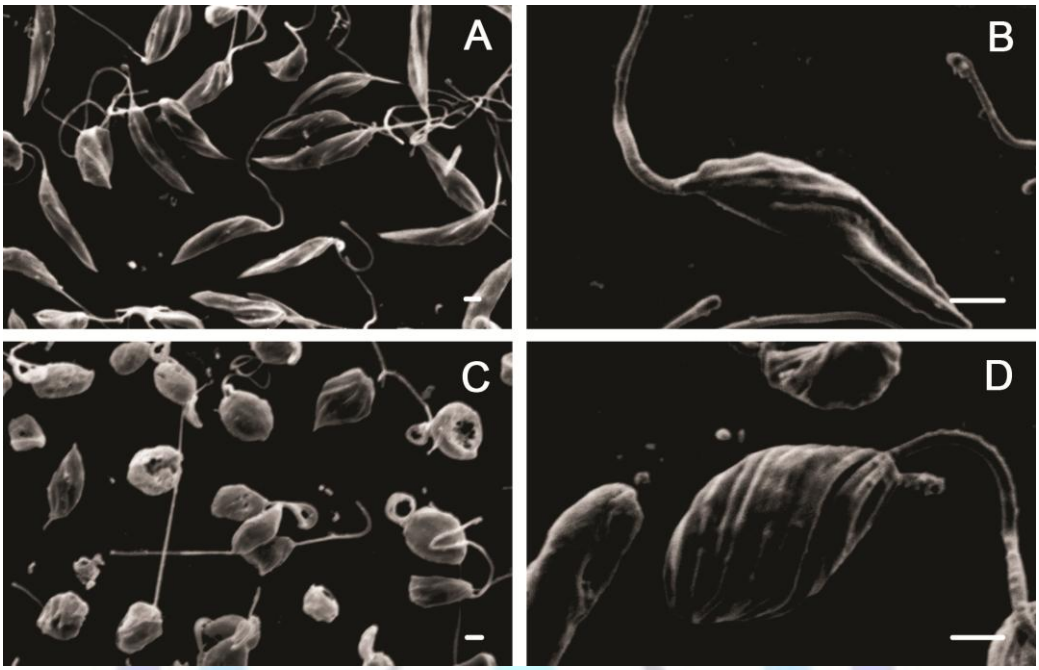

Figure 5. Scanning electron micrography of promastigote forms of *Leishmania amazonensis* not treated (A, B) and treated (C, D) with cis-tetrahydro-β-carboline-1(*m*-nitrophenyl)-3-carbomethoxy for 72 h

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REFERENCES

[1] Alvar, J., Vélez, I.D., Bern, C., Herrero, M., Desjeux, P., Cano, J., Jannin, J., Den Boer, M., the WHO Leishmaniasis Control Team. 2012. Leishmaniasis worldwide and global estimates of its incidence. Plos One. 7 (May 2012), e35671.

[2] Alvar, J., Yactayo, S., Bern, C. 2006. Leishmaniasis and poverty. Trends Parasitol. 2 (Dec. 2006), 552-557.

[3] Kima, P.E. 2007. The amastigote forms of Leishmania are experts at exploiting host cell processes to establish infection and persist. Int J Parasitol. 37 (Aug. 2007) 1087-1096.

[4] Silveira, F. T., Laison, R., Corbett, C. E. P. 2005. Further observations on clinical, histopathological, and immunological features on borderline disseminated cutaneous leishmaniasis caused by *Leishmania (Leishmania) amazonensis*. Mem Inst Oswaldo Cruz. 100 (Aug. 2005), 525-534.

[5] Shaw, J.J., Laison, R. 1987. Ecology and epidemiology: New World. In The Leishmaniases in Bilogy and Medicine. 1, Academic Press, London, 291-363.

[6] Laison, R., Shaw, J. J. 1979. The role of animals in the epidemiology of South American Leishmaniasis. In Biology of the Kinetoplastidae. 2. Academic Press, London, 1-116.

[7] Shaw, J. J., Laison, R., Ward, R. 1972. Leishmaniasis in Brazil: VII. Further observations on the feeding habitats of *Lutzomyia flaviscutellata* (Mangabeira) with particular reference to its biting habits at different heights. Trans R Soc Trop Med Hyg. 66, 718-723.

[8] Murray, H. W., 2000. Treatment of visceral leishmaniasis (kala-azar): a decade of progress and future approaches. Int. J. Infect. Dis. 4, 158-177.

[9] Costa, E. V., Pinheiro, M. L., de Souza, A. D., Barison, A., Campos, F. R., Valdez, R. H., Ueda-Nakamura, T., Filho, B. P., Nakamura, C. V. 2011. Trypanocidal activity of oxoaporphine and pyrimidine-β-carboline alkaloids from the branches of Annona foetida Mart. (Annonaceae). Molecules. 16 (Nov. 2011), 9714-20.

[10] Tonin, L. T., Barbosa, V. A, Bocca, C. C., Ramos, E. R., Nakamura, C. V., da Costa, W. F., Basso, E. A., Nakamura, T. U., Sarragiotto, M. H. 2009. Comparative study of the trypanocidal activity of the methyl 1-nitrophenyl-1,2,3,4-9H-tetrahydro-beta-carboline-3-carboxylate derivatives and benzonidazole using theoretical calculations and cyclic voltammetry. Eur J Med Chem. 44 (Apr. 2009), 1745-1750.

[11] Valdez, R. H., Tonin, L.T., Ueda-Nakamura, T., Silva, S. O., Dias Filho, B. P., Kaneshima, E. N., Yamada-Ogatta, S. F., Yamauchi, L. M., Sarragiotto, M. H., Nakamura, C. V. 2012. *In vitro* and *in vivo* trypanocidal synergistic activity of N-

butyl-1-(4-dimethylamino)phenyl-1,2,3,4-tetrahydro-β-carboline-3-carboxamide associated with benzonidazole. Antimicrob Agents Chemother. 56 (Jan. 2012), 507-512.

[12] Giorgio, C. D., Delmas, F., Olliver, E., Elias, R., Balansard, G., Timon-David, P. 2004. *In vitro* activity of the beta-carboline alkaloids harmane, harmine, and harmaline toward parasites of the species *Leishmania infantum.* Exp Parasitol. 106 (Mar-Apr. 2004), 67-74.

[13] Volpato, H., Desoti, V.C., Cogo, J., Panice, M.R., Sarragiotto, M.H., Silva, S.O., Ueda-Nakamura, T., Nakamura, C.V. 2013. The effects of N-Butyl-1-(4-dimethylamino)phenyl-1,2,3,4- tetrahydro-β-carboline-3-carboxamide against *Leishmania amazonensis* are mediated by mitochondrial dysfunction. Evid Based Complement Alternat Med. (May. 2013).

[14] Pedroso, R. B., Tonin, L. T. D., Ueda-Nakamura, T., Dias Filho, B. P., Sarragiotto, M. H., Nakamura, C. V. 2011. Beta-carboline-3-carboxamide derivatives as promising antileishmanial agents. Ann Trop Med Parasitol. 105 (Dec. 2011), 549-577.

[15] Ueda-Nakamura T, Attias T, de Souza W (2001) Megasome biogenesis in *Leishmania[amazonensis*: a morphometric and cytochemical study. Parasitol Res 87 (Feb. 2001), 89-97.

[16] Monte Neto, R. L., Sousa, M. A., Dias, C. S., Barbosa Filho, J. M., Oliveira, M. R., Figueiredo, R. C. B. Q. 2011. Morphological and physiological changes in Leishmania promastigotes induced by yangambin, a lignan obtained from *Ocotea duckei*. 127 (Aug. 2011), 517-521.

[17] Boursereau, Y., Coldham, I. 2004. Synthesis and biological studies of 1-amino b-carbolines. Bioorg Med Chem Let. 14 (Oct. 2004), 5841–5844.

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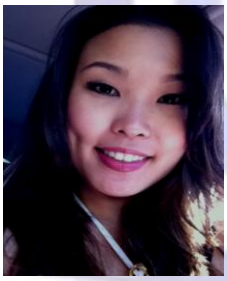

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