Biological activity evaluation of dibenzilbutirolactones lignans derivatives against *Leishmania braziliensis*

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Resumo

Este trabalho apresenta os resultados de ensaios *in vitro* contra formas extracelulares de *Leishmania (viannia) braziliensis* de onze derivados de dibenzilbutirolactonas isolados de plantas ou obtidos através de síntese. Destes, só dois mostraram atividade biológica relativa contra o parasita, as misturas racêmicas de methilpluviatolide,: IC50 = 496 M e (-) -6,6' - dinitrocubebin: IC50 = 510,4 M. Assim, pode-se sugerir que o caminho metabólico responsável para a atividade biológica destas combinações contra estes gêneros de parasita difere do relacionado a *Trypanosoma cruzi* para o qual estas combinações foram bastante ativas. Este fato também sugere fortemente que essa classe de combinações é mais seletivo contra *T. cruzi*. Dessa forma, deveriam ser obtidos outros derivados de lignanas para permitir a completa avaliação desta classe de substâncias contra Leishmaniose.

Abstract

This work reports the results of the *in vitro* assay against extracellular forms of *Leishmania (viannia)* braziliensis of eleven dibenzylbutyrolactone derivatives, either isolated from plants or obtained by synthesis. From these, only two showed relative biological activity against the parasite, the raceme mixtures of methylpluviatolide: IC_{50} = 496 mM and (-)-6,6'- dinitrocubebin: IC_{50} = 510,4 μM. Thus, it can be suggested that the metabolic pathway responsible for the biological activity of these compounds against this parasite genera differs from the one related to *Trypanosoma cruzi*, for which these compounds were quite active. This fact highly also suggests that this class of compounds is more selective against *T. cruzi*. Nevertheles, other lignans derivatives should be obtained to allow the fully evaluation of this class of lignans against Leishmaniosis.

Leishmaniosis is an infection caused by a protozoan of the *Leishmania* gender that presents a high social and economic impact on the world [4]. According to the World Health Organization, 88 countries are now affected by this disease, accounting for 12 million infected people plus around 350 million people who are exposed to the risk of contracting this disease [10]. The annual rate incidence of new cases is approximately 2 million, from which 1.5 million are counted for coetaneous Leishmaniosis and 500 thousand for Visceral Leishmaniosis [6]. Although, those numbers are underestimated, once Leishmaniosis is one of the six most important parasitoses in the world [3].

Leishmania (Viannia) braziliensis, ethyologic agent of American tegumentar Leishmaniosis is a digenetic protozoan whose cycle is accomplished in two hosts, being the vertebrate host the most important one with respect to the dispersion of the disease, since it includes a diversity of mammal groups like: rodents, marsupials, canines and primates, including the man. Transmitted in Brazil by females of the Diptera order, of *Lutzomyia genus, Leishmania* is considered an important zoonosis that imposes, as its main pathogenic characteristic, an infection that varies from benign cases, which can be presented as a located cutaneous lesion, to cases of facial disfiguring in infected individuals. Frequently, these cutaneous-mucous lesions can become more virulent reaching deeper tissues, leading several times the infected individuals to death.

American Tegumentar Leishmaniosis treatment was introduced in 1912 by the Brazilian physician Gaspar Vianna, with the use of antimmonial emetic tartar - drug that was used in therapeutics for many years. Today the pentavalent antimonies are the drugs in use for the Leishmaniasis treatment, being effective in 80% of the cases. Although, it displays some problems, such as toxicity, the need of parenteral administration and difficulty in establishing variable amounts of antimony in the used solution. In addition, the Amphotericin B, medication used in the second treatment line is highly toxic with low tolerance, leading sometimes to parasitic resistance [8].

Due to the arisen of parasitic resistance to drugs, as well as high drug toxicity, several researches have been investigating the possibility of developing alternative drugs holding lower toxicity, mainly from natural origin including vegetal and animal. Also, it should be considered the

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synthetic compounds bearing activity for other diseases, such as the Chagas one.

In this regard, cubebin was initially isolated from the crude hexane extract of the leaves of *Zanthoxylum naranjillo* Griseb by Bastos et *al* [2] and was evaluated for analgesic and anti-inflammatory activities [1]. This compound belongs to the dibenzylbutyrolactone lignan group [2], which is widely distributed in the plant kingdom [5] and has been investigated by researchers from different fields of expertise, leading to the isolation of several compounds holding interesting biological activities.

The aim of this work was to evaluate the leishmanicidal activity of fourteen derivatives of dibenzilbutirolactones, obtained by total and partial-synthesis, against promastigotes forms of *Leishmania (Viannia) braziliensis*.

The obtained results show that compounds 9 and 14 were more active against the promastigotes forms of the parasite. Despite of the lower biological activity showed by the tested compounds against this parasite, it still have potential. Also, it was difficult to establish the correlation between the structure of the tested compounds and its biological activities in this protocol for *Leishmania* On the other hand, Bastos et *al* [1] could find a correlation between chemical structure and trypanocidal activity for this class of compounds, for which the (-)-metilpluviatolide showed a high selectivity activity against *Trypanosoma cruzi*.

Therefore, other dibenzylbutyrolactone derivatives should be obtained to allow a fully investigation of the potential of this class of compound against this parasite. Likewise, the enantiomeric isomers obtained by synthesis should be separated, to allow a proper establishment of the activity. Nevertheless, it is clear that the metabolic pathways of *Leishmania* regarding the activity of these compounds is different from the one in *Trypanosoma cruzi*, once the tested compounds showed a much lower activity for *Leishmania*. On the other hand, it suggests a good selectivity for *T. cruzi*, which is quite interesting.

Table 1. Results of the evaluation of dibenzylbutyrolactone derivatives compounds against promastigote forms of *Leishmania brasiliensis*

Compound	**Concentration (µM) x % de lyses (± SD)**			**IC50 (µM)**
	8,0	**32,0**	**128,0**	
7	16.7 ± 7.0	16.7 ± 7.0	24.6 ± 9.5	38,066.0
8	5.0 ± 3.8	7.8 ± 3.1	12.2 ± 8.1	36,503.0
9	6.9 ± 5.7	8.2 ± 1.0	25.8 ± 4.5	496.0
10	8.9 ± 0.5	11.2 ± 5.0	14.0 ± 6.5	3.9 x 109
11	4.5 ± 5.8	22.8 ± 4.0	23.2 ± 3.9	1,636.0
12	5.9 ± 5.1	17.7 ± 8.3	18.3 ± 2.2	9,607.0
13	5.7 ± 1.0	9.6 ± 3.4	13.6 ± 5.2	4.0 x 107
14	10.8 ± 3.5	19.5 ± 1.7	33.1 ± 4.7	510.4
15	2.5 ± 0.2	7.8 ± 1.8	13.6 ± 1.9	3,567.0
16	9.3 ± 3.1	13.1 ± 4.2	14.9 ± 3.2	1.6 x 106
17	11.0 ± 6.0	11.0 ± 5.6	11.7 ± 1.5	1.2 x 1029

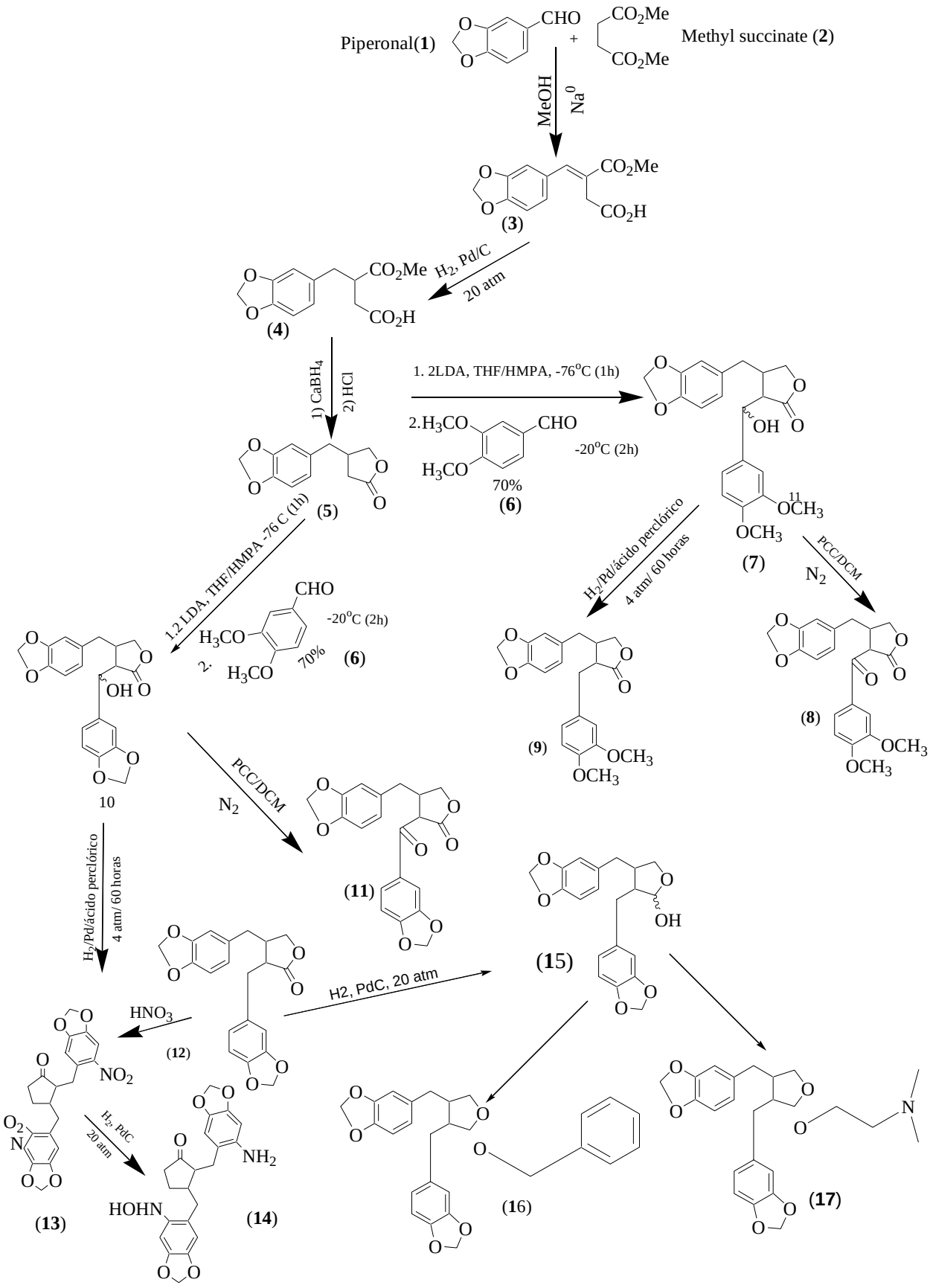
Positive control -amphotericin B (IC_{50}= 31.58 µM)

Negative control - parasites treated with 1% of DMSO solution

Material and Methods

Preparation of dibenzylbutyrolactone derivatives

Synthesis of compounds (7-17): The acid 4-(3',4'-methylenedioxifenil)-3-methoxicarbonyl-3-butenoic (3), acid 4-(3',4'-methylenedioxifenil)-3-methoxicarbonil-3-butanoic (4) preparation was previously described by Landais et al, 1999. Lactone (5), the raceme methylpluviatolide (9) and its intermediates compounds (7) and (8) are displayed in Scheme 1. The Scheme 1 displays the undertaken reactions for obtaining hinokinin and its derivatives by total synthesis, by changing its chemical structure: Compounds (10), (11) and (12) were synthesized according Landais et al. 7. The compound (13) and (14) were prepared by reduction of (-)-6,6'-dinitrohinokinin by using H_2, PdC in 20 atm (Scheme 1). Cubebin (15) derivatives (16 and 17) were synthesized using benzyl bromide and dimethylethylamine chloride in NaH/THF and in sodium ethoxide, respectively.



Scheme 1. Synthesis of dibenzylbutyrolactone derivatives

Drugs and reagents: Gentian violet (Merck, Co.), Amphotericin B (Sigma, Saint Louis, MO), Saline (NaCl 0.9% solution - Glicolabor, Ribeirão Preto, SP), DMSO (Merck, Co.)

Biological assays Strain of Leishmania brasiliensis: The compounds were tested against *Leishmania (Viannia)* brasiliensis, strain that was obtained from a lesion of a sick woman from The Clinic Hospital of Ribeirão Preto, Brazil.

"**In vitro assay":** For the in vitro assay, the axenic culture medium Liver Infusion Tryptose (plus 30% of calf newborn serum) was used. Amastigotes forms obtained of infected mouse were inoculated in the culture medium (10^6 parasites/ml). After 6 days of initial inoculation (exponential growth period) promatisgote forms were obtained. The compounds were added in different concentrations 8, 32 and 128 µM, in microtiter plate (96 wells) and it was incubated for 24 hours at 22°C along with the parasites and culture medium. The determination of the biological activity was quantitatively obtained by colorimetric MTT method[9].

Amphotericin B was used as positive control and dimethylsulfoxide at 1% in 0.85% of sodium chloride solution as negative control. The positive and negative controls were performed using the same dilutions used for tested compounds. Both the *in vitro* percentage of parasitic lysis and the inhibition coefficient of 50% (IC_{50}) were carried out.

References

[1]Bastos, J.K.; Albuquerque, S.; Silva, M.L.A. Evaluation of the trypanocidal activity of lignans isolated from the leaves of *Zanthoxylum naranjillo*. *Planta Med.*, v.65, p.541-544, 1999.

[2]Bastos, J.K.; Gottlieb, O.R.; Sarti, J.S.; Santos Filho, D. Isolation of lignans and sesquiterpenoids from leaves of *Zanthoxylum naranjillo. Nat. Prod. Lett.*, v.9, p.65-70, 1996.

[3]Berman, J.D. *Human leishmaniasis:* clinical, diagnostic, and chemoterapeutic developments in the last 10 years. *Clin. Infec. Dis*., Chicago, v.24, p.684-703, 1997.

[4]Carvalho, P.B.; Arribas, M.A.G.; Ferreira, E.I. Leishmaniasis. What do we know about its chemotherapy ? *Brazilian Journal of Pharmaceutical Sciences*, v.36, n.1, 2000.

[5]Kato, Y.; Munakata. Dibenzylbutirolactones: In: Rao C.B.C. (Ed.) *Chemistry of lignans*, University Press, Andhra Pradesh, Índia, 95, 1978.

[6]Kayser, O.; Kiderlen, F.A. In vitro leishmanicidal activity of naturally occurring chalcones. *Phytotherapy Research*, v.15, p.148-152, 2001.

[7]Landais, Y.; Robin, J.; Lebrun, A. *Tetrahedron*, 47, 3787, 1991.

[8]Marsden, P.D. Mucosal leishmaniasis ("espundia", ESCOMEL, 1911). Trans. Royal Soc. *Trop. Med. Hyg*., v.80, p.859-76, 1985.

[9]Muelas-Serrano, S.; Nogal-Ruiz, J.J.; Gómez-Barrio, A. Setting a Colorimetric method to determine the viability of *Trypanosoma cruzi* epimastigotes. *Parasitol Res.*, v.86, p.999-1002, 2000.

[10]WHO Leishmaniasis. In: *Tropical Disease Research*. Thirteenth Programme Report. Geneva: p.100-111, 1997.

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