***In Vitro* Antiproliferative Effects of Several Diaryl Derivatives on *Leishmania* spp.**

Luciana Vignólio ALVES, Marilene Marcuzzo DO CANTO-CAVALHEIRO, Lea CYSNE-FINKELSTEIN, and Leonor LEON*

Department of Immunology, Oswaldo Cruz, Institute, FIOCRUZ; Av. Brasil, 4365-Manguinhos-CEP 21045-900-Rio de Janeiro, Brasil. Received September 17, 2002; accepted January 15, 2003

In a previous works searching for new drugs with high efficiency, we reported the *in vitro* and *in vivo* antileishmanial activity of a series of diarylheptanoid structurally related to curcumin against *L. amazonensis*. This work describes the *in vitro* antileishmanial activity of a new series of diarylheptanoids and diarylpentanoids derivatives. These drugs were assayed against *Leishmania amazonensis*, *L. braziliensis* and *L. chagasi* promastigotes containing a high percentage of metacyclic forms and the axenic amastigote form of *L. amazonensis* and using Pentamidine Isethionate as reference drug. Parasites in the log late phase culture were incubated with several concentrations of the drugs solubilized in dimethyl sulphoxide (DMSO) and then counted in a Neubauer's chamber. Controls without the drugs and with DMSO were done in parallel. The results showed that all diarylheptanoids and diarylpentanoids had a very good antileishmanial activity.

Key words diarylheptanoid; diarylpentanoid; antileishmanial activity; promastigote; axenic amastigote; *in vitro* analysis

Leishmaniasis is an infection caused by protozoa of the genus *Leishmania* presenting several forms of the disease such as cutaneous (CL), mucocutaneous (MCL) and visceral leishmaniasis (VL), which can be fatal when untreated. The disease is endemic in some tropical areas of the world and in underdeveloped countries, directly affecting about 2 million people per annum, with approximately 350 million people under the risk of contracting the disease worldwide.^{1,2)} The incidence for new cases of leishmaniasis is increasing daily and currently is emerging as a common and serious opportunistic infection in human immunodeficiency virus (HIV)-infected patients.³⁾ The classical treatment is up to now based on the pentavalent antimonials, as sodium stibogluconate (Pentostam), meglumine antimoniate (Glucantime). The long course of treatment allows high levels of the drug to accumulate in the tissues, namely the liver and spleen. The lengthy treatment with Sb^v often causes side effects such as myalgia, pancreatitis, cardiac arrhythmia and hepatitis, leading to the reduction or cessation of treatment. Pentamidine Isethionate, an aromatic diamidine have been used as a second line drug for antimony-resistant cases, but the toxicity has always been a limitation on use with reports of hypoglycemia, diabetes, nephrotoxicity, and pain at site of injection.^{1,2)} The development of new leishmanicidal agents is extremely important, considering the high toxicity of the clinical drugs and in some cases a non completely efficiency. In this work was analyzed the effectiveness of several diaryl (pentanoids and heptanoids) derivatives against promastigote forms of different *Leishmania* species. Was also evaluated the activity of those compounds against axenic amastigotes of *L. amazonensis*.

MATERIALS AND METHODS

Parasites Promastigotes of *L. amazonensis* (MHOM/BR/77/LTB0016 strain), *L. braziliensis* (MHOM/BR/2000/R616 strain) and *L. chagasi* (MHOM/BR/2000/R142 strain) were grown at 26 °C in Schneider's *Drosophila* medium supplemented with 10–20% (v/v) heat-inactivated fetal calf serum (FCS) and pH 7.2. *L. amazonensis* axenic amastigotes

were also cultured in the same medium which was acidified to pH 5.5, supplemented with 20% (v/v) FCS, and incubation temperature of 32 °C.⁴⁾ Parasites from both stages, after been harvested from the medium in the stationary phase, resuspended in fresh medium, counted in Neubauer's chamber, were adjusted to a concentration of 4 × 10⁶ promastigotes/ml and 8 × 10⁵ amastigotes/ml.

Drug Assay Drugs were added to the above parasite cultures for screening, in a concentration range from 320 to 0.16 µg/ml solubilized in dimethyl sulphoxide (DMSO) [the highest concentration used was 1.6% (v/v), which was not hazardous for the parasites]. After 24 h of incubation at specific temperatures, the number of surviving parasites was then counted in a Neubauer's chamber and the percentage of growth inhibition was calculated comparing to the controls, with DMSO without the compounds and the parasites alone. The IC₅₀/24 h values were determined by logarithmic regression from these percentages of inhibition. All tests were done in triplicate and Pentamidine Isethionate was used as reference drug.⁵⁾

Chemicals Diarylheptanoids and diarylpentanoids were synthesized and kindly donated by Dr. A. F. Arieta from University of Leipzig, Germany. Pentamidine was obtained from May & Baker Lab., England and all others chemicals from Sigma.

Statistical Analysis Significance was determined using a non-paired *t* Student test. Differences were considered to be significant when *p* < 0.05. Each experiment was triplicated.

RESULTS AND DISCUSSION

In this report it was evaluated the effect of diarylheptanoids and diarylpentanoids against three different species of *Leishmania* from the New World, as follow: *L. amazonensis* which has been associated to all clinical forms of leishmaniasis^{6–8)}; *L. braziliensis* that usually caused mucocutaneous disease and is endemic in the state of Rio de Janeiro and finally *L. chagasi* which is the causal agent of visceral leishmaniasis. Based in this facts it was expected a different

* To whom correspondence should be addressed. e-mail: lleon@ioc.fiocruz.br

Fig. 1. Effect of Different Concentration of Diarylheptanoids on Growth of Promastigotes of *L. amazonensis*, *L. braziliensis* and *L. chagasi* and Axenic Amastigotes of *L. amazonensis*

Fig. 2. Effect of Different Concentration of Diarylpentanoids on Growth of Promastigotes of *L. amazonensis*, *L. braziliensis* and *L. chagasi* and Axenic Amastigotes of *L. amazonensis*

sensitivity of these parasites to the assayed drugs. Indeed, in a general point of view, these difference was observed and the results showed that both diarylheptanoids and diarylpentanoids derivatives were more effective against *L. braziliensis* promastigotes, comparing to the others studied species, as *L. amazonensis* and *L. chagasi*. It can be observed (Table 2/ Figs. 1, 2) that the activity of these compounds expressed as $IC_{50}/24\text{ h}$ was in a range of 2 to $78\text{ }\mu\text{M}$, while for *L. amazonensis* the IC_{50} values ranging from 1 to $373\text{ }\mu\text{M}$ and 6 to $331\text{ }\mu\text{M}$ for *L. chagasi* promastigotes. Concerning *L. amazonensis* axenic amastigotes the activity variation was of 56 to $435\text{ }\mu\text{M}$, although three compounds presented IC values higher than $500\text{ }\mu\text{M}$. The interesting data is that even *L. braziliensis* strains present usually high invasive capacity resulting in deforming mucocutaneous manifestations, in this

work this species was the most sensitive to the diaryl derivatives assayed. On the other hand, *L. amazonensis* axenic amastigotes showed the worse results with both class of derivatives. It would be explained by the differences in the culture conditions, such as, higher temperature, lower pH and higher concentration of fetal calf serum, what would result in low drug availability, since some amount of drug could be linked to the serum albumin. Pentamidine Isethionate, the drug used as reference, showed an effectiveness similar to those most effective diaryl derivatives against all *Leishmania* species (Table 2).

Comparing the antileishmanial activities of the compounds based in the length of the aliphatic chain, it was observed that diarylheptanoids are more efficient than diarylpentanoids, against the three species of *Leishmania*. It can be suggested

Table 1. Structure and Nomenclature of Compounds

Structure	Diarylheptanoids	Compound
	5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-methoxyphenyl)-1,4,6-heptatrien-3-one	I
	5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-1,4,6-heptatrien-3-one	II
	5-hydroxy-1,7-di(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	III
	5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	IV
	5-hydroxy-7-(4-acetoxy-3-methoxyphenyl)-1-phenyl-1,4,6-heptatrien-3-one	V
	5-hydroxy-1,7-diphenyl-1,4,6-heptatrien-3-one	VI
	3-hydroxy-1,7-diphenyl-2,4,6-heptatrien-3-one	VII
Diarylpentanoids		
	3-hydroxy-5-(4-methoxy-3-hydroxyphenyl)-1-phenyl-2,4-pentadien-1-one	VIII
	3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-1-phenyl-2,4-pentadien-1-one	IX
	3-hydroxy-5-(4-hydroxy-3,5-dimethoxyphenyl)-1-phenyl-2,4-pentadien-1-one	X
	3-hydroxy-5-(4-methoxyphenyl)-1-phenyl-2,4-pentadien-1-one	XI
	3-hydroxy-5-(3,4-dimethoxyphenyl)-1-phenyl-2,4-pentadien-1-one	XII
	3-hydroxy-1,5-di(4-methoxyphenyl)-2,4-pentadien-1-one	XIII
	3-hydroxy-1,5-diphenyl-2,4-pentadien-1-one	XIV
	Pentamidine isethionate	Reference drug

Table 2. IC₅₀/24 h (μ M) of the Compounds

Compounds	IC ₅₀ Values (μ M)			
	<i>L. braziliensis</i>	<i>L. chagasi</i>	<i>L. amazonensis</i>	
	(promastigotes)	(promastigotes)	(promastigotes)	(amastigotes)
I	14 $\pm$ 0.6	22 $\pm$ 2.0	1 $\pm$ 0.6	56 $\pm$ 5
II	11 $\pm$ 1.2	25 $\pm$ 1.5	23 $\pm$ 2.0	80 $\pm$ 3.5
III	11 $\pm$ 1.5	15 $\pm$ 2.0	5 $\pm$ 1.0	76 $\pm$ 2.5
IV	6 $\pm$ 1.2	19 $\pm$ 3.0	8 $\pm$ 1.2	58 $\pm$ 2.0
V	2 $\pm$ 1.0	38 $\pm$ 1.0	21 $\pm$ 2.0	101 $\pm$ 3.5
VI	24 $\pm$ 1.2	331 $\pm$ 4.0	361 $\pm$ 3.5	>500
VII	70 $\pm$ 3.1	231 $\pm$ 3.2	373 $\pm$ 3.0	>500
VIII	24 $\pm$ 3.0	53 $\pm$ 2.5	3 $\pm$ 1.2	69 $\pm$ 1.5
IX	36 $\pm$ 2.5	6 $\pm$ 0.5	11 $\pm$ 2.0	104 $\pm$ 1.5
X	23 $\pm$ 2.0	50 $\pm$ 1.5	16 $\pm$ 1.5	71 $\pm$ 1.2
XI	40 $\pm$ 1.2	88 $\pm$ 2.3	35 $\pm$ 2.0	134 $\pm$ 3.0
XII	23 $\pm$ 0.6	58 $\pm$ 1.2	43 $\pm$ 1.5	88 $\pm$ 2.5
XIII	60 $\pm$ 1.5	128 $\pm$ 3.5	190 $\pm$ 1.2	435 $\pm$ 4.0
XIV	78 $\pm$ 2.0	310 $\pm$ 2.5	352 $\pm$ 3.5	>500
Pentamidine isethionate	14 $\pm$ 1.5	3 $\pm$ 1.2	0.46 $\pm$ 1.2	113 $\pm$ 3.5

that among others factors, these effect would be associated to an increase of lipophilicity of the diarylheptanoids and an ease transport through parasite membrane.

Concerning the substituents in the aromatic ring, the greatest activity was associated to those compounds presenting oxygenated functions in both derivatives. Data from literature described several activities of these classes of compounds in different biological systems and speculate about the potential mode of action of these molecules. Most of these relates pointed for an association of the presence of phenol groups as responsible for the anti-inflammatory activity.^{9–12)} Our data corroborate these results since diarylheptanoids containing an *m*-hydroxyl substituent in the aromatic ring resulted in a significant activity against all parasites. Furthermore, these activities increased by the presence of a *p*-methoxyl substituent. According to this, the most active heptanoid compound against *L. amazonensis* promastigotes, which is [5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-4-methoxyphenyl]-1,4,6-heptatrien-3-one] present those substituents. Also, against *L. braziliensis* the most effective derivative have a *p*-methoxy substituent and an *m*-phenolic group protected by an acetylating reaction [5-hydroxy-7-(4-acetoxy-3-methoxyphenyl)-1-phenyl-1,4,6-heptatrien-3-one] (see Table 1). It is important to notice that when those substituents change their position, which means, when we have *m*-methoxy and *p*-hydroxyl substitutions the activity decreased significantly. The same is true for the diarylpen-tanoids, as can be seen for the compound IX [3-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-1-phenyl-2,4-pentadien-1-one],

which was the most effective derivative against all three *Leishmania* species (Table 1).

Further experiments are being carried out in order to define a mechanism of action, besides a chemical structure and biological activity relationship.

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