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Synthesis and antileishmanial evaluation of 1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazole derivatives

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ABSTRACT

A series of 1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**4a–g**) and 5-amino-1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**5a–g**) were synthesized and evaluated in vitro against three *Leishmania* species: *L. amazonensis*, *L. braziliensis* and *L. infantum* (*L. chagasi* syn.). The cytotoxicity was assessed. Among the derivatives examined, six compounds emerged as the most active on promastigotes forms of *L. amazonensis* with IC_{50} values ranging from 15 to 60 μM. The reference drug pentamidine presented IC_{50} = 10 μM. However, these new compounds were less cytotoxic than pentamidine. Based on these results, the more promising derivative **5d** was tested further in vivo. This compound showed inhibition of the progression of cutaneous lesions in CBA mice infected with *L. amazonensis* relative to an untreated control.

Leishmaniasis is a group of tropical diseases caused by protozoan parasites of the genus *Leishmania*. It is transmitted to human beings and others mammals by the bite of an infected female *Phlebotomine* sandfly.[1] The clinical manifestations are particularly diverse representing a complex of diseases: visceral leishmaniasis (VL) or kala azar is usually fatal when untreated, mucocutaneous (MCL) is a mutilating disease, diffuse cutaneous leishmaniasis (DCL) is a long-lasting disease due to a deficient cellular-mediated immune response and cutaneous leishmaniasis (CL) is disabling when lesions are multiple.[2] Visceral leishmaniasis (VL) is the most severe form, in which vital organs of the body are affected. The cutaneous form often results in self cure, although consequences like permanent scars and deformations of the nose, mouth and throat. Patients with these forms of the disease may also suffer from discrimination and prejudice.[3]

According to WHO estimations, leishmaniasis affect almost 12 million people in 88 countries on five continents (Africa, Asia, Europe, North and South America), representing a worldwide public health problem.[4] The disease is estimated to cause 1.6 million new cases each year, of which an estimated 500 000 are visceral and 1.1 million cutaneous or mucocutaneous. Of the 1.6 million estimated cases, only about 600 000 are reported.[3]

Unfortunately, there is no effective treatment against various forms of leishmaniasis. There are no vaccines and the chemotherapeutic agents for treating these diseases are deficient and most of the drugs cause many adverse and side effects.[3,5] For more than 50 years, pentavalent antimonials have been the first-line drugs in the treatment for all types of leishmaniasis in most countries. Two organic salts, meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam) are mainly used.[5] The treatment is lengthy, potentially toxic and painful.[3] In the case of relapse, patients need treatment with second-line medicines such as pentamidine and amphotericin B. These drugs are toxic and may show a serious of side effects such as nephrotoxicity.[5,6] Newly developed, liposomal amphotericin B is highly effective, has almost no side effects and requires only a short course of treatment but it is too expensive to be a viable treatment option in most developing nations.[3] Other effective medicine is miltefosine, an alkylphosphocholine derivative originally developed as an anticancer drug, which has been registered in India, Germany and Colombia for oral treatment of visceral leishmaniasis (VL). However, it is contraindicated in pregnant women and shows severe gastrointestinal side effects.[5,7] In the light of these facts, there is an urgent need for the development of more efficient, inexpensive, nontoxic and innovative drugs for the treatment of leishmaniases.

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There are several other compounds at various stages of development, such as azoles derivatives, which have been revealed as potential new drugs for therapy or an additional therapy to that already existing.[5,8] Among azoles, many compounds containing pyrazole ring have been synthesized and evaluated. In recent works, we have reported the synthesis of a series of 1-aryl-1*H*-pyrazole-4-carboximidamides derivatives as potential antileishmanial agents.[9] Concomitantly, imidazoline ring, also known as cyclic amidine, has been found in several compounds with antileishmanial activity.[10]

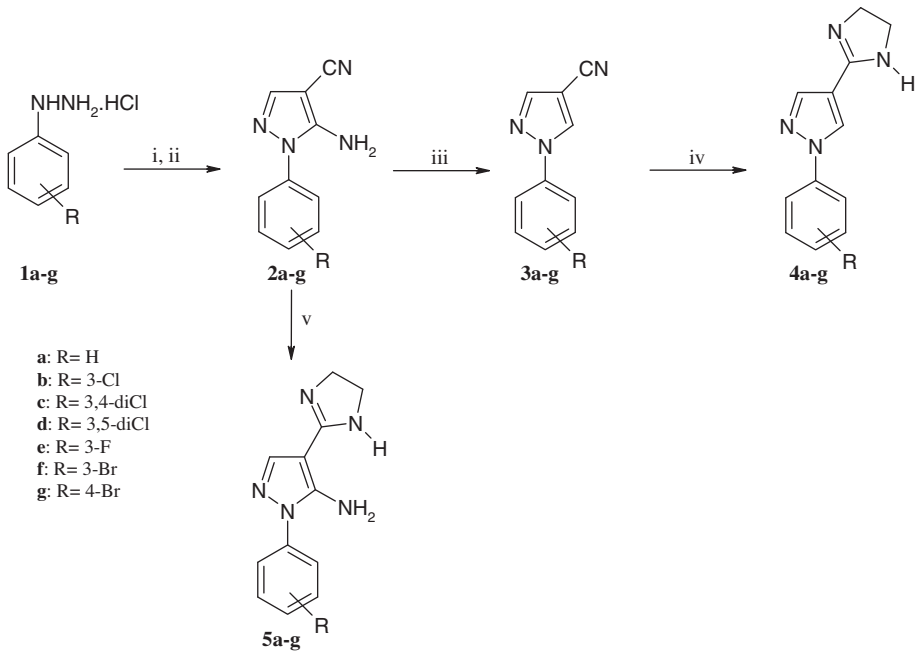
Based on the above report and in the continuation of our studies on chemotherapy of leishmaniasis, herein we described synthesis of some 1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**4a–g**) and 5-amino-1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**5a–g**) and their evaluation against *Leishmania amazonensis*, *Leishmania braziliensis* and *Leishmania infantum* (*Leishmania chagasi* syn.) promastigotes containing a high percentage of metacyclic forms.

The synthesis of the compounds 1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**4a–g**) and 5-amino-1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**5a–g**) is summarized in Scheme 1. Arylhydrazine hydrochlorides (**1a–g**) reacted with ethoxymethylenemalononitrile and sodium acetate in ethanol, under reflux, to form 5-amino-1-aryl-1*H*-pyrazole-4-carbonitriles (**2a–g**).[11] Soon after, the compounds **2a–g** were converted to the 1-aryl-1*H*-pyrazole-4-carbonitriles (**3a–g**) by the aprotic deamination using *t*-butyl nitrite and tetrahydrofuran (THF), under reflux.[12] Finally, the targets 1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**4a–g**) were obtained by the reaction of 1-aryl-1*H*-pyrazole-4-carbonitriles (**3a–g**) with carbon disulfide and 1,2-diamin oethane (ethylenediamine).[13] Similarly, the compounds 5-amino-1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**5a–g**) were synthesized from 5-amino-1-aryl-1*H*-pyrazole-4-carbonitriles (**2a–g**). All these final compounds **4a–g** and **5a–g** are news, except 1-phenyl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazole (**4a**), which was obtained by De La Hoz and co-workers.[14]

The compounds **4a–g** and **5a–g** were evaluated in vitro against the extracellular promastigote stage of *L. amazonensis* (MHOM/BR/77/LTB0016), *L. infantum* (MCAN/BR/98R619) and *L. braziliensis* (MCAN/BR/97/P142) parasites, at various concentrations, taking pentamidine as a control. The cytotoxicity responses were assayed on the mice's peritoneal macrophages. The IC_{50}/24 h values were determined by linear regression from the percentages of inhibition. All tests were done in triplicate. The results are shown in Table 1. In general, these compounds presented lower cytotoxicity in murine macrophages than the reference drug pentamidine. Six derivatives **4a**, **4b**, **4e**, **4g**, **5d** and **5f** were more effective against *L. amazonensis* promastigotes, comparing to the others studied species, as *L. infantum* and *L. braziliensis*. It can be observed that the activity of the derivatives expressed as IC_{50}/24 h was in a range of 15–60 μM, while for the other two species showed IC_{50} >250 μM. In the literature the in vitro inhibitory effect of the pentamidine on *L. amazonensis* is also superior compared to *L. infantum* and *L. braziliensis*.[15,16] Interestingly, the relationships between structure and anti-*L. amazonensis* activity revealed that compound **5d** with chlorine (–Cl) substituents attachment at two *meta*-positions of the aryl nucleus of the aminopyrazole derivatives was most active while similar structure without amino group **4d** presented unsatisfactory activity on the *Leishmania* species assayed. These results also indicated the positive effect of introducing *p*-bromine **4g** and *m*-bromine **5f** on *L. amazonensis* activity. On the other hand, the presence of only one *m*-Cl position attached at aminopyrazole derivative **5b** or without amino group **4b** confers moderate activity. From these results, the most attractive compound **5d** was further evaluated for in vivo evaluated.

To examine the therapeutic efficacy, CBA mice infected with *L. amazonensis* was treated orally with this compound **5d** (29 mg/kg/day, 100 μmol/kg/day),[17] and the reference drug ketoconazole (50 mg/Kg/day).[9c] The use of ketoconazole in the in vivo experiments is due to it similarity to the tested compound, concerning the imidazole ring.

In our study, the treatment was started in the fourth week after infection and continued for 30 days without interruption. It was observed in the groups treated with derivative **5d** or ketoconazole, a smaller development of cutaneous lesion compared to the untreated control group and infected (Fig. 1). Additionally, the tox-



Scheme 1. Reagents and conditions: (i) sodium acetate, ethanol, 0.5 h, reflux; (ii) ethoxymethylene-malononitrile, ethanol, 1 h, reflux; (iii) *t*-butyl nitrite, THF, 2 h, reflux; (iv) ethylenediamine, CS_2, 12–14 h, 110 °C; (v) ethylenediamine, CS_2, 14–15 h, 115 °C.

Table 1
IC_{50}[a] (μM) values of 1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**4a**–**g**) and 5-amino-1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**5a**–**g**) on promastigotes of *Leishmania* spp. and percentage of cytoxicity in murine macrophages

Compd No.	*L. amazonensis* IC_{50} (μM)	*L. braziliensis* IC_{50} (μM)	*L. infantum* IC_{50} (μM)	Cytotoxicity[b]%
4a	43 ± 4.2	>500	>500	11.26
4b	60 ± 3.2	>500	>500	6.46
4c	297 ± 6.0	—	>500	5.32
4d	>500	459 ± 1.69	>500	3.97
4e	54	—	>500	—
4f	>500	—	>500	11.98
4g	30 ± 5.5	—	>500	3.52
5a	365 ± 3.5	433 ± 2.2	>500	—
5b	>500	>500	>500	15.2
5c	381 ± 29	—	—	15.5
5d	15.5 ± 6.8	437 ± 1.6	>500	—
5e	>500	>500	>500	—
5f	31.3 ± 5.4	—	—	—
5g	>500	272 ± 2.9	>500	—
Pentamidine	10 ± 4.44	50	100 ± 2.5	31

[a] IC_{50} values were obtained from the drug concentration-response curve, and the results were expressed as the mean ± standard deviation determined from three independent experiments.
[b] Percentage of cytoxicity values from IC_{50} of *L. amazonensis*.

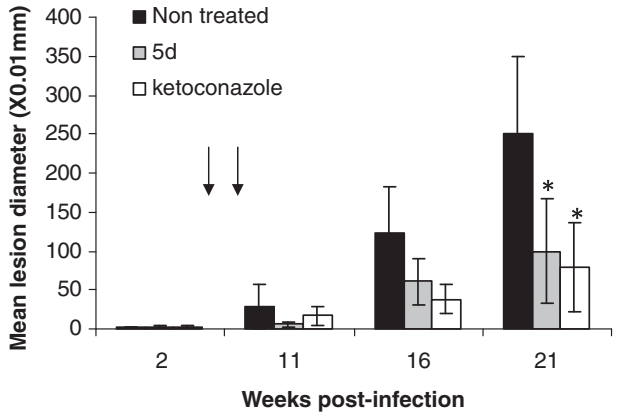


Figure 1. Mean lesion diameter of CBA mice infected with *Leishmania amazonensis* (MHOM/BR/77LTB0016) and treated with 5-amino-1-(3,5-dichlorophenyl)-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazole (**5d**), ketoconazole or left untreated. Treatment started at the fourth week post-infection and continued up to 30 days post-infection (n = 8 per group).

icity of this compound in mice body weight was determined and samples of blood were taken at different times during compound administration from the tails of both uninfected and infected mice left untreated or treated. The total number of leukocytes was estimated by counting in a Neubauer chamber. The sera collected were assayed colorimetrically for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine with commercial kits (Labtest Diagnostica, Brazil). No apparent signs of drug toxicity, weight loss, or lymphocyte, monocyte, or neutrophil alterations were observed in any experiment, and AST, ALT, and creatinine concentrations showed no apparent hepatic or renal toxicity after the treatment with 5-amino-1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**5d**), compared with uninfected mice left untreated. All in vivo data were analyzed by the Student's *t*-test. *P* values <0.05 were considered significant. The InStat program (Graph Pad Software, San Diego, CA) was used for these tests. All experiments were repeated at least three times.

In conclusion, this study showed that the compounds 5-amino-1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**4a–g**) and 1-aryl-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazoles (**5a–g**) presented strong activities against promastigotes of *L. amazonensis* and lower activities against those of *L. braziliensis* and *L. infantum*. In vitro results showed that these derivatives were less cytotoxic than pentamidine. The compound 5-amino-1-(3,5-dichlorophenyl)-4-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-pyrazole (**5d**) was tested further in vivo and exhibited significant inhibition relative to an untreated control. In addition, no apparent hepatic or renal toxicity due to these compounds was found. This finding indicates that derivative should be investigated for the development of selective antileishmanial compounds.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.134.

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