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Short communication

Synthesis and leishmanicidal activities of 1-(4-X-phenyl)-N′-[(4-Y-phenyl)methylene]-1*H*-pyrazole-4-carbohydrazides

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Abstract

1*H*-pyrazole-4-carbohydrazides were synthesized and their leishmanicidal in vitro activities and cytotoxic effects were investigated. The drugs prototypes of these new compounds (ketoconazole, benznidazole, allopurinol and pentamidine) were also tested. It was found that among all the 1*H*-pyrazole-4-carbohydrazides derivatives examined, the most active compounds were those with X = Br, Y = NO_2 (27) and X = NO_2, Y = Cl (**15**) derivatives which showed to be most effective on promastigotes forms of *L. amazonensis* than on *L. chagasi* and *L. braziliensis* species. When tested against murine peritoneal macrophages as mammalian host cell controls of toxicity, 1-(4-Br-phenyl)-N′-[(4-NO_2-phenyl)methylene]-1*H*-pyrazole-4-carbohydrazides (**27**) (EC_{50} = 50 μM l^{-1}) and 1-(4-NO_2-phenyl)-N′-[(4-Cl-phenyl)methylene]-1*H*-pyrazole-4-carbohydrazides (**15**) EC_{50} = 80 μM l^{-1})] was reasonably toxic. However, both compounds were less toxic than pentamidine and ketoconazole. These results provide new perspectives on the development of drugs with activities against *Leishmania* parasite.

Keywords: Synthesis; 1-(4-X-phenyl)-N′-[(4-Y-phenyl)methylene]-1*H*-pyrazole-4-carbohydrazides; Antileishmania activity; Cytotoxicity

1. Introduction

Leishmania is a pathogenic protozoa that causes a broad spectrum of infectious diseases in mammalian hosts ranging from self-healing cutaneous ulceration to progressive and lethal visceral infection that are transmitted by phlebotomine sandflies. *Leishmania* has two evolutive forms: an extracellular flagellated promastigotes in the vector and intracellular amastigotes within mononuclear phagocytes in mammalian host. Leishmaniasis is endemic in 88 countries in the world and affects about 2 million people per year, with approximately 350 million individuals at risk of infection [1]. Lately, in Brazil [2] and in South-western Europe [3], the number of cases of *Leishmania*/HIV co-infection has been increasing, including both the cutaneous and visceral clinical presentation. It has also been considered as an emerging disease with specific difficulties in terms of treatment [4,5].

The difficulty to control the parasitic disease remains a serious problem principally to the diversity of the mammalian reservoirs (wild and domestic animals), the variety of species of vectors and complexity of *Leishmania* species [6]. The severity of clinical manifestations depends, mainly, on the *Leishmania* species involved. In Brazil, *Leishmania* (*Leishmania*) *amazonensis* was found in different regions and it has been described an association with various clinical forms such as: cutaneous, mucosal, diffuse cutaneous and visceral leishmaniasis considered a species with an epidemiological significance [7]. Additionally, *Leishmania* (*Leishmania*) *chagasi* is responsible for visceral clinical form and *Leishmania* (*Viannia*) *braziliensis* is evolved with cutaneous or mucosal lesions [6].

The absence of an alternative chemotherapeutic approach to the treatment of *Leishmania* infection requires urgent attention.

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There are presently no vaccines [8] and chemotherapy is unsatisfactory and high-priced [9]. Therefore, in this work we report the synthesis and the in vitro leishmanial activity of new pyrazolic system series, 1-(4-X-phenyl)-N′-[(4-Y-phenyl) methylene]-1-*H*-pyrazole-4-carbohydrazides (**1**–**30**) have been investigated on their inhibitory effects against the extracellular promastigote stage of *L. amazonensis*, *L. chagasi* and *L. braziliensis* parasites, as its direct toxic effect on macrophage host cells. The drugs prototypes (ketoconazole, benznidazole, allopurinol and pentamidine) were also examined.

2. Chemistry

Drugs containing acylhydrazone derivatives have showed some important biological effects as analgesic, antiplatelets and anti-inflammatory activities. The evaluation of this drug profile leads us to identify a new potent prototype of biological activity [10,11].

In this work it is taking into consideration this molecular characteristic, the new arylpyrazolic-attached acyllhydrazone derivatives were synthesized exploring the molecular hybridization approach. Additionally, the change of para-substituent group of acylhydrazone and arylpyrazolic framework permitted to identify electronic and hydrophobic derivatives effect. The nature of these substituents in phenyl and arylic groups (OH, NCH_3, CH_3, OCH_3, SCH_3, H, F, Cl, Br, CN, NO_2) could be correlated to an important electronic variation (Hammett values σ ranging from –0.18 to 0.78) which could be useful to investigate the contribution of this structural sub-unit on its bioactivity profile. Therefore, we recognized that the aza-vinylogous groups in the molecule pyrazole-carbohydrazides possibly would be functioned as hydrophobic anchors for active sites of protozoa [11].

3. Pharmacology

Pentavalent antimonials, available as sodium stibogluconate (pentostam) or N-methylglucamine antimoniate (glucantime), have been the standard drugs for treatment of leishmaniasis recognized as being expensive and need to be given parenterally, often for prolonged periods [12]. Moreover, the classical and alternative drugs (pentamidine, amphotericin B) often cause toxic side effects and induction of parasitic resistance [13,14]. Miltefosine, is the first effective orally administered treatment for visceral leishmaniasis [15], but possible emergence of miltefosine resistance and side effects should be considered.

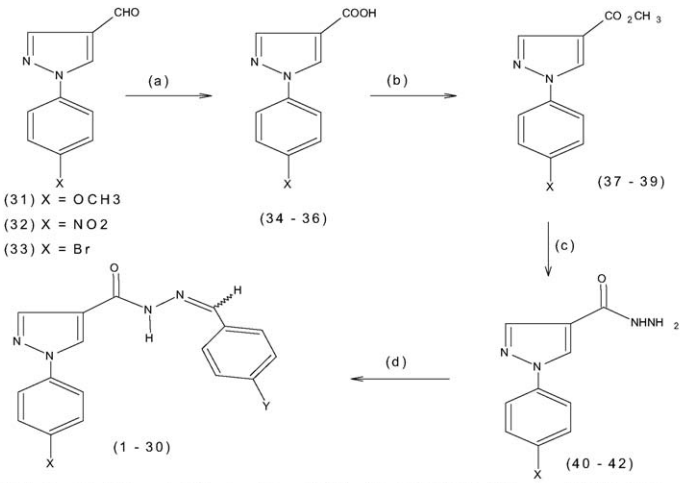
Studies with acylhydrazone series have been showed some important biological effects as analgesic, antiplatelets and anti-inflammatory activities [10]. Some enzymes identified in parasites have been accepted as targets for antiparasite chemotherapy developing protease inhibitors for HIV, *Plasmodium falciparum* and *Trypanosoma cruzi* [16]. It was also recognized that iminic insaturation of acylhydrazone could be had the hydrophobic anchors function for protozoa enzymatic active site. In addition, a novel series of 1-(chlorophenyl)-4-hydroxy-1*H*-pyrazole-3-carboxylic acid hydrazide analogs and some derived 4-substituted-1,2,4-triazolin-3-thiones, 2-substituted-1,3,4-thiadiazole and 2-substituted-1,3,4-oxadiazoles were synthesized and showed antitumoral and antiviral activities [17].

The principal prototype compounds were ketoconazole, allopurinol, pentamidine and benznidazole. Therefore, ketoconazole drug inhibits sterol biosynthesis on ergosterol in *Leishmania* species [18] and is reported that is not active on *L. braziliensis* [19,20]. In trypanosomatids, which are deficient in xanthine oxidase, allopurinol acts as a purine analogue and is incorporated, through hypoxanthine-guanine phosphoribosyltransferase (HGPRT), obstructing the proteins synthesis [21,22]. Benznidazole is a nitroimidazole derivative, which has an important activity against *Trypanosoma*, and it is the drug employed in Brazil for the treatment of *T. cruzi* infection. The anaerobic reduction of benznidazole to reactive intermediates involve covalent bind to proteins and lipids [23] and also induce oxidative stress within the parasite [24]. Pentamidine is the standard drug used in leishmaniasis and early stage of sleeping sickness. Different mechanisms of action have been proposed for pentamidine in kinetoplastid parasites including inhibition of topoisomerase II [25] and serine proteases [26] and disturbance of polyamine metabolism [27]. In this report, new pyrazolic system series, 1-(4-X-phenyl)-N′-[(4-Y-phenyl) methylene]-1*H*-pyrazole-4-carbohydrazides (**1**–**30**) and prototype drugs have been investigated on their inhibitory effects against the extracellular promastigote stage of *L. amazonensis*, *L. chagasi* and *L. braziliensis* parasites, besides its direct toxic effects on macrophage host cells.

4. Results and discussion

The new series of 1-(4-X-phenyl)-N′-[(4-Y-phenyl)methylene]-1*H*-pyrazole-4-carbohydrazides (**1**–**30**) were synthesized following the previous procedure, as showed in Fig. 1. The literature describe synthesis and pharmacological evaluation of some 1-(4-X-phenyl)-N′-[(4-Y-phenyl)methylene]-1*H*-pyrazole-4-carbohydrazides [28–30]. The structure of these new compounds has been characterized by a lateral chain of 1*H*-pyrazole-4-carbohydrazide, which probably contributes to their biological activities [31].

The 1-arilpyrazoles-4carboxaldehydes (X = OCH_3, NO_2 and Br) (**31**–**33**) were prepared by Vilsmeier-Haack reaction [32]. The acids (**34**–**36**) were prepared by Jones oxidation. The **34**–**36** compounds were treated with $SOCl_2$. The freshly prepared 4-(4′-phenyl) pyrazolylchlorides were converted to the corresponding hydrazides intermediates (**40**–**42**) by treatment with hydrazine hydrate. Finally, compounds (**1**–**30**) were obtained by condensing of (**40**–**42**) with functionalized aromatic aldehydes (Y = H, p-OH, p-CN, p-OCH_3, p-Br, p-Cl, p-F, p-SCH_3, p-NO_2, p-CH_3, p-$N(CH_3)_2$, p-OC_2H_5) at reflux in ethanol to produce the desired of 1-(4-X-phenyl)-N′-[(4-Y-phenyl) methylene]-1*H*-pyrazole-4-carbohydrazides (**1**–**30**) in good yields. The structure of these stable and crystalline compounds was fully characterized by usual methods (IR, ^{1}H-, ^{13}C-NMR).



Reagents: (a) CrO_3, H_2SO_4, rt, 30min, 82 – 91%. (b) 1) $SOCl_2$ 30min, 2) MeOH, reflux, 2h, 75 – 82%. (c) $NH_2NH_2.H_2O$, EtOH, reflux, 4h, 72 – 95%. (d) Ar – CHO, EtOH, HCl (cat.), reflux, 2h, 60 – 85%.

(01) X = OCH_3, Y = H	(11) X = OCH_3, Y = $N(CH_3)_2$	(21) X = Br, Y = CN
(02) X = OCH_3, Y = OH	(12) X = OCH_3, Y = OC_2H_5	(22) X = Br, Y = OCH_3
(03) X = OCH_3, Y = CN	(13) X = NO_2, Y = OCH_3	(23) X = Br, Y = Br
(04) X = OCH_3, Y = OCH_3	(14) X = NO_2, Y = Br	(24) X = Br, Y = Cl
(05) X = OCH_3, Y = Br	(15) X = NO_2, Y = Cl	(25) X = Br, Y = F
(06) X = OCH_3, Y = Cl	(16) X = NO_2, Y = F	(26) X = Br, Y = SCH_3
(07) X = OCH_3, Y = F	(17) X = NO_2, Y = $N(CH_3)_2$	(27) X = Br, Y = NO_2
(08) X = OCH_3, Y = SCH_3	(18) X = NO_2, Y = OC_2H_5	(28) X = Br, Y = CH_3
(09) X = OCH_3, Y = NO_2	(19) X = Br, Y = H	(29) X = Br, Y = $N(CH_3)_2$
(10) X = OCH_3, Y = CH_3	(20) X = Br, Y = OH	(30) X = Br, Y = OC_2H_5

Fig. 1. General procedure for the synthesis of 1-(4-X-phenyl)-N′-[(4-Y-phenyl)methylene]-1*H*-pyrazole-4-carbohydrazides.

4.1. Anti-leishmanial assays

The biological effects of 1*H*-pyrazole-4-carbohydrazides (**1**–**30**), on the viability of *Leishmania* sp. were tested and compared with references drugs (ketoconazole pentamidine, benznidazole and allopurinol). Some of these 30 compounds have activities against infective promastigotes forms of *L. amazonensis* (Table 1). Therefore, two substituents, the **27** and **15** have a potent activity, which inhibited the growth of *L. amazonensis* (90% and 66%) at very low concentrations, with EC_{50}/24h of 50 and 80 μM l^{-1}, respectively. Others compounds displayed in vitro activity against promastigotes forms only at very high concentrations, like demonstrated in Table 1. In our experiments, the effect of these principal components (**27** and **15**) were also investigated on others *Leishmania* species like *L. chagasi* and *L. braziliensis* and demonstrated to be less sensitive than on *L. amazonensis*. Taken together, these results indicate that, when compared with reference drug like ketoconazole the compounds **15** and **27** demonstrated similar leishmanicidal activity (Table 2). In the literature there have been studies among azole drugs demonstrating the superior in vitro inhibitory activity on *L. donovani*, *L. braziliensis* and on *L. amazonensis* than on *L. aethiopica*, *L. major*, *L. tropica* and *L. mexicana* promastigotes [18]. In other studies, ketoconazole was considered efficient on treatment of cutaneous leishmaniasis patients with *L. panamensis* [33], *L. major* [34,35] and *L. mexicana* although presented unsatisfactory activity on *L. braziliensis* infection [36].

These differences accentuate the importance of speciation in the treatment of leishmaniasis. American cutaneous leishmaniasis is associated to at least 13 species of *Leishmania* pathogenic to human with biochemical and molecular variation [4,37].

In order to evaluate possible structure–activity relationships of these 1*H*-pyrazole-4-carbohydrazides derivatives it was ob-

Table 1
EC_{50} values of 1-(4-X-phenyl)-N′-[(4-Y-phenyl)methylene]-1*H*-pyrazole-4-carbohydrazides on promastigotes of *L. amazonensis* and murine macrophage

Compound	X	Y	Leishmanicidal activity (μM l^{-1})	Cytotoxicity (μM l^{-1})
1	OCH_3	H	ND	ND
2	OCH_3	OH	> 600	ND
3	OCH_3	CN	> 600	ND
4	OCH_3	OCH_3,	> 600	> 285.4
5	OCH_3	Br	ND	184.1 ± 2.2
6	OCH_3	Cl	480 ± 360	> 281.8
7	OCH_3	F	No activity	> 295.5
8	OCH_3	SCH_3	No activity	> 273.0
9	OCH_3	NO_2	> 600	> 273.7
10	OCH_3	CH_3	ND	ND
11	OCH_3	N $(CH_3)_2$	> 600	> 275.1
12	OCH_3	OC_2H_5	440 ± 5	> 274.4
13	NO_2,	OCH_3	> 320	> 273.7
14	NO_2	Br	560 ± 80	222.6 ± 13.7
15	NO_2	Cl	80 ± 7	> 270.44
16	NO_2	F	> 600	188.8 ± 2.0
17	NO_2	N $(CH_3)_2$	> 600	ND
18	NO_2	OC_2H_5	> 600	> 263.6
19	Br	H	> 600	> 270.8
20	Br	OH	> 600	> 259.6
21	Br	CN	No activity	229.0 ± 28.6
22	Br	OCH_3	> 600	> 250.5
23	Br	Br	> 600	> 223.1
24	Br	Cl	No activity	192.7 ± 9.1
25	Br	F	No activity	203.2 ± 1.7
26	Br	SCH_3	No activity	> 240.8
27	Br	NO_2	50 ± 2	213.9 ± 26.5
28	Br	CH_3	ND	ND
29	Br	N $(CH_3)_2$	340 ± 230	215.6 ± 2.4
30	Br	OC_2H_5	> 600	167.7 ± 16.6

served that the nature and position of substituent on the molecule to improve biological functions. It seems that positions of substituent on molecule **27** which X = Br, Y = NO_2 was more effective in relation to leishmanicidal behavior than what was observed on molecule **14** (X = NO_2, Y = Br). This fact could be associated to the mesomeric electronic effect of Br substituent (where substituent constant σ = 0.23) and NO_2 substituent (where substituent constant σ = 0.78) in Y position to confer increased electrophilic character to iminic group. This last group could be useful to bind on nucleophilic site of protozoa cys-protease [16]. Therefore, it is likely that electronic and hydrophobic factors on molecule structure would be important as hydrophobic anchors to the enzymatic site of parasite.

The toxic effects of these compounds were also evaluated on mouse peritoneal macrophages (Table 1). Compounds **15** and **27** which demonstrated better leishmanicidal activity displayed toxicity index on mammalian cells correspondent to EC_{50} of > 270.4 and 213.9 μM l^{-1}, respectively. When compared with a pattern drug as pentamidine (227 μM l^{-1}) or ketoconazole (130 μM l^{-1}), the results showed these compounds are more toxic to cells than these new compounds increasing the importance to continue this studies.

In conclusion, this study confirms the marked leishmanicidal activity of new 1*H*-pyrazole-4-carbohydrazides on *L. amazonensis* and less important effect on *L. chagasi* and *L. braziliensis*. Fortunately, it will be necessary in vitro and in vivo studies on amastigotes intracellular forms of *Leishmania*.

5. Experimental protocols

Chemicals employed were obtained from commercial supplies and used without purification, unless otherwise noted.

5.1. Chemistry

5.1.1. 1-(4-X-phenyl)-1H-pyrazole-4-carboxilic acids (34–36)

The preparation of these compounds was carried out in accordance with published procedure [32]. The purity of the compounds was verified by means of thin layer chromatography (TLC) using silica gel plate with fluorescent indicator and $CHCl_3$/methanol (8:2) as eluent, melting point, IR spectra and ^{1}H-NMR.

Compound (**34**): IR (KBr, cm^{-1}) 3200–2500 (, OH), 1660 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.9 (s, 1H, OCH_3) 9.1 (s, 1H, H3); 8.2 (s, 1H, H5), 7.2; 7.3 (d, 2H, H2′, H6′); 8.0; 8.1 (d, 2H, H3′, H5′), 12,8 (s, 1H, OH). Melting point: 235–237 °C. Yield: 91%.

Compound (**35**): IR (KBr, cm^{-1}) 3200–2500 (, OH), 1680 (, C=O), 1520, 1340 (NO_2). ^{1}H-NMR (300 MHz; DMSO-d6, δ). Melting point: 163–165 °C. Yield: 82%.

Compound (**36**): IR (KBr, cm^{-1}) 3200–2400 (, OH), 1660 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.2 (s, 1H, H3); 8.2 (s, 1H, H5), 7.8; 7.9 (d, 2H, H2′, H6′); 8,0; 8,1 (d, 2H, H3′, H5′), 12.8 (s. 1H, OH). Melting point: 202–203 °C. Yield: 91%.

5.1.2. Methyl 1-(4-X-phenyl)-1H-pyrazole-4-carboxylates (37–39)

The corresponding freshly prepared 1-(4-X-phenyl)-1*H*-pyrazole-4-carbonylchloride (10 mmol) was added to a stirred methanol (20 ml). The reaction mixture was maintained under

Table 2
Effect of 1H-pyrazole-4-carbohydrazides derivatives and prototypes drugs on promastigotes of *Leishmania* sp and murine peritoneal macrophages

Compound	X	Y	*L. braziliensis*	*L. chagasi*	*L. amazonensis*	Macrophage
15	NO_2	Cl	540 ± 100	270 ± 70	80 ± 7	> 270.4
27	Br	NO_2	210 ± 20	220 ± 20	50 ± 2	210 ± 30
Allopurinol	–	–	370 ± 100	> 600	> 600	> 600
Ketoconazole	–	–	190 ± 20	60 ± 10	70 ± 2	130 ± 10
Benznidazole	–	–	> 600	160 ± 10	No activity	330 ± 20
Pentamidine	–	–	50 ± 6	140 ± 10	10 ± 3	227 ± 80

Mean of EC_{50} (μM l^{-1}) ± S.D. for three determination.

reflux for 2 h. In the next step, the reaction mixture was poured in cold water neutralized with 10% aqueous sodium bicarbonate solution and the precipitate formed was filtered out and dried. The purity of the compounds was checked by means of TLC using silica gel plate with fluorescent indicator and $CHCl_3$/methanol (8:2) as eluent, melting point, IR spectra and ^{1}H-NMR.

Compound (**37**): IR (KBr, cm^{-1}) 1710 (, C=O); 1240, 1130 (, C–O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.86 (s, 1H, OCH_3); 3.85 (s, 1H, OCH_3) 8.3 (s, 1H, H3); 8.0 (s, 1H, H5), 6.9; 7.0 (d, 2H, H2′, H6′); 7.5; 7.6 (d, 2H, H3′, H5′). Melting point: 134–136 °C. Yield: 86%.

Compound (**38**): IR (KBr, cm^{-1}) 1730 (, C=O); 1270, 1110 (, C–O); 1520; 1430 (, NO_2). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 4.1 (s, 1H, OCH_3); 9.5 (s, 1H, H3); 8.9 (s, 1H, H5), 8.3; 8.4 (d, 2H, H2′, H6′); 8.6; 8.4 (d, 2H, H3′, H5′). Melting point: 168–170 °C. Yield: 75%.

Compound (**39**): IR (KBr, cm^{-1}) 1720 (, C=O); 1250, 1140 (, C–O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.8 (s, 1H, OCH_3); 8.3 (s, 1H, H3); 8.0 (s, 1H, H5), 7.6; 7.7 (d, 2H, H2′, H6′); 7.8; 7.9 (d, 2H, H3′, H5′). Melting point: 160–162 °C. Yield: 78%.

5.1.3. General procedure for the preparation of 1-(4-X-phenyl)-1H-pyrazole-4-carbohydrazides (40–42)

To a stirred solution of 1 mmol of the methyl 1-(4-X-phenyl)-1*H*-pyrazole-4-carboxylate (**37**–**39**) in ethanol (10 ml), 2 ml of 80% hydrazine monohydrate was added. The reaction mixture was maintained under reflux for 5–8 hours, until TLC indicated the end of reaction. After this time, the reaction mixture was poured on ice and the solid formed was collected by filtration, washed with cold water and recrystallized from ethanol. As a result of this process the following compounds were prepared (**40**–**42**).

Compound (**40**): IR (KBr, cm^{-1}) 3400–2800 (, NH), 1650 (, C=O); 1260, 1090 (, C–O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.9 (s, 1H, OCH_3); 8.8 (s, 1H, H3); 8.1 (s, 1H, H5), 7.2; 7.3 (d, 2H, H2′, H6′); 7.8; 7.9 (d, 2H, H3′, H5′), 9.5 (s, 1H, NH), 4.5 (s, 1H, NH_2). Melting point: 200–202 °C. Yield: 95%.

Compound (**41**): IR (KBr, cm^{-1}) 3500–3000 (, NH), 1660 (, C=O); 1550, 1440 (, NO_2). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 8.7 (s, 1H, H3); 8.1 (s, 1H, H5), 6.8; 6.9 (d, 2H, H2′, H6′); 7.5; 7.6 (d, 2H, H3′, H5′), 9.5 (s, 1H, NH), 4.6 (s, 1H, NH_2). Melting point: 230–231 °C. Yield: 72%.

Compound (**42**): IR (KBr, cm^{-1}) 3320–3100 (, NH), 1640 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 8.9 (s, 1H, H3); 8.1 (s, 1H, H5), 7.7; 7.73 (d, 2H, H2′, H6′); 7.8; 7.84 (d, 2H, H3′, H5′), 9.5 (s, 1H, NH), 4.4 (s, 1H, NH_2). Melting point: 205–209 °C. Yield: 94%.

5.1.4. General procedure for the preparation of 1-(4-X-phenyl)-N′-[(4-Y-phenyl)methylene]-1H-pyrazole-4-carbohydrazides (1–30)

To a solution of derivatives **(40–42)** (1 mmol) in 10 ml of ethanol, it was added an equimolar amount of the appropriate benzaldehyde derivative in the presence of catalytic amount of 35% hydrochloric acid. The mixture was maintained under reflux for 2 h, until TLC indicated the end of reaction. Then, the reaction mixture was poured in cold water, and the precipitate formed was filtered out washed with ethanol and recrystallized from ethanol to afford crystals. This process was completed and the following compounds (**1**–**30**) were prepared.

Compound (**1**) IR (KBr, cm^{-1}) 3500–2800 (, N–H), 1620 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.9 (s, 3H, OCH_3) 8.8 (s, 1H, H3); 8.2 (s, 1H, H5); 7.2; 7.3 (d, 2H, H2′, H6′); 7.9, 8.0 (d, 2H, H3′, H5′); 7.2–8.0 (m, 1H, N=C–H); 7.6; 8.0 (m, 5H, H2′′, H6′′); 7.6; 8.0 (m, 5H, H3′′, H5′′); 9.0 (s, 1H, N–H). Melting point: 135–137 °C. Yield: 80%.

Compound (**2**): IR (KBr, cm^{-1}) 3500–2500 (, N–H, OH), 1640 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.9 (s, 3H, OCH_3) 9.0 (s, 1H, H3), 8.4 (s, 1H, H5), 6.9; 7.0 (d, 2H, H2′, H6′); 7.7; 7.73 (d, 2H, H3′, H5′); 8.1 (s, 1H, N=C–H); 7.2; 7.3 (d, 2H, H2′′; H5′′); 7.9; 8.0 (d, 2H, H3′′, H5′′); 10.0 (s, 1H, OH); 11.5 (s, 1H, N–H). Melting point: 245–247 °C, 86%.

Compound (**3**): IR (KBr, cm^{-1}) 3500–2600 (, NH), 2220 (, CN) 1640 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.8 (s, 3H, OCH_3); 9.0 (s, 1H, H3); 8.4 (s, 1H, H5); 7.1; 7.2 (d, 2H, H3′, H5′); 7.9; 8.0 (d, 2H, H3′, H5′); 7.8; 7.9 (d, 2H, H2′′, H6′′); 7.9; 8.0 (d, 2H, H3′′, H5′′); 8.2 (s, 1H, N=C–H); 11.5 (s, 1H, N–H). Melting point: 220–222 °C. Yield: 62%.

Compound (**4**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1640 (, C=O), 1255 and 1169 (, C–O). (DMSO-d6, δ): 3.5 (s, 3H, OCH_3); 3.9 (s, 3H, OCH_3); 9.1 (s, 1H, H3); 8.7 (s, 1H, H5); 7.1; 7.2 (d, 2H, H2′, H6′), 7.2; 7.21 (d, 2H, H3′, H5′); 8.2 (s, 1H, N=C–H); 7.7; 7.8 (d, 2H, H2′′; H6′′); 7.9; 7.92 (d, 2H, H3′′, H5′′); 11.9 (s, 1H, N–H). Melting point: 186–188 °C. Yield: 64%.

Compound (**5**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1650 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 4.0 (s, 3H, OCH_3); 9.2 (s, 1H, H3); 8.6 (s, 1H, H5); 7.3; 7.4 (d, 2H, H2′, H6′); 7.9; 8.0 (d, 2H, H3′, H5′); 7.9; 8.1 (d, 2H, H2′′, H6′′); 8.0 8.1 (d, 2H, H3′′, H5′′); 8.0 (s, 1H, N=C–H); 11.9 (s, 1H, N–H). Melting point: 205–207 °C. Yield: 75%.

Compound (**6**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1650 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 4.0 (s, 3H, OCH_3); 9.1 (s, 1H, H3); 8.6 (s, 1H, H5); 7.3; 7.34 (d, 2H, H2′, H6′); 8.1; 8.12 (d, 2H, H3′, H5`); 7.7; 7.8 (d, 2H, H2′′, H6′′); 7.9; 8.0 (d, 2H, H3′′, H5′′); 8.0 (s, 1H, N=C–H); 11.9 (s, 1H, N–H). Melting point: 205–208 °C. Yield: 67%.

Compound (**7**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1660 (ν, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.9 (s, 3H, OCH_3); 9.1 (s, 1H, H3); 8.5 (s, 1H, H5); 7.2; 7.3 (d, 2H, H2′, H6′); 7.4; 7.5 (d, 2H, H3′, H5′); 7.9; 8.0 (d, 2H, H2′′, H6′′); 7.9; 8.0 (d, 2H, H3′′, H5′′), 8.4 (s, 1H, N=C–H); 11.8 (s, 1H, N–H). Melting point: 180–182 °C. Yield: 67%.

Compound (**8**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1638 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.5 (s, 3H, OCH_3); 9.0 (s, 1H, H3); 8.4 (s, 1H, H5); 7.2; 7.25 (D, 2h, h2′, h6′); 7.4; 7.43 (d, 2H, H3′, H5′); 7.7; 7.8 (d, 2H, H2′′,

H6′′); 7.9; 8.0 (d, 2H, H3′′, H5′′); 8.2 (s, 1H, N=C–H); 11.7 (s, 1H, N–H). Melting point: 143–145 °C. Yield: 71%.

Compound (**9**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1670 (, C=O), 1530 and 1350 (, NO_2). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.8 (s, 3H, OCH_3); 8.8 (s, 1H, H3); 8.3 (s, 1H, H5); 7.1; 7.2 (d, 2H, H2′; H6′); 7.8; 7.9 (d, 2H, H3′, H5′); 7.7; 7.8 (d, 2H, H2′′, H6′′); 8.1; 8.2 (d, 2H, H3′′, H5′′); 8.1 (s, 1H, N=C–H); 9.0 (s, 1H, N–H). Melting point: 225–226 °C. Yield: 83%.

Compound (**10**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1643 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.3 (s, 3H, CH_3); 3.7 (s, 3H, OCH_3); 8.9 (s, 1H, H3); 8.3 (s, 1H, H5); 7.0; 7.1 (d, 2H, H2′, H6′); 7.7; 7.8 (d, 2H, H3′, H5′); 7.2; 7.3 (d, 2H, H2′′, H6′′); 7.6, 7.63 (d, 2H, H3′′, H5′′); 8.0 (s, 1H, N=C–H); 11.5 (s, 1H, N–H). Melting point: 194–196 °C. Yield: 75%.

Compound (**11**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1652 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.3 (s, 3H, CH_3); 3.8 (s, 3H, OCH_3); 8.9 (s, 1H, H3); 8.2 (s, 1H, H5); 6.8; 6.83 (d, 2H, H2′, H6′); 7.1; 7.2 (d, 2H, H3′, H5′); 7.5; 7.6 (d, 2H, H2′′, H6′′); 7.8; 7.8 (d, 2H, H3′′, H5′′); 8.0 (s, 1H, N=C–H); 11.3 (s, 1H, N–H). Melting point: 170–172 °C. Yield: 76%.

Compound (**12**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1637 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 1.3; 4.0 (m, 5H, OCH_2CH_3); 3.2 (s, 3H, OCH_3); 8.8 (s, 1H, H3); 8.2 (s, 1H, H5); 6.8; 6.9 (d, 2H, H2′, H6′); 7.63; 7.6 (d, 2H, H3′, H5′); 7.0; 7.1 (d, 2H, H2′′, H6′′); 7.7; 7.8 (d, 2H, H3′′, H5′′); 8.0 (s, 1H, N=C–H); 11.4 (s, 1H, N–H). Melting point: 163–165 °C. Yield: 71%.

Compound (**13**): IR (KBr, cm^{-1}) 3500–2900 (, NH), 1650 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.9 (s, 3H, OCH_3); 9.1 (s, 1H, H3); 8.7 (s, 1H, H5); 7.1; 7.2 (d, 2H, H2′, H6′); 7.5; 7.6 (d, 2H, H3′; H5′); 7.6; 7.7 (d, 2H, H2′′, H6′′); 7.8; 7.83 (d, 2H, H2′′, H6′′); 8.3 (s, 1H, N=C–H); 12.0 (s, 1H, N–H). Melting point: 221–223 °C. Yield: 60%.

Compound (**14**): IR (KBr, cm^{-1}) 3500–2500 (, NH), 1650 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.2 (s, 1H, H3); 8.9 (s, 1H, H5); 7.1; 7.2 (d, 2H, H2′, H6′); 7.95; 7.97 (d, 2H, H3′, H5′); 7.8; 7.9 (d, 2H, H2′′, H6′′); 8.0; 8.1 (d, 2H, H3′′; H5′′); 8.6 (s, 1H, N=C–H); 12.1 (s, 1H, N–H). Melting point: 230–232 °C. Yield: 73%.

Compound (**15**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1640 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.1 (s, 1H, H3); 8.7 (s, 1H, H5); 7.1; 7.2 (d, 2H, H2′, H6′); 7.5; 7.6 (d, 2H, H3′; H5′); 7.6; 7.7 (d, 2H, H2′′, H6′′); 7.8; 7.83 (d, 2H, H2′′, H6′′); 8.3 (s, 1H, N=C–H); 12.0 (s, 1H, N–H). Melting point: 240–241 °C. Yield: 68%.

Compound (**16**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1650 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.2 (s, 1H, H3); 8.9 (s, 1H, H5); 6.9; 6.91 (d, 2H, H2′, H6′); 8.0; 8.1 (d, 2H, H3′; H5′); 7.4; 7.5 (d, 2H, H2′′, H6′′); 7.6; 7.7 (d, 2H, H3′′, H5′′); 8.5 (s, 1H, N=C–H); 11.7 (s, 1H, N–H). Melting point: 200–202 °C. Yield: 61%.

Compound (**17**): IR (KBr, cm^{-1}) 3500–280 (, NH), 1640 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.0 (s, 6H, $N(CH_3)_2$8.4 (s, 1H, H3); 8.2 (s, 1H, H5); 6.6; 6.7 (d, 2H, H2′, H6′); 7.8; 7.9 (d, 2H, H3′, H5′); 6.7, 6.8 (d, 2H, H2′′, H6′′); 7.6; 7.7 (d, 2H, H3′′, H5′′); 8.1(s, 1H, N=C–H); 11.9 (s, 1H, N–H). Melting point: 235–237 °C. Yield: 70%.

Compound (**18**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1650 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 4.2; 1.4 (m, 5H, OCH_2CH_3); 8.9 (s, 1H, H3); 8.7 (s, 1H, H5); 6.9; 7.0 (d, 2H, H2′, H6′); 7.0; 7.1 (d, 2H, H3′; H5′); 7.7; 7.73 (d, 2H, H2′′, H6′′); 7.9; 8.0 (d, 2H, H3′′, H5′′); 8.3 (s, 1H, N=C–H); 11.5 (s, 1H, N–H). Melting point: 231–232 °C. Yield: 40%.

Compound (**19**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 1653 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 8.7 (s, 1H, H3); 8.1 (s, 1H, H5); 7.5; 7.6 (d, 2H, H2′, H6′); 7.9; 8.0 (d, 2H, H3′, H5′); 7.4–7.7 (m, 5H, H′Ar); 7.5 (s, 1H, N=C–H); 9.1 (s, 1H, N–H). Melting point: 228–229 °C. Yield: 75%.

Compound (**20**): IR (KBr, cm^{-1}) 3500–2400 (, NH), 1628 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.2 (s, 1H, H3); 8.5 (s, 1H, H5); 7.0; 7.1 (d, 2H, H3′, H6′); 7.8; 7.9 (d, 2H, H3′, H5′); 7.96; 7.93 (d, 2H, H2′′; H6′′); 8.0; 8.1 (d, 2H, H3′′, H5′′); 8.1 (s, 1H, N=C–H); 10.1 (s, 1H, OH); 11.6 (s, 1H, N–H). Melting point: 258–261 °C. Yield: 76%.

Compound (**21**): IR (KBr, cm^{-1}) 3500–2800 (, NH), 2222 (, CN) 1640 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.3 (s, 1H, H3); 8.6 (s, 1H, H5); 7.9; 8.0 (d, 2H, H2′; H6′); 8.0; 8.1 (d, 2H, H3′, H5′); 8.0; 8.1 (d, 2H, H2′′, H6′′); 8.0; 8.1 (d, 2H, H3′′, H5′′); 8.2 (s, 1H, N=C–H); 12.1 (s, 1H, N–H). Melting point: 250–252 °C. Yield: 65%.

Compound (**22**): IR (KBr, cm^{-1}) 3500–2700 (, NH), 1652 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.8 (s, 3H, OCH_3); 9.1 (s, 1H, H3); 8.4 (s, 1H, H5); 7.07; 7.1 (d, 2H, H2′, H6′); 7.8; 7.7 (d, 2H, H3′, H5′); 7.72; 7.75 (d, 2H, H2′′, H6′′); 7.9; 7.94 (d, 2H, H3′′; H5′′); 8.1 (s, 1H, N=C–H); 11.5 (s, 1H, N–H). Melting point: 227–232 °C. Yield: 85%.

Compound (**23**): IR (KBr, cm^{-1}) 3500–2500 (, NH), 1633 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.0 (s, 1H, H3); 8.3 (s, 1H, H5); 7.6; 7.7 (d, 2H, H2′, H6′); 7.8, 7.83 (d, 2H, H3′; H5′); 7.6; 7.7 (d, 2H, H2′′, H6′′); 7.7; 7.8 (d, 2H, H3′′, H5′′); 8.0 (s, 1H, N=C–H); 11.6 (s, 1H, N–H). Melting point: 248–250 °C. Yield: 78%.

Compound (**24**): IR (KBr, cm^{-1}) 3500–2900 (, NH), 1634 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.2 (s, 1H, H3); 8.4 (s, 1H, H5); 7.6; 7.64 (d, 2H, H2′; H6′); 7.9; 8.0 (d, 2H, H3′, H5′); 7.8; 7.83 (d, 2H, H2′′, H6′′), 7.8; 7.7 (d, 2H, H3′′, H5′′); 8.0 (s, 1H, N=C–H); 11.8 (s, 1H, N–H). Melting point: 215–216 °C. Yield: 70%.

Compound (**25**): IR (KBr, cm^{-1}) 3500–3000 (, NH), 1651 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.1 (s, 1H, H3); 8.7 (s, 1H, H5); 7.3; 7.37 (d, 2H, H2′, H6′); 7.75; 7.78 (d, 2H, H3′; H5′); 7.8; 7.9 (d, 2H, H2′′, H6′′), 8.0; 7.9 (d, 2H, H3′′, H5′′); 8.1 (s, 1H, N=C–H); 11.7 (s, 1H, N–H). Melting point: 255–258 °C. Yield: 60%.

Compound (**26**): IR (KBr, cm^{-1}) 3500–2600 (, NH), 1650 (, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.4 (s, 3H, CH_3); 9.2 (s, 1H, H3); 8.4 (s, 1H, H5); 7.4; 7.5 (d, 2H, H2′, H6′); 7.8; 7.9 (d, 2H, H3′; H5′); 7.7; 7.8 (d, 2H, H2′′; H6′′);

7.9; 8.0 (d, 2H, H3′′, H5′′); 8.2 (s, 1H, N=C–H); 11.7 (s, 1H, N–H). Melting point: 258–260 °C. Yield: 62%.

Compound (**27**): IR (KBr, cm^{-1}) 3300–2700 (□, NH), 1660 (□, C=O), 1550 and 1320 (□, NO_2). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 9.3 (s, 1H, H3); 8.7 (s, 1H, H5); 7.9; 8.0 (d, 2H, H2′, H6′); 8.5; 8.6 (d, 2H, H3′, H5′); 8.0; 8.1 (d, 2H, H2′′, H6′′); 8.2; 8.24 (d, 2H, H3′′; H5′′); 8.6 (s, 1H, N=C–H); 12.1 (s, 1H, N–H). Melting point: 273–275 °C. Yield: 67%.

Compound (**28**): IR (KBr, cm^{-1}) 3500–2600 (□, NH), 1637 (□, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.4 (s, 3H, CH_3); 9.3 (s, 1H, H3); 8.5 (s, 1H, H5); 7.4; 7.5 (d, 2H, H2′; H6′); 7.8; 7.9 (d, 2H, H3′; H5′); 7.9; 8.0 (d, 2H, H2′′; H6′′); 8.1; 8.11 (d, 2H, H3′′; H5′′); 8.3 (s, 1H, N=C–H); 11.8 (s, 1H, N–H). Melting point: 228–231 °C. Yield: 68%.

Compound (**29**): IR (KBr, cm^{-1}) 3500–2600 (□, NH), 1600 (□, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 3.5 (s, 6H, $N(CH_3)_2$); 9.2 (s, 1H, H3); 8.5 (s, 1H, H5); 6.9; 7.0 (d, 2H, H2′, H6′); 7.8; 7.9 (d, 2H, H3′; H5′); 7.6; 7.7 (d, 2H, H2′′, H6′′); 8.0; 8.1 (d, 2H, H3′′; H5′′); 8.4 (s, 1H, N=C–H); 11.5 (s, 1H, N–H). Melting point: 245–246 °C. Yield: 82%.

Compound (**30**): IR (KBr, cm^{-1}) 3500–2800 (□, NH), 1639 (□, C=O). ^{1}H-NMR (300 MHz; DMSO-d6, δ): 1.4; 4.2 (m, 5H, OCH_2CH_3); 9.1 (s, 1H, H3); 8.4 (s, 1H, H5); 7.1; 7.13 (d, 2H, H2′; H6′); 7.7; 7.8 (d, 2H, H3′; H5′); 7.8; 7.9 (d, 2H, H2′′, H6′′); 7.9; 8.0 (d, 2H, H3′′; H5′′); 8.3 (s, 1H, N=C–H); 11.6 (s, 1H, N–H). Melting point: 228–233 °C. Yield: 90%.

Melting points were obtained with a Fischer apparatus and were not corrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity plus 300 MHz, employing tetramethysilane as the internal reference at room temperature. The chemical shifts (δ) are reported in ppm and the coupling constant (J) in hertz. Infrared (IR) spectra were recorded on a Perkin–Elmer model 1420 FT-IR Spectrophotometer in KBr pellets. Microanalyses were performed on a Perkin–Elmer Model 2400 instrument and all values were within ± 0.4% of the calculated compositions.

5.2. Pharmacology

5.2.1. Leishmanicidal assays

Parasites: *L. amazonensis* (MHOM/BR/77LTB0016 strain), *L. chagasi* (MCAN/BR/97/P142 strain) and *L. braziliensis* (MCAN/BR/98/R619 strain) were maintained by animal passage and cryopreserved in liquid nitrogen. Promastigotes were cultured in Schneider's Drosophila medium pH 7.2 (Sigma, MO, USA), supplemented with 10–20% (v/v) of heat-inactivated foetal calf serum (FCS). The characterization of the strains was performed by molecular techniques such as isoenzyme electrophoresis [38].

5.2.2. Animals

The mice acquired from Laboratory Animals Nucleus (NAL-UFF) were first killed to obtain peritoneal macrophages and for both infection and isolation of *Leishmania*. The experiments were conducted using a protocol approved by the Institutional Committee of the Center for Biological Evaluation and Care of Research Animals (CEUA-Fiocruz).

5.2.3. Drug assay

The drugs tested within a concentration range of 40–320 μg ml^{-1} were solubilized in dimethylsulfoxide (DMSO, Sigma Chemical Co.), with the final concentration of the solvent in the experiments never exceeding 1.6%, then added to a 96 wells micro plate and incubated at 26 °C for 24 h with the parasites in their metacyclic phase in a concentration of 4×10^6 cells per ml [39].

The procedure used to observe the drug effects was a counting of the remaining parasites in Neubauer's chamber. The percentage of inhibition was estimated compared with the control and the results were expressed as EC_{50}/24h, i.e. the concentration of a compound that caused a 50% reduction in survival/viability in comparison to identical cultures without this compound. All tests were carried out in triplicate and ketoconazole, allopurinol or pentamidine was used as the reference drugs.

5.2.4. Cytotoxicity assays

The cytotoxicity effect of the main compounds: **27** and **15** expressed as cell viability was assayed on the mice's peritoneal macrophages. The cells were isolated from peritoneal cavity of Balb/c mice with cold RPMI 1640 medium, supplemented with 1 mmol l^{-1} L-glutamine, 1 mol l^{-1} HEPES, penicillin G (10^5 IU l^{-1}), streptomycin sulfate (0.10 g l^{-1}). The 2×10^5 cells per well were cultivated on microplate and incubated at 37 °C in a humidified 5% CO_2 atmosphere. After 2 h of incubation no adherent cells were then removed, and the adhered macrophages were washed twice with RPMI. Compounds were added to the cell culture at the respective EC_{50}/24h for *L. amazonensis* and cells incubated at 24 h. Then, the 3-[4,5-dymethilthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, MTT (Sigma) was added and the results could be read on an ELISA reader [40].

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