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Short Report

Synthesis and Antileishmanial Activity of New 1-Aryl-1*H*-Pyrazole-4-Carboximidamides Derivatives

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A quimioterapia para as leishmanioses, doenças causadas por protozoários do gênero *Leishmania*, ainda permanece ineficiente em diversos tratamentos. Portanto, existe a necessidade de pesquisa por novos fármacos. Nesse trabalho, foram sintetizados derivados 1-aril-1*H*-pirazol-4-carboximidamidas, avaliadas as atividades leishmanicida e os efeitos citotóxicos *in vitro*, e realizado um estudo de relação estrutura-atividade (REA) com a série de compostos. O composto **2** apresentou um perfil de atividade que pode ser melhorado através de estratégias de modificação molecular da química medicinal.

Chemotherapy for leishmaniasis, diseases caused by protozoa of the genus *Leishmania*, remains inefficient in several treatments. So there is a need to search for new drugs. In this work, we have synthesized 1-aryl-1*H*-pyrazole-4-carboximidamides derivatives and evaluated antileishmanial activities *in vitro*, as well as cytotoxic effects. Structure-activity relationship (SAR) studies were carried out with all the compounds of the series. Compound **2** showed an activity profile that can be improved through medicinal chemistry strategies.

Keywords: synthesis, 1-aryl-1*H*-pyrazole-4-carboximidamides, leishmaniasis

Introduction

Leishmaniasis is a group of vector-borne diseases caused by species of the genus *Leishmania* that affects about 12 million people in 88 countries in the world. These life-threatening diseases are of medical, social and economic importance in endemic areas, particularly in subtropical and tropical regions. *Leishmania* parasites exist in two forms: amastigote in the mammalian host and a flagellated promastigote in the insect vector.[1,2] Clinical manifestations occur in four major forms in humans including: *i*) visceral leishmaniasis (VL) that is usually fatal when untreated, *ii*) muco-cutaneous leishmaniasis (MCL) that is a mutilating disease, *iii*) diffuse cutaneous leishmaniasis (DCL), which is a long-lasting disease due to a deficient cellular-mediated immune response and, *iv*) cutaneous leishmaniasis (CL) that is disabling when there are multiple lesions.[3]

The difficulty to control this parasitic disease remains a serious problem mainly due to the diversity of mammalian reservoirs (wild and domestic animals), species of vectors and *Leishmania* species.[4]

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Chemotherapy for leishmaniasis is generally ineffective mainly due to the emergence of drug-resistant strains and toxicity of the therapeutics agents. The pentavalent antimonials compounds are widely used as primary therapy whereas other drugs such as amphotericin B, pentamidine, paromomycin, azole derivatives and glucantime are also used.[5]

Pyrazoles are a class of heterocyclic compounds that exhibit a broad spectrum of biological activities such antiinflamatory, antimicrobial and antitumor.[6] Consequently, a large number of synthetic routes to pyrazoles have been reported and summarized in some monographs and reviews.[7,8] These reports have been useful for biologists and chemists engaged in the development of new drugs and/or synthetic routes. Our group has synthesized pyrazole carbohydrazides with anti-*Leishmania in vitro*[9] and *in vivo*[10] activity. Simultaneously, substances containing the amidine group and affecting large number of pathogens (*i.e., Giardia lamblia*, *Leishmania sp*., *Pneumocystis carinii*, *Candida albicans*, *Aspargillus sp*. and *Trypanosoma sp.*) have been reported,[11] as well as some reviews about synthetic approaches.[12] Pentamidine (Figure 1) is clinically used in the treatment of pneumonia caused by the opportunistic fungus, *Pneumocystis jirovecii*, early stage human african tripanosomiasis (HAT) and when treatment with pentavalent antimonials or amphotericin B has failed against *Leishmania*.[5,13]

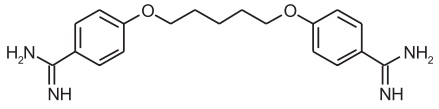


Figure 1. Structural formula of pentamidine.

In the present work, we prepared five new 1-aryl-1*H*-pyrazole-4-carboximidamides derivatives **1-5** and evaluated their leishmanicidal acitivity, cytotoxicity and theoretical profiles (Scheme 1).

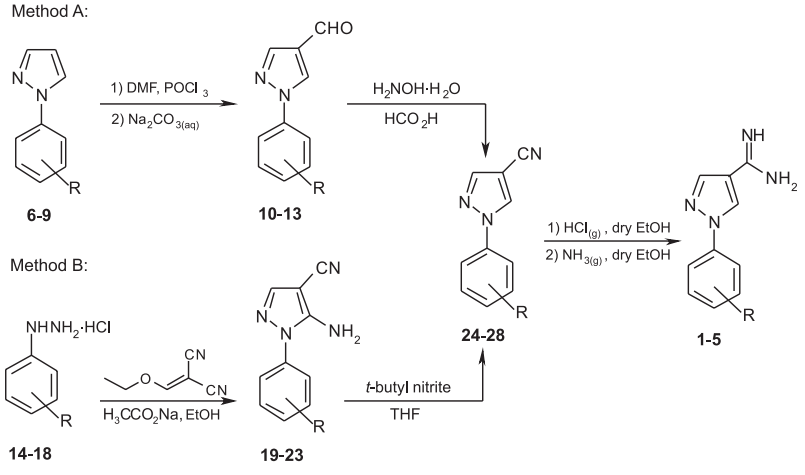
Results and Discussion

The key intermediates 1-aryl-1*H*-pyrazole-4-carbonitriles **24-28** were obtained from two alternate routes (methods A and B). In method A, 1-aryl-1*H*-pyrazole-4-carbaldehydes **10-13** were synthesized through a Vilsmeyer-Haack reaction involving 1-aryl-1*H*-pyrazoles **6-9**, dimethylformamide (DMF) and $POCl_3$.[14] The aldehydes formed were converted to key intermediates from the "one-pot reaction" with hydroxylamine and methanoic acid.[15] In method B, arylhydrazine hydrochlorides **14-18** reacted with ethoxymethylenemalononitrile in ethanol and the resulting 5-amino-1-aryl-1*H*-pyrazole-4-carbonitriles **19-23** were subjected to aprotic deamination with *t*-butyl nitrite in tetrahydrofuran to generate the key intermediates.[16,17] Finally, the targets 1-aryl-1*H*-pyrazole-4-carboximidamides derivatives **1-5** were obtained by the reaction of the key intermediates **24-28** with gaseous hydrochloric acid followed by treatment with ammonia.[18]

The compounds **1-5** were identified by proton nuclear magnetic resonance (^{1}H NMR), carbon nuclear magnetic resonance (^{13}C NMR), Fourier transform infrared (FTIR) spectroscopies and elementary analysis.

Biological and cytotoxity assays

The effect of different concentrations (40, 80, 160 and 320 μg mL^{-1}) of the 1-aryl-1*H*-pyrazole-4-carboximidamides



Scheme 1.

derivatives against *L. amazonensis* promastigotes growth inhibition was monitored microscopically at the end of the exponential growth phase. Interestingly, an antiproliferative effect was observed for compounds **2** and **3** (IC_{50} = 105 ± 45 μmol L^{-1} or 279 ± 12 μg mL^{-1} and 112 ± 41 μmol L^{-1} or 259 ± 12 μg mL^{-1} respectively) on *L .amazonensis* in contrast to compounds **1** (IC_{50} > 1720 μmol L^{-1} or > 320 μg mL^{-1}), **4** (IC_{50} > 1480 μmol L^{-1} or > 320 μg mL^{-1}) and **5** (IC_{50} > 1250 μmol L^{-1} or > 320 μg mL^{-1}) (Figure 2). Despite the lower profile of **2** and **3** compared to pentamidine effect (IC_{50} = 3.6 ± 1.6 μmol L^{-1}), their activity is still promising since new substitutions may be performed to improve it. The major pyrazolic compounds presented a cytotoxicity profile better than pentamidine (Figure 2).

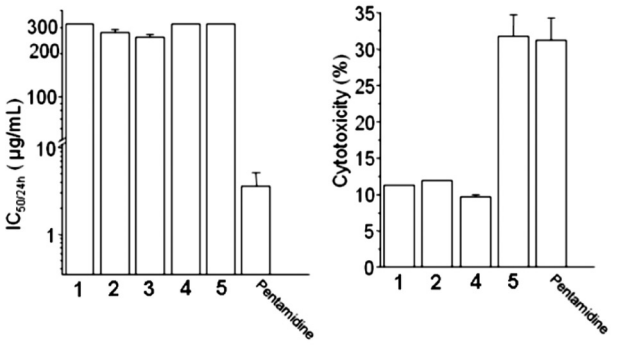


Figure 2. Biological features of 1-aryl-1*H*-pyrazole-4-carboximidamides compounds compared with pentamidine. Antiparasitic effect against *Leishmania amazonensis* (left) and cytotoxicity profile against mice peritoneal macrophages (right).

These biological results may suggest that the two amidine groups of the pentamidine (Figure 1) are important for binding in a non-intercalative way to the minor grove regions of DNA (kDNA) in the *Leishmania.*[19]

Literature reports described monoamidine derivatives with three hydrophobic phenyl groups with potential effects against *L. amazonensis*.[20] This effect may be associated to an increase of lipophilicity of these compounds, which facilitates the transport through parasite membrane. In addition, it has been demonstrated the inhibitory effect of these monoamidine derivatives on the phosphorylating activity of cAMP-dependent (cyclic adenosine monophosphate) protein kinase (PKA)[21] and on nitric oxide production by promastigotes and axenic amastigotes forms of *L. amazonensis*.[22]

Concerning the 1-aryl-1*H*-pyrazole-4-carboximidamides compounds, the pyrazoles nucleus have displayed an impressive array of biological activities, among which antiprotozoa, anti-malarial, anti-inflammatory, immunomodulatory, nitric oxide inhibition, cytotoxic and anti-cancer activities.[23]

Molecular modeling and Lipinski rule of five studies

The minimum energy conformations of **1**-**5** derivatives, calculated by the AM1 semiempirical Hamiltonian,[24] showed that, as expected, all rings of these compounds are co-planar except for **5** where the two chlorines lead to a rotation of the ring (Figure 3). Despite of this conformational difference, this structural feature did not contribute for a biological activity for **5**. Subsequently, a single-point energy *ab initio* calculation was performed at the 6-311G* level in order to derive electronic properties, such as highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy values, volume, molecular dipole moment (μ), and molecular electrostatic potential (MEP), which could be related to the variation of the antileishmanial activity of these compounds.[25] The results showed that, although the substitution pattern into the 1-aryl-1*H*-pyrazole-4-carboximidamides structures lead to different antileishmanial profiles, the molecular dipole moment, volume and TPSA (total polar surface area) values did not present any direct correlation with it, as shown in Table 1 and Figure 3.

Differently, HOMO and LUMO energy values apparently seem to be related to these derivatives biological profile since compounds **2** and **3** showed an antileishmanial profile and also presented the lowest values for these orbital energies (Table 1). This suggests a different reactivity for these molecules compared to the other derivatives that probably allowed their active profile.

As receptors recognize stereo-electronic effects and not atom *per se*, studies of molecular electronic properties

Table 1. Theoretical evaluation of 1-aryl-1*H*-pyrazole-4-carboximidamides compounds

Compound	R	$IC_{50\ (\mu g\ mL^{-1})}$	Energy (eV)		Dipole (Debie)	Volume (A^3)	TPSA	Lipinski rule of five			
			HOMO	LUMO				M_w	HBA	HBD	clogP
1	H	> 320	–8.38	2.60	4.17	215.37	67.7	186.22	4	3	–0.025
2	Br	279	–9.33	–0.79	2.88	240.04	67.7	265.11	4	3	0.784
3	NO_2	259	–9.20	0.85	3.59	246.52	113.5	231.22	7	3	–0.066
4	OMe	> 320	–7.99	2.78	5.19	246.64	76.9	216.24	5	3	0.032
5	2,6Cl	> 320	–8.97	2.34	5.50	250.98	67.7	255.11	4	5	1.658

could be very effective in interpreting the electronic structure in a comprehensive way.[26] Therefore, the MEP is a useful approach for understanding the electrostatic contribution for the receptor-ligand binding process that has been used in different reports for elucidating this issue. In this work, the analysis of R position on MEPs generated for these compounds revealed that the substitution lead to a different electrostatic distribution depending on the added group mainly on compound **2** (Figure 3). However this MEP difference is not directly expressed in the biological activity as expected.

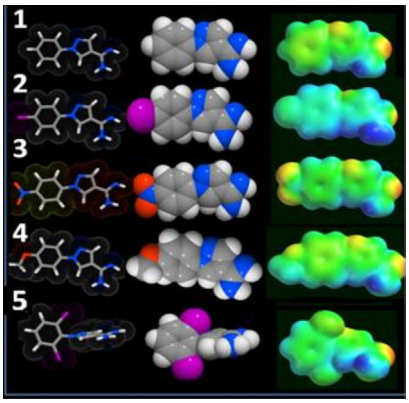


Figure 3. Conformational and electronic properties of 1-aryl-1*H*-pyrazole-4-carboximidamides derivatives. Tube (left) and CPK (middle) representation of the most stable conformation are represented by atom color. On the right, the molecular electrostatic potential energy isosurfaces (MEP) is superimposed onto total electrons density of 0.002 e au^{-3}. The color code (see online) is in the range of –25 to +55 kcal mol^{-1}.

Since the compounds are considered for oral delivery, in this work we submitted them to the analysis of Lipinski rule of five,[27] which indicates if a chemical compound could be an orally active drug in humans. The rule states that most "drug-like" molecules have clogP ≤ 5, molecular weight (M_w) ≤ 500, PSA ≤ 140, and number of hydrogen bond acceptors (HBA) ≤ 10 and donors (HBD) ≤ 5. Molecules violating more than one of these rules may have problems with bioavailability.[27] Our results pointed all compounds as fulfilling this rule and therefore with a good theoretical biodisponibility (Table 1).

Experimental

Unless otherwise noted, all the reagents and solvents were obtained from the market and used without further purification. Melting points were obtained with a Fisher apparatus and were uncorrected. ^{1}H NMR spectra were recorded at room temperature on a Varian Unity plus 300 MHz employing tetramethylsilane as the internal reference. The chemical shifts (δ) are reported in ppm and the coupling constants (J) in hertz. Infrared (IR) spectra were recorded as potassium bromide (KBr) pellets on a Perkin-Elmer Model 1420 FT IR Spectrophotometer. Microanalyses were performed on a Perkin-Elmer Model 2400 instrument and all values were within ± 0.4% of the calculated compositions. Purity of the reaction products were checked by means of thin layer chromatography (TLC) using silica gel plates with fluorescent indicator and hexane/ethyl acetate (1:1, v/v) as eluent, melting points, IR and ^{1}H NMR spectra.

*General procedure for the preparation of 1-aryl-1H-pyrazole-4-carbaldehydes compounds **10-13***

$POCl_3$ (0.023 mol) was added to DMF (0.033 mol) at 0 °C and the mixture was stirred for 15 min. After this time 1-aryl-1*H*-pyrazoles **6-9** (0.003 mol) dissolved in DMF were added dropwise with stirring. The reaction mixture was then heated 3 h at 110 °C. The solution was then poured slowly into 5 mL saturated sodium carbonate aqueous solution and stirred 30 min. The organic layer was diluted with ether, washed with saturated Na_2CO_3 aqueous solution, and dried with $MgSO_4$ anhydrous. Evaporation of the organic extract under reduced pressure gave the corresponding products. The recrystallization was made from ethanol/water. **10** (R = H): mp 84-85 °C, yield 73%; **11** (R = 4-Br): mp 124-126 °C, yield 86%; **12** (R = 4-NO_2): mp 150-151 °C, yield 75%; **13** (R = 4-OCH_3): mp 91-92 °C, yield 59%.

*General procedure for the preparation of 5-amino-1-aryl-1H-pyrazole-4-carbonitriles compounds **19-23***

Arylhydrazine hydrochlorides **14-18** (0.01 mol) were reacted with ethoxymethylenemalononitrile (0.01 mol) and sodium acetate (0.02 mol) in ethanol (40 mL), under reflux, during 40 min. Afterwards, the mixture was poured in cold water and the precipitate formed was filtered out and recrystallized from ethanol/water. The reactions were accomplished by means of TLC using silica gel plate with fluorescent indicator and hexane/ ethyl acetate (1:1) as eluent. **19** (R=H): mp 135-136 °C, yield 90%; **20** (R=4-Br): mp 168-169 °C, yield 86%; **21** (R=4-NO_2): mp 220-221 °C, yield 68%; **22** (R=4-OCH_3): mp 135-136 °C, yield 75%; **23** (R=2,6-diCl): mp 190-191 °C, yield 78%.

*General procedure for the preparation of 1-aryl-1H-pyrazole-4-carbonitriles compounds **24-28***

Method A

The reaction mixture of the aldehydes **10-13** (7.8 mmol L^{-1}) with hidroxylamine and methanoic acid

(10 mL) was maintained under reflux form 6 h, until the end of reaction was indicated by TLC. Then the reaction mixture was poured in cold water and the precipitate formed was filtered out washed with ethanol and recrystallized from ethanol/water to afford crystals. The purity of the compounds was checked by means of TLC using silica gel plate with fluorescent indicator and hexane/ethyl acetate (1:1, v/v) as eluent, melting point, IR spectra and ^{1}H NMR. **24** (R = H): mp 91-92 °C, yield 85%; **25** (R = 4-Br): mp 198-199 °C, yield 80%; **26** (R = 4-NO_2): mp 185-186 °C, yield 65%; **27** (R = 4-OCH_3): mp 138-141 °C, yield 79%; **28** (R = 2,6-diCl): not synthesized.

Method B

The reaction mixture of *t*-butyl nitrite (4 mL) with dry THF (10 mL) was stirred and refluxed under 20 min. Then, 0.005 mol of 5-amino-1-aryl-1*H*-pyrazole-4-carbonitriles **19**-**23** was added. The mixture was stirred and refluxed about 2 h. Afterwards, the mixture THF and *t*-butyl nitrite was evaporated. The precipitate was recrystallized with the mixture of ethanol/water. The purity of the compounds was checked by means of TLC using silica gel plate with fluorescent indicator and hexane/ethyl acetate (1:1, v/v) as eluent, melting point, IR spectra and ^{1}H NMR. **24** (R = H): mp 90-91 °C, yield 82%; **25** (R = 4-Br): mp 198-200 °C, yield 86%; **26** (R = 4-NO_2): mp 187-188 °C, yield 62%; **27** (R = 4-OCH_3): mp 138-141 °C, yield 82%; **28** (R = 2,6-diCl): mp 141-142 °C, yield 81%.

*General procedure for the preparation of 1-aryl-1H-pyrazole-4-carboximidamides compounds **1**-**5***

A mixture of derivatives **24**-**28** (0.01 mol) and 20 mL of dry ethanol was cooled at 0-5 ºC and saturated with chloridric acid gas. The mixture was sealed and stirred at room temperature for 5 days. After this, bubble ammonium gas was added at mixture reaction and stirred for 7 days. The solvent was evaporated and the crystals was purify with ethanol/water. The purity of the compounds was checked by means of TLC using silica gel plate with fluorescent indicator and hexane/ethyl acetate (1:3) as eluent, melting point, IR Spectra and ^{1}H NMR.

*1-Phenyl-1H-pyrazole-4-carboximidamide (**1**)*

mp 220-221 °C, yield 67%; FT IR (KBr) ν_{max}/cm^{-1} 3303 to 3077, 1682; ^{1}H NMR (DMSO, δ in ppm): H_3 8.63 (s), H_5 9.64 (s), H_2' and H_6' 7.93 (d; 8.1 Hz), H_3' and H_5' 7.71 (t; 7.8 Hz), H_4' 7.56 (t; 7.8 Hz), $NHNH_2$ 4.08 (br); ^{13}C NMR (DMSO, δ in ppm): C_3 141.3, C_4 113.3, C_5 131.4, C_1' 138.7, C_2' and C_6' 119.3, C_3' and C_5' 130.1, C_4' 128.0, C(NH)NH_2 158.1; MS: *m/z* 186.2022 (M^+, 100%). Found: C, 64.34; H, 5.28; N, 29.95. Calc. for $C_{10}H_{10}N_4$: C, 64.50; H, 5.41; N, 30.09%.

*1-(4'-Bromophenyl)-1H-pyrazole-4-carboximidamide (**2**)*

mp 270-271 °C, yield 66%; FT IR (KBr) ν_{max}/cm^{-1} 3400 to 3096, 1660; ^{1}H NMR (DMSO, δ in ppm): H_3 9.23 (s), H_5 10.00 (s), H_2' and H_6' 7.93 (d; 8.7 Hz), H_3' and H_5' 7.21 (d; 9.0 Hz), $NHNH_2$ 3.48 (br); ^{13}C NMR (DMSO, δ in ppm): C_3 143.1, C_4 114.2, C_5 132.5, C_1' 145.8, C_2' and C_6' 124.1, C_3' and C_5' 125.0, C_4' 142.5, C(NH)NH_2 152.0; MS: *m/z* 265.1107 (M^+, 100%). Found: C, 45.21; H, 3.29; N, 21.06; Br, 29.98. Calc. for $C_{10}H_9N_4Br$: C, 45.30; H, 3.42; N, 21.14; Br, 30.14%.

*1-(4'-Nitrophenyl)-1H-pyrazole-4-carboximidamide (**3**)*

mp 257-258 °C, yield 48%; FT IR (KBr) ν_{max}/cm^{-1} 3400 to 3100, 1658; ^{1}H NMR (DMSO, δ in ppm): H_3 8.53 (s), H_5 9.26 (s), 7.20-7.40 (m), $NHNH_2$ 3.86 (br); ^{13}C NMR (DMSO, δ in ppm): C_3 145.1, C_4 117.5, C_5 133.2, C_1' 147.7, C_2' and C_6' 126.0, C_3' and C_5' 127.1, C_4' 146.5, C(NH)NH_2 156.0; MS: *m/z* 231.2101 (M^+, 100%). Found: C, 51.81; H, 3.79; N, 30.06; O, 14.34. Calc. for $C_{10}H_9N_5O_2$: C, 51.95; H, 3.92; N, 30.29; O, 13.84%.

*1-(4'-Methoxyphenyl)-1H-pyrazole-4-carboximidamide (**4**)*

mp 239-240 °C, yield 65%; FT IR (KBr) ν_{max}/cm^{-1} 3211 to 3100, 1634; ^{1}H NMR (DMSO, δ in ppm): H_3 8.06 (s), H_5 8.77 (s), H_2' and H_6' 7.74 (d; 9.0 Hz), H_3' and H_5' 7.06 (d; 8.7 Hz), $NHNH_2$ 4.62 (br), OCH_3 3.88 (s); ^{13}C NMR (DMSO, δ in ppm): C_3 143.5, C_4 92.6, C_5 120.4, C_1' 131.2, C_2' and C_6' 121.1, C_3' and C_5' 114.9, C_4' 149.8, C(NH)NH_2 158.8, OCH_3 55.7; MS: *m/z* 216.2193 (M^+, 100%). Found: C, 60.96; H, 5.49; N, 25.75; O, 7.80. Calc. for $C_{11}H_{12}N_4O$: C, 61.10; H, 5.59; N, 25.91; O, 7.40%.

*1-(2',6'-Dichlorophenyl)-1H-pyrazole-4-carboximidamide (**5**)*

mp 222-223 °C, yield 60%; FT IR (KBr) ν_{max}/cm^{-1} 3400 to 3189, 1647; ^{1}H NMR (DMSO, δ in ppm): H_3 8.28 (s), H_5 9.05 (s), H_3' and H_5' 7.93 (d; 8.7 Hz), H_4' 7.55 (t; 8.7 Hz), $NHNH_2$ 4.65 (br); ^{13}C NMR (DMSO, δ in ppm): C_3 144.0, C_4 95.9, C_5 132.1, C_1' 136.8, C_2' 112.4, C_3' and C_5' 118.3, C_4' 128.3, C_5' 130.1, C_6' 112.4, C(NH)NH_2 156.3; MS: *m/z* 255.0925 (M^+, 100%). Found: C, 46.94; H, 3.02; N, 21.88; Cl, 28.16. Calc. for $C_{10}H_8N_4Cl_2$: C, 47.09; H, 3.16; N, 21.96; Cl, 27.79%.

Biological and cytotoxity assays

Leishmania amazonensis (MHOM/BR/LTB0016 strain) promastigotes were grown at 26 °C in Schneider'

Drosophila medium[21] supplemented with 10% v/v heat-inactivated foetal calf serum (FCS) at pH 7.2. Parasites were harvested from the medium on day 4, when there was a high percentage of infective forms (metacyclic promastigotes), were counted in a Neubauer' Chamber and adjusted to a concentration of 4×0^6 parasites mL^{-1}, for the drug assay.[22,28]

The assay was carried out in 96-well flat-bottom microplate with a volume of 200 µL/well. The compounds **1**-**5** solubilized in dimethyl sulfoxide (DMSO) (the highest concentration used was 1.6% v/v, not hazardous to the parasite) were added to the culture, in a concentration range from 320 from 80 µg mL^{-1}. After 24 h incubation in a temperature of 26 ºC, the remaining parasites were counted in a Neubauer's chamber and compared with the controls with DMSO, without the drugs and with the parasites alone. All tests were done in triplicate and pentamidine isethionate was used as reference drug. The IC_{50}/24 h was calculated by means of dose-response curves at a wider range of concentrations, and the results were expressed as the mean ± standard deviation determined from three independent experiments.

The cytotoxicity effect of the derivatives **1**-**5** expressed as cell viability was assayed on 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 (10^5IUI^{-1}), streptomycin sulfate(0.10 g L^{-1}). The 2×10^5 cells *per* well were cultivated on microplate and incubated at 37 ºC 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} /24 h for *L. amazonensis* and cells incubated for 24 h. Then, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide, MTT was added and after 2-4 h the reaction was interrupted with DMSO. The results could be read in spectrophotometer with wavelength of 570 nm.[23,28,29]

Molecular modeling

The molecular modeling study was performed using SPARTAN'06 software package (Wavefunction Inc. Irvine, CA, 2000).[30] The minimum energy conformation of the derivatives was obtained by the AM1 semiempirical Hamiltonian. In order to better evaluate the electronic properties of the AM1 minimum energy conformations, they were submitted to a single-point energy *ab initio* calculation at the 6-311G* level.

In order to perform structure-activity relationship (SAR) studies, some electronic properties, such as HOMO and LUMO energy values, HOMO and LUMO orbital coefficients distribution, molecular dipole moment (µ), and molecular electrostatic potential (MEP) were calculated. MEP isoenergy surface maps were generated in the range from −25.0 (deepest red color) to +30.0 (deepest blue color) kcal mol^{-1} and superimposed onto a molecular surface of constant electron density of 0.002 e au^{-3}. Each point of the three dimensional molecular surface map expresses the electrostatic interaction energy value evaluated with a probe atom of positive unitary charge providing an indication of the overall molecular size and location of attractive (negative) or repulsive (positive) electrostatic potentials.

Since the compounds are considered for oral delivery, they were also submitted to the analysis of Lipinski rule of five, which evaluate some properties of a compound that would make it a likely orally active drug in humans. These structural parameters were performed using Molispiration program.[31]

Conclusions

In this work we described a new set of 1-aryl-1*H*-pyrazole-4-carboximidamide compounds synthesized in good yields that presented an antileishmanial activity profile. This series can be scaled up and easily produce new analogues. Compound **2** (Br-substituted) also presented a low cytotoxicity profile that pointed it as a lead compound for further substitutions to improve its biological profile. All compounds showed a good theoretical biodisponibility and the molecular modelling evaluation showed that HOMO and LUMO energies of **2** and **3** led to a different reactivity profile that seem to be related to their antileishmanial profile. The hydrophobic substituents in phenyl-pyrazolic groups may be useful to investigate the contribution of this structural unit on its bioactivity profile. Further experiments are being carried out in order to define better chemical structure and biological activity relationships.

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